Hereditary X-Linked Thrombocytopenia Maps to the Same Chromosomal Region as the Wiskott-Aldrich Syndrome

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Hereditary X-linked thrombocytopenia occurs either as isolated thrombocytopenia or as a part of the Wiskott-Aldrich syndrome (WAS). We studied X-linked thrombocytopenia in a family with eight affected male members, none of whom exhibited the increased susceptibility to infection that occurs in WAS. We found a significant linkage between thrombocytopenia and DXS 146, a marker on the proximal part of the short arm of the X-chromosome. WAS has previously been mapped to the same chromosomal region. The present findings indicate that X-linked thrombocytopenia and WAS are closely related and may even be caused by different mutations of the same gene. This view is supported by our findings of atopic symptoms and minor deviations in immunologic variables among some of the affected subjects.

HEREDITARY thrombocytopenia occurs in several forms differing from each other in inheritance pattern. The commonest forms of transmission are autosomal dominant[1-4] and X-linked recessive.[5-12] The X-linked form has been considered by some researchers to be a separate genetic entity,[7,9,13] or by others[6,8,12,14] to be a milder variant of the Wiskott-Aldrich syndrome (WAS), an X-linked disease characterized by thrombocytopenia and abnormally small platelets, profound immunodeficiency, eczema, and a poor prognosis.[15-17]

The WAS locus has recently been mapped to the proximal short arm of the X-chromosome.[18] We report studies in a family with hereditary thrombocytopenia in eight male members, none of whom had severe immunodeficiency. Thrombocytopenia showed significant linkage with a polymorphic DNA sequence on the proximal part of the short arm of the X-chromosome, suggesting that X-linked thrombocytopenia and WAS are caused by different mutations of the same gene. This hypothesis is further supported by the finding of deviations in immunologic variables among some of the affected members.

MATERIALS AND METHODS

Family members were examined and checked for any history of abnormal bleeding, infections, eczema, or other diseases. Blood samples were obtained for studies of hematologic and immunologic variables, liver enzymes, serum creatinine, and DNA extraction.

Hematologic variables were determined with standard methods. Platelets were counted by phase microscopy, and platelet volume distribution data were obtained with a cell counter (Coulter S-plus, Hialeah, FL). Platelet-bound IgG was quantified with the method of Shaw et al,[19] using radiolabeled protein A as described by Donnér et al.[20]

Serum Igs and the complement components, C3 and C4, were measured with standard techniques. IgG subclasses were determined with monospecific polyclonal antisera, as outlined by Oxelius.[21] Circulating immune complexes were measured as Clq-binding material. Immunologic phenotyping of lymphocytes was performed using commercial mouse monoclonal antibodies against CD2, CD3, CD4, CD8 (Ortho, Raritan, NJ) and CD16 and CD19 (Becton Dickinson, Mountain View, CA). Two hundred cells were counted with immunofluorescence microscopy.

DNA Studies

DNA was extracted from EDTA-stabilized peripheral blood with routine methods; 5 μg DNA was digested with the appropriate restriction enzyme, as recommended by the manufacturer (Boehringer, Mannheim). The resulting fragments were separated on 0.7% agarose gel (BioRad, Richmond, CA), followed by blotting onto Gene Screen Plus membranes (New England Nuclear, Boston) by alkali transfer. Radioactive probes were prepared by oligonucleotide-primed DNA synthesis, using ^{32}P-dCTP.[22] Before labeling was done, the insert was excised from the plasmid and purified on agarose gel. The filters were hybridized overnight at 68°C in 0.5 mol/L Na-phosphate, pH 7.2, 7% sodium dodecyl sulfate (SDS) and 1 mmol/L EDTA. Excess probe was washed from the filters first with 0.04 mol/L phosphate buffer, pH 7.2, 1% SDS, and then with 0.02 mol/L phosphate buffer, 0.5% SDS. Signals were detected by autoradiography with Kodak X-O-MAT AR 5 films and intensification screens.

Five probes for five different polymorphic DNA loci were used. Four of these loci (OTC, DXS7, DXS146, DXS14) have previously been mapped to the proximal short arm of the X-chromosome, whereas DXS1 has been mapped to Xq11-13.[23] The DNA probes were provided by investigators from different laboratories: L 1.28 was from P. Pearson, TAK 8a from T. Kruse, p8 from G. Bruns, p58.1 from L. Kunkel, and pH0731 from A. Horwitz. Table 1 lists the probes and their characteristics.

Linkage Analysis

Lod scores at different recombination fractions were estimated with the computer program LIPED.

Patients

The pedigree of the patients is given in Fig 1.

