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MHC Susceptibility Genes to IgA Deficiency Are Located in Different Regions on Different HLA Haplotypes

Emilio G. De la Concha,1* Miguel Fernandez-Arquero,1 Lorena Gual,1 Patricia Vigil,1 Alfonso Martinez,1 Elena Urcelay,1 Antonio Ferreira,1 María C. García-Rodríguez,1 and Gumersindo Fontan2

Familial predisposition to IgA deficiency (IgAD) suggests that genetic factors influence susceptibility. Most studies support a polygenic inheritance with a susceptibility locus (designated IGAD1) in the MHC, but its exact location is still controversial. This study aimed to map the predisposing IGAD1 locus (or loci) within the MHC by investigating the pattern of association of the disease with several markers in the region. DNA-based techniques were used to type individual alleles of four polymorphic HLA genes (HLA-DR, -DQA1, -DQB1, and HLA-B), six microsatellites (all located between HLA-DR and HLA-B), and three single nucleotide polymorphisms on the TNF genes. The frequencies of these alleles were compared among ethnically matched populations comprising 182 patients and 343 controls. Additionally, we investigated parents and siblings of 100 of these patients. All four parental haplotypes were established in each family (n = 400), and transmission disequilibrium tests were performed. Surprisingly, our results did not support the hypothesis of a unique susceptibility gene being shared by all MHC susceptibility haplotypes. On HLA-DR1 and -DR7-positive haplotypes IGAD1 mapped to the class II region, whereas on haplotypes carrying HLA-DR3 the susceptibility locus mapped to the telomeric end of the class III region, as reported previously. Our results show how, in complex diseases, individuals may be affected for different genetic reasons and a single linkage signal to a region of a chromosome may actually be the result of disease-predisposing alleles in different linked genes in different pedigrees.

Immunoglobulin A deficiency (IgAD) is the most common form of primary immunodeficiency and affects approximately one of every 600 Caucasian individuals. The familial predisposition to IgAD suggests that genetic factors influence susceptibility. Most studies support a polygenic inheritance with a susceptibility locus (designated IGAD1) in the MHC. Associations of IgAD were first described with HLA class I alleles, most consistently with A1 and B8 (2–4) but also with A28, B14 (5–7), and B44 (8, 9). Subsequently, an association with the class II allele HLA-DR3 was repeatedly observed (4, 9–14), in addition to associations with DR1 (7, 9–11) and DR7 (6, 9, 10, 12). Some early studies already signaled that the association was with particular combinations of MHC alleles rather than with individual alleles per se (5, 7) and that these combinations reflected extended, conserved, or “ancestral” haplotypes A1-B8-DR3, A28-B14-DR1, and B44-DR7 (7, 8, 11, 15). The conservation of these haplotypes, which appear derived from a common remote ancestor (16, 17), has rendered difficult the identification of the individual genes responsible for IgAD.

Within the MHC, primary association with both class II HLA genes (10, 18) and class III genes (8, 11, 12, 15) has been claimed. In recent years, despite the development of high-density genetic

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Materials and Methods

The study group consisted of 182 IgAD patients (as defined by the World Health Organization Group on Primary Immunodeficiencies (24), all ascertained at the Immunology Unit of La Paz Hospital (Madrid, Spain)) and 343 healthy controls. All patients and controls were unrelated Spanish
Caucasians. Recurrent infections were present in 24 patients, allergy was present in 26 patients, autoimmune diseases were present in 27 patients, and celiac disease was present in 14 patients. Additionally, we investigated parents and siblings of 100 of these patients. Of the 100 families, 13 were found to have a second (or more) IgAD patient (nine siblings, four fathers, and eight mothers of the probands). In all, 412 family members were studied and all parental haplotypes were established in each family (n = 400).

