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Refinement of Linkage of Human Severe Combined Immunodeficiency (SCIDX1) to Polymorphic Markers in Xq13

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Summary

The most commmon form of human severe combined immunodeficiency (SCID) is inherited as an X-linked recessive genetic defect, MIM 300400. The disease locus, SCIDX1, has previously been placed in Xq13.1-q21.1 by demonstration of linkage to polymorphic markers between DXS159 and DXS3 and by exclusion from interstitial deletions of Xq21.1-q21.3. We report an extension of previous linkage studies, with new markers and a total of 25 SCIDX1 families including female carriers identified by nonrandom X chromosome inactivation in their T lymphocytes. SCIDX1 was nonrecombinant with DXS441, with a lod score of 17.96. Linkage relationships of new markers in the SCIDX1 families were consistent with the linkage map generated in the families of the Centre d'Etude du Polymorphisme Humain (CEPH) and with available physical map data. The most likely locus order was DXS1–(DXS159,DXS153)–DXS106–DXS132–DXS453–(SCIDX1,PGK1, DXS325,DXS347,DXS441)–DXS447–DXS72–DXYS1X–DXS3. The SCIDX1 region now spans approximately 10 Mb of DNA in Xq13; this narrowed genetic localization will assist efforts to identify gene candidates and will improve genetic management for families with SCID.

Introduction

Severe combined immunodeficiency (SCID) is a phenotype characterized by susceptibility to severe and persistent infections due to lack of both cell-mediated and humoral immune responses. Although previously lethal in the first year of life, SCID in many patients is now successfully treated with bone marrow transplantation. Several distinct autosomal defects can give rise to the SCID phenotype, but the most common form of SCID in the United States is caused by X-linked recessive mutations of the SCIDX1 locus (Conley et al. 1990; Puck et al. 1992; WHO Scientific Group 1992). SCIDX1 (formerly IMD4) was first localized to proximal Xq by de Saint Basile et al. (1987); we subsequently confirmed this map position and determined SCIDX1 to lie in Xq13.1-q21.1, by tight linkage to PGK1 and DXS72 and by deletion analysis (Puck et al. 1989).

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Efforts to localize SCIDX1 have been hampered by several factors, including (i) the rarity of SCID, estimated to occur on the order of 1 per 10^4–10^5 births (Stiehm 1993); (ii) an array of known and unknown confounding autosomal genocopies; (iii) lack of affected patients with recognized cytogenetic abnormalities, such as translocations, to pinpoint the genetic region; (iv) early lethality in affected males, universal in previous generations; and (v) the lack of any immunologically detectable abnormality which would identify female carriers. Only the last of these difficulties has been overcome, with the discovery that female carriers of SCIDX1 have nonrandom X chromosome inactivation in cell lineages specifically targeted by the gene defect (Puck et al. 1987, 1992; Conley et al. 1988). Thus, women at risk of being SCIDX1 carriers can be identified because the active X chromosome in their mature T lymphocytes is uniformly the nonmutant X.

Since our first report of SCIDX1 linkage in six pedigrees, we have accumulated 25 unrelated kindreds segregating this disorder. The incorporation of newly available polymorphic markers into our original panel has enabled us to narrow the region in which SCIDX1 lies and has helped us to order loci in Xq13.

Material and Methods

Subjects

Records from all subjects, recruited throughout the United States through immunologists and geneticists, were reviewed to establish the diagnosis of SCID. Subjects or their parents gave informed consent for genetic evaluation of DNA samples prepared from blood, amniocytes, or lymphoblastoid cell lines. Of the 25 SCIDX1 pedigrees included in linkage analysis, 17 had more than one affected boy in at least two generations and demonstrated X-linked inheritance. Of these multigeneration pedigrees, eight have been published, including A–F in our original linkage report (Puck et al. 1989) and the families reported by Fireman et al. (1966) and Gatti et al. (1968); the seven most informative new multigeneration pedigrees are G–N, illustrated in the top portion of figure 1.

The remaining 8 of the 25 total pedigrees, of which 5, P–T, are shown in the bottom portion of figure 1, were ascertained either through a sporadic affected male or, in families P and R, two affected male siblings with otherwise negative family history. A sixth pedigree has been published previously (Puck et al. 1990). These families were considered to have SCIDX1 mutations because mothers of affected boys demonstrated nonrandom X chromosome inactivation in T cells (see Evaluation of Carrier Status subsection below). In both family P and family T, an affected male was born after genetic workup and prenatal diagnosis, in each case confirming that the genotype had been correctly assigned by the X inactivation carrier test (Himelstein et al. 1993).