II:4. II:4 is a 55-year-old man who has not been unusually susceptible to infections. At age 7 years, however, he developed slowly progressing chronic glomerulonephritis which recently led to uremia. For ~10 years, beginning at age 22 years, he had recurrent episodes of deep vein thrombosis in the legs and of pulmonary emboli and has since been on dicumarol treatment. He also has severe bronchial asthma requiring corticosteroid treatment, severe arterial

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Table 1. X-Chromosome Markers Examined for Linkage to Thrombocytopenia

Marker	Locus	Enzyme	Polymorphic Fragments (kb)	Allele Frequencies	Alleles
pH0731	OTC	*Msp*I	6.6/6.2	0.61/0.39	A, B, C, D
			5.1/4.4	0.73/0.27	(haplotypes)
L1.28	DXS7	*Pst*I	12/9	0.68/0.32	T1 T2
TAK8a	DXS146	*Xba*I	3.3/5.0	0.77/0.23	X1 X2
58.1	DXS14	*Msp*I	4/2.5	0.65/0.35	M1 M2
p8	DXS1	*Taq*	15/9	0.84/0.16	P1 P2

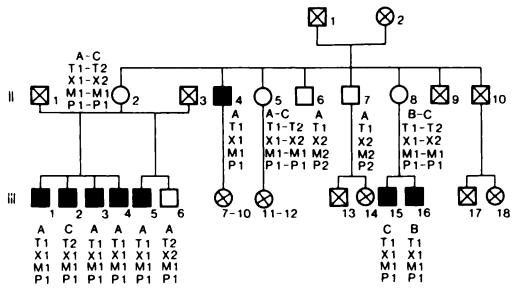

Fig 1. Pedigree. Letters indicate alleles of the X-chromosomal polymorphic loci OTC, DXS7, DXS146, DXS14, and DXS1, as given in Table 1. (⊠ ⊗), not tested; (■), thrombocytopenic males.

hypertension, and type II diabetes mellitus. In the last 2 years, he developed cardiomyopathy with heart dilatation. He has had no bleeding symptoms.

III:1. III:1 is a 16-year-old boy who has been consistently healthy apart from easy bruising.

III:2. III:2 is a 13-year-old boy who bruises easily. He has been admitted to the hospital twice because of bleeding in his left knee associated with trauma but received no treatment apart from ε-aminocaproic acid.

III:3. III:3 is a 10-year-old boy with atopic eczema. At age 10 years, he was admitted to the hospital because of a superficial thrombophlebitis of the right thigh. He bruises easily but otherwise has no bleeding symptoms.

III:4. III:4 is an 8-year-old boy who bruises easily, but is otherwise healthy.

III:5. III:5 is a 4-year-old boy who was examined for the first time at age 3 months; his platelet count was normal. At age 6 months, he had petechiae, and his platelet count has since been consistently low. Bleeding symptoms have been mild and have consisted mostly of skin hematomas.

III:15. III:15 is a 26-year-old man with bronchial asthma and allergic rhinoconjunctivitis since childhood. He bruises easily but is otherwise free of severe bleeding symptoms.

III:16. III:16 is a 24-year-old man subject to recurrent nose bleeds and skin hematomas.

RESULTS

The pedigree (Fig 1) shows eight thrombocytopenic male family members and clearly indicates an X-linked mode of inheritance. Table 2 lists the platelet counts of the eight patients. All patients also had decreased mean platelet volume. Platelet-bound IgG was high in two individuals (III:1 and III:2) (Table 2). In the same two cases, Coombs' direct test was weakly positive, and circulating immune complexes could be detected in plasma. Serum creatinine was normal in all patients except II:4, who had chronic glomerulonephritis (Table 2). Liver enzymes were normal in all cases.

Serum Igs and complement components are given in Table 3. No major abnormality was found. IgM levels were borderline in most cases but still within normal range. IgA was within normal limits. IgG subclasses were normal with two exceptions: In III:15, IgG 1 was 4.35 g/L (normal 4.62 to 12.92 g/L) and IgG 3 was 0.39 g/L (normal 0.41 to 1.29 g/L); in III:16, IgG 3 was 0.18 g/L. IgE was increased in three patients. The complement component C3 was slightly increased in three patients, and C4 was slightly increased in two.

All patients had normal granulocyte and lymphocyte counts. Data on lymphocyte phenotyping are given in Table 4. Two patients had a slightly decreased proportion of CD2 cells, and one had a slightly decreased proportion of CD3 cells. Three patients had a low proportion of CD8 cells leading to increased ratios of CD4/CD8. Five patients had a low proportion of CD16 cells (natural killer cells), and one had a low proportion of CD19 (pan-B) cells.

Five DNA markers were examined for linkage with thrombocytopenia. Segregation of the markers is shown in Fig 1 in the established gene sequence: Xter-OTC-DXS7-DXS146-DXS14-cent-DXS1. The most informative marker was DXS146 (alleles X1 and X2). No recombinants were detected in 11 informative meioses (Fig 2).