**Molecular biology methods**

DNA was isolated by standard methods from peripheral blood leukocytes by a salting-out procedure with 6 M NaCl after overnight incubation with proteinase K (25). HLA-B was typed using the Low Resolution SSP Typing kit by Biosynthesis (Lewisville, TX). DRB1 typing (and subtyping) and DQA1 and DQB1 typing were conducted by PCR amplification and hybridization with allele-specific oligonucleotides; both primers and probes were used according to the Eleventh International Histocompatibility Workshop (26). Because we were interested in positive associations, only DR1 was subtyped. Positive associations were observed (as previously published) with DR3 and DR7, but in our population only one subtype of DR3 and one subtype of DR7 are found; therefore, no subtyping was performed on them. TNFα alleles at positions −376, −308, and −238 were investigated by PCR and dot blot hybridization according to the method of Brinkman et al. (27). The TNFα and β microsatellite typing was performed as described by Nedosposav et al. (28). PCR amplification and sizing of DNA fragments containing TNFα and β microsatellites separately and of a larger fragment containing both microsatellites made TNFab haplotype assignment possible; the two longer fragment lengths were a combination of the sizes of the two individual microsatellites in each DNA molecule plus the intervening 22-bp sequence (28). MICA transmembrane exonic microsatellite was amplified using primers and conditions described by Ota et al. (29). The MICA R primer was labeled with FAM. D6S273 microsatellite was amplified using primers described by Martin et al. (30). The 142-3 primer was labeled with tetrachloro-6-carboxyfluorescein. BAT2 (821) and 9N2-1 primer was labeled with FAM. The PCR fragments were subsequently run on an ABI Prism 310 automatic sequencer (PE Applied Biosystems, Foster City, CA). The microsatellite samples were analyzed using the Genescan software (PE Applied Biosystems) with the TAMRA 500-pb marker (added to each sample) and the local Southern size-calling method under denaturing conditions.

**Statistical analysis**

The joint segregation of DRB1, DQA1, DQB1, 9N2, D6S273, BAT2, TNF-376, −308, −238, TNFab, MICA, and HLA-B in the families led to the establishment of the haplotypes involved.

Allele or haplotype transmission from heterozygous parents to affected offspring was studied using the transmission disequilibrium test. The distributions of transmitted and nontransmitted alleles were compared using a Monte Carlo computer simulation with 20,000 iterations under the null hypothesis of a probability of transmission of 0.5. These were confirmed with a χ² test (or Fisher’s exact test) for each individual allele. When a difference between the two methods was found we preferred the Monte Carlo simulation, although the p values were slightly higher, because it is considered more exact.

For stratified transmission test (when the null hypothesis of a probability of transmission of 0.5 is no longer feasible) and for all case control comparisons, a χ² test (or Fisher’s exact test when an expected cell value was <5) was used. Strength of associations was given as odds ratios (OR). A standard statistical software package (EPI-INFO version 6.02; World Health Organization, Geneva, Switzerland) was used.

Because IgAD was associated with more than one allele or haplotype in the MHC region, the relative predispositional effects (RPE) of some alleles or haplotypes could not be readily determined. A primary association could mask a second positive association because of the expected decreased frequency of the latter imposed by the former. Therefore, we used the RPE method (31) to identify several associations sequentially. Thus, the possibility that an association with an allele or haplotype could create misleading deviations in the frequencies of other alleles was eliminated.

**Results**

**Association with HLA-DR alleles**

Table I shows the frequency of HLA-DR alleles in IgAD patients and controls. All three alleles (DR1, DR3, and DR7) previously