Evaluation of Carrier Status

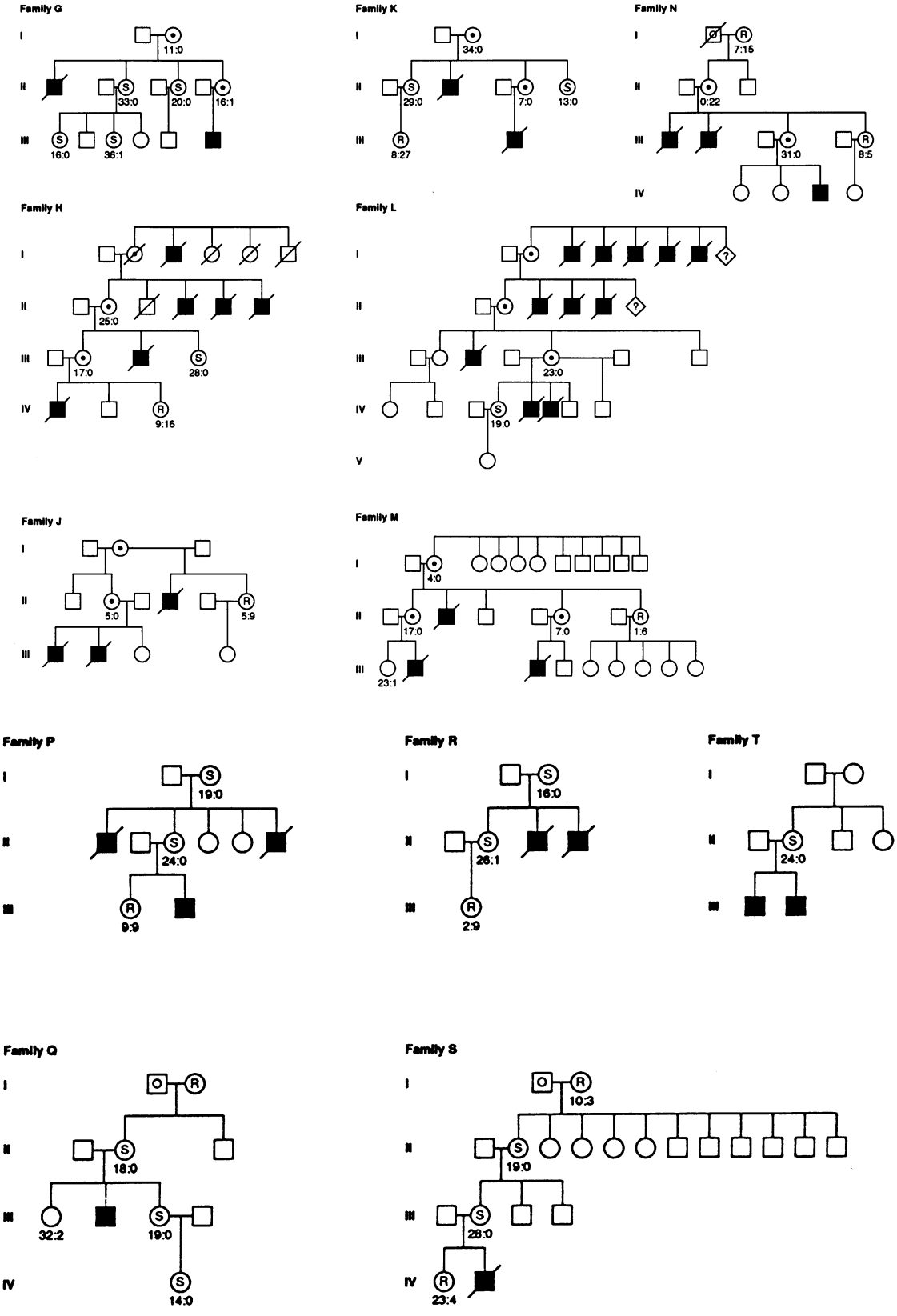
All available females were assayed for X chromosome inactivation pattern in T cells, by the somatic cell hybrid technique reported elsewhere (Puck et al. 1992). In brief, peripheral blood T cells, highly purified after polyclonal phytohemagglutinin stimulation, were fused with $HPRT^-$ hamster fibroblasts and cultured with hypoxanthine and azaserine to select for retention of the human active X chromosome. In over 90% of hybrid clones arising from such fusions, a single human X chromosome, the active X, was found. DNA from a panel of independent hybrids was analyzed by Southern blot, with an X chromosome RFLP probe for which the subject was heterozygous, to identify the active human X chromosome. The numbers of hybrids containing a human active X chromosome from each parent (paternally derived:maternally derived) are shown below each tested female's symbol in figure 1. For females who had no sons affected with SCID, assignment of status as either a noncarrier, for those with random patterns (R), or a carrier, for those with skewed patterns (S), was made according to a maximum-likelihood analysis published previously (Puck et al. 1992).

Genotyping at Marker Loci

Genotypes at marker loci in the pericentromeric region and proximal long arm of the X chromosome were determined by Southern blot analysis of genomic DNA as reported elsewhere (Puck et al. 1989). In addition, for many females, DNA samples prepared from the cloned hybrid cell lines containing a single active human X chromosome were included to confirm accuracy and to establish phase. RFLP probes were obtained from the American Type Culture Collection (Rockville, MD) or with permission of the originators, for DXS14 in Xp11.21; DXS1 in Xq11.2; DXS159, DXS153, DXS106, and DXS132 in Xq12; DXS347, DXS325, and DXS441 in Xq13.2-q13.3; PGK1 in Xq13.3; DXS447 and DXS72 in Xq21.1; DXYS1X in Xq21.31; and DXS3 in Xq21.3 (Williamson et al. 1991). All but DXS1, DXS347, and DXS325 are included in the Reference Marker List from the Human Gene Mapping 11 Workshop (Davies et al. 1991). Poly-TG microsatellite polymorphisms at DXS441 (Ram et al. 1992) and DXS453 (Weber et al. 1990) were also used, with primers and PCR conditions as described.

