Genetic aspects of Multiple Sclerosis
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Chapter 4

Inheritance mode of multiple sclerosis: the effect of HLA class II alleles is stronger than additive

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Gerard J. te Meerman, PhD

4.1 Abstract

We previously identified on chromosome 6 an interval of 51 kb as the most likely interval in the HLA-region for a disease-susceptibility locus for multiple sclerosis (MS). The interval was located between markers G511525 and D6S1666 and identified by the Haplotype Sharing Statistic (HSS). The study comprised 124 patients with ancestry within the northeastern part of the Netherlands. Haplotype clustering indicated that two different ancestral haplotypes likely include a polymorphism involved in susceptibility to MS. To investigate the dominance characteristics of the MS susceptibility locus in the HLA class II region we reanalyzed our data performing genotype association analyses for both marker loci separately and for the two-locus haplotype. The two-locus genotype association analysis showed that in individuals who carry only one of the risk haplotypes the risk for MS is moderately increased (OR 2.82; 95%CI 1.50 - 5.31). However, in individuals carrying two risk haplotypes the risk for MS is highly increased compared with individuals who carry no risk haplotypes (OR 37.00; 95%CI 8.31-164.74). This susceptibility locus for MS seems to follow an intermediate mode of inheritance. Fitting additive, multiplicative and third power risk models to the data, the effect appears to be significantly stronger than additive.

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4.2 Introduction

There are several indications for genetic factors playing a role in susceptibility for multiple sclerosis (MS). Among these is an association between MS and specific HLA-alleles, reported in the early 1970s and later confirmed. Whole genome screens have supported the presence of a susceptibility gene in the HLA-region. Investigations into the nature of the genetic susceptibility, however, gave conflicting results and no specific mode of inheritance could be established. Analysis of pedigrees excluded a single-locus Mendelian inheritance with complete penetrance.

In order to gain information on the inheritance mode of MS, we reanalyzed our data by studying the genotypic distribution among patients and controls of risk haplotypes as defined by our previous analysis. We reported earlier on the results of an HLA screen in a population of Dutch MS patients using 22 microsatellite markers. Three methods of analysis were used: allelic association analysis, a transmission/disequilibrium test (TDT) and the Haplotype Sharing Statistic (HSS). We found strong indications for the presence of an MS susceptibility gene in the HLA-region situated most likely in the interval between marker loci G511525 (also known as D6S2663) and D6S1666. Haplotype clustering showed that in the population under investigation two extended ancestral haplotypes were likely to include a polymorphism involved in susceptibility to MS. The corresponding G511525-D6S1666-subhaplotypes were 217-136 and 223-132. In the current paper, we infer a model of inheritance for this susceptibility locus from the results of genotypic association analysis for both marker loci separately and for the two-locus haplotype and the associated risks.
4.3 Subjects and Methods

Subjects
DNA of 124 MS patients with ancestry within the three northeast provinces of the Netherlands was collected as reported previously. All patients were diagnosed with MS according to standard criteria. Both patients with relapse-onset MS and patients with primary progressive MS were included. In part of the families of these patients, haplotypes that were not transmitted from the parents to the affected child served as (pseudo-)control genotypes. These haplotypes were either directly available (n=46) or derived from sibs (n=27). In the other families the genotype of the spouse, either directly available (n=37) or derived from children (n=7) was used as control. This resulted in a total of 117 patient and 98 control genotypes. Missing genotypes caused by errors of PCR or non-fitting segregation reduced the resulting numbers for analysis. DNA of the available relatives was used to determine linkage phase between the two loci. The Ethical Committees of the University Hospital Groningen and the Martini Hospital in Groningen approved the study. All participants gave their informed consent; children under 18 were excluded.

Association analysis
In addition to the analyses presented earlier, we performed genotype association analyses for the two markers G51525 and D6S1666, separately and combined, by means of chi-square tables. For the single locus genotype association analysis, patients and controls were divided in three categories based on genotypes, i.e. carrying zero, one or two of either risk alleles. For the combined genotype of the two-locus haplotypes of loci G51525 and D6S1666, patients and controls were divided in three categories, i.e. carrying zero, one or two risk haplotypes. The possible combinations of two identical or two different risk alleles or risk haplotypes were calculated separately to determine the contribution of specific combinations to the results. Odds ratios (ORs) and the 95% confidence intervals (CI) were calculated from the raw numbers without adjusting for external variables like age at diagnosis or sex.

