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High-Resolution Mapping of the X-linked Hypohidrotic Ectodermal Dysplasia (EDA) Locus

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Summary

The X-linked hypohidrotic ectodermal dysplasia (EDA) locus has been previously localized to the subchromosomal region Xq11-q21.1. We have extended our previous linkage studies and analyzed linkage between the EDA locus and 10 marker loci, including five new loci, in 41 families. Four of the marker loci showed no recombination with the EDA locus, and six other loci were also linked to the EDA locus with recombination fractions of .009–.075. Multipoint analyses gave support to the placement of the PGK1P1 locus proximal to the EDA locus and the DXS453 and PGK1 loci distal to EDA. Further ordering of the loci could be inferred from a human/rodent somatic cell hybrid derived from an affected female with EDA and an X;9 translocation and from studies of an affected male with EDA and a submicroscopic deletion. Three of the proximal marker loci, which showed no recombination with the EDA locus, when used in combination, were informative in 92% of females. The closely linked flanking polymorphic loci DXS339 and DXS453 had heterozygosities of 72% and 76%, respectively, and when used jointly, they were doubly informative in 52% of females. The human DXS732 locus was defined by a conserved mouse probe pcos169E/4 (*DXCrc169* locus) that cosegregates with the mouse *tabby* (*Ta*) locus, a potential homologue to the EDA locus. The absence of recombination between EDA and the DXS732 locus lends support to the hypothesis that the *DXCrc169* locus in the mouse and the DXS732 locus in humans may contain candidate sequences for the *Ta* and EDA genes, respectively.

Introduction

X-linked hypohidrotic ectodermal dysplasia (EDA), a disorder that disrupts normal morphogenesis of several ectodermal derivatives—including hair, teeth, and eccrine sweat glands—is inherited as an X-linked recessive trait with full expression in affected males and with variable expression in carrier females (Clarke et al. 1987). We previously have localized EDA to the general region of Xq11-q21.1, by linkage analysis, specifically to band q13.1, on the basis of the cytogenetic breakpoints in an X;9 balanced translocation from a female with full manifestations of EDA (Zonana et al. 1988*a*, 1988*b*). Localization of the locus has permitted a molecular-genetic diagnostic approach to carrier testing and to both pre- and early-postnatal diagnosis (Zonana et al. 1989, 1990). In order to construct a high-resolution genetic map around the EDA locus, we have continued our linkage studies of the disorder, studying both new marker loci and new families. The addition of new marker loci to the map, especially highly informative microsatellite polymorphisms, was sought to improve diagnostic capabilities and to aid in positional cloning of the EDA locus. Additional families were included to identify key recombinants with marker loci that previously showed little or no recombination with the EDA locus, as well as to address further the issue of genetic heterogeneity.

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Finally, we wished to explore further possible homology between the *tabby* (*Ta*) mutation in the mouse and EDA in humans. In the mouse, *Ta* is inherited as an X-linked trait that affects teeth, hair, and sweat glands, producing a phenotype similar to EDA (Blecher 1986). Comparative mapping data indicates a conserved syntenic relationship of gene order between mouse and human X-chromosomes in the Xq12-q13 region (Searle et al. 1987). pcos169E/4 is a subclone of EM169, an EMBL3 lambda linking clone isolated from a mouse X-chromosome library, located at the mouse *DXCrc169* locus (Brockdorff et al. 1990). In previous studies, no recombinants had been observed between the mouse *DXCrc169* and *Ta* loci, and a probe from the *DXCrc169* locus (pcos169E/4) displayed significant homology on hybridization to human DNA (Brockdorff et al. 1991*a*, 1991*b*). The *DXCrc169* locus is also deleted in the Ta^{25H} mutation, a deletion that includes both the *Tfm* and *Ta* loci (Brockdorff et al. 1991*a*). We wished to map the homologous human locus identified by hybridization with the mouse genomic DNA probe (pcos169E/4) to further the comparative mapping of the *Ta* and EDA loci and to examine whether the conserved locus might be a candidate locus for the EDA gene.

Subjects, Material, and Methods

Families Studied

We studied 16 American families and 25 British families, each of which had multiple affected individuals. Except for seven of the American families, including one extensive kindred with five affected generations, the remaining families had been analyzed in our linkage study published elsewhere (Zonana et al. 1988*a*). Families were ascertained by referral from medical and dental specialists, through genetic counseling centers, and with the cooperation of the National Foundation for Ectodermal Dysplasias. Informed consent for blood sampling and participation in the study was obtained in accordance with guidelines approved by the Human Research Review Board of the Oregon Health Sciences University.

