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The Gene for Severe Combined Immunodeficiency Disease in Athabascan-Speaking Native Americans Is Located on Chromosome 10p

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Summary

Severe combined immunodeficiency disease (SCID) consists of a group of heterogeneous genetic disorders. The most severe phenotype, T^-B^- SCID, is inherited as an autosomal recessive trait and is characterized by a profound deficiency of both T cell and B cell immunity. There is a uniquely high frequency of T^-B^- SCID among Athabascan-speaking Native Americans (A-SCID). To localize the A-SCID gene, we conducted a genomewide search, using linkage analysis of ~300 microsatellite markers in 14 affected Athabascan-speaking Native American families. We obtained conclusive evidence for linkage of the A-SCID locus to markers on chromosome 10p. The maximum pairwise LOD scores 4.53 and 4.60 were obtained from two adjacent markers, D10S191 and D10S1653, respectively, at a recombination fraction of $\theta = .00$. Recombination events placed the gene in an interval of ~6.5 cM flanked by D10S1664 and D10S674. Multipoint analysis positioned the gene for the A-SCID phenotype between D10S191 and D10S1653, with a peak LOD score of 5.10 at D10S191. Strong linkage disequilibrium was found in five linked markers spanning ~6.5 cM in the candidate region, suggesting a founder effect with an ancestral mutation that occurred sometime before 1300 A.D.

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Introduction

Severe combined immunodeficiency disease (SCID) represents a group of genetically and phenotypically heterogeneous disorders, all of which result in T cell with or without B cell immunodeficiency (Fischer 1992; Rosen et al. 1995). SCID with the T^-B^+ phenotype accounts for ~45% of cases, two-thirds of which are inherited as X-linked, common γ-chain-receptor deficiency (Noguchi et al. 1993; Mathews et al. 1995). The remaining one-third of T^-B^+ SCID cases have autosomal recessive inheritance and include JAK3 protein-kinase deficiency (Russell et al. 1995) and ZAP70 deficiency (Elder et al. 1994). The most severe form of SCID is characterized by the T^-B^- phenotype and represents ~30% of cases. Genes encoding proteins that affect the V(D)J (i.e., variable, diversity, joining) recombination reaction have been suggested as potential targets for T^-B^- SCID (Taccioli and Alt 1995), and some T^-B^- SCID patients have been found to carry mutations in the recombinase-activating genes (RAGs) (Schwarz et al. 1996), whereas some have been found to show irregular V(D)J recombination events (Schwarz et al. 1991; Abe et al. 1992). However, the underlying pathogenesis for the majority of T^-B^- SCID patients is still unknown. The remaining 25% of SCID cases include patients with Omenn syndrome, adenosine deaminase deficiency (Hirschhorn 1993), and reticular dysgenesis.

An increased incidence of T^-B^- SCID is found among Athabascan-speaking (A-SCID), Navajo, and Apache Native Americans (Murphy et al. 1980; Hu et al. 1988; Jones et al. 1991). We also have identified the same phenotype in two related families (three affected patients) among the Diné Indians, a small tribe of Athabascan speakers living in the Northwest Territories. The incidence of T^-B^- SCID among Navajo Indians is ~1/2,000 live births, with no known etiology (Hu et al. 1988; Jones et al. 1991). Autosomal recessive transmission of the disorder is supported by the incidence of multiple affected siblings with unaffected parents and by other results from pedigree and population-genetics

