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Close linkage of the locus for X chromosome-linked severe combined immunodeficiency to polymorphic DNA markers in Xq11-q13

(X chromosome/restriction fragment length polymorphic markers/multipoint linkage analysis/carrier and prenatal diagnosis)

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ABSTRACT **The gene for X chromosome-linked severe combined immunodeficiency (SCID), a disease characterized by a block in early T-cell differentiation, has been mapped to the region Xq11–q13 by linkage analysis with restriction fragment length polymorphisms. High logarithm of odds (lod) scores were obtained with the marker 19.2 (DXS3) ($z = 5.51$ at a recombination fraction $\theta = 0.11$) and with the marker cpX73 (DXS159) that showed complete cosegregation with the disease locus in the informative families analyzed ($z = 5.27$ at $\theta = 0.00$). Other significant linkages were obtained with several markers from Xq11 to q22. With the help of a recently developed genetic map of the region, it was possible to perform multipoint linkage analysis, and the most likely genetic order is DXS1–(SCID, DXS159)–DXYS1–DXYS12–DXS3, with a maximum multipoint logarithm of odds score of 11.0. Our results demonstrate that the SCID locus (gene symbol *IMD4*) is not closely linked to the locus of Bruton's agammaglobulinemia (a defect in B-cell maturation). They also provide a way for a better estimation of risk for carrier and antenatal diagnosis.**

Severe combined immunodeficiency (SCID) is a syndrome in which affected infants lack both cellular and humoral immunity and die in early life from overwhelming infection if bone marrow transplantation is not performed. X chromosome-linked severe combined immunodeficiency (McKusick no. 30040, also designated *IMD4* in the nomenclature of Human Gene Mapping Workshops) is characterized by a complete absence of mature T lymphocytes, suggesting that the disease results from a block in early T-cell differentiation (1). In autosomal recessively transmitted SCID, an absence of both T and B lymphocytes is most often found, although cases of female SCID patients with a complete absence of T cells and normal B-cell numbers have been described (2). Obligatory carrier females of X-linked SCID are immunologically normal, and this impairs genetic counseling. The gene has not previously been localized on the X chromosome. Many restriction fragment length polymorphisms (RFLPs) have been detected on the human X chromosome, and they are increasingly used to establish a genetic map that includes disease genes (3). We have performed a genetic linkage analysis in nine families with clear-cut X-linked SCID by using several RFLP markers, and this has allowed us to map the *IMD4* gene to the region Xq11–q13. This should be an important step toward carrier and prenatal diagnosis and toward isolation of the gene itself.

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METHODS

Families. Families of X chromosome-linked SCID were chosen according to the following two criteria. The diagnosis of SCID in at least one affected sibling was ascertained by immunological investigations according to the criteria of the World Health Organization Committee on Immunodeficiency (4). They included the determination of T- and B-lymphocyte numbers by indirect immunofluorescence using specific monoclonal antibodies to T cells (T3, T11, T4, and T8) and anti-immunoglobulin heavy-chain antisera as well as the study of mitogen-induced T-lymphocyte proliferation (1). In these patients, SCID diagnosis was based on the absence of T lymphocytes and a lack of T-cell proliferation, whereas B lymphocytes were detected in normal or increased numbers as previously published (1). An unequivocal X-linked transmission was established in the families, as all the tested families showed segregation of the disease in at least three generations (see Fig. 1).

DNA Analysis. Total genomic DNA was extracted from human peripheral leukocytes collected in EDTA. Ten micrograms was digested to completion with restriction endonuclease, fractionated by electrophoresis on 0.9% agarose gels. and blotted onto diazobenzyloxymethyl paper as described (5, 6). The filters were screened, using DNA probes radioactively labeled by nick-translation or random-primed synthesis, in a 40% formamide hybridization solution at 42°C (6). For families analyzed in London, 0.8% agarose gels were used; blotting was on nitrocellulose filters and hybridization was in 4× SSC (1× SSC = 0.15 M sodium chloride/0.015 M sodium citrate, pH 7) at 65°C.