Linkage Analysis

Linkage calculations were performed on a MicroVAX 3400 under VAX/VMS 5.4 using version 4.9 of the LINKAGE analysis package (Lathrop et al. 1985). Allele frequencies for published RFLP loci were obtained from Williamson et al. (1991). Every pair of loci was analyzed by MLINK using the 25 SCIDX1 pedigrees to determine lod score *(Z)* values at specified recombination fraction (θ) values; the θ yielding the greatest Z by this method was used as a seed for iteration by ILINK to determine the θ of maximum likelihood, $\hat{\theta}$, for each pair of loci. The SCIDX1 locus was placed on the map of marker loci by using the LINKMAP program. The values for distances between fixed loci in our data set were combined published values as described below in Results. Order of marker loci was established by genetic recombination and physical mapping data. Mutation rate and allele frequency for SCIDX1 were assigned to be 10^{-6} and 2×10^{-6}, respectively. For diagnosed or obligate female carriers of SCIDX1 whose parents were not available, the genotype of the active X,


Family G
I
II
III
11:0
S
33:0
S
20:0
16:1
S
16:0
S
36:1
Family K
I
II
III
34:0
S
29:0
7:0
S
13:0
R
8:27
Family N
I
II
III
IV
Ø
R
7:15
0:22
31:0
R
8:5
Family H
I
II
III
IV
25:0
17:0
S
28:0
R
9:16
Family L
I
II
III
IV
V
?
?
23:0
S
19:0
Family J
I
II
III
R
5:9
5:0
Family M
I
II
III
4:0
17:0
7:0
R
1:6
23:1
Family P
I
II
III
S
19:0
S
24:0
R
9:9
Family R
I
II
III
S
16:0
S
26:1
R
2:9
Family T
I
II
III
S
24:0
Family Q
I
II
III
IV
O
R
S
18:0
32:2
S
19:0
S
14:0
Family S
I
II
III
IV
O
R
10:3
S
19:0
S
28:0
R
23:4

isolated in a human/hamster hybrid, was encoded as a parental X.

Results

In the families included in this study, carrier status was assayed by X inactivation analysis in 71 women at risk who had no affected offspring. Odds ratios, calculated according to the method of Puck et al. (1992), of either >90:1 or <1:100 for being a carrier were found in all but four women, one each in family C and family D (odds ratios 1.5:1 and 2.3:1, respectively [Puck et al. 1992]) and, as shown in figure 1, one each in the third generations of family M (odds ratio 60:1) and family Q (odds ratio 20:1). These women were classified as unknown SCIDX1 genotype.

Two-point linkage of SCIDX1 to pericentromeric and proximal Xq polymorphic marker loci is shown in table 1. SCIDX1 was closely linked to markers in Xq13, showing no recombination with DXS441, DXS325, DXS347, or PGK1. There was also no recombination with the more distal marker DXS72. The use of both RFLP and microsatellite polymorphisms at DXS441 made this the most informative locus, which showed no recombination with SCIDX1 with $Z = 17.96$. The relative order of DXS347, DXS325, DXS441, and PGK1 has not been established by recombination, but the first three of these markers are proximal to the Menke syndrome translocation breakpoints, while PGK1 is distal (Verga et al. 1991; Tumer et al. 1992).

Pairwise linkage of loci was determined for the SCIDX1 pedigrees, and all θ values supported by $Z > 3$ are shown in table 2, along with the $\hat{\theta}$ and the peak Z ($\hat{Z}$) at that $\hat{\theta}$. Because Z values for two-point linkage are additive, we combined our scores with those of Mahtani et al. (1991) for the CEPH 40-family panel, wherever common pairs of markers were tested. The more precise Z values based on both data sets are shown in boldface type in table 2.

Multipoint linkage analysis was performed with multiple sets of six stationary loci, moving the SCIDX1 locus from proximal Xp through Xq21.3. The genetic distances used for the fixed loci were $\hat{\theta}$ values at $\hat{Z}$ values underlined in table 2, where available; others were obtained from our own data and from Keats et al. (1989). When no recombination was documented but order was established by physical map data, such as the placement of DXS447 distal to DXS441, a genetic distance of 0.001 Morgan was assigned. A representative run is shown in figure 2, in which SCIDX1 was placed at intervals throughout the map: DXS159–.05 M–DXS106–.03 M–DXS132–.13 M–DXS441–.06 M–DXY S1X–.09 M–DXS3. The $\hat{Z}$ values indicated for each interval show that the maximum-likelihood location for SCIDX1 was at DXS441. SCIDX1 was 1.3×10^9–fold more likely to be distal, rather than proximal, to DXS159 and was 2×10^{11}–fold more likely to be distal to DXS132. Similarly, SCIDX1 was 6.6×10^7–fold more likely to be proximal, rather than distal, to DXYS1X. Other stationary marker sets gave substantially the same results; for example, in calculations not illustrated, SCIDX1 was 6.2×10^4–fold more likely proximal to DXS447.

The 19 recombinant X chromosomes from our families which most narrowly localized SCIDX1 are shown in figure 3. Individuals who inherited a maternal X chromosome with a normal SCIDX1 allele (as assessed either by non-SCID phenotype, for males, or by random X inactivation, in at-risk females) are shown on the left, and those with a mutant SCIDX1 allele are shown on the right, with the resultant SCIDX1 critical interval shaded. Two X chromosomes, one each in families N and E, had double crossovers, and one, in family P, had three crossovers in the interval illustrated. All recombinations were confirmed by more than one marker allele on each side of a crossover, except for the SCIDX1 carrier female from family E, whose mother was homozygous at all loci tested between DXS447 and DXS132. Two non-SCID phenotype chromosomes, one each in families N and P, and one affected individual's chromosome in family K have crossovers which mark the proximal boundary of the SCIDX1 region as DXS132. These plus non-SCID chromosomes from families C and B (fig. 3, *left*) and SCID chromosomes in families