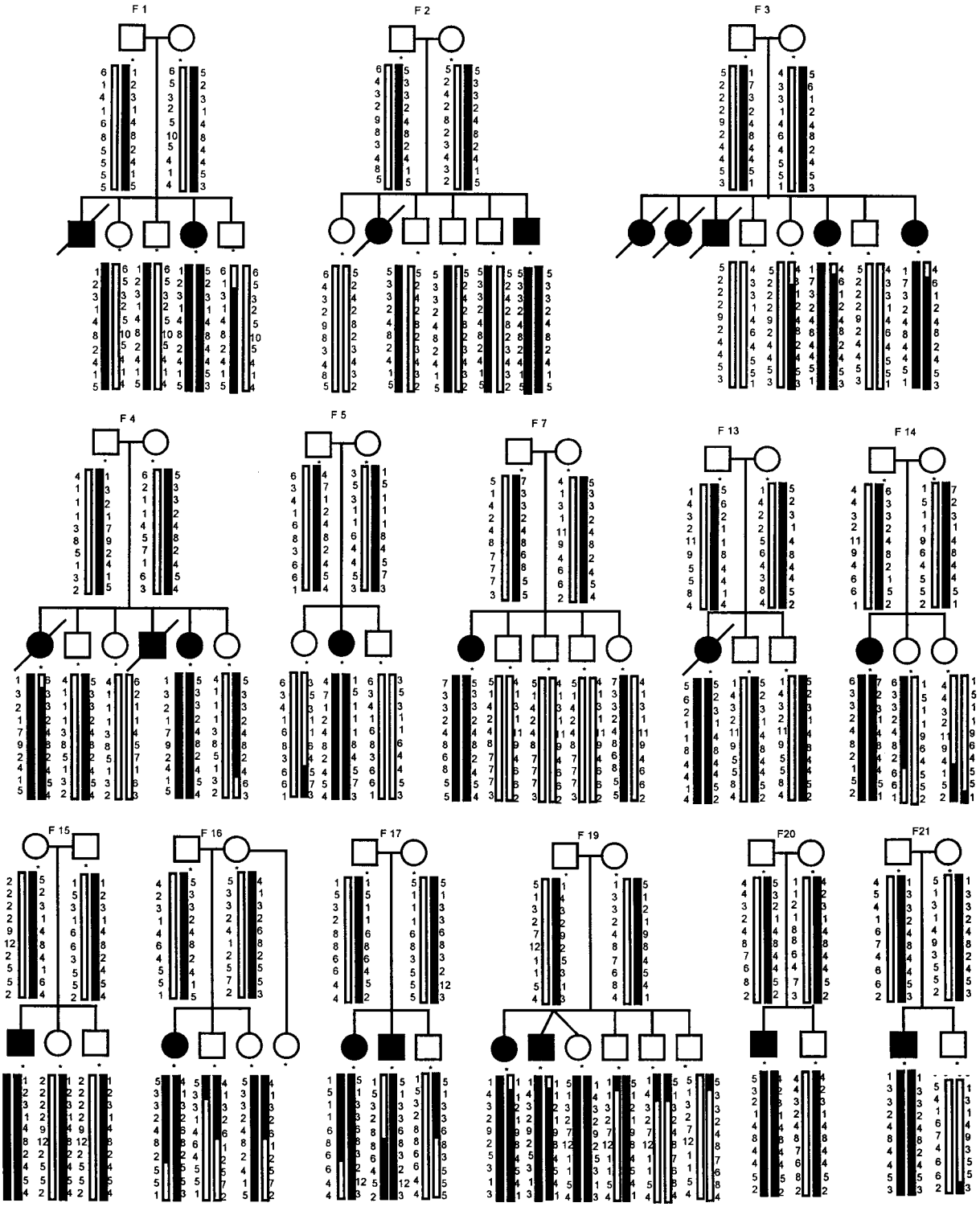

Figure 1 Pedigrees of 14 families with A-SCID. An asterisk (*) below a circle or a square indicates an individual who participated in the study. Blackened circles and squares denote affected individuals; a diagonal line indicates that the individual is deceased. Family 21 was of Apache origin, and family 17 was of Diné origin, whereas all others were Navajo. The genotypes for 10 linked microsatellite markers on chromosome 10p are presented in the following order (from top to bottom): D10S585, D10S1430, D10S223, D10S1725, D10S1664, D10S191, D10S1653, D10S674, D10S1477, and D10S1423. The genotypes of D10S585 for the healthy sibling in family 21 were not determined, which is indicated by hyphens (-).

Table 1

Candidate Genes Excluded for Genetic Linkage with A-SCID

Gene(s)	Function	Location
IL-7	Stimulates pre–B cells and pre–T cells in bone marrow	8q12-13
IL-7 receptor	Expressed in pre-T and pre-B cells	5p13
RAG1, RAG2	Activates V(D)J recombination	11p12-13
XRCC-7	Encodes p350, which regulates V(D)J recombination	8q11
XRCC-5	Encodes Ku80, which is involved in V(D)J recombination	2q33-35
XRCC-4	Encodes a protein involved in V(D)J recombination	5q13-14
XRCC-6	Encodes Ku70, which is involved in V(D)J recombination	22q13, 10q22-24[a]

[a] XRCC-6 was also found to be weakly hybridized to 10q22-24 (Cai et al. 1994).

studies, which estimate the gene frequency to be 2.1% in the Navajo (Jones et al. 1991). All patients have similar laboratory findings of severe T and B lymphopenia (lymphocyte counts <500, with <5% T and B cells) and very low (<5% of normal) to absent lymphocyte proliferation in response to mitogens and alloantigens. No other hematopoietic cell lineage is affected in this disease. Clinically, affected children present with oral/genital ulcers (Murphy et al. 1980; Rotbart et al. 1986; Hu et al. 1988) and life-threatening infections, within the first 3 mo of life. Without a bone-marrow transplant, a large majority die by 6 mo of age (Dror et al. 1993).

To localize the A-SCID gene, we conducted a genomewide search, using ~300 polymorphic microsatellite markers in 14 Athabascan-speaking Native American families with at least one affected individual. Here we report the results of our linkage and genetic analyses, which allowed us to map the gene for A-SCID to a region on chromosome 10p.