<table>
<thead>
<tr>
<th>HLA-DR</th>
<th>Patients n = 182 (%)</th>
<th>Controls n = 383 (%)</th>
<th>p value OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR1*01</td>
<td>1.4 × 10⁻³</td>
<td>2.33 NS</td>
<td></td>
</tr>
<tr>
<td>DR1*0101</td>
<td>12.1</td>
<td>13.3</td>
<td>NS</td>
</tr>
<tr>
<td>DR1*0102</td>
<td>27.5</td>
<td>6.8</td>
<td>2.2 × 10⁻¹⁰</td>
</tr>
<tr>
<td>DR1*0103</td>
<td>1.1</td>
<td>2.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HLA-DR</th>
<th>Patients n = 182 (%)</th>
<th>Controls n = 383 (%)</th>
<th>p value OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR3</td>
<td>14.8</td>
<td>21.1</td>
<td>1.7 × 10⁻¹⁴</td>
</tr>
<tr>
<td>DR1*01</td>
<td>3.8</td>
<td>26.4</td>
<td>NS</td>
</tr>
<tr>
<td>DR1*0101</td>
<td>36.3</td>
<td>24.5</td>
<td>0.004</td>
</tr>
<tr>
<td>DR1*0102</td>
<td>14.8</td>
<td>21.1</td>
<td>NS</td>
</tr>
<tr>
<td>DR1*0103</td>
<td>9.9</td>
<td>24.0</td>
<td>7.4 × 10⁻³</td>
</tr>
<tr>
<td>DR7</td>
<td>28.6</td>
<td>29.0</td>
<td>NS</td>
</tr>
<tr>
<td>DR1*01</td>
<td>44.0</td>
<td>28.2</td>
<td>0.0002</td>
</tr>
<tr>
<td>DR1*0101</td>
<td>2.7</td>
<td>7.8</td>
<td>0.02</td>
</tr>
<tr>
<td>DR1*0102</td>
<td>0</td>
<td>1.5</td>
<td>NS</td>
</tr>
<tr>
<td>DR1*0103</td>
<td>2.2</td>
<td>1.8</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Table I. HLA-DR (and DRB1*01 subtypes) phenotypic frequencies in IgAD patients and healthy controls**

FIGURE 1. Schematic presentation of the MHC from HLA-DQ to HLA-B and relative positions of the genes and microsatellites studied. The studied genes and the physical distances between them are indicated in the upper part. The tested microsatellites and the physical distances between them are indicated in the lower part. Approximate distances are given in kilobases. Extended conserved haplotypes predisposing to IgAD, with the haplotype alleles of the tested markers, are also shown within a frame. The different susceptibility regions have been underlined and susceptibility alleles are shown in boldface.
Table II. Frequencies of B8-DR3 haplotypes, recombinant forms of the B8-DR3 haplotype, and other DR3-positive haplotypes in transmitted (T) vs nontransmitted (NT) haplotypes

<table>
<thead>
<tr>
<th>HLA-DR3</th>
<th>D6S273</th>
<th>TNF-308A</th>
<th>TNF-238G</th>
<th>TNFa2b3</th>
<th>MICA A5.1</th>
<th>HLA-B8</th>
<th>T</th>
<th>NT</th>
<th>p Value</th>
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<tr>
<td>+</td>
<td>140</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>16</td>
<td>6</td>
<td>+</td>
<td>0.031</td>
</tr>
<tr>
<td>+</td>
<td>136</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>7</td>
<td>1</td>
<td>+</td>
<td>0.037</td>
</tr>
<tr>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>16</td>
<td>19</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>5</td>
<td>2</td>
<td>+</td>
<td>NS†</td>
</tr>
</tbody>
</table>

† a, Presence of the allele; —, absence of the allele; blank, not defined.

† RPE: OR = 4.2, p = 0.08.

reported to be associated with IgAD were found to be increased in frequency in patients. When HLA-DR1-positive patients were subtyped (Table I), a very strong association with DRB1*0102 was observed (OR = 6.35). DR3 and DR7 were not subtyped, because only one subtype for each group is present in our population (DRB1*0301 and DRB1*0701, respectively).

HLA-DR2, DR5, and DR8 showed a negative association with the disease.

DR3-positive haplotypes

We analyzed the 400 haplotypes from the 100 IgAD patients’ families studied to identify the extended haplotypes and to look for transmission disequilibrium of the associated marker allele or haplotype from a heterozygous parent to an affected offspring. Sixty-eight haplotypes carried HLA-DR3. Of these DR3-positive haplotypes, we found 32 carrying haplospecific markers of the B8-DR3 haplotype (TNF-308A, TNFa2b3, and HLA-B8) in addition to DR3; therefore, they were considered to be B8-DR3 conserved haplotypes. Additionally, 12 were found carrying DR3, TNF-376A, TNFa1b5, and HLA-B18, all of them haplotypic markers of the B18-DR3 conserved haplotype. All other DR3-positive haplotypes present had multiple combinations of alleles at the tested loci not shared by more than four of them. The B8-DR3 haplotype was transmitted more frequently than not from heterozygous parents to IgAD patients (23 vs 7; p = 0.027), whereas no statistically significant difference was seen for the B18-DR3 haplotype or for all other DR3-positive haplotypes.

