Linkage of the Wiskott–Aldrich syndrome with polymorphic DNA sequences from the human X chromosome

(Immunodeficiency/mapping/logarithm of odds)

MONICA PEACOCKE*† and KATHERINE A. SIMINOVITCH‡§

*Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20055; and ‡Department of Medicine, Toronto Western Hospital, Toronto, ON MST 2S8, Canada

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ABSTRACT The Wiskott–Aldrich syndrome (WAS) is one of several human immunodeficiency diseases inherited as an X-linked trait. The location of WAS on the X chromosome is unknown. We have studied 10 kindreds segregating for WAS for linkage with cloned, polymorphic DNA markers and have demonstrated significant linkage between WAS and two loci, DXS14 and DXS7, that map to the proximal short arm of the X chromosome. Maximal logarithm of odds (lod) scores for WAS–DXS14 and WAS–DXS7 were 4.29 (at θ ≈ 0.03) and 4.12 (at θ = 0.00), respectively. Linkage data between WAS and six marker loci indicate the order of the loci to be (DXYS1–DXS1–WAS–DXS14–DXS7–DXS84–OTC). These results suggest that the WAS locus lies within the pericentric region of the X chromosome and provide an initial step toward identifying the WAS gene and improving the genetic counseling of WAS families.

Although a number of human immunodeficiency syndromes are inherited by X-linked transmission, the molecular basis of these deficiencies is entirely unknown, and clinically useful linkage information is not available. The Wiskott–Aldrich syndrome (WAS) is one such X-linked disease. It is characterized by thrombocytopenia, eczema, and profound immunodeficiency (1–3). B- and T-lymphocyte functions are impaired and recurrent infections and lymphoreticular malignancies are major causes of early mortality of affected boys (4).

The immune defects in WAS are poorly understood but include depressed serum IgM, elevated IgA and IgE, lack of response to polysaccharide antigens and thus absent iso-haemagglutinins, reduced T-cell functions such as delayed hypersensitivity and graft rejection, and production of low numbers of abnormal small platelets that are rapidly eliminated, resulting in thrombocytopenia (5, 6). Despite extensive research efforts, the underlying biochemical defect and molecular basis of WAS remain unknown. Selective inactivation of the X chromosome carrying the WAS gene renders the heterozygous females serologically and clinically normal, thereby precluding detection of the carrier state (7). Thus, no direct test is available for prenatal diagnosis or carrier screening.

In the absence of information on the primary product of a gene, one approach to locating and eventually cloning the gene is to use restriction fragment length polymorphic (RFLP) markers in conjunction with linkage analysis to study families in which the disease segregates.

As a first step in providing diagnostic information for screening and prenatal diagnosis, and in identifying the primary molecular defect in WAS, we have used genetic linkage analysis with a series of X chromosome-specific cloned DNA markers to determine the subchromosomal location of the WAS locus. We report here close linkage between WAS and two DNA marker loci, DXS14 and DXS7, that map to the proximal short arm of the X chromosome. Linkage analysis with six markers has enabled us to infer gene order and conclude that the WAS locus maps within the pericentric region of the X chromosome.

MATERIALS AND METHODS

Subjects. With the assistance of clinical geneticists in Canada and the United States, we were able to obtain detailed pedigrees, clinical data, and blood samples on the families of a number of boys affected with WAS. To exclude cases of spontaneous mutation, only those families with at least two WAS patients and in which X-linked segregation could be demonstrated unequivocally were studied. Ten families fulfilling these criteria were identified and blood samples were obtained from 140 individual members of these kindreds.

DNA Extraction and Analysis. Total genomic DNA was extracted from peripheral blood or lymphoblastoid cell lines. Ten micrograms of DNA was digested to completion with the appropriate restriction endonucleases, size-fractionated over 1% agarose gels, and transferred by the method of Southern to nylon membranes (8).

Probes. Six X-chromosome-specific cloned DNA probes, corresponding to loci DXS1, DXS1, DXS14, DXS7, DXS84, and OTC, were used in the linkage analysis (9–17). As shown in Table 1, each probe detects two allelic fragments, with the exception of probe pH731, which defines a four-allele Map I polymorphism at the ornithine transcarbamoylase locus (16). For hybridization, the probes were radiolabeled by random priming to a specific activity of 2 × 10⁶ cpm/µg (18).