Subjects and Methods

Subjects

The identification, clinical assessment, and sample collection of A-SCID families were achieved in collaboration with pediatricians at the University of Colorado Health Science Center (for family 19), the University of Alberta (for family 17), the White River Apache Health Service (for family 21), and the Tuba City Indian Health Services (for all other families and the normal controls). For all cases, the clinical and laboratory diagnoses were confirmed by M.J.C. Blood samples and skin biopsies for fibroblast cultures (for A-SCID patients only) were collected from affected and unaffected individuals from the participating families, after informed consent was obtained. The study was approved by the Committee on Human Research at the University of California San Francisco (UCSF) and the Navajo Nation Health Board. Fourteen families were used for the linkage analysis (fig. 1), including 12 Navajo families, 1 Apache family, and 1 Diné family. These 14 kindreds comprised 18 affected children, 28 parents, and 35 healthy full siblings. The parents of kindred 1 were very distant cousins (five generations removed), and the parents of kindred 16 were from the same clan, although there was no known consanguineous relationship; all other families were not explicitly consanguineous. No cross-consanguinity with other tribes could be traced among the families. Finally, we performed cytogenetic analyses of fibroblast-cell cultures from three Navajo children with A-SCID; all had normal karyotypes (data not shown).

Genetic Markers

A total of ~300 microsatellite markers were typed. The majority of them were chosen from published genetic maps (Gyapay et al. 1994; Murray et al. 1994; Dib et al. 1996), whereas others were selected from published polymorphic markers in the Genome Database (http://www.gdb.org). The markers covered all the autosomal chromosomes, with an average separation of 10–20 cM. The areas containing genes with known functions related to the features of T^-B^- SCID were covered with more densely spaced (1–10 cM) markers. The general map positions of markers were based on published information (Murray et al. 1994; Dib et al. 1996). For markers in the vicinity of the candidate region, the map positions were determined by use of the Généthon linkage map (Dib et al. 1996), with additional information taken from the Massachusetts Institute of Technology (MIT)/Whitehead Institute sequence-tagged site (STS) map (Hudson et al. 1995). Approximately one-third of the markers were synthesized by Dr. Bruce Shiramizu's laboratory at UCSF, and two-thirds were purchased from Research Genetics.

Table 2

Pairwise LOD Scores between A-SCID and Candidate Regions

Gene(s) and Marker	LOD Score at θ = .00	.05	.10	.15	.20	.25	.30	θ_{max}
IL-7 and XRCC-7:								
D8S531	−∞	−2.31	−1.27	−.75	−.45	−.26	−.14	.5
D8S519	−∞	−3.34	−1.98	−1.27	−.82	−.52	−.31	.5
D8S538	−∞	−.45	−.20	−.09	−.03	.00	.00	.5
D8S285	−∞	−3.06	−1.80	−1.12	−.69	−.41	−.23	.5
D8S166	−∞	−2.18	−1.21	−.70	−.40	−.22	−.11	.5
CRH	−∞	−1.55	−.60	−.18	.02	.10	.11	.4
D8S84	−∞	−.98	−.33	−.07	.05	.08	.08	.3
D8S88	−∞	−1.30	−.53	−.23	−.10	−.04	−.02	.5
RAG1 and RAG2:								
D11S2001	−∞	−6.81	−3.83	−2.34	−1.44	−.87	−.49	.5
D11S2014	−∞	−4.55	−2.54	−1.51	−.89	−.50	−.27	.5
D11S907	−∞	−2.39	−1.43	−.91	−.57	−.35	−.20	.5
D11S935	−∞	−2.15	−1.30	−.84	−.54	−.34	−.20	.5
D11S1360	−∞	−1.60	−.86	−.48	−.26	−.13	−.06	.5
D11S905	−∞	−3.09	−1.75	−1.06	−.64	−.38	−.21	.5
D11S1313	−∞	−3.99	−2.47	−1.62	−1.07	−.68	−.41	.5
D11S1314	−∞	−.43	−.12	.01	.07	.08	.07	.25
D11S873	−∞	−3.14	−1.61	−.87	−.46	−.23	−.10	.5
XRCC-5:								
CTLA4	−∞	−3.42	−2.06	−1.34	−.89	−.58	−.35	.5
D2S325	−∞	−2.22	−1.22	−.73	−.44	−.26	−.14	.5
D2S137	−∞	−.83	−.12	.11	.17	.16	.12	.2
D2S173	−∞	−1.57	−.76	−.42	−.25	−.15	−.09	.5
XRCC-4:								
D5S647	−∞	−2.78	−1.64	−1.09	−.74	−.50	−.32	.5
D5S351	−∞	−4.95	−3.05	−2.03	−1.37	−.90	−.55	.5
D5S357	−∞	−3.73	−2.14	−1.38	−.93	−.61	−.38	.5
D5S626	−∞	−1.81	−1.04	−.66	−.42	−.26	−.16	.5
XRCC-6:								
D22S301	−∞	−3.03	−1.77	−1.13	−.73	−.46	−.27	.5
D22S343	−∞	−1.01	−.05	.07	.11	.11	.08	.25
D22S281	−∞	−2.68	−1.35	−.75	−.42	−.22	−.11	.5
IL2RB	−∞	−.67	−.41	−.28	−.19	−.13	−.08	.5

