Linkage Disequilibrium Between HLA-DPB1 Alleles and Retinoid X Receptor β Haplotypes

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ABSTRACT: The human retinoid X receptor β (RXRB) gene maps to the major histocompatibility complex (MHC) region, between KE4 and COL11A2, approximately 130-kb centromeric to HLA-DPB1. We have recently reported a new polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method to detect the G to T single nucleotide polymorphism (SNP) located seven nucleotides after the tenth exon of the RXRB gene, or 3′end+7 position according to existing nomenclature. We also reported strong linkage disequilibrium between the HLA-DPB1*0401 and RXRB 3′end+7*T alleles. In the present study, we describe two PCR-RFLP methods to detect additional SNPs in the RXRB gene, T to A, at exon10+378 and A to T at 3′end+140. This new methodology permitted the unambiguous assignment of three distinct SNPs at RXRB exon10+378, 3′end+7 and 3′end+140 to form an “RXRB haplotype.” The data generated from this study were used to determine linkage disequilibrium between several MHC markers and the RXRB alleles and haplotypes. Family studies revealed significant linkage disequilibrium between the RXRB alleles and a number of HLA-DPB1 alleles. Human Immunology 63, 771–778 (2002). © American Society for Histocompatibility and Immunogenetics, 2002. Published by Elsevier Science Inc.

KEYWORDS: HLA-DPB1; linkage disequilibrium; polymorphism; retinoid X receptor; SNP

ABBREVIATIONS

HLA human leukocyte antigen
H-2IIIB murine retinoid X receptor β
H-2 murine major histocompatibility complex
MHC major histocompatibility complex
PCR polymerase chain reaction
RFLP restriction fragment length polymorphism
RXR retinoid X receptor
RXRB retinoid X receptor β
SNP single nucleotide polymorphism
TDT transmission disequilibrium test
T1DM type 1 diabetes mellitus

INTRODUCTION

The retinoid X receptor β (RXRB) is a member of the retinoid X receptor (RXR) family of nuclear receptors (reviewed in [1–3]). The murine RXRB gene product, H-2RIIBP, has been reported to bind the major histocompatibility (H-2) class I promoter [4, 5]. The analogous human RXRB gene is located on the short arm of chromosome 6 about 130-kb centromeric to the human leukocyte antigen (HLA) marker HLA-DPB1 [6–8] (Figure 1A). This nuclear receptor may play a role in the activation of RXRB target genes regulated by 9-cis retinoic acid [9–12].

To date, using sequence analysis, four distinct DNA variants of human RXRB have been identified. The first is a single nucleotide polymorphism (SNP) within exon 10 at position +378 [13]. Three other polymorphisms are located in the 3′ untranslated region after exon 10, at position +7 [14], +140, and +561 [13] (Figure 1B).

In a recent report [14] we described a polymerase chain reaction–restriction fragment length polymor-
phism (PCR-RFLP) method to detect the RXRB 3’end+7 SNP, and evaluated linkage disequilibrium between the two RXRB 3’end+7 alleles and the HLA markers, HLA-A,B,DRB1,DQB1, and DPB1. Significant positive linkage was observed between HLA-DPB1*0401 and RXRB 3’end+7*T alleles.

In this present study, we report the development of two new PCR-RFLP methods for the detection of the RXRB 3’end+7 SNP also in the 3’ untranslated region distal to exon 10, and RXRB exon10+378 within the last exon of RXRB. Using family data, RXRB haplotypes were assigned and two-point linkage disequilibrium between the three pairs of RXRB markers was determined. Finally, linkage analysis of the RXRB exon10+378, 3’end+7, +140 individual alleles and the HLA markers HLA-A, -B, -DRB1, -DQB1, and -DPB1 indicated strong linkage disequilibrium between specific RXRB alleles as well as the RXRB haplotypes and a number of HLA-DPB1 alleles.