Affected males and possible carrier females in all of the British families were personally examined by one of the authors (A.C.), while the American families were either personally examined by one of us (J.Z.) or their diagnoses were confirmed by a review of the clinical records and a personal telephone interview of the family. Males were scored as affected if they had the typical findings of hypodontia, hypohidrosis, and hypotrichosis. Women were scored as manifesting carriers on the basis of the findings of abnormal dentition as defined elsewhere by Zonana et al. (1988*a*). The status of one female, a 60-year-old edentulous woman with a history of missing two of her permanent teeth and whose three offspring (two sons and one daughter) were normal, was changed from affected, as scored in our previous study, to unknown in this study, since we have been unable to confirm her dental history.

DNA Probes and RFLP Loci

Ten polymorphic marker loci were utilized in the linkage study. The location, allele sizes, and frequencies of the seven RFLP loci are given in table 1. The allele sizes and frequencies listed are based on published data (Kidd et al. 1989). An eighth marker locus, DXS732, was defined by the use of a conserved mouse genomic DNA probe, pcos169E/4 (Brockdorff et al. 1991*a*). The probe was hybridized to Southern blots containing human genomic DNAs from 16 unrelated X-chromosomes that had been digested with 14 restriction enzymes (*Pst*I, *Rsa*I, *Hae*III, *Hin*dIII, *Bcl*I, *Bgl*II, *Bgl*I, *Bam*HI, *Msp*I, *Sac*I, *Pvu*II, *Eco*RI, *Taq*I, and *Xba*I).

Southern Blot Analyses

Four micrograms of DNA were digested with the appropriate restriction endonuclease, electrophoresed on a 0.8% agarose gel for approximately 640 V/h, and subsequently transferred in 0.4 N NaOH to nylon filters (Hybond N+; Amersham). Filters were prehybridized at 65°C for 4 h in a solution of 7% PEG, 10% SDS, 50 mM NaH_2PO_4, and 2 mM EDTA. Denatured human placental DNA (250 μg/ml) was added to the mixture just prior to prehybridization. A sample of 80–100 ng of probe DNA were oligolabeled with ^{32}P by standard methods and were added to the hybridization solution to a final concentration of 1.5×10^6cpm/ml. Filters were hybridized at 65°C overnight and sequentially washed for 15 min at room temperature in 2 × SSC/0.1% SDS and 0.1 × SSC/0.1% SDS, with a final wash at 60°C for 5–30 min in 0.1% SSC/0.1% SDS. Filters were then exposed to Kodak X-OMAT AR film overnight at −80°C with an intensifying screen.

Identification and Analysis of Microsatellite Loci

The allele sizes and frequencies, primers, and their annealing temperatures for the three microsatellite loci (PGK1P1, DXS339, and DXS453) are shown in table

Table 1

RFLP Loci

Locus (Probe) and Enzyme	Location	Allele Length (kb)	Allele Frequency
DXS14 (p58-1) *Msp*I.....	Xp11.21	4.0	.75
		2.5	.25
DXS159 (cpX289) *Pst*I	Xq12	5.5	.59
		1.6	.41
DXS339 (RX-21) *Pst*I.....	Xq11.2-q13	5.0	.58
		3.9	.42
DXS732 (pcos169 E/4) *Xba*I....	Xq12-q13.1	13.3	.43
		8.4	.57
DXS348 (RX-97) *Bgl*II.....	Xq11.2-q13	16.0	.68
		11.5	.32
PGK1 (pSPT/PGK) *Bgl*I.....	Xq13	12.0	.79
		5.0	.21
DXS72 (pX65H7) *Hin*dIII.....	Xq21.1	7.2	.54
		.7	.46
DXYS1X (pDP34) *Taq*I.....	Xq21.31	12.0	.35
		11.0	.65

2. Details of the characterization and amplification of the microsatellite polymorphism at the PGK1P1 locus have been published elsewhere (Browne et al. 1991, 1992). A similar strategy was used in an attempt to characterize microsatellite polymorphisms at the DXS339 and DXS348 loci (Barker et al. 1989; Goldgar et al. 1989). A cosmid library, constructed with DNA from flow-sorted X chromosomes and arrayed