All DR3, TNF-308A, TNFa2b3, and HLA-B8 haplotypes were carrying MICA allele A5.1, except in one instance where allele A5 was present. Concerning D6S273 and BAT2 microsatellites, two different patterns were present: allele 136 at D6S273, always coupled with allele 2 at BAT2, was found in eight instances, whereas all other 24 haplotypes were carrying allele 140 at D6S273 coupled with allele 3 at BAT2 in most instances (allele BAT2-2 was present on two haplotypes and alleles 3 and 4 were present on each). Transmission from the 30 heterozygous parents was significantly increased for both groups of DR3, TNF-308A, TNFa2b3, and HLA-B8 haplotypes (Table II).

Moreover, seven HLA-DR3-negative haplotypes carried the haplospecific markers of the telomeric portion of the class III region of the B8-DR3 haplotype (TNF-308A and TNFa2b3) and were therefore considered recombinant forms of the B8-DR3 extended haplotype. Five of them were transmitted and two were nontransmitted (Table II). Table III shows these seven haplotypes. Only three carried D6S273 and BAT2 alleles present on the B8-DR3 haplotype (two carried D6S273 (140) and BAT2 (1) and the third one carried D6S273 (140) and BAT2 (3)). Therefore, in four recombinant haplotypes (three transmitted and one nontransmitted) recombination appears to have taken place between D6S273 and TNF. Moreover, one transmitted haplotype lacked HLA-B8 and another one lacked both MICA A5.1 and HLA-B8, suggesting that the susceptibility locus could be centromeric to these two genes.

DRB1*0102-positive haplotypes

Of the 44 DRB1*0102-positive haplotypes, 26 B14-DRB1*0102 conserved extended haplotypes (i.e., carrying DRB1*0102, TNF-308G, TNFa2b1, HLA-B14) were identified (Table IV). All were D6S273-132, and most were BAT2-3 positive (all but four) and MICA-A6 positive (except two, carrying MICA-A5.1). Two more haplotypes carried TNF-308G, DRB1*0102, and TNFa2b1 but not HLA-B14 or the other tested markers present on the B14-DRB1*0102 extended haplotype. The other 16 DRB1*0102-positive haplotypes present lacked TNFa2b1 and HLA-B14 and had multiple combinations of alleles at the tested loci not corresponding to any known conserved haplotype. As seen in Table IV, B14-DRB1*0102 conserved extended haplotype was transmitted more frequently to IgAD offspring. Moreover, DRB1*0102-positive haplotypes not carrying B14-DRB1*0102 haplotype TNF or HLA-B alleles were also transmitted with increased frequency to IgAD patients (11 transmitted vs 5 nontransmitted) (Table IV).

Table III. Recombinant, HLA-DR3-negative forms of the B8-DR3 extended haplotype, carrying haplospecific markers of the telomeric portion of the class III region of the B8-DR3 haplotype (TNF-308A, TNFa2b3)†

<table>
<thead>
<tr>
<th>Family</th>
<th>Transmitted Haplotype</th>
<th>HLA-DR</th>
<th>D6S273</th>
<th>BAT2</th>
<th>TNF-308</th>
<th>TNF-238</th>
<th>TNFa</th>
<th>TNFb</th>
<th>MICA</th>
<th>HLA-B8</th>
</tr>
</thead>
<tbody>
<tr>
<td>578</td>
<td>Yes</td>
<td>6</td>
<td>140</td>
<td>1</td>
<td>A</td>
<td>G</td>
<td>2</td>
<td>3</td>
<td>A5.1</td>
<td>Yes</td>
</tr>
<tr>
<td>599</td>
<td>No</td>
<td>5</td>
<td>140</td>
<td>1</td>
<td>A</td>
<td>G</td>
<td>2</td>
<td>3</td>
<td>A5.1</td>
<td>Yes</td>
</tr>
<tr>
<td>649</td>
<td>Yes</td>
<td>1</td>
<td>140</td>
<td>3</td>
<td>A</td>
<td>G</td>
<td>2</td>
<td>3</td>
<td>A5.1</td>
<td>Yes</td>
</tr>
<tr>
<td>622</td>
<td>No</td>
<td>5</td>
<td>126</td>
<td>2</td>
<td>A</td>
<td>G</td>
<td>2</td>
<td>3</td>
<td>A5.1</td>
<td>Yes</td>
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<tr>
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<td>Yes</td>
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<td>134</td>
<td>7</td>
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<td>G</td>
<td>2</td>
<td>3</td>
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<td>Yes</td>
</tr>
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<td>6</td>
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<td>G</td>
<td>2</td>
<td>3</td>
<td>A5.1</td>
<td>No</td>
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<td>551</td>
<td>Yes</td>
<td>6</td>
<td>134</td>
<td>2</td>
<td>A</td>
<td>G</td>
<td>2</td>
<td>3</td>
<td>A6</td>
<td>No</td>
</tr>
</tbody>
</table>