Data Analysis. Linkage analysis and inference of gene order were performed with the LINKAGE programs (7). Logarithm of odds (lod) scores for two-point linkages were calculated using MLINK version 3.4, and multipoint analysis was performed with ILINK and LINKMAP (versions 3.5) on a compatible PC-AT computer.

RESULTS

Four large families with X chromosome-linked SCID were analyzed for segregation of 23 polymorphic markers located throughout the X chromosome (see Table 1 for the markers located in Xq11–q22). The markers that were chosen detect RFLPs with either *Taq* I or *Pst* I. All the available DNAs were digested with these enzymes and blotted onto diazobenzyloxymethyl paper, and the blots were tested sequentially with the probes (as many as 14 probes could be

Abbreviations: SCID, severe combined immunodeficiency; lod, logarithm of odds; RFLP, restriction fragment length polymorphism.

Table 1. Probes belonging to the Xq11–q21 region tested in this study

Probe	Locus	Enzyme	Hetero-zygosity	Ref.
p8	DXS1	*Taq* I	0.21	8
cpX73	DXS159	*Pst* I	0.37	9
DP31	DXYS1	*Taq* I	0.49	10
St25/1	DXYS12	*Taq* I	0.35	11
7b	DXYS2	*Pst* I	0.33	11
19–2	DXS3	*Taq* I	0.45	8
pXG12	DXS94	*Pst* I	0.48	12
p212	DXS178	*Taq* I	0.42	13
S21	DXS17	*Taq* I	0.45	14

Heterozygosities are those given in the references or by Willard *et al.* (15) and appeared valid in the families studied here. Additional information on the probes and associated RFLPs can be found in Willard *et al.* (15).

tested on a single blot). After the first results demonstrated a linkage in the proximal region of the long arm, five additional families were tested for *Taq* I and *Pst* I RFLPs from the q11–q22 region.

The results of the segregation analysis using eight probes are presented in Fig. 1 for four of the families. The lod scores for the linkage of SCID to each of these markers are shown in Table 2. A high lod score was obtained with probe 19.2 (DXS3) ($z = 5.51$ at a recombination fraction θ of 0.11). This probe has been previously localized to region q21.3–q22 (3). The polymorphic locus DXS159, which we have recently characterized [*Pst* I RFLP detected by probe cpX73(9)], showed no recombination with the disease, with a highly significant lod score of 5.27. We have localized this marker to the region q11–q12 (23). Other significantly positive lod scores were obtained with markers localized in the XY homologous region in q13–q21 (DXYS1 and DXYS12) with p8 (DXS1), pXG12 (DXS94), and S21 (DXS17).

In order to locate more precisely the disease locus with respect to these markers, it is important to first consider their respective positions on the X chromosome map. From linkage data, weak suggestion for the order DP31–19.2–S21 was obtained by Drayna and White (16). We have recently performed additional physical mapping and linkage analysis on the various markers used in this study and have derived the order p8–cpX73–DP31–St25–19.2–pXG12–S21 (23). DP31 and St25 are both in the XY homologous region (11, 17, 18), whereas the flanking probes cpX73 and 19.2 detect X sequences only. Probe 212 also used here maps very close to both pXG12 and S21 (23).

