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Genetic Mapping of the Wiskott–Aldrich Syndrome with Two Highly-Linked Polymorphic DNA Markers

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The Wiskott–Aldrich syndrome (WAS) is an X-linked recessive genetic disease in which the molecular defect is unknown. In 15 families with WAS, seven restriction fragment length polymorphic loci from the X chromosome were used to map the disease locus. Of the eight intervals studied, the likelihood of the WAS gene lying between DXS7 (Xp11.3) and DXS14 (Xp11) was at least 128 times higher than that for any other interval. The most likely gene order is DXS84–OTC–DXS7–WAS–DXS14–DXS1–PGK–DXYS1. Close genetic linkage to DXS7 and DXS14 permits accurate prenatal diagnosis and carrier detection with greater than 98% confidence in fully informative WAS families.

The Wiskott–Aldrich syndrome is an X-linked recessive disease characterized by severe immunodeficiency, eczema, and thrombocytopenia with platelets of reduced size and function. The disease has an estimated incidence of 4 per million live male births. Recurrent infections, hemorrhage, and lymphoreticular malignancies are common causes of early mortality in affected males; the median age of survival is 6 years (Perry *et al.*, 1980). Heterozygous females are clinically normal and show selective inactivation of the X chromosome carrying the WAS gene (Gealy *et al.*, 1980). Therefore, no widely accepted test for detection of the female carriers or individuals at risk for WAS is available.

So far, attempts to isolate and characterize the WAS gene directly have been unsuccessful. Two well-characterized membrane glycoproteins are structurally abnormal in patients with WAS: sialophorin in T lymphocytes and gpIb in platelets (Remold-O'Donnell *et al.*, 1984). Recent studies with scanning electron microscopy revealed a unique morphologic abnormality in T lymphocytes and platelets. These morphologic alterations are evident in umbilical cord blood before any clinical manifestations of WAS are evident (Kenney *et al.*, 1986).

As an alternative approach for determining the precise location of any gene (Drayna *et al.*, 1984), restriction fragment length polymorphic (RFLP) markers can be used in combination with multipoint linkage analysis. In a recent linkage study of patients with WAS, Peacocke and Siminovitch (1987) showed that the pericentric region of the X chromosome is the most likely location for the gene. However, their study was unable to specify the exact position of the locus within a region defined by three X-chromosome-specific cloned DNA probes, corresponding to the loci DXYS1, DXS14, and DXS7. These loci span a distance of approximately 27 cM, or approximately 15% of the genetic length of the X chromosome (Arveiller *et al.*, 1987). In this paper we present a more precise localization of WAS. These results will be valuable both to facilitate attempts to identify the WAS gene and for reliable prenatal diagnosis and carrier detection.

MATERIALS AND METHODS

Families

Diagnosis of WAS was established according to the criteria of the World Health Organization Committee on Immunodeficiency (Rosen *et al.*, 1983). To avoid cases due to spontaneous mutation, only families with at least two afflicted members were included in the analysis. After informed consent was obtained from the propositi or their relatives, blood samples of 15–40 ml were taken from 195 individuals in 15 families.

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DNA Isolation and Hybridization

Total genomic DNA was extracted from human peripheral blood as described previously by Kwan *et al.* (1986). Two micrograms of genomic DNA was digested in the appropriate buffer with various restriction endonucleases. Electrophoresis was carried out in 0.8% agarose gels, and DNA was transferred onto Hy-bond nylon membranes according to the method of Southern (1975).

DNA Probes

Of the 19 X-chromosome DNA markers, 11 were used in gene ordering (Aldridge *et al.*, 1984; Bruns *et al.*, 1982; Davies *et al.*, 1983; Drayna *et al.*, 1984; Francke *et al.*, 1984; Horwich *et al.*, 1984; Michelson *et al.*, 1983; Middlesworth *et al.*, 1985; Monaco *et al.*, 1985; Page *et al.*, 1984). DNA probes were ^{32}P labeled either by using T4 DNA polymerase according to the method of O'Farrell (1981) or by the procedure of random primed synthesis. Genomic DNA that bound to nylon filters was hybridized to radiolabelled probes in the presence of 50% formamide solution at 42°C.