In order to investigate whether an additive, multiplicative or third power risk model would better fit the data, the observed genotype numbers were compared with the ones expected under the assumed risk model fixing the marginals of the crosstable. We optimized the (single) risk parameter by maximizing the p-value of the difference between the observed and the expected genotype numbers. This yields the best fitting risk model.
Calculations were made under the assumption of an additive, multiplicative and third power risk effect separately. If the maximal p-value of the best fitting risk model is smaller than 0.05, this indicates that the assumed risk model can be rejected.
4.4 Results

From our former results, it appeared that the risk alleles for marker locus G511525 were 217 and 223. For marker locus D6S1666 they were 136 and 132. The common haplotypes constructed of these alleles were 217-136 and 223-132. The haplotype 217-132, as likely resulting from recombination, was found in one patient and in one control individual. The haplotype 223-136 was not observed in our population.

The results of single locus genotypic association analysis for the markers G511525 and D6S1666 are shown in Table 1. For both marker loci, individuals with two risk alleles were over-represented among patients (p=1.04x10^-5 for G511525, p=1.08x10^-8 for D6S1666). The corresponding OR associated with a genotype consisting of two risk alleles for locus G511525 was 5.09 (95%CI 2.33-11.12) and for locus D6S1666 it was 16.85 (95%CI 5.87-48.37). The OR for genotypes consisting of one risk allele and one non-risk allele was equal to 2.99 (95%CI 1.58-5.68) for locus D6S1666. For locus G511525, the OR for a genotype of one risk allele and one non-risk allele was not significantly increased (OR 1.30; 95%CI 0.62-2.69).

Table 2 shows the results of the two-locus analyses for the combined haplotypic genotypes at markers G511525 and D6S1666. Two-locus genotypic association analysis revealed an even larger difference between patients and controls (p=1.01x10^-9) than the separate single locus analyses. Individuals with two risk haplotypes were over-represented among patients, while those with no risk haplotypes were over-represented among controls. The OR for carriers of one risk haplotype was 2.82 (95%CI 1.50-5.31) and for carriers of two risk haplotypes it was 37.00 (95%CI 8.31-164.74).

The best fitting additive model to the data of Table 2 was the one with an OR of 3.93 for carriers of one risk haplotype and 6.86 for carriers of two risk haplotypes. The resulting expected genotype numbers however differ significantly from the observed ones (p=0.01). Under a multiplicative risk model, the respective ORs yielding the best fitting model were 3.95 and 15.60. This model could not be rejected (p=0.20). A third power risk model best fitted the data with an OR for carriers of one risk haplotype of 3.25 and for carriers of two risk haplotypes of 34.33 (p=0.89).
Table 1a

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients</th>
<th>Controls</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>No risk alleles</td>
<td>18</td>
<td>29</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>One risk allele</td>
<td>37</td>
<td>46</td>
<td>1.30</td>
<td>0.62 - 2.69</td>
</tr>
<tr>
<td>Two risk alleles</td>
<td>60</td>
<td>19</td>
<td>5.09</td>
<td>2.33 - 11.12</td>
</tr>
<tr>
<td>Total</td>
<td>115</td>
<td>94</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

χ² = 22.95; df = 2; p = 1.04 x 10⁻¹

Table 1b

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients</th>
<th>Controls</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>No risk alleles</td>
<td>23</td>
<td>51</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>One risk allele</td>
<td>54</td>
<td>40</td>
<td>2.99</td>
<td>1.58 - 5.68</td>
</tr>
<tr>
<td>Two risk alleles</td>
<td>38</td>
<td>5</td>
<td>16.85</td>
<td>5.87 - 48.37</td>
</tr>
<tr>
<td>Total</td>
<td>115</td>
<td>96</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

χ² = 36.69; df = 2; p = 1.08 x 10⁻¹

Table 1: Observed numbers of genotypes and odds ratios for the loci G511525 (1a) and D6S1666 (1b) separately among MS patients and controls.