**MATERIALS AND METHODS**

**Nomenclature**

According to Numasawa et al. [13], the RXRB DNA variants were called RXRB exon10+378 T-A, 3’end+140 A-T and 3’end+561 C-CC (see Figure 1B). In the present study, we used the same nomenclature and define the allele by denoting the specific nucleotide substitution that position following an asterisk. The two possible alleles for RXRB exon10+378 are RXRB exon10+378*T and RXRB exon10+378*A. For RXRB 3’end+7 the alleles are RXRB 3’end+7*G and RXRB 3’end+7*T. Finally, The RXRB 3’end+140 alleles are RXRB 3’end+140*A and RXRB 3’end+140*T. The RXRB haplotype assignment includes eight theoretical allele combinations and they are designated as follows:

1. RXRB exon10+378*T, 3’end+7*G, +140*A or *T*G*A,

DNA Samples
Genomic DNA samples were prepared from human whole blood using either Qiagen (Valencia, CA, USA) spin columns or phenol-chloroform extraction.

PCR Amplification
Based on the published nucleotide sequence [13] we designed a specific primer pair, EX10-RXRBFOR (5’-GGCCATGTTCCCAGAACCTTGAGG-3) and EX10-RXRBRREV (5’-AAAGAGAATTTGGACAGTGACA GGG-3), to amplify a 444-bp DNA fragment of the RXRB gene that contains the SNPs located at Exon10+378. Another specific primer pair, 140-RXRBFOR (5’-CCATGATTTGGGTACAGTGACA GG-3) and 140-RXRBRREV (5’-GCAGATCGGTTCCTACATCTC-3) was designed to amplify a 368-bp DNA fragment of the RXRB gene that contains the SNPs located at 3’end+140 (see Figure 1B). Amplification of approximately 200 ng of genomic DNA was performed in a 50-μl reaction mixture containing 2.5 units of Taq DNA polymerase (Roche Diagnostics, Indianapolis, IN, USA) in 10-mM Tris-HCl, 50-mM KCl, pH 8.3 (Roche Diagnostics), 200 μM of each deoxynucleotide triphosphate (dATP, dCTP, dGTP, and dTTP; Promega, Madison, WI, USA), 3.0-mM MgCl2 (Roche Diagnostics), and 0.5 μM of each primer.

The PCR was performed using a Perkin-Elmer 9600 Thermal Cycler (Perkin-Elmer, Norwalk, CT, USA) by first denaturing for 60 seconds at 96°C followed by: five cycles at 96°C for 25 seconds, 70°C for 50 seconds, and 72°C for 45 seconds; 21 cycles at 96°C for 25 seconds, 65°C for 50 seconds, and 72°C for 45 seconds; four cycles at 96°C for 25 seconds, 55°C for 60 seconds, and 72°C for 80 seconds; and a final extension at 72°C for 8 minutes. Finally, 10 μl of each PCR product was analyzed by electrophoresis in a 3% agarose gel (Pel-Freez, Brown Deer, WI, USA).

RFLP Analysis
The RXRB Exon10+378 variant was determined by digesting 30–40 μl of the 444-bp PCR amplicon with 4 units of BspCNI restriction endonuclease (New England BioLabs, Beverly, MA, USA), overnight at room temperature (around 25°C) in a 500 μl reaction containing 100 μg/ml bovine serum albumin (BSA) and 20 μM S-adenosylmethionine (SAM).

The RXRB 3’end+140 variant was determined by digesting 20–25 μl of the 368-bp PCR amplicon with 5 units of MboI restriction endonuclease (New England BioLabs), for 2 hours at 37°C in a 30-μl reaction. The digested samples were then subjected to electrophoresis on 3% agarose gel and the restriction enzyme patterns produced were detected by staining with ethidium bromide (GibcoBRL LifeTechnologies, Rockville, MD, USA).