Data Analysis

Linkage analysis and inference of gene order were performed using the MLINK computer program (LINKAGE Version 3.5) (Lathrop *et al.*, 1984).

RESULTS AND DISCUSSION

Linkage analysis was performed on a total of 195 individuals in 15 families. Only families with at least 2 WAS offspring were included in the analysis. Four of the families were part of a previous study (Peacocke and Siminovitch, 1987). In several cases, family members from three or four generations were available for testing. We first tested each obligate WAS carrier with a series of 19 DNA probes, spanning both the short and the long arms of the X chromosome. Segregation analysis with a particular marker was then performed only on those families that were informative for that marker. Probes derived from distal regions of the X chromosome were eliminated early in the analysis because of extensive recombination with the WAS gene. Thus, linkage analysis was performed with seven loci (DXS84–OTC–DXS7–DXS14–DXS1–PGK–DXYS1) defined by 11 RFLP markers that are located on the proximal portion of the short and long arms of the X chromosome. No recombinations were observed among the five cloned DNA fragments: pDp7a, P1, p47n, P31, PDP34 (DXYS1 complex); the group was collectively regarded as a single marker system in the analysis because of the physical proximity of the fragments on the long arm of the X chromosome (D. Page *et al.*, in preparation). Each of the families studied proved to be informative for 2 or more RFLP markers with an average of 4 informative markers per family. In some families, persons in the generations preceding the first obligate carrier were available to be tested. Such persons were regarded as unknown with respect to the WAS gene, but their genotypes frequently enabled us to deduce the phase of the markers in the next generation.

The results for the segregation of one RFLP in one family are presented in Fig. 1; *Msp*I alleles were detected by DXS14. One of two total recombinational events with this marker is evident in Fig. 1. As person II_3 is homozygous for the 4-kb allele, it can be concluded that the unaffected grandfather (I_1) also had the 4-kb allele. The WAS gene, coming from the grandmother (I_2), cosegregates with the 2.7-kb *Msp*I allele in two of three obligate carriers, II_1 and II_4. We conclude that in this family, at least one recombination between DXS14 and the WAS locus has occurred. The most likely location for recombination is person II_3, although phase in I_2 is unknown and nonpaternity could confuse the issue.

Results of marker tests were first analyzed in two-point linkage analyses for several recombination frequencies between the WAS locus and each RFLP marker (Table 1). Recombinations were observed between the WAS locus and all markers except DXS1. A significant lod score (logarithm of the odds for linkage) was obtained only for linkage between marker DXS14 and WAS. One recombination with WAS was observed for marker DXS7, and two recombinations for marker DXS14.

The next step in our analysis involved the use of the multipoint linkage program (Lathrop *et al.*, 1984) to establish the order of the loci on the chromosome and

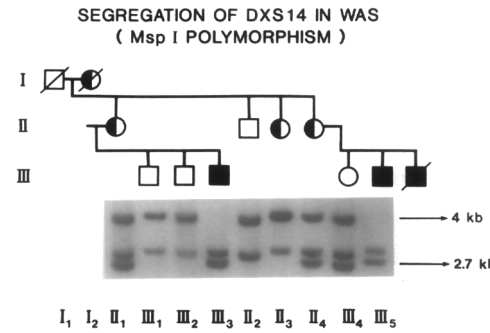

FIG. 1. Segregation of DXS14 and the locus for WAS with the restriction enzyme *Msp*I. High-molecular-weight genomic DNA was isolated from peripheral blood and subjected to *Msp*I digestion prior to electrophoresis on 0.85% agarose gel. DNA was transferred to Hy-bond membrane hybridized to probe 58-1 (DXS14). The restriction pattern shows 4- and 2.7-kb bands with an autosomal band of 3 kb present in all lanes. Darkened squares represent males affected with WAS, and half-darkened circles denote obligate heterozygote females. The affected offspring of II_3 is not shown.