Table 2

<table>
<thead>
<tr>
<th>Genotype (G511525-D6S1666)</th>
<th>Patients</th>
<th>Controls</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>No risk haplotypes</td>
<td>28</td>
<td>56</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>One risk haplotype</td>
<td>48</td>
<td>34</td>
<td>2.82</td>
<td>1.50 - 5.31</td>
</tr>
<tr>
<td>Two risk haplotypes</td>
<td>37</td>
<td>2</td>
<td>37.00</td>
<td>8.31 - 164.74</td>
</tr>
<tr>
<td>Total</td>
<td>113</td>
<td>92</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

χ² = 41.42; df = 2; p = 1.01 x 10⁻³

Table 2: Observed numbers of genotypes and odds ratios for the loci G511525 and D6S1666 combined among MS patients and controls.
Table 3 shows ORs for specific alleles at loci G511525 and D6S1666. Both at locus G511525 (Table 3a) and at locus D6S1666 (Table 3b) all possible combinations of two risk alleles led to significantly increased ORs. For locus D6S1666 also carriers of one risk allele had an increased OR, although this increase was modest.

### Table 3a

<table>
<thead>
<tr>
<th>Allele 1/allele 2</th>
<th>Patients</th>
<th>Controls</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other / other</td>
<td>18</td>
<td>29</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>217 / other</td>
<td>9</td>
<td>11</td>
<td>1.32</td>
<td>0.46-3.80</td>
</tr>
<tr>
<td>223 / other</td>
<td>28</td>
<td>35</td>
<td>1.29</td>
<td>0.60-2.78</td>
</tr>
<tr>
<td>217 / 217</td>
<td>8</td>
<td>0</td>
<td>27.11</td>
<td>1.48-498</td>
</tr>
<tr>
<td>217 / 223</td>
<td>24</td>
<td>8</td>
<td>4.83</td>
<td>1.79-13.1</td>
</tr>
<tr>
<td>223 / 223</td>
<td>28</td>
<td>11</td>
<td>4.10</td>
<td>1.65-10.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>115</td>
<td>94</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( \chi^2=25.11; \text{df}=5; p=1.3\times10^{-4} \)

### Table 3b

<table>
<thead>
<tr>
<th>Allele 1/allele 2</th>
<th>Patients</th>
<th>Controls</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other / other</td>
<td>23</td>
<td>51</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>136 / other</td>
<td>15</td>
<td>13</td>
<td>2.56</td>
<td>1.05-6.24</td>
</tr>
<tr>
<td>132 / other</td>
<td>39</td>
<td>27</td>
<td>3.20</td>
<td>1.60-6.42</td>
</tr>
<tr>
<td>136 / 136</td>
<td>7</td>
<td>0</td>
<td>32.87</td>
<td>1.80-600</td>
</tr>
<tr>
<td>136 / 132</td>
<td>16</td>
<td>3</td>
<td>11.83</td>
<td>3.13-44.6</td>
</tr>
<tr>
<td>132 / 132</td>
<td>15</td>
<td>2</td>
<td>16.63</td>
<td>3.51-78.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>115</td>
<td>96</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( \chi^2=37.45; \text{df}=5; p=4.88\times10^{-7} \)

*Table 3: Observed numbers and odds ratios for combinations of specific alleles at locus G511525 (3a) and at locus D6S1666 (3b) among MS patients and controls.*
In Table 4, the possible combinations of risk haplotypes and non-risk haplotypes are compared. When these combinations were calculated separately, carriers of one risk haplotype and one non-risk haplotype did not have significantly increased ORs. For homozygotes for the haplotype consisting of alleles 217 at locus G511525 and 136 at locus D6S1666 the OR was 29.74, which, as a result of small numbers, was not significant. For homozygotes 223-132 the OR was 30 (95%CI 1.20-753). The combination of haplotypes 217-136 and 223-132 led to the same OR of 30 (95%CI 1.20-753).

Table 4

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Patients</th>
<th>Controls</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>No risk haplotype</td>
<td>28</td>
<td>56</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>217-136 / other</td>
<td>16</td>
<td>13</td>
<td>2.46</td>
<td>0.65-9.38</td>
</tr>
<tr>
<td>223-132 / other</td>
<td>32</td>
<td>21</td>
<td>3.05</td>
<td>1.01-9.23</td>
</tr>
<tr>
<td>217-136 / 217-136</td>
<td>7</td>
<td>0</td>
<td>29.74</td>
<td>0.33-2682</td>
</tr>
<tr>
<td>217-136 / 223-132</td>
<td>15</td>
<td>1</td>
<td>30.00</td>
<td>1.20-753</td>
</tr>
<tr>
<td>223-132 / 223-132</td>
<td>15</td>
<td>1</td>
<td>30.00</td>
<td>1.20-753</td>
</tr>
<tr>
<td>Total</td>
<td>113</td>
<td>92</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