Table 2

$(CA)_n$ Polymorphisms

Locus (location)	No. of Alleles	Allele Size (bp)	Allele Frequency	Reference
DXS339(Xq11.2-q13)[a]	1	214	.01	This paper
	2	212	.12	
	3	210	.22	
	4	208	.11	
	5	206	.04	
	6	204	.06	
	7	202	.43	
	8	200	.01	
PGK1P1(Xq12)[b]	1	204	.05	Browne et al. (1992)
	2	202	.72	
	3	200	.01	
	4	198	.22	
DXS453(Xp11.3-q21.1)[c]	1	170	.21	Weber et al. (1990)
	2	168	.48	
	3	166	.18	
	4	164	.01	
	5	160	.12	

[a] Primers were 5′ ATGAAATAGCCCAGTACTCC 3′ and 5′ TCTGCTATAACCACACCATC 3′, with an annealing temperature of 54°C.

[b] Primers were 5′ TCTACATGTACTTAACCTGC 3′ and 5′ AACTCAGTTTTGAGCTCCTA 3′, with an annealing temperature of 51°C.

[c] Primers were 5′ GCCCCTACCTTGGCTAGTTA 3′ and 5′ AACCTCAGCTTATACCCAAG 3′, with an annealing temperature of 54° C.

on high density filters containing four equivalents of the human X chromosome (Nizetic et al. 1991), was screened by hybridization with ^{32}P-oligolabeled probes from the DXS339 (RX21) and DXS348 (RX97) loci. Potentially positive clones were identified only with the RX21 probe. DNA from the cosmids was extracted, digested with several restriction enzymes, electrophoresed, and blotted to nylon filters. These filters were hybridized with both the RX21 probe and a [^{32}P]end-labeled (CA)15 oligonucleotide probe. Cosmid ICRFc104E001154 hybridized strongly with both probes. This cosmid was digested with *Sau3a* and was subcloned into plasmid pTZ18u (U.S. Biochemical). Subclones were rescreened with the $(CA)_{15}$ oligonucleotide probe, and a positive clone (pRX21/A) was partially sequenced by using the dideoxy-chain-termination method, with Sequenase (U.S. Biochemical). Oligonucleotide primers flanking the dinucleotide repeat sequences (table 2) were designed and prepared for PCR amplification according to methods described elsewhere (Luty et al. 1990).

Previously boiled human genomic DNA samples (50 ng) were amplified in a total volume of 13 μl containing 10 pmol of each primer; 0.2 mM each of dATP, dCTP, dGTP, and dTTP; 1.5 mM $MgCl_2$; 2.5 mM spermidine; 20 mM Tris-HCl pH 8.3; 50 mM KCl; 0.1% (w/v) gelatin; and 0.25 units of *Taq* polymerase (AmpliTaq; Perkin Elmer Cetus). The reaction was carried out in a Perkin-Elmer System 9600 thermocycler utilizing a touchdown type of protocol (Don et al. 1991). All cycles commenced with a denaturation of 60 s at 94°C and an extension of 60 s at 72°C. The initial cycle utilized an annealing temperature of 62°C, with the temperature lowered by 2°C for subsequent cycles (2 cycles each), followed by 25 cycles at the locus-specific annealing temperatures for 30 s. A final extension time of 5 min at 72°C was employed. An equal volume of sequencing loading buffer was mixed with the amplified products, and the samples were denatured for 5 min at 94°C. The alleles were separated by electrophoresis for 3 h at 80 W on a 6% denaturing polyacrylamide gel and transferred overnight by capillary blotting to a Hybond N+ (Amersham) membrane that had been previously soaked in distilled water. The membrane was removed, soaked in 0.4 N NaOH for 15 min, neutralized in 2 × SSC pH 7.5, and prehybridized in 5 × SSPE/0.1% SDS for 15 min at 60°C. A $(CA)_{15}$ oligonucleotide probe was kinased with [γ^{32}P] ATP and was added to the prehybridization mixture to a final concentration of 1 pmol/ml. Hybridization was carried out at 60°C for 2 h, and the filters were subsequently washed twice for 5–15 min in 6 × SSC/0.1% SDS at 60°C. Membranes were dried and exposed to Kodak X-OMAT AR film with an intensifying screen overnight at −80°C.