† Alleles present on the B8-DR3 conserved haplotype are underlined.

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In addition, 13 DRB1*0102-negative haplotypes carried the haplo-
typic markers of the telomeric portion of the B14-DRB1*0102 con-
served haplotype (TNF-308G, TNFα2b1, MICA-A6, B14). For these 
recombinant forms of the B14-DRB1*0102 extended haplotype, no trans-
mission disequilibrium was observed (Table IV).

Because D6S273, BAT2, TNFab, and MICA microsatellite al-
leles present on the B14-DRB1*0102 haplotype were among the most common alleles present in our population (and therefore were not very informative), we looked for another microsatellite that could be considered as a more specific marker of the B14-
DRB1*0102 haplotype. We found that allele BAT2-3, D6S273-132,
and microsatellite 9N2-5, B14 positive and DRB1*0102 negative, were present. All six carried the alleles D6S273-132, BAT2-3, TNF-308G, TNFα2b1, and MICA-A6, haplo-
typic of the B14-DRB1*0102 extended haplotype. As observed 
previously for all DRB1*0102-negative haplotypes, no transmis-
sion disequilibrium was seen (three transmitted vs three nontrans-
mitted).

**DR7-positive haplotypes**

**DR7-positive** haplotypes were increased in frequency in transmitted 
haplotypes (62 transmitted vs 19 nontransmitted; \( p < 10^{-5} \)). Because HLA-DR7 is present on two DR-DQ haplotypes, both 
were analyzed separately and a similar transmission disequilibrium 
was observed (DR7, DQA1*0201, DQB1*0202: 53 transmitted vs 
18 nontransmitted; \( p < 10^{-5} \); DR7, DQA1*0201, DQB1*0303: 9 
transmitted vs 1 nontransmitted; \( p = 0.01 \)). HLA-B and microsat-
ellite typing showed combinations of alleles corresponding to five 
different DR7-positive conserved extended haplotypes (Table V).

In all instances, transmission of these haplotypes to IgAD offspring 
was increased (Table V) and no statistical differences were found 
between them. HLA-DR7-positive haplotypes not belonging to any 
known conserved extended haplotype were also found more fre-
cently in transmitted haplotypes (14 transmitted vs 6 nontrans-
mitted; \( p = 0.056 \)).

Some haplotypes, carrying markers telomeric to DR7 but not 
DR7, of the aforementioned extended haplotypes were found. 
More than one was found for B50-DR7, B14-DR7, B44-DR7, and 
B57-DR7 extended haplotypes. None of these putative recombin-
ant forms was predominantly transmitted (Table V).

**Relative predispositional effects**

Results presented above showed that the B8-DR3 and B14-
DRB1*0102 extended haplotypes, as well as all haplotypes carry-
ing HLA-DR7, were preferentially transmitted to the IgAD pa-
tients. These primary associations could mask other positive 
associations because of the expected decreased frequency of the 
former imposed by the latter. Therefore, we used the RPE sequen-
tial study (31) to identify several associations sequentially. Ex-
cluding all B8-DR3, B14-DRB1*0102, and DR7-carrying haplo-
types, the centromeric fragment of the B14-DRB1*0102 extended 
haplotype (the DRB1*0102-carrying fragment) was preferentially 
transmitted (Table IV) and with this sequential study the difference 
between transmitted and nontransmitted fragments appeared sta-
tistically significant (11 of 99 transmitted vs 5 of 160 nontrans-
mitted; \( p = 0.009; \ OR = 3.88 \) (Table IV). Conversely, no pref-
rential transmission of the telomeric fragment was observed. For 
the B8-DR3 fragments, those carrying the telomeric haplospec-
ific markers (TNF-308A, TNFα2b3) were preferentially transmitted 
(Table II) and, in the RPE sequential study, the OR, reflecting the 
strength of the association, was high; the difference between trans-
mitted and nontransmitted telomeric fragments approached, but 
did not reach, statistical significance (5 of 99 transmitted vs 2 of 
160 nontransmitted; \( p = 0.08; \ OR = 4.20 \)).