I:2 is clearly a carrier, having one affected son and two

Table 2. Laboratory Data in Thrombocytopenic Subjects

Patient	Platelet Count × 10^9/L	Mean Platelet Volume (fL)	Platelet-Bound IgG PABS/Platelet	Circulating Immune Complexes (μg/mL)	Serum Creatinine (μmol/L)
II:4	82	4.6	474	<100	274
III:1	57	4.6	2133	149	76
III:2	26	4.8	2072	145	59
III:3	33	4.4	293	<100	62
III:4	50	4.8	250	<100	54
III:5	58	4.1	192	<100	50
III:15	68	4.7	ND	167	73
III:16	79	4.5	ND	<100	71
Normal range	125-400	8.0-10.3	≤500	<100	19-115

PABS, protein A binding sites.

Table 3. Serum Immunoglobulins and Complement Components in Thrombocytopenic Subjects

Patient	Age (yr)	IgG (g/L)	IgM (g/L)	IgA (g/L)	IgE (kU/L)	C3 (%)	C4 (%)
II:4	55	10.2	0.6	2.0	784	131	246
III:1	16	10.5	1.1	2.72	8	108	102
III:2	13	10.7	0.41	1.66	116	146	99
III:3	10	7.39	0.36	1.77	20	139	214
III:4	8	7.0	0.36	2.5	7	144	121
III:5	4	5.32	0.28	0.51	3	86	73
III:15	26	8.27	0.85	1.52	109	83	145
III:16	24	8.56	0.71	1.87	25	81	109
Normal range (>age 5 yr)		5-15	0.3-1.5	0.5-3.0	<100	70-136	53-207

Table 4. Immunologic Phenotyping of Lymphocytes in Thrombocytopenic Individuals

Patient	CD2 (%)	CD3 (%)	CD4 (%)	CD8 (%)	CD16 (%)	CD19 (%)	CD4/CD8
II:4	72.5	75	52.5	22	8	2.5	2.3
III:1	76.5	70.5	53.5	20	12.5	7	2.7
III:2	80	70.5	60	15.5	2.5	8	3.8
III:3	79	48	43.5	25	2	5	1.7
III:4	82.5	70.5	57	19.5	16	16	2.9
III:5	80	73	57.5	7.5	6.5	9	7.6
III:15	80	69	54.5	20	4.5	10	2.7
III:16	71.5	68	52	13	4	11.5	4.0
Normal range	75-92	59-89	38-58	17-39	8-22	5-15	1-3.4

carrier daughters. By inference, she is heterozygous for the OTC, DXS146, DXS14, and DXS1 alleles. Although her linkage phase is not known, a recombination between the disease locus, TBP (thrombocytopenia), and the OTC locus must have occurred, since II:4, who is affected, has received the same OTC allele as his two nonaffected brothers, II:6 and II:7. These meioses showed a cosegregation of the TBP/DXS146/DXS14/DXS1 haplotype placing TBP proximal to OTC.

II:2 is a carrier and heterozygous for the OTC, DXS7, and DXS146 markers. Recombinations are detected in III:2 and III:6. The haplotype in III:2 showed a recombination between the disease locus and DXS7 and cosegregation of the DXS146/TBP haplotype and the DXS7/OTC haplotype, respectively. TBP must therefore map proximal both to DXS7 and OTC.

The lod scores between TBP and the different X-chromosome marker loci are shown in Table 5. There was a significant linkage between TBP and DXS146, which was maximum at zero recombination. Owing to the lack of family members simultaneously heterozygous for all markers, however, we were unable to establish the disease locus in respect to DXS146, DXS14, and DXS1.

DISCUSSION

The thrombocytopenia in this family was clearly inherited as an X-chromosomal recessive trait. In addition, affected members had abnormal platelet volume distribution curves with low mean platelet volume. The most common X-linked disorder associated with thrombocytopenia is WAS, which is characterized by abnormally small platelets but also by extensive immunologic alterations,[24] leading in most cases to an early death owing to fulminant infections or to development of lymphoreticular malignancies. However, X-linked inheritable thrombocytopenia without profound immunodeficiency has been reported in several families.[6-10,12-14] In some of these studies,[12,14] as in our kindred, thrombocytopenia was associated with reduced platelet volume, thus resembling WAS.