Figure 1 Previously unpublished pedigrees of multigeneration SCIDX1 families (Family G–Family M) and families ascertained through affected males in a single generation whose mothers were found to carry SCIDX1 mutations by X inactivation carrier testing (Family P–Family T). Blackened boxes denote affected males; unblackened boxes denote unaffected males; and circles with central dots denote obligate carrier females. A slash mark denotes that the individual is deceased. R = females at risk for carrying SCIDX1 mutations with random (noncarrier) T cell X chromosome inactivation; and S = females at risk for carrying SCIDX1 mutations with skewed (carrier) T cell X chromosome inactivation. Paternal:maternal ratios of hybrids scored are shown below the circles representing females who were tested. In families N, Q, and S, squares with unblackened circles denote the chromosome of origin of the SCIDX1 mutation, determined by linkage study. In families P and T, a generation-III affected male was born subsequent to genetic prediction and prenatal diagnosis by linkage. Families P, Q, and S were originally reported by Conley et al. (1990).

Table 1

Z Values, for Increasing θ Values, between SCIDX1 and Pericentromeric and Proximal Long-Arm Loci on the X Chromosome

	Z AT θ = [a]												
LOCUS	0	.001	.01	.05	.10	.15	.20	.25	.30	.35	.40	$\hat{\theta}$[b]	$\hat{Z}$[c]
DXS14	$-\infty$	.61	3.50	5.06	<u>5.26</u>	5.05	4.65	4.12	3.49	2.76	1.94	.092	5.27
Centromere													
DXS1	−15.40	−4.18	−2.75	2.11	2.77	<u>2.91</u>	2.80	2.55	2.19	1.74	1.21	.148	2.91
DXS159[d]	$-\infty$	2.99	7.78	10.30	<u>10.52</u>	10.50	9.18	8.09	6.80	5.33	3.68	.087	10.73
DXS153[d]	2.39	5.08	5.98	<u>6.24</u>	5.92	5.44	4.84	4.18	3.44	2.65	1.81	.038	6.26
DXS106	$-\infty$	2.47	5.32	6.76	<u>6.79</u>	6.41	5.82	5.09	4.24	3.29	2.24	.074	6.86
DXS132	$-\infty$	3.95	6.79	<u>8.19</u>	8.16	7.69	6.99	6.13	5.13	4.00	2.74	.071	8.26
DXS347[e]	<u>8.89</u>	8.88	8.75	8.19	7.45	6.67	5.86	4.99	4.08	3.10	2.08	.0	8.89
DXS325[e]	<u>2.69</u>	2.69	2.65	2.49	2.28	2.06	1.83	1.58	1.31	1.02	.71	.0	2.69
DXS441[e]	<u>17.96</u>	17.94	17.68	16.54	15.05	13.48	11.83	8.24	6.31	1.90	.94	.0	17.96
PGK1	<u>8.73</u>	8.71	8.60	8.06	7.36	6.61	5.82	4.99	4.10	3.15	2.15	.0	8.73
DXS447	4.18	6.87	7.73	<u>7.79</u>	7.27	6.59	5.82	4.98	4.07	3.11	2.09	.027	7.89
DXS72	<u>10.38</u>	10.36	10.21	9.53	8.65	7.73	6.76	5.74	4.68	3.55	2.38	.0	10.38
DXYS1X	−3.58	4.02	6.85	<u>8.20</u>	8.14	7.64	6.94	6.09	5.12	4.03	2.82	.067	8.26
DXS3	$-\infty$	−7.80	−.95	3.27	4.49	<u>4.79</u>	4.67	4.30	3.73	3.01	2.14	.158	4.79

[a] For each marker, the $\hat{Z}$ is underlined.
[b] Most likely θ.
[c] Z at $\hat{\theta}$.
[d] The relative order of DXS159 and DXS153 has not been established.
[e] The relative order of DXS347, DXS325, and DXS441 has not been established.

G and E (fig. 3, *right*) could narrow the SCIDX1 region proximal boundary further, with additional informative markers. Similarly, there are five potentially informative meioses for definition of distal boundaries with new markers.

In an attempt to further localize the SCIDX1 critical region, the position of DXS453 in proximal Xq (Weber et al. 1990) was defined more precisely. This PCR-based poly-TG polymorphic locus was amplified, proving that it was present, in a hybrid cell line containing only Xq distal to the AnLy translocation breakpoint at the ectodermal dysplasia locus in Xq12-q13.1 (Zonana et al. 1988); DXS132 was proximal to this breakpoint. Furthermore, limited genotyping with DXS453 in our laboratory revealed four crossovers not informative for SCIDX1 but, nonetheless, placing DXS453 proximal to DXS441. Thus DXS453 was shown to lie between DXS132 and DXS441.

When SCIDX1-informative meioses were analyzed at DXS453, only one recombinant X chromosome was found which enabled the SCIDX1 critical region to be narrowed. The maternally derived X chromosome of a noncarrier female in family B, which is second from the left in figure 3, had the DXS453 allele from the maternal mutation-bearing X. Thus a crossover is likely to have occurred distal to DXS453 and proximal to SCIDX1. DXS453 may therefore represent a new, narrower proximal boundary for SCIDX1.