---

**Table IV. Frequencies of the B14-DRB1*0102 haplotype, possible recombinant forms of the B14-
DRB1*0102 haplotype, and other DRB1*0102-positive haplotypes in transmitted (T) vs nontransmitted (NT) 
haplotypes**

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>DQB1*</th>
<th>HLA-DR</th>
<th>D6S273</th>
<th>TNF-308A</th>
<th>TNF-238G</th>
<th>TNFα2b1</th>
<th>MICA</th>
<th>HLA-B</th>
<th>T</th>
<th>NT</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>B44-DR7</td>
<td>0202</td>
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<td>132</td>
<td>–</td>
<td>–</td>
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<td>A6</td>
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<td>18</td>
<td>8</td>
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<td>+</td>
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<td>A9</td>
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<td>8</td>
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<td>130</td>
<td>–</td>
<td>–</td>
<td>7.4</td>
<td>A5.1</td>
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<td>–</td>
<td>4.7</td>
<td>A5</td>
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<td>5</td>
<td>1</td>
<td>NS</td>
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<td>B50-DR7</td>
<td>0202</td>
<td>7</td>
<td>128</td>
<td>–</td>
<td>+</td>
<td>5.7</td>
<td>A6</td>
<td>50</td>
<td>6</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td>B44-DR7</td>
<td>Xc</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>7.4</td>
<td>A6</td>
<td>44</td>
<td>3</td>
<td>6</td>
<td>NS</td>
</tr>
<tr>
<td>B57-DR7</td>
<td>Xc</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>2.5</td>
<td>A9</td>
<td>57</td>
<td>0</td>
<td>2</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>B14-DR7</td>
<td>Xc</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>4.7</td>
<td>A5</td>
<td>14</td>
<td>0</td>
<td>2</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>B50-DR7</td>
<td>Xc</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>5.7</td>
<td>A6</td>
<td>50</td>
<td>1</td>
<td>2</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

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**Table V. Frequencies of DR7 conserved extended haplotypes and their recombinant forms in transmitted (T) vs nontransmitted (NT) haplotypes**

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>DQB1*</th>
<th>HLA-DR</th>
<th>D6S273</th>
<th>TNF-308A</th>
<th>TNF-238A</th>
<th>TNFα2b1</th>
<th>MICA</th>
<th>HLA-B</th>
<th>T</th>
<th>NT</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>B44-DR7</td>
<td>0202</td>
<td>7</td>
<td>132</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>A6</td>
<td>44</td>
<td>18</td>
<td>8</td>
<td>0.038</td>
</tr>
<tr>
<td>B57-DR7</td>
<td>0303</td>
<td>7</td>
<td>136</td>
<td>–</td>
<td>+</td>
<td>2.5</td>
<td>A9</td>
<td>57</td>
<td>8</td>
<td>1</td>
<td>0.04</td>
</tr>
<tr>
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<td>0202</td>
<td>7</td>
<td>130</td>
<td>–</td>
<td>–</td>
<td>7.4</td>
<td>A5.1</td>
<td>13</td>
<td>6</td>
<td>1</td>
<td>0.06</td>
</tr>
<tr>
<td>B14-DR7</td>
<td>0202</td>
<td>7</td>
<td>128</td>
<td>–</td>
<td>–</td>
<td>4.7</td>
<td>A5</td>
<td>14</td>
<td>5</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
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<td>0202</td>
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<td>128</td>
<td>–</td>
<td>+</td>
<td>5.7</td>
<td>A6</td>
<td>50</td>
<td>6</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
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<td>Xc</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>A6</td>
<td>44</td>
<td>3</td>
<td>6</td>
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<tr>
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<td>+</td>
<td>4.7</td>
<td>A5</td>
<td>14</td>
<td>0</td>
<td>2</td>
<td>NS</td>
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<tr>
<td>B50-DR7</td>
<td>Xc</td>
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<td>+</td>
<td>5.7</td>
<td>A6</td>
<td>50</td>
<td>1</td>
<td>2</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

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\( ^a \) +, Presence of the allele; –, absence of the allele; blank, not defined.