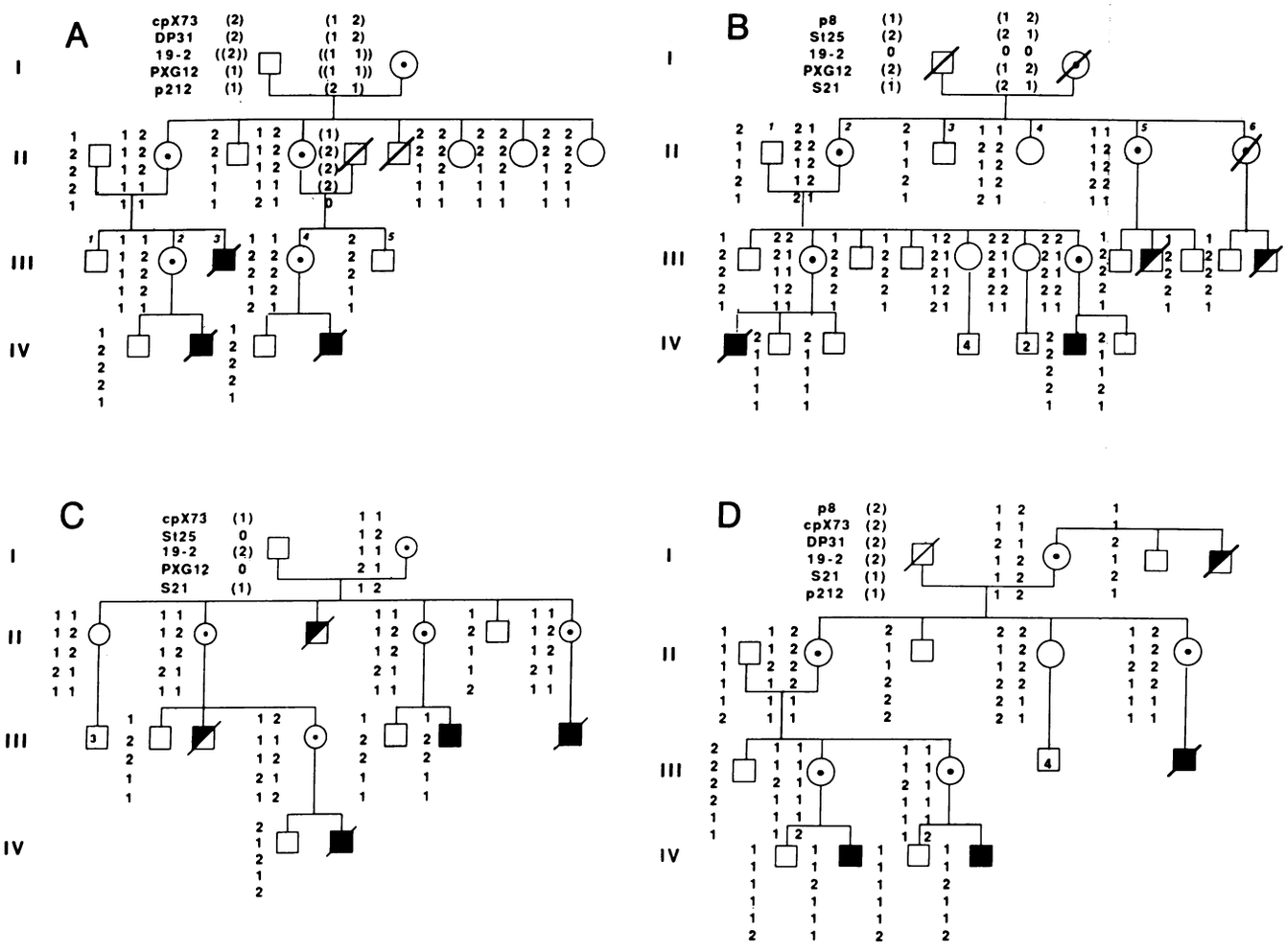

FIG. 1. Pedigrees and genotypes of four X chromosome-linked SCID families. The vertical arrangement of alleles indicates the most likely haplotype. Paternal haplotype is always indicated on the right for females. In parentheses, deduced genotype; in double parentheses, most likely genotype (based on minimum recombination between closely linked markers). Alleles 1 and 2 stand for the largest and smallest fragments, respectively. 0, Genotype unknown at the marker locus. Identification numbers have been given only to individuals in families A and B, for generations explicitly cited in the text. □, Male; ○, female; ■, affected male; ⊙, obligatory carrier; ⌀ and ⌀, deceased; ⌀, deceased from infection in infancy; ⌀, deceased affected male.