Genotyping

DNA was prepared by use of standard methods (Ford et al. 1989), from peripheral blood samples, fibroblast cultures, or Epstein-Barr virus–transformed lymphocytes (the transformation was performed for family 19 by A.H.). The microsatellite markers were typed by PCR, followed by separation on 6% polyacrylamide sequencing gels. The method used is similar to that described by Weber and May (1989). The PCR reactions were performed with 30–60 ng of genomic DNA as templates; 10–30 pmol of each primer; 1 μCi of α-[^{33}P]dATP; 200 μM dGTP, dTTP, and dCTP; 2.5 μM dATP; and 0.5 U of *Taq* polymerase (Boehringer Mannheim), in a final volume of 10–15 μl. The PCR profile was as follows: 95°C for 5 min, then 35 cycles of 95°C for 30 s, 55–60°C for 1–2 min, and 72°C for 30–60 s, followed by extension at 72°C for 7 min.

Linkage Analysis

Two-point and multipoint linkage analyses were performed by use of the MLINK and LINKMAP programs, respectively, of the LINKAGE package, version 5.1 (Terwilliger and Ott 1994). The genetic model used in the analysis was that for a fully autosomal recessive trait. Calculations assumed a disease-gene frequency of .021 and no sex difference, as reported elsewhere (Jones et al. 1991). Allele frequencies for all markers were kept equal, while every genotype at a locus in a pedigree was required to be known, for analysis. The map distances were calculated by use of the Haldane mapping function. The marker order and genetic distances used for the multipoint analysis were determined by use of the Généthon linkage map (Dib et al. 1996), and the following mapping information for D10S674 was taken from the MIT/Whitehead Institute map (Hudson et al. 1995):

D10S1664–.019–D10S191–.025–D10S1653–.021–D10S674.

Allele-Association Analysis

The most frequent allele of each marker detected in disease chromosomes was assumed to be the disease-associated allele, and all others were classified as non-associated alleles. Control allele distributions were determined from 30 unrelated individuals from unaffected families of 100% Navajo origin. The significance of allelic association for the 32 Navajo A-SCID chromosomes at each locus within the candidate region was determined by the statistics χ^2 and δ (Lehesjoki et al. 1993; Devlin and Risch 1995): $\delta = P_{excess} = (P_{affected} - P_{normal})/(1 - P_{normal})$, in which $P_{affected}$ is the proportion of disease-associated alleles in disease chromosomes and P_{normal} is the proportion in control chromosomes. Haplotypes were inferred so that recombinants were minimized.

Results

Exclusion of Genetic Linkage with Candidate Regions

The areas containing genes with known functions related to the features of T^-B^- SCID were typed with densely spaced markers and analyzed. The candidate genes consisted of those for interleukin-7 (IL-7) and the IL-7 receptor and those involved in V(D)J recombination. Their locations are summarized in table 1 (Oettinger et al. 1990, 1992; Jeggo et al. 1992; Lynch et al. 1992; Mombaerts et al. 1992; Shinkai et al. 1992; Muegge et al. 1993; Cai et al. 1994; Peschon et al. 1994; Taccioli et al. 1994; Blunt et al. 1995; Kirchgessner et al. 1995; Li et al. 1995; Otevrel and Stamato 1995). The pairwise LOD scores are presented in table 2. No linkage was found between markers in these eight locations and the A-SCID gene.

Genetic Linkage between A-SCID and Chromosome 10p Markers

Before linkage with the 10p markers was found, there were several regions (1q21-31, 4q28-33, 5q33-35, 7p21-15, 9p24-21, 10q11-21, and 11q23-24) with markers showing a degree of linkage with A-SCID. However, additional studies, using more markers closely spaced around the suspected region, excluded linkage (data not shown). After ~300 markers were typed, linkage at 10p was detected. The first evidence was found with D10S1430. Additional markers typed in the region proximal to D10S1430 confirmed the linkage, with no evidence of heterogeneity (table 3). The highest LOD scores, 4.53 and 4.60, were observed for markers D10S191 and D10S1653, respectively, at a recombination fraction (θ) of .00. The haplotypes are shown in figure 1. Recombination events in families 5, 14, 16, and 17 positioned the A-SCID gene between D10S1664 and D10S674 (a 6.5-cM interval). The two loci with the highest LOD scores, D10S191 and D10S1653, are located within this interval. Multipoint analysis placed the A-SCID locus between markers D10S191 and D10S1653, with a maximum multipoint LOD score (Z_{max}) of 5.10 at D10S191 (fig. 2).