Linkage and Heterogeneity Analyses

The computer package LINKAGE, version 5.0, was used to perform pairwise (MLINK) and multipoint analyses (ILINK and LINKMAP programs) (Lathrop et al. 1984). Since EDA is a congenital defect diagnosable in early childhood, no age correction was incorporated in the analysis. X-linked recessive inheritance, with a gene frequency of less than .0001 and with complete penetrance in males, was assumed. Two schemes were employed for the designation of affection status of females. In one scheme, females with phenotypic manifestations were designated as "affected." In the second method, these females were considered "unknown" with respect to disease status, in order to minimize any errors in phenotyping, since errors in classification of females based on dental exam may occasionally occur because of the occurrence of hypodontia in the normal population (Crawford et al. 1991). In both cases, "possible carrier" females, those with a normal phenotype but with an affected male relative, were designated as "unknown."

To determine whether closely linked marker loci, which showed recombination with the EDA locus, were more likely to be either proximal or distal to the EDA locus, a four-point multipoint analysis (ILINK) was performed utilizing two flanking marker loci (DXS14 and DXSY1X) and the two internal test loci. These loci were chosen since, on the basis of physical mapping, they were known to bracket the Xq12-q13.1 region.

The HOMOG program was used to test for linkage heterogeneity among the EDA families (Ott 1991, pp. 203–213). This program employs an admixture test in which one assumes that the disease is linked to a common locus in a proportion (α) of families, and unlinked in the remaining portion, $(1 - \alpha)$. The likelihood of the hypothesis of linkage heterogeneity (H_2) is compared to the null hypothesis (H_1) of linkage homogeneity in which $\alpha = 1.0$ (Hodge et al. 1983; Ott 1983). The likelihood ratio of the two hypotheses (H_2/H_1) is the test statistic. In order to take advantage of the known map relationships between several of the linked markers, we conducted a multipoint heterogeneity test based on the map generated from an analysis of Centre d'Etude du Polymorphisme Humain reference families (Mahtani et al. 1991). Specifically, we

used LINKMAP to calculate, for each family, multipoint lod scores at each of three marker loci and at the midpoint in the interval between loci. This series of lod scores served as the input to the HOMOG program. The map distances and loci used in the analysis were DXS14–0.031 cM–DXS159–0.094 cM–PGK1.

Results

Identification of New Polymorphisms

Three new polymorphic systems were identified in the Xq12-Xq13 region.

PGK1P1.—In an attempt to find a microsatellite polymorphism at the PGK1 locus, a cosmid (ICRFc100-F0599) from an X-chromosome library was identified by hybridization with the pSPT/PGK genomic probe, which contains the 5′ end of the PGK1 gene (Keith et al. 1986). A $(CA)_n$ dinucleotide repeat from this cosmid was initially characterized, and a polymorphism thought to be at the PGK1 locus was identified (Browne et al. 1991). We subsequently realized, after a single individual who was recombinant between the new $(CA)_n$ polymorphism and the *BglI* polymorphism at the PGK1 locus was identified, that the pSPT/PGK probe may have had sufficient homology to hybridize to the PGK1P1 locus at Xq12 and that the cosmid identified may have been derived from that locus. A primer pair specific for sequence at the PGK1P1 locus was designed for PCR, and amplification of the cosmid produced the expected product size, which contained the expected restriction site (Browne et al. 1992). Thus, this is the first polymorphism identified at the PGK1P1 locus.

DXS339.—Partial sequencing of the subclone pRX21/A from the DXS339 locus revealed a (dTdG)19 repeat that proved to be polymorphic. Eight different alleles were observed in 140 unrelated X chromosomes studied (table 2). Alleles were inherited in an X-linked codominant manner, and the heterozygosity was 72%. The informativeness of the locus was not improved by haplotyping with the *PstI* RFLP polymorphism, since marked linkage disequilibrium was observed (table 3). The 5.0-kb allele of the RFLP was associated only

Table 3

Linkage Disequilibrium Observed at the DXS339 Locus

$(CA)_n$ Alleles by RFLP Alleles	No. Expected[a]	No. Observed[b]
1:		
1	1.2	0
2	.8	2
2:		
1	9.8	0
2	7.1	17
3:		
1	17.4	0
2	12.6	30
4:		
1	8.7	0
2	6.3	15
5:		
1	3.5	0
2	2.5	6
6:		
1	4.6	8
2	3.4	0
7:		
1	34.8	60
2	25.2	0
8:		
1	1.2	0
2	.8	2

[a] Based on individual allele frequency.
[b] In 140 unrelated X chromosomes.

with alleles 6 and 7 of the $(CA)_n$ polymorphism, while the 3.9-kb RFLP allele was associated exclusively with the other 6 $(CA)_n$ alleles. Thus, all females heterozygous for the RFLP were also heterozygous with the $(CA)_n$ polymorphism.