Discussion

This study indicates that the SCIDX1 locus, mutations of which cause the majority of human SCID, lies on the proximal long arm of the X chromosome, between the markers DXS132 and DXS447. A single recombinant suggests placement of SCIDX1 in an even narrower interval, on the order of 10 Mb, between DXS453 and DXS447. SCIDX1 did not recombine with a cluster of markers in Xq13 which include PGK1, DXS325, DXS347 and DXS441, for which an RFLP and a multiallele poly-TG polymorphism were nonrecombinant with SCIDX1 with $Z = 17.96$. To date, link-

Table 2

Z Values, for Increasing θ Values, between Pericentromeric and Proximal Long-Arm Marker Loci on the X Chromosome

		Z AT θ = [a]											
Locus 1	Locus 2	.00	.01	.02	.03	.04	.05	.10	.20	.30	.40	$\hat{\theta}$[b]	$\hat{Z}$[c]
DXS14	DXS1	3.34[c]	3.28	3.23	3.17	3.12	3.06	2.78	2.18	1.53	0.81	.00	3.34
DXS14	DXS159	−∞	2.52	2.77	2.89	2.96	3.00	2.99	2.59	1.94	1.08	.07	3.02
		−∞	**12.08**	**12.45**	**12.62**	**12.58**	**12.53**	**11.85**	**9.66**	**6.81**	**3.44**		
DXS14	DXS347	5.10	5.02	4.93	4.85	4.76	4.68	4.24	3.32	2.33	1.23	.00	5.10
DXS14	DXS447	3.66	3.61	3.55	3.49	3.43	3.37	3.06	2.39	1.67	0.88	.00	3.66
DXS1	DXS441	−∞	1.15	1.96	2.38	2.66	2.85	3.23	3.01	2.27	1.23	10.12	3.25
DXS159	DXS153	7.05	6.94	6.83	6.72	6.60	6.49	5.90	4.62	3.17	1.59	.00	7.05
		26.62	**26.21**	**25.79**	**25.37**	**24.94**	**24.52**	**22.31**	**17.50**	**12.06**	**5.95**	**.00**	**26.62**
DXS159	DXS106	−∞	.85	2.20	2.92	3.38	3.70	4.36	4.05	2.92	1.40	.12	4.41
		−∞	**12.20**	**13.65**	**14.35**	**14.73**	**14.94**	**14.85**	**12.54**	**8.95**	**4.60**		
DXS159	DXS132	−∞	1.33	2.68	3.40	3.87	4.19	4.86	4.55	3.40	1.75	.12	4.91
		−∞	**13.28**	**14.73**	**15.42**	**15.81**	**16.01**	**15.90**	**13.54**	**9.90**	**5.30**		
DXS159	DXS347	−∞	6.23	6.42	6.48	6.48	6.46	6.12	5.01	3.56	1.84	.04	6.48
DXS159	PGK1	4.97	4.91	4.84	4.78	4.71	4.64	4.27	3.43	2.46	1.33	.00	4.97
		−∞	**19.98**	**20.50**	**20.66**	**20.65**	**20.55**	**19.44**	**15.92**	**11.43**	**6.05**		
DXS159	DXS441	−∞	9.20	9.34	9.35	9.31	9.24	8.66	7.03	5.00	2.62	.03	9.35
DXS159	DXS72	−∞	2.28	3.35	3.91	4.27	4.51	4.96	4.49	3.29	1.66	.11	4.97
DXS153	DXS106	4.21	4.14	4.07	3.99	3.92	3.84	3.45	2.61	1.70	.77	.00	4.21
		−∞	**5.94**	**6.39**	**6.59**	**6.68**	**6.71**	**6.47**	**5.25**	**3.58**	**1.67**		
DXS153	DXS132	7.37	7.25	7.13	7.01	6.88	6.76	6.11	4.71	3.17	1.53	.00	7.37
		−∞	**14.32**	**14.37**	**14.30**	**14.16**	**14.02**	**12.99**	**10.39**	**7.31**	**3.80**		
DXS153	DXS347	3.28	3.22	3.17	3.12	3.07	3.02	2.74	2.14	1.48	7.60	.00	3.28
DXS153	DXS441	−∞	4.14	4.37	4.47	4.52	4.54	4.43	3.77	2.79	1.55	.06	4.54
DXS153	PGK1	4.15	4.09	4.03	3.97	3.91	3.85	3.53	2.83	2.03	1.10	.00	4.15
		−∞	**12.21**	**12.55**	**13.12**	**13.24**	**13.26**	**12.79**	**10.68**	**7.77**	**4.18**		
DXS153	DXYS1X	−∞	3.84	4.06	4.16	4.20	4.22	4.09	3.44	2.54	1.40	.05	4.22
DXS106	DXS132	−∞	9.66	9.78	9.77	9.70	9.61	8.94	7.13	4.96	2.49	.02	9.78
		−∞	23.59	24.04	24.10	24.00	23.83	22.30	17.92	12.52	6.27		
DXS106	DXS441	−∞	3.21	3.97	4.37	4.60	4.75	4.94	4.29	3.09	1.58	.04	4.95
DXS106	DXS72	−∞	2.87	3.65	4.04	4.28	4.44	4.65	4.04	2.89	1.42	.09	4.65
DXS106	DXYS1X	−∞	2.01	2.53	2.80	2.97	3.08	3.26	2.93	2.23	1.25	.10	3.26
DXS132	DXS441	−∞	.67	1.76	2.35	2.73	3.00	3.60	3.48	2.67	1.43	.13	3.68
DXS132	DXS72	−∞	3.75	4.23	4.46	4.59	4.66	4.62	3.85	2.69	1.28	.07	4.70
DXS132	DXS3	−∞	−.14	1.24	1.99	2.49	2.84	3.66	3.66	2.81	1.52	.14	3.81
DXS347	DXS441	−∞	2.76	3.26	3.50	3.65	3.73	3.78	3.21	2.28	1.14	.08	3.81
DXS347	DXS447	3.22	3.17	3.12	3.07	3.02	2.96	2.68	2.07	1.38	.64	.00	3.22
DXS347	DXS72	5.70	5.61	5.51	5.41	5.32	5.22	4.72	2.65	2.49	1.21	.00	5.70
DXS347	DXYS1X	5.38	5.29	5.20	5.12	5.03	4.94	4.48	3.51	2.45	1.30	.00	5.38
DXS325	DX572	4.50	4.43	4.37	4.30	4.23	4.17	3.81	3.06	2.19	1.19	.00	4.50
DXS325	DXYS1X	−∞	3.03	3.26	3.37	3.42	3.45	3.37	2.86	2.11	1.17	.06	3.45
PGK1	DXS441	12.37	12.19	12.00	11.81	11.62	11.43	10.44	8.28	5.85	3.10	.00	12.37
PGK1	DXS72	4.21	4.14	4.07	4.00	3.93	3.85	3.49	2.71	1.88	.98	.00	4.21
PGK1	DXYS1X	−∞	4.02	4.51	4.74	4.88	4.95	4.94	4.24	3.13	1.72	.07	5.00
DXS441	DXS447	4.95	4.87	4.79	4.70	4.62	4.53	4.08	3.13	2.10	1.01	.00	4.95
DXS441	DXS72	6.59	6.48	6.37	6.26	6.14	6.03	5.44	4.20	2.85	1.40	.00	6.59
DXS441	DXYS1X	−∞	5.85	6.31	6.52	6.63	6.67	6.51	5.49	4.02	2.20	.06	6.68
DXS447	DXS72	−∞	3.36	3.57	3.66	3.09	3.71	3.55	2.88	1.99	9.82	.05	3.72