Linkage Analysis. Detection of linkage and estimation of the recombination fractions between pairs of marker loci were performed with the computer program package LINKAGE (19). Penetrance in males was assumed to be 100%. Gene order was examined by multilocus likelihood calculations using the ILINK program from LINKAGE to determine the support for each possible gene order.

RESULTS

Linkage Analysis. To identify families informative for the polymorphic probes, we initially screened each obligate WAS carrier with a series of DNA markers that have been mapped to loci spanning the short and long arms of the X chromosome. Genotypic determinations were then performed on all informative families.

Abbreviations: lod, logarithm of odds; RFLP, restriction fragment length polymorphism; WAS, Wiskott–Aldrich syndrome.

*Present address: USDA Human Nutrition Research Centre, Tufts University, Boston, MA 02111.
§To whom reprint requests should be addressed.

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Table 1. X chromosome markers used in this study

<table>
<thead>
<tr>
<th>Marker</th>
<th>Locus</th>
<th>Chromosome location</th>
<th>Enzyme</th>
<th>Polymeric fragments, kb</th>
<th>Allele frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>pHO731</td>
<td>OTC</td>
<td>Xp21</td>
<td>Msp I</td>
<td>6.6/6.2</td>
<td>0.61/0.39</td>
</tr>
<tr>
<td>754</td>
<td>DXS84</td>
<td>Xp21.1-Xp21.2</td>
<td>Pst I</td>
<td>5.1/4.4</td>
<td>0.73/0.27</td>
</tr>
<tr>
<td>L1.28</td>
<td>DXS7</td>
<td>Xp11-Xp11.3</td>
<td>Tag I</td>
<td>12/9</td>
<td>0.62/0.38</td>
</tr>
<tr>
<td>58.1</td>
<td>DXS14</td>
<td>Xp11-Xcen</td>
<td>Msp I</td>
<td>4.2/5.5</td>
<td>0.65/0.35</td>
</tr>
<tr>
<td>p8</td>
<td>DXSI</td>
<td>Xcen-XqXq13</td>
<td>Tag I</td>
<td>15/9</td>
<td>0.84/0.16</td>
</tr>
<tr>
<td>pDP34</td>
<td>DXYS1</td>
<td>Xq13-Xq21.1</td>
<td>Tag I</td>
<td>11/12</td>
<td>0.60/0.40</td>
</tr>
</tbody>
</table>

kb, Kilobases.

Early in the study, it became apparent that recombination occurred extensively between WAS and markers mapping to the distal ends of the X chromosome, whereas very little recombination was evident between the disease and proximal marker loci. Linkage analysis was thus performed utilizing six polymorphic markers that, as detailed in Table 1, are located proximally on the X chromosome. Each of the 10 WAS families was informative for at least one of these probes.

Table 2 gives the logarithm of odds of linkage (lod scores) and maximum recombination fractions between WAS and the six markers. The data indicate close linkage between WAS and the loci DXS14 and DXS7. One recombination was observed between WAS and DXS14 in 45 meiotic events and no recombintants were detected between the disease and DXS7. The maximum lod score for WAS–DXS14 was estimated to be 4.29 (odds ratio of 20,000:1) at a recombination fraction (θ) of 0.03 (confidence limits of θ = 0.00–0.16). The maximal lod score between the WAS and DXS7 loci was 4.12 (odds ratio of 30,100:1) at θ = 0.00 (confidence limits of θ = 0.00–0.15).

Although measurable linkage was evident between WAS and some of the other marker loci, lod scores clearly decreased and recombinations increased when markers mapping distally to DXS14 and DXS7 were used as test probes. Thus, recombination was most frequently observed between the disease and the two most distally located marker loci studied, the OTC locus (six recombinants in 41 informative meioses) and the DXYS1 locus (four recombinants in 20 meioses).