The RXRB 3’end+7 alleles were detected using PCR-RFLP analysis as previously described [14]. In brief, the two primers, 7FORRXRB 5’T-TTGGCAAGCTGCTG CGTCGTTCCCTCTG-3 and 7REVRXB 5’-CCTGTC AGGGCCCTACTCTATGTC-3 generated a 593-bp PCR product. This amplicon was digested with BsaI and subjected to electrophoresis on 3% agarose gel.

HLA Typing
DNA typing for DRB1, DQB1, and DPB1 alleles was performed using sequence-specific oligonucleotide probe hybridization (SSOPH) according to the protocols of the 11th International Histocompatibility Workshop [15]. HLA-A, -B, and -C antigens were assigned by the standard microlymphocytotoxicity assay [16].

Statistical Analysis
Non-random association, or two-point linkage disequilibrium between the individual RXRB exon10+378, 3’ end +7, +140 alleles or the RXRB haplotype and each of the following MHC markers, i.e., HLA-A, -B, -DRB1, -DQB1, and -DPB1, was determined by comparing the observed (obs) to the expected (exp) haplotype frequency. The p values were determined by Fisher’s exact test analysis and corrected by multiplying by the number of alleles or haplotypes compared. Corrected p values <0.05 were considered statistically significant. The RXRB-HLA linkage disequilibrium analysis was limited to alleles with observed frequencies >5.0%. The Δ value was determined by calculating the difference between the observed haplotype frequency and the expected haplotype frequency. Linkage disequilibrium was calculated using SAS Software, Version 8 (SAS Institute, Inc., Cary, NC, USA). Three-point linkage disequilibrium was not computed because of sample size consideration.
RESULTS

PCR-RFLP Analysis for RXRB Exon10+378

A 444 bp RXRB exon10+378 PCR amplicon was generated using the primers EX10-RXRB-FOR and EX10-RXRB-REV described in Materials and Methods. This SNP previously described using single strand conformational polymorphism [13], was detected by digestion of the PCR products with the endonuclease BspCNI. This enzyme cleaves the nucleotide sequence 5'-CTCAG(N)_{10}-3' but not 5'-CTCTG(N)_{10}-3'.

As expected, no digestion of the 444-bp band was observed with homozygous T/T samples (Figure 2A, lane 1), giving a pattern identical to the undigested controls.

<table>
<thead>
<tr>
<th>Population</th>
<th>Number</th>
<th>+378*T %</th>
<th>+378*A %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian</td>
<td>192</td>
<td>90.7</td>
<td>9.3</td>
</tr>
<tr>
<td>African-American</td>
<td>86</td>
<td>97.9</td>
<td>2.1</td>
</tr>
</tbody>
</table>
TABLE 2  RXRB 3’end+140 allele frequency

<table>
<thead>
<tr>
<th>Population</th>
<th>Number</th>
<th>+140*T %</th>
<th>+140*A %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian</td>
<td>214</td>
<td>36.0</td>
<td>64.0</td>
</tr>
<tr>
<td>African-American</td>
<td>86</td>
<td>30.2</td>
<td>69.8</td>
</tr>
</tbody>
</table>

(Exon 2A, lane 4). Accordingly, homozygous A/A samples produced two distinct fragments of 282 bp and 162 bp (Figure 2A, lane 3). Finally, for heterozygous T/A samples, a restriction pattern with bands at 444 bp, 282 bp, and 162 bp was obtained after BspCNI digestion (Figure 2A, lane 2).

PCR-RFLP Analysis for RXRB 3’end+140

A 368-bp RXRB 3’end+140 PCR amplicon was generated using the primers 140-RXRBFOR and 140-RXRBEV described in Materials and Methods. This SNP was detected by digestion of the PCR products with the endonuclease MboI. This enzyme cleaves the nucleotide sequence 5’-GATC-3’ but not 5’-GAAC-3’ located at position +140 in the 3’ untranslated region.