NOTE.—Values in boldface type are the sum of SCIDX1 and CEPH 40-family Z values from Mahtani et al. (1991).

[a] For each marker, the $\hat{Z}$ is underlined.

[b] Most likely θ.

[c] Z at $\hat{\theta}$.

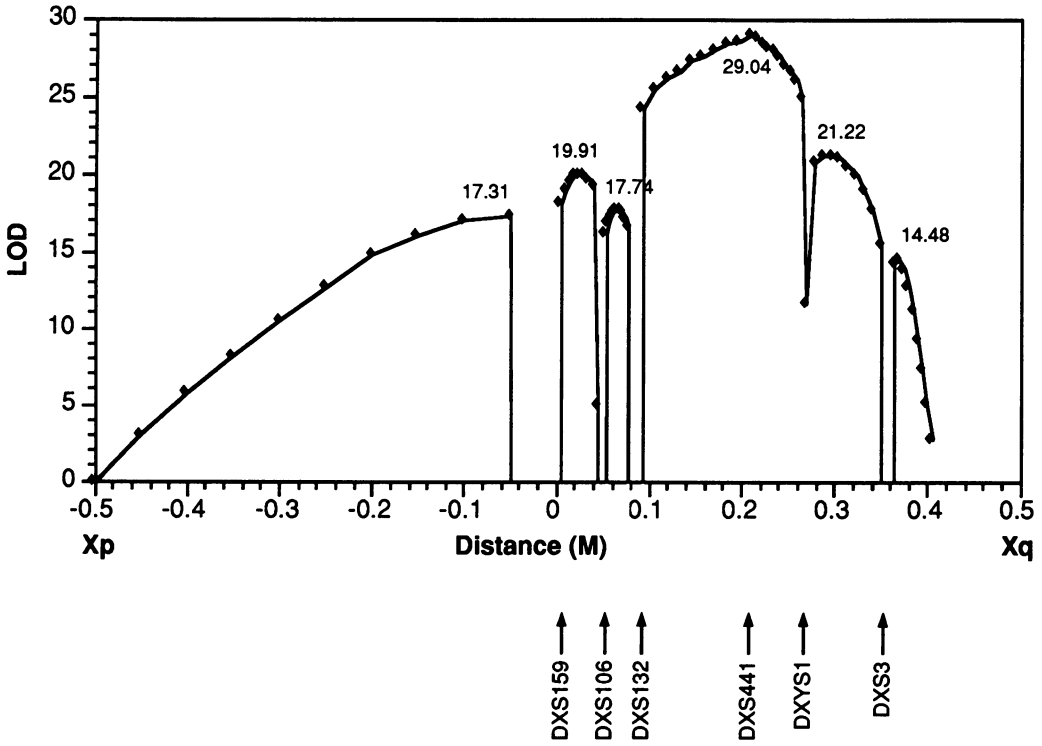


Figure 2 Seven-point multipoint linkage analysis of SCIDX1 with respect to markers in the proximal long arm of the X chromosome. Lod scores represent the $\log_{10}$ likelihood that SCIDX1 lies at each position shown, relative to an unlinked position on Xp.

age studies by our group and others (de Saint Basile et al. 1987), in over 30 unrelated families segregating this disorder, have shown no evidence of genetic heterogeneity for the X-linked form of SCID.