χ²=41.71; df=5; p=6.73x10⁻⁸

Table 4: Observed numbers and odds ratios for specific combinations of risk haplotypes and/or non-risk haplotypes at the loci G511525 and D6S1666.
4.5 Discussion

Single-locus Mendelian inheritance cannot explain the observed patterns in multiplex MS-pedigrees. Ample investigations have shown that both environmental and genetic factors play a role. There are indications that there is a major locus\(^13,14\), although there is no consensus as to the etiological fraction of this locus\(^15-18\). The major locus is most likely situated in the HLA-region. Concerning the mode of inheritance of this locus, evidence has been conflicting.

Using different parametric and nonparametric methods, many studies aimed to investigate the presence of linkage between MS and loci in the HLA-region or association with specific alleles\(^14-29\). These investigators applied dominant, intermediate and recessive models, based on former studies or theoretical arguments. Although most studies found indications for the presence of linkage, many were inconclusive as to the mode of inheritance. Some studies statistically rejected the dominant\(^14,25\) or the recessive\(^19,21,22\) model, but most could not reject either of the models.

We have previously identified the 51 kb interval between marker loci G511525 and D6S1666 as the most likely interval in the HLA-region for a disease locus involved in MS by means of HSS, a method of haplotype sharing analysis\(^9\). At these marker loci, we identified two risk haplotypes for MS in a subpopulation of The Netherlands. Allelic association analysis pointed out that the alleles of the two risk haplotypes at these loci were common, implying that both haplotypes are likely to contain low penetrance mutations in this region.

As shown in the present study, both single locus and two-locus genotypic association analysis revealed a highly significant difference between patients and controls in the observed number of carriers of two risk alleles or haplotypes (ORs ranging from 5.09 to 37.00). In particular, 37 of 113 patients carried two risk haplotypes, as opposed to only 2 of 92 controls. Carrying one risk haplotype already seems to modestly increase susceptibility to MS (OR 2.82; 95% CI 1.50-5.31). However, the difference in risk between carriers of one versus two haplotypes (OR 2.82 vs. OR 37) indicates that the effect is stronger than additive.

Our results are compatible with those of Rasmussen et al.\(^30\). They compared patients and controls who were homozygous or heterozygous for HLA-DR2 with carriers of all other alleles. However, the difference in OR between heterozygotes and homozygotes for DR2 that they found is in line with an additive effect. Part of the difference in results may be caused by the fact that we calculated ORs for carriers of combinations of two risk haplotypes...
and not only DR2. However, the combination of DR2 with another allele (DR3) has previously been found associated with MS.

Recent papers suggested a dose effect of specific HLA haplotype combinations on susceptibility for MS and disease course. Barcellos et al. found an increased disease risk for carriers of one DR2 haplotype, but a higher risk for carriers of two DR2 haplotypes. Moreover, DR2 homozygotes were less frequent in the subgroup of patients with a mild disease course. Although their data supported a DR2 dose effect for disease risk, distinction between an additive and a multiplicative model could not be made.

De Jong et al. investigated the HLA-region for loci other than HLA-DR2 that contribute to susceptibility for relapse-onset MS. They found that microsatellite-allele C1_3_2*354, encoded within an ancestral haplotype linked to HLA-DR3, was increased among patients. In combination with HLA-DR2 the risk to develop relapse-onset MS was eightfold higher. However, comparison of their results and ours is hampered by the fact that they only determined carrierhip for HLA-DR2 and to our knowledge did not discriminate between homozygous and heterozygous individuals.

Summarizing, for individuals carrying one risk haplotype, the risk to become affected with MS is increased compared with individuals carrying no risk haplotypes. However, the risk for individuals carrying any combination of two risk haplotypes is much higher than the sum of risks of the separate haplotypes. These findings support an intermediate model of inheritance for MS susceptibility alleles in the HLA-region. Our analyses showed that no additive model could be fitted to the data. A multiplicative model could not be rejected, but a third power method best fitted the data. Therefore, we can conclude with more than 95% confidence that the inheritance of MS susceptibility alleles in the HLA class II region follows a risk model with an effect stronger than additive.
References