Fig. 1 illustrates the segregation of the markers in one WAS kindred that was informative for five of the six test probes. Within this family, the disease segregates with DXS14, DXS7, and DXSI alleles; a single recombination event is evident in individual 12 at the DXS84 and OTC loci.

Homogeneity Test. Although genetic heterogeneity in WAS is unlikely on clinical grounds, we tested for heterogeneity using the WAS–DXS14 linkage data with the HOMOG computer program (21). Linkage analysis on our 10 families revealed no evidence of genetic heterogeneity (x² = 0.00; α = 1.0), thus indicating the existence of only a single WAS locus. To fully exclude genetic heterogeneity, however, studies on additional WAS families will be required.

Table 2. Lod scores between WAS and X chromosome marker loci

<table>
<thead>
<tr>
<th>Marker locus</th>
<th>No. of informative families</th>
<th>No. of informative meioses</th>
<th>Lod scores at varying recombination fractions (θ)</th>
<th>Peak estimate</th>
<th>δ</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>OTC</td>
<td>7</td>
<td>41</td>
<td>0.00 0.001 0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4</td>
<td>0.23</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>DXS84</td>
<td>5</td>
<td>36</td>
<td>-10.03 -6.28 -8.00 0.01 0.33 0.45 0.46 0.39 0.29 0.18</td>
<td>0.23</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>DXS7</td>
<td>5</td>
<td>34</td>
<td>-0.23 0.04 1.32 1.62 1.72 1.69 1.55 1.33 1.06 0.73</td>
<td>0.16</td>
<td>1.73</td>
<td></td>
</tr>
<tr>
<td>DXS14</td>
<td>7</td>
<td>45</td>
<td>4.12 4.12 3.79 3.43 3.10 2.65 2.23 1.78 1.32 0.84</td>
<td>0.00</td>
<td>4.12</td>
<td></td>
</tr>
<tr>
<td>DXSI</td>
<td>3</td>
<td>23</td>
<td>3.66 3.76 4.24 3.90 3.42 2.89 2.33 1.76 1.20 0.68</td>
<td>0.03</td>
<td>4.29</td>
<td></td>
</tr>
<tr>
<td>DXYS1</td>
<td>4</td>
<td>20</td>
<td>0.56 0.56 0.99 1.19 1.20 1.11 0.97 0.80 0.61 0.41</td>
<td>0.13</td>
<td>1.21</td>
<td></td>
</tr>
</tbody>
</table>

Our data establish close linkage between WAS and two loci, DXS14 and DXS7, that map to the proximal short arm of the X chromosome. The probes that define these loci (58.1 and L1.28, respectively) are of potential use in the genetic diagnosis of WAS families. Given that the allele frequencies for the RFLPs detected by each probe are 0.65 and 0.35, 45% of carriers would be heterozygous at one or the other locus, making these two probes useful in the segregation analysis of the majority of WAS families. However, linked markers that flank the disease locus provide much more diagnostic reliability than analysis with linked but nonflanking markers, as

Order of Loci. To obtain information on gene order, we examined the linkage relationships between the marker loci. As indicated in Table 3, the number of individuals doubly heterozygous for the marker loci was, in general, small, and very little linkage information could be obtained for establishing gene order and a localization for WAS. Because multilocus analysis is more efficient in deriving gene order from family data, we next utilized the ILINK program to examine WAS and several marker loci jointly and to thereby determine the most likely order (22). A three-point linkage analysis of WAS and the two closely linked loci DXS14 and DXS7 gave greatest support for the order WAS–DXS14–DXS7, but the evidence was very weak, as the support for this order was only slightly greater than the other possible orders (2:1 odds). To derive more information on order, the analysis was expanded to include three, four, and five marker loci at a time. In Table 4, the maximal likelihood order is given in the top line of each analysis, and the odds in favor of this order over other possible orders is given beside each alternate order. Six-factor analysis revealed two most favored orders: (DXYS1–DXSI)–WAS–DXS14–DXS7–(DXS84–OTC) and (DXYS1–DXSI)–DXS14–WAS–DXS7–(DXS84–OTC). The alternate possible orders were excluded by odds of >100:1. The two favored orders could not be distinguished by this analysis, which provides only slightly greater support (2:9:1) for the first of the two orders. Also the relative placement of locus DXSI to DXYS1 and DXS84 to OTC remained uncertain, although physical mapping data place DXSI proximal to DXYS1 (11).