Table 2. Lod scores for various recombination fractions between the SCID locus and the listed loci

	Lod scores						
Locus (probe)	$\theta =$ 0.00	$\theta =$ 0.01	$\theta =$ 0.05	$\theta =$ 0.10	$\theta =$ 0.15	$\theta =$ 0.20	$\theta =$ 0.30
DXS1 (p8)	−0.51	1.46	2.04	2.14	2.07	1.91	1.40
DXS159 (cpX73)	5.27	5.16	4.70	4.11	3.50	2.90	1.71
DXYS1 (DP31)	−4.21	0.94	2.05	2.27	2.21	2.03	1.45
DXYS12 (St25)	−3.87	−0.16	1.05	1.39	1.44	1.36	0.99
DXS3 (19.2)	$-\infty$	2.22	4.97	5.51	5.37	4.95	3.60
DXS94 (pXG12)	−6.86	−1.43	0.59	1.29	1.51	1.52	1.18
DXS178 (p212)	−2.41	−0.73	−0.10	0.10	0.18	0.21	0.19
DXS17 (S21)	−7.00	−1.87	0.20	0.90	1.12	1.10	0.73

Lod scores are presented for different values of the recombination fraction (θ). Close linkage was excluded for loci on the short arm (DXS7, DXS84, and DXS164) and for loci on the distal long arm (DXS51, DXS52, and DXS102).

The two-point linkage data suggested that the SCID gene is close to the cpX73 (DXS159) marker. Examination of haplotypes defined by the set of ordered markers (Fig. 1) led us to the following conclusions. In family A, the obligate carrier III*4* shows segregation of the disease with cpX73 but recombination with DP31 and 19.2. This suggests that the disease locus is located proximal to DP31 in a region around cpX73. In family B, meioses that show recombination between p8 and St25 are compatible with a localization of the disease gene between p8 and St25 (compare individuals II*2*, II*3*, and II*5*). Furthermore, in the same sibship, St25 cosegregates with S21 but not with SCID in three meioses, and it is unlikely that the disease locus is between these two markers (located about 10 centimorgans apart) since this would imply double recombination events within this relatively small region. This is also confirmed by the relatively high recombination fraction found between the disease locus and S21 or pXG12 (0.17 and 0.18, respectively, with several proven recombinants, see Table 2).

The order was tested further by formal multipoint analysis using the ILINK program from the LINKAGE package (7). ILINK calculates the likelihoods for alternate orders of loci and the recombination fractions that best fit the data for each order tested, taking into account the segregation of alleles at all loci considered. It is not dependent on a genetic map determined prior to the analysis. Several three-point analyses showed with very high relative likelihood that the SCID gene was on either side of cpX73 but proximal to DP31, 19.2, and S21. Four-point analysis confirmed this order (with relative odds of more than 2000 to 1) and yielded more accurate estimates of the recombination fractions since they are constrained by the linkage relationships between markers (Table 3). Other four-point tests suggested that the SCID gene is located distal to p8, although the relative likelihood with respect to a location proximal to p8 is only 5.6 to 1 or 2.9 to 1, depending on the markers tested. The final most likely order is thus p8–(SCID, cpX73)–DP31–St25–19.2. The best estimate of the recombination fraction between the flanking markers p8–DP31 is 0.22 when only the SCID families are considered and 0.15 when marker-to-marker data from a larger set of normal families is included in the multilocus analysis (calculated from Table 3).

An additional test was performed with the LINKMAP program, which calculates the likelihood (expressed as multipoint lod scores) for the position of the disease locus with respect to a fixed map of markers. We used the map recently constructed by us for the region between DXS1 and DXS17. A maximum multipoint lod score of 11.0 was obtained for SCID being at a distance of 0 centimorgans from cpX73. Lower lod scores were obtained for a localization of SCID proximal to p8 ($z \leq 10.22$) or distal to DP31 ($z \leq 7.32$). This further supports the localization of SCID close to cpX73, within the interval p8–DP31.

Table 3. Multipoint analysis

Loci	$\theta 1$	$\theta 2$	$\theta 3$	−2ln(L)	Relative probability
SCID–cpX73–S21	0.001	0.214	—	422.5	297
cpX73–SCID–S21	0.001	0.213	—	422.5	298
cpX73–S21–SCID	0.093	0.130	—	433.9	1
SCID–cpX73–DP31–19.2	0.001	0.111	0.048	506.5	2831
cpX73–SCID–DP31–19.2	0.001	0.111	0.047	506.5	2875
cpX73–DP31–SCID–19.2	0.126	0.064	0.103	522.4	1
cpX73–DP31–19.2–SCID	0.198	0.045	0.099	521.9	1.2
SCID–p8–St25–19.2	0.054	0.111	0.001	480.3	1
p8–SCID–St25–19.2	0.129	0.115	0.001	476.8	5.6
	(0.096)	(0.096)	(0.022)		
SCID–p8–DP31–19.2	0.08	0.047	0.076	510.2	1
p8–SCID–DP31–19.2	0.125	0.096	0.056	508.0	2.9
	(0.076)	(0.074)	(0.070)		