Linkage-Disequilibrium Analysis

Allele frequencies for Navajo disease-bearing (n = 32) and control (n = 60) chromosomes, with results of the χ^2 test and the disequilibrium parameter δ for the associated allele, are given in table 4. Significant linkage disequilibrium was found in five adjacent marker loci in the candidate region ($P < .01$). The evidence of strongest disequilibrium was found at D10S1653, where there is a striking excess of allele 2 or allele 4 on A-SCID chromosomes versus that on control chromosomes. There is also a significant excess of alleles 4, 8, 4, and 5 at markers D10S1664, D10S191, D10S674, and D10S1477, respectively. Marker D10S1653 is associated with two different alleles, allele 2 in 50% of A-SCID chromosomes and allele 4 in 41% of A-SCID chromosomes. The likely explanation is a mutation at the D10S1653 locus, most likely an early mutational event, since the rare ancestral alleles were significantly retained at both loci flanking D10S1653. It is also possible that the mutation from allele 4 to allele 2 (and from allele 2 to allele 4) occurs at a frequency high enough that the two alleles are effectively the same.

The linkage disequilibrium with these five loci, covering a relatively large region, suggests a founder effect. The two Apache A-SCID–bearing chromosomes carry the same disease-associated alleles 4, 8, 4/2, 4, and 5 at loci D10S1664, D10S191, D10S1653, D10S674, and D10S1477, respectively, and the four Diné A-SCID chromosomes carry the common allele 8 at D10S191 (data not shown).

Discussion

In a genomewide scan for linkage to A-SCID, we analyzed ~300 microsatellite markers in 14 families from three different Athabascan-speaking Native American tribes. We studied 80 individuals, 18 of whom were affected with A-SCID of an identical phenotype. Fifteen of the affected individuals were of Navajo descent, one was an Apache Indian, and two were Diné Indians from the Northwest Territories. We excluded most of the autosomal genome, including regions encoding products involved in V(D)J recombination or IL-7 and its receptor.

Table 3

Pairwise LOD Scores between A-SCID and 10p Markers

Marker and Tribe	LOD Score at θ = .00	.02	.04	.06	.08	.10	Z_{max}	θ_{max}	Distance (cM)
D10S585:	−∞	3.50	3.51	3.39	3.22	3.02	3.53	.03	0[a]
Navajo	−∞	3.39	3.42	3.31	3.15	2.96			
Apache	.12	.11	.09	.08	.07	.06			
Diné[b]	…	…	…	…	…	…			
D10S223:	−∞	2.32	2.45	2.44	2.37	2.27	2.45	.05	3.1[a]
Navajo	−∞	3.20	3.07	2.91	2.74	2.57			
Apache	.12	.11	.09	.08	.07	.06			
Diné	−∞	−.99	−.71	−.55	−.44	−.36			
D10S1725:	−∞	1.51	1.64	1.65	1.60	1.53	1.65	.05	5.5[a]
Navajo	−∞	2.34	2.26	2.12	1.97	1.83			
Apache	.12	.11	.09	.08	.07	.06			
Diné	−∞	−.99	−.71	−.55	−.44	−.36			
D10S1664:	−∞	2.93	2.97	2.88	2.74	2.57	2.98	.03	7.4[a]
Navajo	−∞	4.20	3.92	3.66	3.39	3.11			
Apache	−.18	−.16	−.14	−.13	−.12	−.10			
Diné	−∞	−1.11	−.81	−.65	−.53	−.44			
D10S191:[c]	4.53	4.23	3.94	3.65	3.37	3.09	4.53	0	7.4[a]
	(5.20)	(4.91)	(4.62)	(4.32)	(4.03)	(3.75)	(5.20)	(0)	
Navajo	4.41	3.96	3.83	3.55	3.28	3.01			
Apache[b]	…	…	…	…	…	…			
Diné	.12	.12	.11	.10	.09	.08			
D10S1653:	4.60	4.32	4.04	3.77	3.50	3.24	4.60	0	9.9[a]
Navajo	4.48	4.21	3.95	3.69	3.43	3.18			
Apache	.12	.11	.09	.08	.07	.06			
Diné[b]	…	…	…	…	…	…			
D10S674:	−∞	1.31	1.71	1.86	1.89	1.86	1.89	.08	12[d]
Navajo	−∞	3.41	3.25	3.07	2.88	2.69			
Apache	−∞	.12	.11	.09	.08	.07			
Diné	−∞	−2.21	−1.63	−1.29	−1.06	−.89			
D10S1477:	−∞	2.04	2.35	2.41	2.37	2.29	2.40	.06	12[a]
Navajo	−∞	4.14	3.89	3.62	3.36	3.12			
Apache	.12	.11	.09	.08	.07	.06			
Diné	−∞	−2.21	−1.63	−1.29	−1.06	−.89			
D10S1423:	−∞	.26	.79	1.05	1.18	1.24	1.25	.12	16[d]
Navajo	−∞	2.63	2.56	2.47	2.36	2.23			
Apache	−.18	−.16	−.14	−.13	−.12	−.10			
Diné	−∞	−2.21	−1.63	−1.29	−1.06	−.89			

[a] Distance from D10S585, as given in the Généthon linkage map (Dib et al. 1996).