In contrast to WAS, the major symptom in X-linked thrombocytopenia is abnormal bleeding and not severe infec-

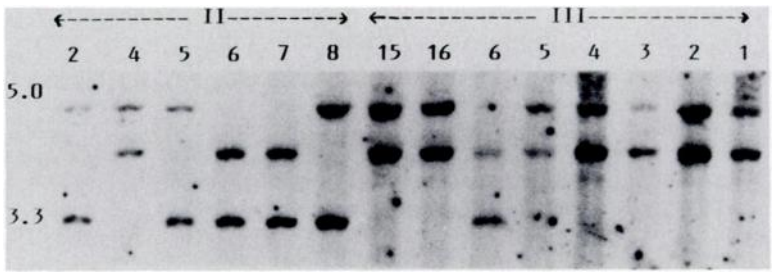

Fig 2. Southern blot of *Xba*I-digested DNA from the investigated family, hybridized with *TAK*8a. Blot shows cosegregation of the 5.0-kilobase fragment and the disease. Lanes 1 through 6, generation II; lanes 7 through 14, generation III.

Table 5. Lod Scores Between TBP and X-Chromosome Marker Loci

Marker Locus	Lod Scores at Varying Recombination Fractions (θ)							
	0.00	0.05	0.10	0.15	0.20	0.25	0.30	0.40
OTC	$-\infty$	−3.05	−1.71	−1.00	−0.56	−0.27	−0.09	−0.07
DXS7	$-\infty$	0.93	1.06	1.07	1.01	0.92	0.78	0.44
DXS146	3.42	3.13	2.82	2.50	2.15	1.79	1.41	0.59
DXS14	1.20	1.09	0.97	0.85	0.72	0.58	0.43	0.15
DXS1	1.20	1.09	0.97	0.85	0.72	0.58	0.43	0.15

tions. The bleed episodes are generally mild, consisting of excessive bruising, hematomas, epistaxis, and hematuria.[6-8,10,12] In the family, we studied easy bruising was the commonest symptom, though one patient had recurrent hemarthroses after trauma.

Although X-linked thrombocytopenia as reported here is not generally associated with increased susceptibility to infection, the question has, nevertheless, been raised of whether it is related to WAS, because some patients with benign X-linked thrombocytopenia have also had chronic eczema,[6,7,12,14] a finding typical of WAS. In addition, minor immunologic aberrations have been detected in a few patients, eg, high serum IgA and low isoagglutinin titers.[8,12,13]

Of our eight patients, one had chronic eczema and two had bronchial asthma, both of which are also common findings in WAS. One patient (II:4), although not unduly susceptible to infection, manifested clinical evidence of dysregulation of his immune system; besides severe bronchial asthma, he had progressive glomerulonephritis, diabetes mellitus, and recurrent deep vein thrombosis. Relationships have previously been reported[12,13] between nephropathy and X-linked thrombocytopenia, as well as between nephropathy and WAS,[25] although the mechanism of renal damage has not been clarified. In some members of our family, laboratory investigations also revealed deviations in immunologic variables, eg, increased levels of IgE, circulating immune complexes, and increased platelet-associated IgG. Slight abnormalities of cellular components of the immune system could also be demonstrated.

Although immunologic abnormalities in X-linked thrombocytopenia suggest a relationship to WAS, the evidence is circumstantial. More direct evidence could be obtained by demonstrating linkage to the DNA markers that are also linked to WAS. Recently, Peacocke and Siminovitch[18] demonstrated significant linkage between WAS and two loci, DXS14 and DXS7, that map to the proximal short arm of the X chromosome. They found one recombination between WAS and DXS14 in 45 meiotic events, and no recombination between the disease locus and DXS7. Arveiler et al[26] demonstrated linkage of WAS to DXS1 (at Xq11-q13), suggesting a paracentromeric location of WAS. The published data favor the gene sequences Xter-OTC-DXS7-DXS14-WAS-cent-DXS1 or Xter-OTC-DXS7-WAS-DXS14-cent-DXS1.[18] Our data show close linkage between X-linked thrombocytopenia and the locus, DXS146, which has been placed between DXS7 and DXS14 (T. Kruse, personal communication, May 1987). Furthermore, we found recombinations between thrombocytopenia and OTC and one recombination between thrombocytopenia and DXS7. We detected no recombinations between thrombocytopenia and the loci DXS14 and DXS1, but the few informative meioses observed with these two markers provided little information regarding linkage. The data clearly demonstrate that the locus for X-linked thrombocytopenia should be placed proximally to OTC and DXS7, but its order in relation to DXS14 and DXS1 could not be decided. The locus maps to the same chromosomal region as the WAS locus, however, supporting the hypothesis that the two disorders are caused by different mutations within the same gene.

The gene product or gene products lacking or defective in WAS have not yet been identified. Remold-O'Donnell et al[27] reported a deficiency of the surface protein, sialophorin, in lymphocytes of patients with WAS, but whether this is a primary or secondary event and how it is related to primary T-lymphocyte and platelet defects is not yet known. Even less is known about X-linked thrombocytopenia without profound immunodeficiency. Precise location and exploration of the thrombocytopenia and WAS loci by means of molecular biology techniques may be one way of elucidating the pathogenesis of these disorders.

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