$\theta 1$, $\theta 2$, and $\theta 3$ are the maximum likelihood recombination fractions between the adjacent loci, for the order tested as determined using the ILINK program for the set of nine SCID families, and −2ln(L) is the maximum likelihood for each order tested. The likelihoods for other orders are not given, since the marker order has been established in a separate study using a larger set of non-SCID families (23). The recombination fractions given in parentheses were calculated by including the latter set of families in order to have a better estimation of the genetic map distances between loci in this region.

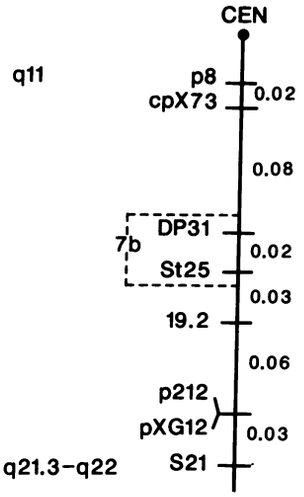

FIG. 2. Genetic localization of markers. Map distances between successive markers were estimated by Arveiler *et al.* (23) and are indicated on the right (in morgans). Correspondence with the cytogenetic map of the X chromosome is indicated on the left.

DISCUSSION

These results allow us to map unambiguously the locus for X chromosome-linked SCID in the q11–q13 region with a most probable localization between DXS1 and the group of XY homologous loci DXYS1 and DXYS12. There was a complete cosegregation between the disease and the marker DXS159 defined by probe cpX73, which maps in q11–q12, proximal to DXYS1, and the relatively high lod score of 5.27 was obtained. The 90% confidence limit for the recombination fraction between SCID and DXS159 is 0.09. A lod score of 5.51 at $\theta = 0.11$ was also obtained for the linkage between SCID and DXS3. This suggests that the SCID locus is indeed very close to DXS159, since the recombination fraction between DXS159 and DXS3 analyzed in a larger set of families is about 0.13 (ref. 23; Fig. 2). This is further supported by multipoint analysis, which gave a maximum lod score of 11.0 for linkage to DXS159 at $\theta = 0$. Additional data would be welcome to give more precision on the genetic distance between SCID and the markers analyzed here and especially to substantiate the relative position of p8 (DXS1) and cpX73 (DXS159) with respect to the disease locus. We feel, however, that our data are sufficient to validate the careful use of DXS159 and of the (probably) flanking markers—DXS1 (on the centromeric side) and the XY homologous probes DXYS1, DXYS12, and also DXYS2 (on the distal side)—for genetic counseling in SCID families, especially since no alternate methods of carrier diagnosis are available at the present time. In addition, these probes will make feasible an earlier prenatal diagnosis, in informative families, than by using immunological methods on fetal blood (19). However, because of the possibility of recombination, a control of the presence of T cells in fetal blood should be performed if RFLP analysis suggests that the fetus has a low risk of being affected, knowing that in >90–95% of the cases (depending on the informative markers that could be used) the fetus will be effectively normal and pregnancy will be allowed to come to term. It should be noted that there was no evidence for genetic heterogeneity since linkage with probes in the q11–q21 region was observed in all nine families.

Our results clearly show that X chromosome-linked SCID, which is thought to involve a defect in T-cell differentiation, is located in a different region than Bruton's agammaglobulinemia (a defect in B-cell maturation) since the latter disease has been mapped very close to markers S21 and pXG12 (20–22). Analysis of additional families together with a refinement of the genetic map in this area of the human X chromosome should result in a more precise localization of the SCID gene and should thus provide more accurate tools for prenatal and carrier diagnosis and for the ultimate isolation of the SCID gene.

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