No digestion of the 368-bp band was observed with homozygous A/A samples (Figure 2, lane 3), giving a pattern identical to the undigested controls (Figure 2, lane 7). Homozygous T/T samples produced two distinct fragments of 238 bp and 130 bp (Figure 2, lane 6). Finally, for heterozygous T/A samples, a restriction pattern with bands at 368 bp, 238 bp, and 130 bp was obtained after MboI digestion (Figure 2, lanes 1, 2, 4, and 5).

TABLE 3  Linkage disequilibrium between the three RXRB alleles (values of observed, expected, Δ, and p value for each RXRB exon10,3’end+7,+140 allele combination) in family studies

<table>
<thead>
<tr>
<th>Exon10</th>
<th>3’end+7</th>
<th>3’end+140</th>
<th>Observed number</th>
<th>Observed frequency</th>
<th>Expected number</th>
<th>Expected frequency</th>
<th>Δ</th>
<th>X²</th>
<th>p value (corrected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*A</td>
<td>*G</td>
<td>16</td>
<td>0.066</td>
<td>9.9</td>
<td>0.041</td>
<td>0.025</td>
<td>3.76</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>*T</td>
<td>*G</td>
<td>132</td>
<td>0.550</td>
<td>138.1</td>
<td>0.575</td>
<td>-0.025</td>
<td>0.27</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>*A</td>
<td>*T</td>
<td>0</td>
<td>0</td>
<td>6.1</td>
<td>0.025</td>
<td>-0.025</td>
<td>6.10</td>
<td>&lt; 2 × 10⁻²</td>
<td></td>
</tr>
<tr>
<td>*T</td>
<td>*T</td>
<td>92</td>
<td>0.383</td>
<td>85.8</td>
<td>0.358</td>
<td>0.025</td>
<td>0.45</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>240</td>
<td></td>
<td></td>
<td>10.58</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*A</td>
<td>*A</td>
<td>16</td>
<td>0.066</td>
<td>11.3</td>
<td>0.046</td>
<td>0.020</td>
<td>1.95</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>*T</td>
<td>*A</td>
<td>153</td>
<td>0.637</td>
<td>157.7</td>
<td>0.657</td>
<td>-0.020</td>
<td>0.14</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>*A</td>
<td>*T</td>
<td>0</td>
<td>0</td>
<td>4.7</td>
<td>0.020</td>
<td>-0.020</td>
<td>4.70</td>
<td>&lt; 5 × 10⁻²</td>
<td></td>
</tr>
<tr>
<td>*T</td>
<td>*T</td>
<td>71</td>
<td>0.296</td>
<td>66.2</td>
<td>0.276</td>
<td>0.020</td>
<td>0.35</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>240</td>
<td></td>
<td></td>
<td>7.14</td>
<td></td>
<td></td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>*G</td>
<td>*A</td>
<td>77</td>
<td>0.321</td>
<td>104.2</td>
<td>0.434</td>
<td>-0.113</td>
<td>7.1</td>
<td>&lt; 10⁻²</td>
<td></td>
</tr>
<tr>
<td>*G</td>
<td>*T</td>
<td>71</td>
<td>0.296</td>
<td>43.7</td>
<td>0.182</td>
<td>0.113</td>
<td>17.1</td>
<td>&lt; 10⁻⁴</td>
<td></td>
</tr>
<tr>
<td>*T</td>
<td>*A</td>
<td>92</td>
<td>0.383</td>
<td>64.6</td>
<td>0.270</td>
<td>0.113</td>
<td>11.6</td>
<td>&lt; 10⁻³</td>
<td></td>
</tr>
<tr>
<td>*T</td>
<td>*T</td>
<td>0</td>
<td>0</td>
<td>27.1</td>
<td>0.113</td>
<td>-0.113</td>
<td>25.1</td>
<td>&lt; 10⁻⁶</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>240</td>
<td></td>
<td></td>
<td>60.9</td>
<td></td>
<td></td>
<td>&lt; 10⁻¹⁶</td>
<td></td>
</tr>
</tbody>
</table>