\( ^b \) RPE: \( p < 0.05 \).

\( ^c \) Not HLA-DR7.
Discussion

An important feature of the MHC is the strong linkage disequilibrium between the various alleles of highly polymorphic loci. This is especially so in the region extending from HLA-B to HLA-DR, where haplotypes seem to have been conserved from remote ancestors and have been called conserved, extended, or ancestral haplotypes. It has been estimated that ~70% of the human haplotypes are conserved, extended haplotypes or recombinants of no more than two of them (17, 32, 33).

It is generally accepted that a small number of these MHC conserved haplotypes are found in the majority of IgAD patients (5, 7, 8, 10, 11, 13, 19) and that these haplotypes share a susceptibility locus designated IGAD1. To track down this MHC susceptibility locus, most studies have either focused on the B8-DR3 haplotype (13, 14) or looked for a shared feature between different haplotypes associated with the disease (8, 10–12, 15, 18, 19). Conclusions from different groups map the susceptibility locus either to the telomeric class III region (8, 11–13, 15) or to the telomeric class II region (10, 18, 19).

Our patients showed, in agreement with previous reports, IgAD association with MHC haplotypes carrying HLA-DRB1*0102, DRB1*03, or DRB1*07. Therefore, we studied all three haplotypes groups to map the IGAD1 locus region in patients’ MHC haplotypes positive for any of these HLA-DR alleles. We typed polymorphic genes (HLA-DR, -DQA1, -DQB1, and -B), some single nucleotide polymorphisms (TNF-238, -308 and -376), and several microsatellites. These, in many instances, can be considered as markers of conserved haplotypes, because only one allele for each microsatellite is observed in most instances on these conserved extended haplotypes (34).

Moreover, HLA-DR2, DR5, and DR8 showed a negative association with the disease. HLA-DR2, the most significant negative association, has already been reported (10). The other two were seen to be secondary to the decreased frequency imposed by the well-known positive associations with HLA-DRB1*0102, DR3, and DR7 (data not shown).

Surprisingly, our results did not support the hypothesis of a unique susceptibility gene being shared by all the MHC susceptibility haplotypes. All HLA-DRB1*0102- and DR7-carrying haplotypes appeared to be preferably transmitted to IgAD offspring, independently of the markers present on the class III region, whereas for HLA-DR3-positive haplotypes transmission distortion was present only when the haplotype contained the alleles TNF-308A, TNFa2, and TNFb3. These three alleles at the TNF region are haplospecific markers of the B8-DR3 haplotype; therefore, our results indicated that a susceptibility allele was present in the B8-DR3 haplotype but not in other DR3-positive haplotypes. Although this ruled out as susceptibility alleles the HLA class II alleles present on all DR3-positive haplotypes (HLA-DR3, DQA1*0501, and DQB1*0201), it did not give any more clues on where the susceptibility locus was located.