Early lethality of SCID, clearly evident in the pedigrees in figure 1, has reduced the number of individuals available for linkage analysis. The present linkage study was substantially aided by the performance of carrier testing by X chromosome inactivation in T lymphocytes. The number of SCIDX1-informative meioses more than doubled in the multigeneration pedigrees after carrier determination. Moreover, those pedigrees ascertained by affected males in a single generation could not otherwise have been included. Results of X inactivation testing by the hybrid method gave a definitive result in 93% of women at risk. Additional phase information was obtained from the hybrids of female carriers segregating the active, non–mutation-bearing X chromosomes.

The most likely locus order in the pericentromeric and proximal long-arm regions of the human X chromosome for SCIDX1 families was DXS1–(DXS159, DXS153)–DXS106–DXS132–DXS453–(SCIDX1,PGK1, DXS325,DXS347,D XS441)–DXS447–DXS72–DXYS1X–DXS3. This order and the genetic distances between loci shown in table 2 are consistent with previous maps (Davies et al. 1991; Mahtani et al. 1991) and provide additional marker localization. With these new linkage boundaries for SCIDX1, positional cloning approaches and evaluation of candidate genes will be facilitated. Moreover, 11 SCIDX1-informative meioses with crossovers are available which could potentially narrow the SCIDX1 critical region further when new polymorphic markers become available.

Survival of SCID patients after bone marrow transplantation has improved significantly during the past 2 decades, although important questions remain about both optimization of posttransplantation B lymphocyte function and long-term outcome (Fischer et al. 1990; Conley 1991). Genetic management of families with SCID has been greatly facilitated by carrier determination for SCIDX1 mutations and by the currently available array of closely linked marker loci. The poly-TG polymorphism at DXS441 alone, with a heterozygosity of .76 (Ram et al. 1992), is a powerful predictor of SCIDX1 status, and the present delineates abundant

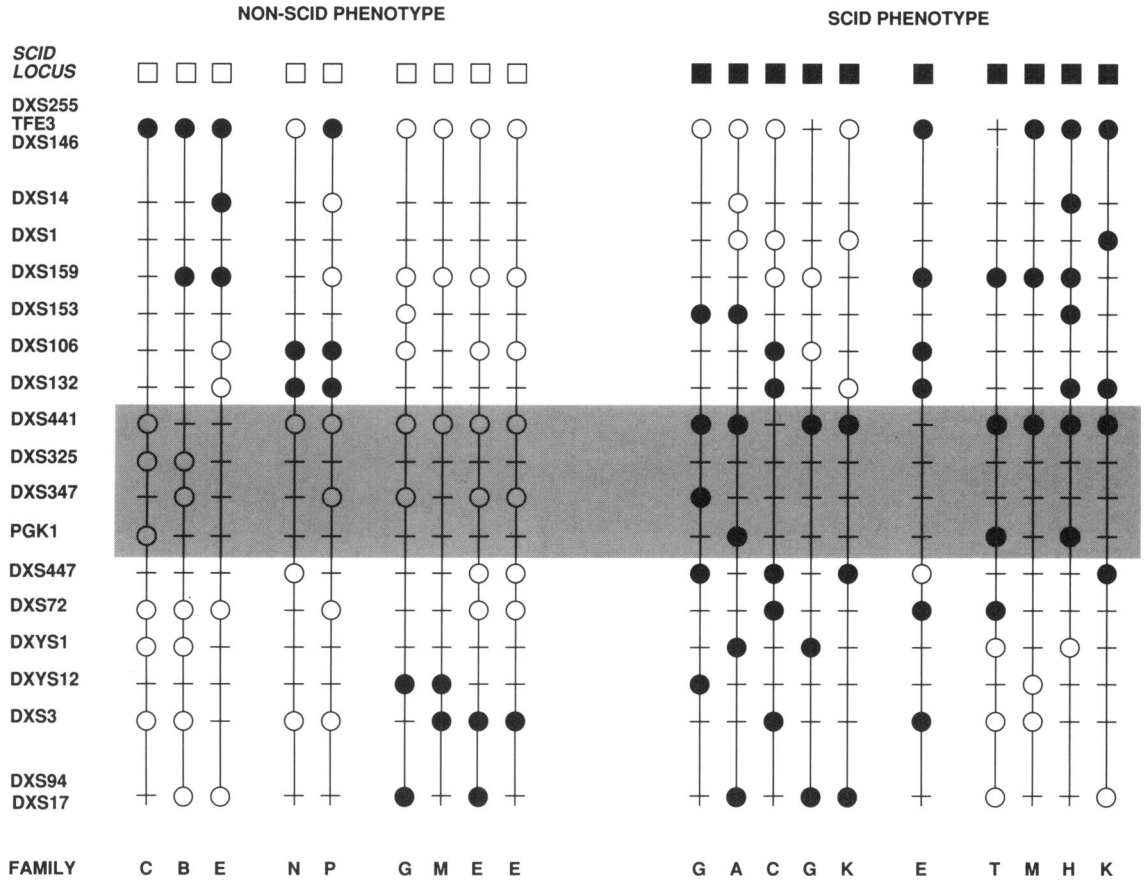


Figure 3 Representation of SCIDX1-informative X chromosomes with meiotic crossovers near the SCIDX1 region. Blackened squares and circles denote alleles derived from the maternal mutation-bearing X chromosome; and unblackened squares and circles denote the maternal nonmutant X chromosome. Horizontal dashes at a locus denote maternal homozygosity.

tightly linked flanking markers. To date, successful prenatal diagnosis using chorionic villus or amniocyte DNA has made possible perinatal bone marrow transplantation in three affected boys (Himelstein et al. 1993; Puck 1993); their excellent outcome underscores the value of linkage analysis for this disorder.