The p value was calculated by Fisher’s exact test and was corrected multiplying by the number of comparisons. The combinations {RXRB exon10*A, 3’end+7*T}, {RXRB exon10*A, 3’end+140*T}, and {RXRB 3’end+7*T, +140*T} did not appear on the same chromosome in any of the samples tested.
TABLE 5  Transmission of RXRB haplotypes in T1DM affected and unaffected siblings

<table>
<thead>
<tr>
<th>RXRB exon10, 3’end+7, +140 haplotypes</th>
<th>T1DM haplotypes</th>
<th>Unaffected haplotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T</td>
<td>NT</td>
</tr>
<tr>
<td><em>T</em>G*A</td>
<td>27</td>
<td>38</td>
</tr>
<tr>
<td><em>A</em>G*A</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td><em>T</em>G*T</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td><em>T</em>T*A</td>
<td>37</td>
<td>25</td>
</tr>
</tbody>
</table>

Haplotypes present in 70 parents heterozygous for RXRB were analyzed using TDT. In this limited number of T1DM families studied, there was no significant difference between the RXRB haplotypes transmitted to T1DM patients and unaffected siblings.

Abbreviations: T = transmitted; NT = not transmitted; TDT = transmission disequilibrium test.

casians were 36.0% and 64.0%, and in the 43 African-Americans 30.2% and 69.8%, respectively (Table 2). No statistical difference in RXRB 3’ end+140 allele frequency between the two test populations was detected.

**RXRB Segregation and Linkage**

We defined RXRB exon10+378, RXRB 3’ end+7 and RXRB 3’ end+140 genotypes in 236 individuals from 53 Caucasian type 1 diabetes mellitus (T1DM) families from the Boston area, who were well-characterized for the MHC markers, HLA-A, -B, -DRB1, -DQB1, and -DPB1. These 53 families contained 212 chromosomes from 106 unrelated parents and 28 chromosomes from first-degree family members for a total of 240. The RXRB exon10+378, 3’ end+7 and 3’ end+140 alleles were assigned to a haplotype that included the MHC markers described above. As expected, these family studies confirmed the segregation of RXRB exon10+378, RXRB 3’ end+7 and 3’ end+140 with the HLA complex, and no crossover or recombination events were detected.

The combined segregation and linkage analysis of the three RXRB markers demonstrated that instead of the eight theoretical haplotypes, there were only four different RXRB haplotypes detected in the 240 chromosomes tested: (1) RXRB *T*G*A; (2) RXRB *A*G*A; (3) RXRB *T*G*T; and (4) RXRB *T*T*A (Table 3). Table 3 illustrates the two-point linkage disequilibrium between the three combinations of RXRB markers.

**Association With T1DM**

An association between each of the RXRB exon10+378, 3’ end+7, +140 loci as well as the combined RXRB haplotype and T1DM was analyzed using the data from our 53 well-characterized Caucasian families. For this study, the parental alleles or haplotypes present in the patient were considered to be ‘T1DM alleles’ or ‘T1DM haplotypes’ and those that did not appear in the patient were considered ‘family control alleles or haplotypes’ [17, 18]. No significant difference in RXRB exon10+378, 3’ end+7, +140 allele or haplotype frequencies was found when we compared those observed in T1DM patients with those in non-T1DM family controls (Table 4).

A comparison between the transmission disequilibrium test (TDT) [19] of the RXRB haplotypes in T1DM patients and the unaffected siblings is illustrated in Table 5. No significant difference in TDT between the T1DM patients and the unaffected siblings was found.

**Linkage Disequilibrium Between the RXRB Haplotypes and HLA Markers**

In addition, we evaluated two-point linkage disequilibrium between the RXRB exon10+378, 3’ end+7, +140 alleles and the individual HLA markers, HLA-A, -B, -DRB1, -DQB1, and -DPB1. Because there was no significant difference in the frequencies of the RXRB alleles or haplotypes between the T1DM and family controls, we combined these two data sets. No significant difference between the individual RXRB alleles or haplotypes and HLA-A, -B, -DRB1, or -DQB1 markers was found.