Allele 140 of the D6S273 microsatellite (D6S273-140) has been considered as a haplospecific marker of the B8-DR3 haplotype (34, 35). We found it in 75% of the B8-DR3 haplotypes (coupled with BAT2-1), but in eight instances allele D6S273-136 (always coupled with BAT2-2) was present. This suggested the presence of a double recombination at this level. Because in both instances a statistically significant transmission disequilibrium was observed, these two polymorphisms did not appear to play any role in susceptibility to IgAD. Moreover, all genes lying between them, and indeed all genes present in the whole fragment that had been recombined, should also be excluded as the predisposing locus. Still, the susceptibility gene on the B8-DR3 haplotype could be either centromeric or telomeric to that fragment. However, in seven families we have seen incomplete copies, or fragments, of the B8-DR3 haplotype, positive for TNF-308A and TNFa2b3, but not for DR3. In most instances (71%) these haplotypes were transmitted to the IgAD offspring, suggesting that the susceptibility locus was in linkage disequilibrium with these markers and not with HLA-DR3, DQA1*0501, and DQB1*0201. Still, the DR3-negative haplotypes could have a class II gene that is involved in the deficiency. Because this gene should be in linkage disequilibrium with the telomeric class III markers of the B8-DR3 haplotype, but not with DR3, recombination in these DR3-negative B8-DR3 haplotype should have taken place in the class II region. This was unlikely, because between HLA-DR and HLA-B the preferential recombination site has been located between D6S273 and TNF (36). Moreover, of the seven haplotypes, only three carried D6S273 and BAT2 alleles present on the B8-DR3 haplotype (two carried D6S273-140 and BAT2-1 and the third one carried D6S273-140 and BAT2-3). Therefore, in four recombinant haplotypes (three transmitted and one nontransmitted) recombination appears to have taken place between D6S273 and TNF (Table III) and the susceptibility locus appears to be telomeric to D6S273. This was in agreement with results from most reports (8, 11–13, 15), notably from Schroeder et al. (13), which in a large family study concluded that the susceptibility locus was located between the class III markers D8S21/ D8S23 and HLA-B8.

The B14-DRB1*0102 haplotype was also observed to be preferably transmitted to IgAD offspring. Both “complete” copies of the B14-DRB1*0102 haplotype and fragments, recombinant B14-DRB1*0102 haplotypes, carrying DRB1*0102, showed transmission disequilibrium, whereas this was not so for the telomeric end of the B14-DRB1*0102 haplotype, encompassing from 9N2 and D6S273 microsatellites to HLA-B but lacking DRB1*0102. Although this does not necessarily mean that DRB1*0102 is the susceptibility allele, it does establish that the predisposing locus on this haplotype is in strong linkage disequilibrium with DRB1 and not located in the telomeric class III region. Therefore, the susceptibility locus to IgAD on the B14-DRB1*0102 haplotype appears located centromeric to the predisposing region mapped on the B8-DR3 haplotype (Fig. 1).

The HLA-DRB1*07 allele was also seen preferably transmitted to IgAD offspring. It is carried by the Spanish white population on several conserved extended haplotypes (B13-DR7, B44-DR7, B50-DR7, B57-DR7, B64-DR7), all carrying different complementopeptides (17, 34, 37) and different alleles at the microsatellites typed in this and previous studies (34, 37). Complete copies (from HLA-DR to HLA-B) of all these haplotypes were found in our IgAD families and all of them were preferentially transmitted, irrespective of any other marker. We do not know whether all of these haplotypes have any locus in common outside HLA-DRB1. On the centromeric end this is very unlikely, because transmission of two different DR-DQ haplotypes (DR7-DQA1*0201-DQB1*0202 and DR7-DQA1*0201-DQB1*0303) was significantly increased. Therefore, the susceptibility locus must be either HLA-DRB1*07 or any other in strong linkage with it and present in most DR7-positive conserved extended haplotypes, and definitely not in the telomeric end of the class III region (Fig. 1).

Our data point to the predisposing locus on the DRB1*0102- and DR7-positive haplotypes located on the telomeric end of the class II MHC region. This was advocated by Oelerup and colleagues (10, 18) more than 10 years ago. They found positive associations of IgAD with DR1-DQ5, DR7-DQ2, and DR3-DQ2 haplotypes and suggested that susceptibility and resistance to IgAD was associated with different amino acids at position 57 of the HLA-DQβ chain. However, our results do not point to the DQB1 gene, even for
Any linked locus, more than one predisposing gene and/or allele may be affected for different genetic reasons (38). Functionally related genes are frequently linked to each other (38) and a single linkage signal to a region of a chromosome may actually be the result of several genetic defects in the same chromosomal region. This genetic complexity may result in the same chromosomal region. This genetic complexity may be even greater within the HLA region and for diseases in which negative selection is not acting on phenotypes that do not strongly influence reproduction. But we stress that genetics of complex diseases must always be investigated under the assumption that, at any linked locus, more than one predisposing gene and/or allele may be present.

Acknowledgments
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References