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References

Conley M (1991) X-linked severe combined immunodeficiency. Clin Immunol Immunopathol Suppl 61:s94–s99

Conley M, Buckley R, Hong R, Guyerra-Hanson C, Roifman C, Brockstein J, Pahwa S, et al (1990) X-linked severe combined immunodeficiency: diagnosis in males with sporadic severe combined immunodeficiency and clarification of clinical findings. J Clin Invest 85:1548–1554

Conley M, Lavoie A, Briggs C, Brown P, Guerra C, Puck J (1988) Nonrandom X chromosome inactivation in B cells from carriers of X chromosome-linked severe combined immunodeficiency. Proc Natl Acad Sci USA 85:3090–3094

Davies K, Mandel J, Monaco A, Nussbaum R, Willard H (1991) Report of the Committee on the Genetic Constitu-

tion of the X Chromosome. Human Gene Mapping 11. Cytogenet Cell Genet 58:853–966

de Sainte Basile G, Arveiler B, Oberle I, Malcolm S, Levinsky R, Lau Y, Hofker M, et al (1987) Close linkage of the locus for X chromosome-linked severe combined immunodeficiency to polymorphic DNA markers in Xq11-13. Proc Natl Acad Sci USA 84:7576–7579

Fireman P, Johnson H, Gitlin D (1966) Presence of plasma cells and gamma$_1$ M-globulin synthesis in a patient with thymic alymphoplasia. Pediatrics 37:485–492

Fischer A, Landais P, Friedrich W, Morgan G, Gerritsen B, Fasth A, Porta F, et al (1990) European experience of bone-marrow transplantation for severe combined immunodeficiency. Lancet 336:850–854

Gatti R, Meuwissen H, Allen H, Hong R, Good R (1968) Immunological reconstitution of sex-linked lymphopenic immunological deficiency. Lancet 2:1366–1369

Himelstein B, Puck J, August C, Pierson G, Bunin N (1993) T-cell depleted maternal bone marrow transplant for siblings with X-linked severe combined immunodeficiency. J Pediatr 122:289–291

Keats B, Ott J, Conneally M (1989) Report of the Committee on Linkage and Gene Order. Human Gene Mapping 10. Cytogenet Cell Genet 51:459–502

Lathrop GM, Lalouel JM, Julier C, Ott J (1985) Multilocus linkage analysis in humans: detection of linkage and estimation of recombination. Am J Hum Genet 37:482–498

Mahtani M, Lafreniere R, Kruse T, Willard H (1991) An 18-locus linkage map of the pericentromeric region of the human X chromosome: genetic framework for mapping X-linked disorders. Genomics 10:849–857

Puck J (1993) Prenatal diagnosis and genetic analysis of X-linked immunodeficiency disorders. Pediatr Res Suppl 33:s29–s34

Puck J, Krauss C, Puck S, Buckley R, Conley M (1990) Prenatal test for X-linked severe combined immunodeficiency by analysis of maternal X-chromosome inactivation and linkage analysis. N Engl J Med 322:1063–1066

Puck J, Nussbaum R, Conley M (1987) Carrier detection in X-linked severe combined immunodeficiency based on patterns of X chromosome inactivation. J Clin Invest 79:1395–1400

Puck J, Nussbaum R, Smead D, Conley M (1989) X-linked severe combined immunodeficiency: localization within the region Xq13.1-q21.1 by linkage and deletion analysis. Am J Hum Genet 44:724–730

Puck J, Stewart C, Nussbaum R (1992) Maximum likelihood analysis of human T-cell X chromosome inactivation patterns: normal women versus carriers of X-linked severe combined immunodeficiency. Am J Hum Genet 50:742–748

Ram K, Barker D, Puck J (1992) Dinucleotide repeat polymorphism at the locus DXS441. Nucleic Acids Res 20:1428

Stiehm R (1993) New and old immunodeficiencies. Pediatr Res Suppl 33:s2–s8

Tumer Z, Chelly J, Tommerup N, Ishikawa-Brush Y, Tonnensen T, Monaco A, Horn N (1992) Characterization of a 1.0 Mb YAC contig spanning two chromosome breakpoints related to Menkes disease. Hum Mol Genet 1:483–489

Verga V, Hall B, Wang S, Johnson S, Higgins J, Glover T (1991) Localization of the translocation breakpoint in a female with Menkes syndrome to Xq13.2-q13.3 proximal to PGK-1. Am J Hum Genet 48:1133–1138

Weber J, Kwitek A, May P, Polymeropoulos M, Ledbetter S (1990) Dinucleotide repeat polymorphisms at the DXS453, DXS454 and DXS458 loci. Nucleic Acids Res 18:4037

Williamson R, Bowcock A, Kidd K, Pearson P, Schmidtke J, Ceverha P, Chipperfield M, et al (1991) Report of the DNA committee and catalogues of cloned and mapped genes, markers formatted for PCR and DNA polymorphisms. Human Gene Mapping 11. Cytogenet Cell Genet 58:1190–1832

WHO Scientific Group (1992) Primary immunodeficiency diseases. Immunodefic Rev 3:195–236

Zonana J, Roberts S, Thomas N, Harper P (1988) Recognition and reananlysis of a cell line from a manifesting female with X linked hypohidrotic ectodermal dysplasia and an X;autosome balanced translocation. J Med Genet 25:383–386