TABLE 1

Lod Scores between WAS and X Chromosome Marker Loci

	Lod scores at varying recombination fractions (θ)								
Marker	0	0.01	0.05	0.10	0.20	0.30	0.40	θ	Z
DXS84	∞	−4.17	−1.01	0.08	0.69	0.63	0.32	0.23	0.71
OTC	∞	−2.62	−0.73	−0.09	0.27	0.24	0.09	0.23	0.28
DXS7	∞	1.78	2.23	2.19	1.76	1.16	0.50	0.06	2.24
DXS14	∞	3.16	4.04	3.97	3.19	2.12	0.96	0.07	4.06
DXS1	1.96	1.92	1.76	1.55	1.11	0.65	0.21	0	1.96
PGK	∞	−2.93	−0.99	−0.28	0.21	0.32	0.23	0.30	0.32
DXYS1	∞	−0.83	1.22	1.78	1.76	1.30	0.68	0.14	1.87

Note. Calculations were carried out with the MLINK program (LINKAGE Version 3.5; Lathrop *et al.*, 1984) with the following assumptions: gene frequency for WAS 0.000003 (other values do not influence the results to any extent); penetrance for WAS in hemizygous males, 100%; carrier females, 0%; homozygous affected females, 100%. Allele frequencies for markers: DXS84, 0.38, 0.62; OTC, 0.39, 0.61; DXS7, 0.68, 0.32; DXS14, 0.35, 0.65; DXS1, 0.16, 0.84; PGK, 0.5, 0.5; all markers in DXYS1 complex, 0.5 and 0.5. Data for the five markers in the DXYS1 complex were analyzed in two ways: once in a six-point analysis, where the five markers were regarded as separate markers with no possibility of recombination between the markers and an arbitrary order within the marker group; and once in a two-point analysis, where data from the five markers were condensed into one locus with three alleles (frequencies 0.25, 0.25, and 0.5). Lod scores obtained in both calculations were identical.

to estimate the genetic distances between loci. The order of the marker loci as used in the multipoint linkage analysis is based on the following previously established order of the probes: DXS84–OTC–DXS7–DXS14–DXS1–PGK–DXYS1 (Bakker *et al.*, 1985; Goodfellow *et al.*, 1985). For most distances between these markers, reliable estimates have been obtained in large-scale linkage studies in "standard" families from the published database (Drayna and White, 1985; Arveiller *et al.*, 1987). No information is available to determine the exact position of DXS14, the most important marker in our analysis, although it has been established that DXS14 is located between DXS7 and DXS1 on the short arm of the X chromosome (Goodfellow et al., 1985). Estimates for the distance between DXS7 and DXS14 obtained by Wirth *et al.* (1988) and Bhattacharya *et al.* (1988) in relatively small data sets vary from 5 to 35 cM. Values exceeding 25 cM are inconsistent with the best estimate for the total distance between DXS7 and DXS1, previously shown to range from 20 to 25 cM (Arveiller *et al.*, 1987). We carried out all calculations for each of five arbitrary positions of DXS14 between DXS7 and DXS1 (at 4, 8, 12, 16, 20 cM from DXS7), while keeping the distance between DXS7 and DXS1 constant at 24 cM. For each position of DXS14, multipoint likelihoods were calculated for 86 arbitrary positions of the WAS gene relative to the map of the other markers. For each assumed position of DXS14, the most likely location of the WAS gene is between DXS7 and DXS14. Highly significant lod scores supporting this order (DXS7–WAS–DXS14) vary between 6.42 and 6.65, depending on the positioning of DXS14. The overall maximum likelihood is obtained for the following distances: DXS7–10 cM–WAS–6 cM–DXS14–8 cM–DXS1. For this position of DXS14, the odds favoring location of WAS between DXS7 and DXS14 over the next most likely location (i.e., between OTC and DXS7) are 128 to 1 (Table 2). For the position of DXS14 farther away from DXS7 (at 20 cM) the odds increase to 203 to 1. For positions of DXS14 closer to DXS7, the odds decrease: 44 to 1 when DXS14 is

TABLE 2

Odds against Each Order, When Compared with the Order of Maximum Likelihood: DXS84–OTC–DXS7–WAS–DXS14–DXS1–PGK–DXYS1