**TABLE 6  Linkage disequilibrium between RXRB and HLA-DPB1 alleles, and values of observed, expected, Δ, and p value for the RXRB exon10+378-HLA-DPB1, RXRB 3’ end+7-DPB1, and RXRB 3’ end+140-DPB1 combinations in total family haplotypes**

<table>
<thead>
<tr>
<th>RXRB allele</th>
<th>HLA DPB1 allele</th>
<th>Observed number</th>
<th>Observed frequency</th>
<th>Expected number</th>
<th>Expected frequency</th>
<th>Δ</th>
<th>p Value (corrected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3’ end+140*T</td>
<td>DPB1*0101</td>
<td>10</td>
<td>0.049</td>
<td>3.06</td>
<td>0.015</td>
<td>0.034</td>
<td>&lt; 5 × 10^{-3}</td>
</tr>
<tr>
<td>3’ end+7*G</td>
<td>DPB1*0201</td>
<td>27</td>
<td>0.132</td>
<td>18.97</td>
<td>0.093</td>
<td>0.039</td>
<td>&lt; 1 × 10^{-2}</td>
</tr>
<tr>
<td>3’ end+140*T</td>
<td>DPB1*0201</td>
<td>25</td>
<td>0.123</td>
<td>9.18</td>
<td>0.045</td>
<td>0.078</td>
<td>&lt; 5 × 10^{-9}</td>
</tr>
<tr>
<td>3’ end+140*A</td>
<td>DPB1*0301</td>
<td>37</td>
<td>0.181</td>
<td>25.50</td>
<td>0.125</td>
<td>0.057</td>
<td>&lt; 5 × 10^{-6}</td>
</tr>
<tr>
<td>3’ end+7*T</td>
<td>DPB1*0401</td>
<td>59</td>
<td>0.289</td>
<td>31.21</td>
<td>0.153</td>
<td>0.136</td>
<td>&lt; 1 × 10^{-15}</td>
</tr>
<tr>
<td>3’ end+140*T</td>
<td>DPB1*0401</td>
<td>18</td>
<td>0.088</td>
<td>28.56</td>
<td>0.140</td>
<td>-0.052</td>
<td>&lt; 5 × 10^{-2}</td>
</tr>
<tr>
<td>Exon10+378*A</td>
<td>DPB1*0402</td>
<td>5</td>
<td>0.025</td>
<td>0.61</td>
<td>0.003</td>
<td>0.022</td>
<td>&lt; 1 × 10^{-3}</td>
</tr>
</tbody>
</table>

The p value was calculated by Fisher’s exact test and corrected by multiplying by the number of comparisons (total number = 15). Only the RXRB-HLA-DPB1 combinations with significant linkage disequilibrium are shown.
detected. Specific HLA-DPB1 alleles revealed strong positive linkage disequilibrium with the following RXRB haplotypes: DPB1*0401-RXRB *T*T*A (Δ = 0.114, p < 5 × 10^{-11}), DPB1*0301-RXRB *T*G*A (Δ = 0.076, p < 5 × 10^{-9}), DPB1*0201-RXRB *T*G*T (Δ = 0.072, p < 5 × 10^{-9}), DPB1*0101-RXRB *T*G*G*T (Δ = 0.032, p < 1 × 10^{-4}), and DPB1*0402-RXRB *A*G*A (Δ = 0.019, p < 1 × 10^{-2}).

Furthermore, strong positive linkage disequilibrium between specific individual RXRB alleles and common specific HLA-DPB1 alleles was found. The HLA-DPB1-RXRB allele linkage analysis results are shown in Table 6.