[b] Uninformative for the marker.

[c] The data omit the information from family 19, in which two affected and one unaffected offspring have nonpaternal alleles that appear to be from a mutation at the marker locus. The data determined after replacement with paternal alleles are given in parentheses.

[d] As given in the MIT/Whitehead Institute STS map (Hudson et al. 1995).

A total of eight regions showed preliminary evidence for linkage, and seven of them also were excluded after studies with a denser array of markers. Only markers on chromosome 10p were confirmed for linkage with A-SCID, and the conclusive pairwise LOD scores 4.53 and 4.60 were achieved with marker loci D10S191 and D10S1653, respectively, at $\theta = 0$.

Analysis of recombinants placed the gene in a 6.5-cM interval that contains the two loci with the highest LOD scores. There is no evidence of clinical or laboratory heterogeneity for A-SCID. Genetic homogeneity is suggested by combination of the phenotypic identity and the results of linkage and haplotype analyses. However, the genetic evidence for the Diné group is not conclusive, given that the disease-associated allele is present only at one locus and that a limited number of disease chromosomes were available for analysis. Studies of more A-SCID families from the Apache and Diné are needed to confirm this observation.

Our results from allele-association studies of linked markers further confirmed the location of the A-SCID gene and indicated that the gene must be genetically close

to D10S1653. This allelic association suggests that most patients carry the same mutation of identical origin. The results are very interesting when we combine information from the history and migration pattern of these Indian tribes. The Navajo and Apache originated from the same Na-Dene subdivision of the Athabascan-speaking linguistic group that migrated to the U.S. Southwest around 1300 A.D. and that separated into two groups around 1500 A.D. (Krauss 1979; Murphy et al. 1980; Kehoe 1981*a*, 1981*b*). In our study, the two Apache A-SCID–bearing chromosomes (from an affected family that, on the basis of family history, was determined to be unrelated to the Navajo) carry the same disease-associated alleles on all five loci in the candidate region. The best explanation for the identity of their phenotype and genotype seems to be that they are carrying the same founder mutation, which occurred before their separation around 1500 A.D. In addition, before the migration of the Na-Dene group, around 1300 A.D., the Athabascan-speaking people were hunting-gathering groups living in the Northwest Territories.

The Diné tribe is a small Athabascan-speaking Indian tribe that lives in the Northwest Territories. We identified two related families with the A-SCID phenotype and were able to include one family (with two affected children) in our study. These two families are not related to the Navajo or Apache, and the four A-SCID–bearing chromosomes carry the same disease-associated allele 8 at D10S191. Therefore, it is possible that they also carry the same mutation as that of the Navajo and Apache A-SCID patients.

Thus, when the phenotypic identity of A-SCID is combined with the results from our linkage, linkage-disequilibrium, and haplotype analyses, as well as with the information from population history, the best explanation for our results is that the Navajo, Apache, and Diné descended from the same ancestors and carry the same ancestral mutation for A-SCID, which occurred in one of their common ancestors sometime before 1300 A.D. It will be difficult to assign a more precise age to the mutation until additional studies of the Diné and other Athabascan-speaking populations in the Northwest Territories and Alaska are completed. The founder effect for the incidence of A-SCID has been suspected before (Murphy et al. 1980). Our results provide the evidence to support the founder-effect theory.

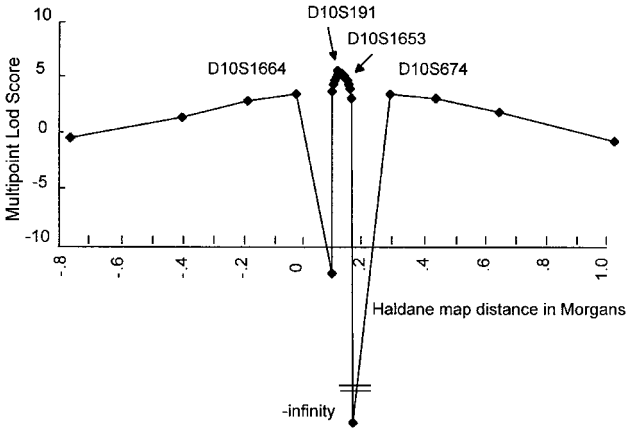

Figure 2 Multipoint linkage analysis between A-SCID locus and selected markers on chromosome 10p, for 14 families. D10S1664 was arbitrarily assigned position 0, and the order and genetic distances of the marker loci were determined by use of the Généthon genetic map (Dib et al. 1996).

The 6.5-cM candidate interval is a large region for positional cloning. There have been five genes and ~50 transcripts mapped to the region within which this interval is found. None of these genes have known functions obviously related to features of A-SCID. Additional fine mapping, with more markers within the candidate interval and with more disequilibrium studies, is necessary. This work is in progress in our laboratory, and it should refine the candidate region to a size more suitable for positional cloning.