Location of WAS	Odds against (compared to maximum likelihood)
Unlinked to markers	4.52×10^6
29 cM distal of DXS84	4.49×10^5
Between DXS84 and OTC	9.6×10^9
Between OTC and DXS7	128
Between DXS7 and DXS14	1
Between DXS14 and DXS1	228
Between DXS1 and PGK	826
Between PGK and DXYS1	2.8×10^6
19 cM distal ot DXYS1	1862
Maximum lod score	6.655

Note. For these comparisons, the distance between DXS7 and DXS14 was fixed at 16 cM (for all situations the distance of maximum likelihood). The locations at 29 cM distal of DXS84 and 19 cM distal of DXYS1 represent the most likely locations within the distal ranges. All calculations were carried out with the MLINK program (LINKAGE Version 3.5), using Haldane's mapping function. Distances (in cM) were fixed at DXS84–OTC, 5; OTC–DXS7, 18; DXS7–DXS1, 24; DXS1–PGK, 5; and PGK–DXYS1, 5. Loci in the DXYS1 complex were analyzed as one locus.

located at 8 cM from DXS7. However, positions of DXS14 far away from its maximum likelihood location (at 16 cM from DXS7) are unlikely (Fig. 2).

Confidence intervals were constructed for the jointly estimated locations of WAS and DXS14 (Fig. 2). In linkage analysis, a confidence interval for the maximum likelihood location corresponding to a confidence coefficient of approximately 99% includes locations with less than 2 units difference in log likelihood. Since we estimated the positions of two loci simultaneously (DXS14 and WAS), we obtained a confidence area (Fig. 2) within the line indicated by −2, comprising pairs of locations for WAS and DXS14 around the point of maximum likelihood at ML (Fig. 2). Only locations for WAS between DXS7 and DXS14 fall within the confidence area; other locations appear unlikely. As was to be expected with this small number of families, the confidence area is rather large. It covers almost all locations for DXS14 between 5 and 20 cM from DXS7, and almost all locations for WAS between DXS7 and DXS14. To obtain more accurate estimates of the recombination frequency between the WAS locus and its flanking markers, it would be beneficial to obtain a better estimate of the recombination frequency between DXS7 and DXS14 in normal families rather than studying additional WAS families.

We conclude that the WAS gene is most likely located between DXS7 and DXS14. Questions regarding the order of these loci therefore appear to be solved. Markers DXS7 and DXS14 can be used as flanking markers for WAS in prenatal diagnosis and carrier detection. Questions regarding the frequency of recombination between the WAS gene and its flanking markers cannot be answered completely, so long as the exact distance between DXS7 and DXS14 is not known. For the moment, an average recombination frequency of 8% (range 4–12%) can be used in calculations of genetic risk. When both markers are informative, the accuracy of conclusions will be greater than 98%.

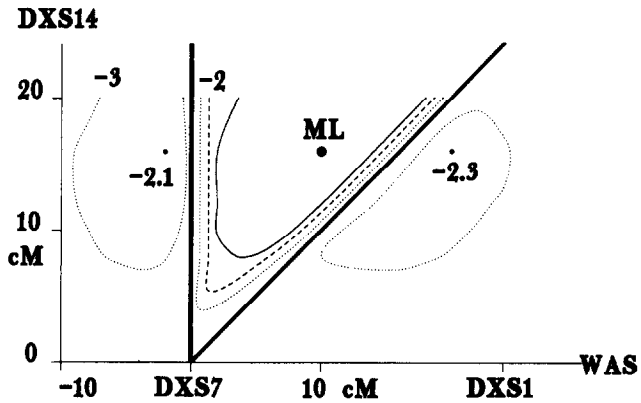

FIG. 2. Contour lines of $\log_{10}$ likelihood for varying locations of marker DXS14 and WAS on the map DXS84–OTC–DXS7–DXS1–PGK–DXYS1. *X*-axis, distance between DXS7 and WAS; *Y*-axis, distance between DXS7 and DXS14. ML, the point of overall maximum likelihood. Thin solid line, max log(L) −3; heavy solid lines, log(L) of minus infinity (recombination between WAS and DXS7 or DXS14). Area inside the dashed line, labeled −2, forms a confidence region (corresponding approximately to a confidence coefficient of 99%) for joint location of DXS14 and WAS.

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