**DISCUSSION**

We previously described an RXRB polymorphism at position 3’end+7, as well as a PCR-RFLP method to detect the genetic variants. Our prior study found significant linkage disequilibrium only between RXRB 3’end+7*T and HLA-DPB1*0401 [14]. In the present study, we describe similar methodology to detect two additional RXRB polymorphic sites at position exon10+378 and 3’end+140. Using family studies, the RXRB exon10+378, RXRB 3’end+7, and RXRB 3’end+140 allele assignments were combined to form an “RXRB haplotype.”

The data generated here permitted linkage studies between the three individual RXRB alleles. No significant positive linkage disequilibrium between the RXRB exon10+378 alleles and the two RXRB 3’end+7 and RXRB 3’end+140 alleles was found. However, strong positive linkage disequilibrium was detected between RXRB 3’end+7*G and RXRB 3’end+140*T (Δ = 0.113, p < 10^{-4}), as well as between RXRB 3’end+7*T and RXRB 3’end+140*A (Δ = 0.113, p < 10^{-3}). There was a complete lack of the RXRB exon10+378, 3’end+7, +140*A*T* and *T*T*T haplotypes (Table 3). This is due to a very strong negative linkage disequilibrium between the RXRB 3’end+7*T and 3’end+140*T alleles in our study population (Δ = -0.113, p < 10^{-6}). The distance between RXRB 3’end+7 and RXRB 3’end+140 is only 133 bp and the strong linkage disequilibrium (Δ = 0.113, p < 10^{-16}) observed between these two markers was expected. By contrast, the alleles for RXRB exon10+378 and 3’end+7, just 12 bp apart, reveals marginal linkage disequilibrium (Δ = 0.025, p < 2 × 10^{-2}).

There was no detectable linkage disequilibrium between RXRB alleles or haplotypes and HLA-A, -B, -DRB1, and -DQB1. However, strong linkage disequilibrium between the following RXRB and HLA-DPB1 alleles was observed:

- RXRB exon10+378*A and HLA-DPB1*0402,
- RXRB 3’end+7*T and HLA-DPB1*0401,
- RXRB 3’end+7*G and HLA-DPB1*0201,
- RXRB 3’end+140*A and HLA-DPB1*0501,
- RXRB 3’end+140*T and HLA-DPB1*0201 or DPB1*0101.

The distance between the HLA-DPB1 locus and the three loci for RXRB is almost identical. Yet, linkage disequilibrium between the alleles of HLA-DPB1 and RXRB appears to be both locus and allele specific. This suggests that linkage disequilibrium at least in this case is not determined by distance alone.

The RXRB 3’end+7 and 3’end+140 loci are located in a GC-rich region within the 3’ untranslated region of the RXRB gene. In this region, DNA binding sites that act as a terminal signal of transcription have already been identified [13].

The degree of linkage disequilibrium between HLA-DPB1 and RXRB alleles was similar to that between HLA-DPB1 and RXRB haplotypes (see Results and Table 6).

In this work, we report two new methods for the detection of SNPs in the RXRB gene using simple PCR-RFLP analysis. This methodology allowed us to further investigate linkage disequilibrium between four RXRB haplotypes and the HLA markers. Analysis was limited to RXRB exon10+378, RXRB 3’end+7, and RXRB 3’end+140 because the methodology for these three markers is now well defined. Methodology to detect a fourth SNP in the 3’ untranslated region of RXRB [13] is currently being developed.

Previous data has revealed association between HLA-DPB1 alleles and T1DM [20-22]. We were interested in examining RXRB alleles as possible markers of T1DM. The TDT of the different RXRB haplotypes demonstrated no significant difference between the T1DM and the control haplotypes. However, further studies of RXRB may reveal linkage with other diseases, specifically those that are HLA-DPB1 associated.

**ACKNOWLEDGMENTS**

This work was supported by grant HL-29583 from the National Heart, Lung, and Blood Institute of the National Institutes of Health, and a Translational Research Grant (6674-02) from the Leukemia and Lymphoma Society to Dr. P.A. Fraser. We also want to thank Dr. Alvin Davis and Dr. Robert Mandle for their helpful comments and suggestions.

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