Table 4

Allelic Association between Navajo A-SCID and Chromosome 10p Markers

Marker	Associated-Allele No.	No. of Associated Alleles/*n* (%)		χ^2	*P*	δ[a]
		A-SCID Chromosomes	Control Chromosomes			
D10S1725	2	19/32 (59)	27/58 (46)	1.36	.244	.24
D10S1664	4	23/32 (72)	11/60 (18)	25.68	<.001	.66
D10S191	8	28/32 (88)	14/58 (24)	33.27	<.001	.84
D10S1653	2/4	29/32 (91)	21/60 (35)	26.03	<.001	.86
D10S674	4	22/32 (69)	8/58 (14)	28.03	<.001	.64
D10S1477	5	20/32 (63)	20/60 (33)	7.22	.007	.45
D10S1423	4	10/38 (26)	8/60 (13)	2.43	.119	.15

[a] $\delta = P_{excess} = (P_{affected} - P_{normal})/(1 - P_{normal})$.

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References

Abe T, Tauge I, Kamachi Y, Torii S, Utsumi K, Akahori Y, Ichihara Y, et al (1992) Evidence for defects in V(D)J rearrangements in patients with severe combined immunodeficiency. J Immunol 152:5504–5513

Blunt T, Finnie NJ, Taccioli GE, Smith GCM, Demengeot J, Gottlieb TM, Mizuta R, et al (1995) Defective DNA-dependent protein kinase activity is linked to V(D)J recombination and DNA repair defects associated with the murine SCID mutation. Cell 80:813–823

Cai Q-Q, Plet A, Imbert J, Lafage-Pochitaloff M, Cerdan C, Blanchard J-M (1994) Chromosomal localization and expression of the genes coding for Ku70 and Ku80 in human cell lines and normal tissues. Cytogenet Cell Genet 65: 221–227

Devlin B, Risch N (1995) A comparison of linkage disequilibrium measures for fine-scale mapping. Genomics 29: 311–322

Dib C, Fauré S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, et al (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 380:152–154

Dror Y, Wara DW, Gallagher R, Colombe BW, Merino A, Benkerrou M, Cowan MJ (1993) Immune reconstitution in severe combined immunodeficiency disease following lectin-treated, T-cell depleted haplocompatible bone marrow transplantation. Blood 81:2021–2030

Elder ME, Lin D, Clever J, Chan AC, Hope TJ, Weiss A, Parslow TG (1994) Human severe combined immunodeficiency due to a defect in Zap-70, a T cell tyrosine kinase. Science 264:1596–1599

Fischer A (1992) Severe combined immunodeficiencies. Immunodefic Rev 3:83–100

Ford N, Nolan M, Ferguson M, Ockler M (1989) Isolation of high-molecular-weight DNA from mammalian cells. In: Sambrook J, Fritsch EF, Maniatis T (eds) Molecular cloning: a laboratory manual, 2d ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp 9.16–9.23

Gyapay G, Morissette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc S, et al (1994) The 1993–1994 Généthon human genetic linkage map. Nat Genet 7:246–339

Hirschhorn R (1993) Overview of biochemical abnormalities and molecular genetics of adenosine deaminase deficiency. Pediatr Res 29, Suppl 1:S35–S41

Hu DC, Gahagan S, Wara DW, Hayward A, Cowan MJ (1988) Congenital severe combined immunodeficiency disease (SCID) in American Indians. Pediatr Res 24 (4, pt 2): 239

Hudson TJ, Stein LD, Gerety SS, Ma MJ, Castle AB, Silva J, Slonim DK, et al (1995) An STS-based map of the human genome. Science 270:1945–1954

Jeggo PA, Hafezparast M, Thompson AF, Broughton BC, Kaur GP, Zdsienicka MZ, Athwal RS (1992) Localization of a DNA repair gene (XRCC5) involved in double-strand break rejoining to human chromosome 2. Proc Natl Acad Sci USA 89:6423–6427

Jones J, Ritenbaugh C, Spence A, Hayward A (1991) Severe combined immunodeficiency among the Navajo: characterization of phenotypes, epidemiology and population genetics. Hum Biol 63:669–682

Kehoe AB (1981*a*) The Apacheans. In: North American Indians: a comprehensive account. Prentice-Hall, Englewood Cliffs, NJ, pp 133–140

——— The arctic and subarctic (1981*b*) In: North American Indians: a comprehensive account. Prentice-Hall, Englewood Cliffs, NJ, pp 449–458

Kirchgessner CU, Patil CK, Evans JW, Cuomo CA, Fried LM, Carter T, Oettinger MA, et al (1995) DNA-dependent kinase (p350) as a candidate gene for the murine SCID defect. Science 267:1178–1182

Krauss ME (1979) Na-Dene. In: TA Sebeok (ed) Native languages of the Americas. Plenum Press, New York, pp 258–283