DISCUSSION

Our data establish close linkage between WAS and two loci, DXS14 and DXS7, that map to the proximal short arm of the X chromosome. The probes that define these loci (58.1 and L1.28, respectively) are of potential use in the genetic diagnosis of WAS families. Given that the allele frequencies for the RFLPs detected by each probe are 0.65 and 0.35, 45% of carriers would be heterozygous at one or the other locus, making these two probes useful in the segregation analysis of the majority of WAS families. However, linked markers that flank the disease locus provide much more diagnostic reliability than analysis with linked but nonflanking markers, as

Table 3. Two-point linkage data between X chromosome markers

<table>
<thead>
<tr>
<th>Locus</th>
<th>OTC</th>
<th>DXS84</th>
<th>DXS7</th>
<th>DXS14</th>
<th>DXS1</th>
<th>DXYS1</th>
</tr>
</thead>
<tbody>
<tr>
<td>OTC</td>
<td>0.21</td>
<td>3.65</td>
<td>1.29</td>
<td>0.37</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>DXS84</td>
<td>0.5</td>
<td>0.92</td>
<td>0.06</td>
<td>0.86</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>DXS7</td>
<td>0.10</td>
<td>0.26</td>
<td>1.71</td>
<td>0.20</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>DXS14</td>
<td>0.23</td>
<td>0.58</td>
<td>0.17</td>
<td>0.65</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>DXSI</td>
<td>0.64</td>
<td>0.14</td>
<td>0.68</td>
<td>0.74</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>DXYS1</td>
<td>0.55</td>
<td>0.21</td>
<td>0.55</td>
<td>0.18</td>
<td>0.50</td>
<td></td>
</tr>
</tbody>
</table>

Maximal likelihood estimates of the recombination fraction are given below the diagonal and corresponding lod scores are given above the diagonal.

in the former instance, double cross-overs would need to occur to produce errors in risk calculations. In the present study, we were unable to establish with certainty the relative positions of the WAS, DXS7, and DXS14 loci but studies on these and additional WAS families with other polymorphic probes will hopefully resolve this issue.

To establish the order of the markers on the X chromosome and the relative position of the WAS locus, we analyzed our data by pairwise and multipoint linkage analysis. Although the pairwise analysis revealed close linkage between the WAS locus and loci DXS14 and DXS7 and measurable linkage between the disease and loci DXSI and DXS84, information on linkage between the markers was limited because of low numbers of informative meioses. Thus, the localization of WAS relative to the marker loci could not be established by pairwise analysis alone. Ultimately, five- and six-factor analyses were necessary to position WAS relative to the marker loci. Not only the low number of meioses, but paradoxically, the low number of recombinations between the marker loci rendered assessment of gene order difficult. As others have reported, these problems are not infrequent in linkage analysis and emphasize the value of multipoint analysis in establishing gene order (23).

Although ambiguities of order were not entirely resolved, the multipoint analysis did provide us with an approximate localization for WAS in the pericentric region or proximal short arm of the X chromosome. Interestingly, two other human immunodeficiency syndromes, X-linked agammaglobulinemia and X-linked lymphoproliferative syndrome, have been mapped to different loci on the long arm of the X chromosome (24, 25). In addition, Nurmi et al. (26) have observed immunodeficiency in a female patient with an interstitial deletion in the short arm of the X chromosome, a deletion that maps distally to WAS. These observations suggest that a number of genes or gene families on the X chromosome are involved in immunoregulation. The existence of such genes has been predicted by several studies that implicate X chromosome genes in human immune function (27–29) and by the recent cloning of an X-linked gene family linked to the murine immunodeficiency disorder zid (30). Thus, the ultimate significance of localization for WAS is a first step toward the identification of the gene or genes on the human X chromosome involved in regulation of lymphocyte development and function.

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