Lehesjoki AE, Koskiniemi M, Norio R, Tirrito S, Sistonen P, Lander ES, de la Chapelle A (1993) Localization of the EPM1 gene for progressive myoclonus epilepsy on chromosome 21: linkage disequilibrium allows high resolution mapping. Hum Mol Genet 2:1229–1234

Li Z, Otevrel T, Gao Y, Cheng H-L, Seed B, Stamato TD, Taccioli GE, et al (1995) The XRCC4 gene encodes a novel protein involved in DNA double-strand break repair and V(D)J recombination. Cell 83:1079–1089

Lynch M, Baker E, Park LS, Sutherland GR, Goodwin RG (1992) The interleukin-7 receptor gene is at 5p13. Hum Genet 89:566–568

Mathews DJ, Clark PA, Herbert J, Morgan G, Armitage RJ, Callard R (1995) Function of the interleukin-2 (IL-2) receptor γ-chain in biologic responses of X-linked severe combined immunodeficient B cells to IL-2, IL-4, IL-13 and IL-15. Blood 85:38–42

Mombaerts O, Iacomini J, Jackson RS, Herrup K, Tonewage S, Papaionnou VE (1992) RAG-1 deficient mice have no mature B and T lymphocytes. Cell 68:859–867

Muegge K, Vila MP, Durum SK (1993) Interleukin-7: a cofactor for V(D)J rearrangement of the T cell recepter β gene. Science 261:93–95

Murphy S, Hayward A, Troup G, Devor E, Coon T (1980) Gene enrichment in an American Indian population: an excess of severe combined immunodeficiency disease. Lancet 2:502–505

Murray JC, Buetow KH, Weber JL, Ludwigsen S, Scherpbier-Heddema T, Manion F, Quillen J, et al (1994) A comprehensive human linkage map with centimorgan density. Science 265:2049–2054

Noguchi M, Yi YH, Rodenblatt HM, Pilipovich AH, Adelstein S, Modi WS, McBride OW, et al (1993) Interleukin-2 receptor gamma chain mutation results in X-linked severe combined immunodeficiency. Cell 73:147–157

Oettinger MA, Schatz DG, Gorka C, Baltimore D (1990) RAG-1 and RAG-2, adjacent genes that synergistically activate V(D)J recombination. Science 248:1517–1523

Oettinger MA, Stanger B, Schatz DG, Glaser T, Call K, Housman D, Baltimore D (1992) The recombination activating genes, RAG 1 and RAG 2, are on chromosome 11p in humans and chromosome 2p in mice. Immunogenetics 35: 97–101

Otevrel T, Stamato TD (1995) Regional localization of the XRCC4 human radiation repair gene. Genomics 27: 211–214

Peschon JJ, Morrissey PJ, Grabstein KH, Ramsdell FJ, Maraskovsky E, Gliniak BC, Park LS, et al (1994) Early lymphocyte expansion is severely impaired in interleukin-7 deficiency mice. J Exp Med 180:1955–1960

Rosen FS, Cooper MD, Wedgwood RJP (1995) The primary immunodeficiency. N Engl J Med 333:431–440

Rotbart HA, Levin MJ, Jones JF, Hayward AR, Allan J, McLane MF, Essex M (1986) Noma in children with severe combined immunodeficiency. J Pediatr 109:596–600

Russell SM, Tayebi N, Nakajima H, Riedy MC, Roberts JL, Aman MJ, MigoneTS, et al (1995) Mutation of Jak-3 in a patient with SCID: essential role of Jak-3 in lymphoid development. Science 270:797–800

Schwarz K, Gauss GH, Ludwig L, Pannicke U, Li Z, Lindner D, Friedrich W, et al (1996) RAG mutations in human B cell–negative SCID. Science 274:97–99

Schwarz K, Hansen-Hagge TE, Knobloch C, Friedrich W, Kleihauer E, Bartram CR (1991) Severe combined immunodeficiency (SCID) in man: B cell–negative (B−) SCID patients exhibit an irregular recombination pattern at the JH locus. J Exp Med 174:1039–1048

Shinkai Y, Rathbun G, Lam K, Oltz EM, Stewart V, Mendelsohn M, Charon J, et al (1992) RAG-2 deficient mice lack mature lymphocyte due to inability to initiate V(D)J rearrangement. Cell 68:855–867

Taccioli GE, Alt FW (1995) Potential targets for autosomal SCID mutation. Curr Opin Immunol 7:436–440

Taccioli GE, Gottlieb TM, Blunt T, Priestley A, Demengeot J, Mizuta R, Lehmann AR, et al (1994) Ku80: product of the XRCC 5 gene and its role in DNA repair and V(D)J recombination. Science 265:1442–1445

Terwilliger JD, Ott J (1994) Handbook of human genetic linkage. Johns Hopkins University Press, Baltimore and London

Weber JL, May PE (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 44:388–396