DXS732.—Hybridization of human genomic DNA on Southern blot with the mouse genomic probe pcos169E/4 at the DXS732 locus revealed a single-copy band that proved to be polymorphic after digestion of the DNA with *Xba*I. Two alleles were observed, and they were inherited as an X-linked codominant trait (table 1). Forty-nine percent of 38 unrelated females were heterozygous, and all males were hemizygous, with no male-to-male transmission of the trait.

Linkage Analysis Results

All 41 families were informative for at least one of the marker loci. The DXS348 locus was less informative (32%) in our sample than anticipated from the published allele frequencies (Kidd et al. 1989), while the DXS453 locus was more informative (76%) than expected from the published 63% heterozygosity (Weber et al. 1990).

Heterogeneity Analysis

The multipoint test of linkage heterogeneity resulted in a likelihood ratio of only 1.98:1 in favor of heterogeneity ($\chi^2 = 1.37$; $P = .12$). Although P values are unreliable for multipoint heterogeneity tests (Ott 1991, pp. 203–213), the nonsignificant value of P, along with the low likelihood ratio, fails to provide definitive evidence for heterogeneity among these 41 families.

However, the value of α, the proportion of families linked to the same locus, was estimated at 0.95. According to recent recommendations (Merette et al. 1991), we calculated the support limits, or confidence interval, for α for the entire set of families, and for each family, the conditional probability of belonging to the linked type and the associated confidence interval. The 99% support interval for α extends from 0.70–1.00. With two exceptions, all families fell within this interval and had conditional probabilities greater than .95 of being in the linked type. The two families that fell outside this range had conditional probabilities of belonging to the linked type of .20 (family EDA-1029) and .36 (family EDA-67) and associated confidence intervals extending from .02–1.0 and .06–1.0, respectively. In family EDA-67, which is a small pedigree (four individuals), a crossover occurred between EDA and the DXS14 locus, while the DXS159 and PGK1 loci, the other two loci in the LINKMAP analysis, were uninformative. The pedigree was also uninformative at the remaining seven marker loci. Review of the clinical features of this family revealed a phenotype typical for X-linked EDA.

In family EDA-1029, DNA analysis revealed that the mother was informative at six marker loci flanking the EDA locus, and both an affected (III-4) and unaffected brother (III-3) shared the same marker haplotypes, while the other affected brother (III-2) had the alternate haplotype (fig. 1). For the three mapped markers used in the heterogeneity analysis, crossovers occurred at the DXS14 and PGK1 loci, and the DXS159 locus was uninformative. New DNA samples were obtained from the family, but the results of the repeated DNA analyses were unchanged. This family consisted of two affected and one normal brother, a

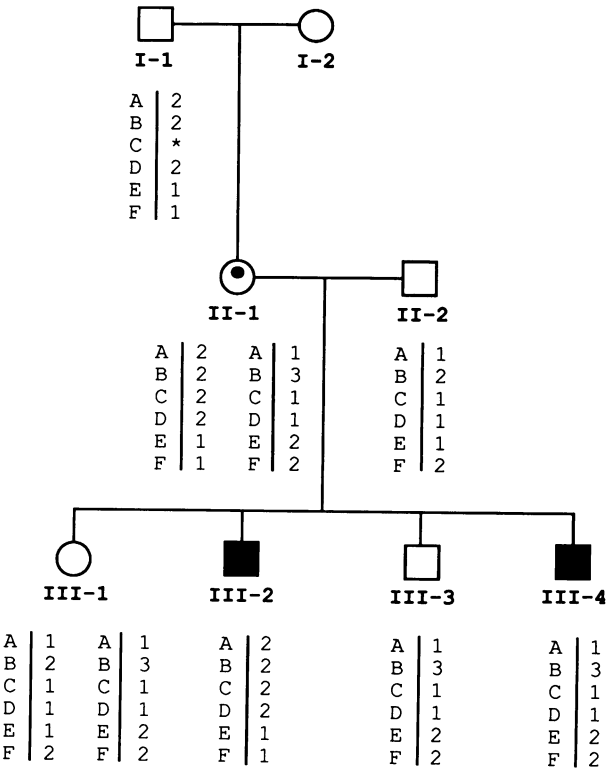

Figure 1 Results at informative marker loci for family EDA-1029. Blackened squares denote affected males. Unblackened squares denote unaffected males. Unblackened circles denote unaffected females. The circle with a dot denotes a manifesting female. A = DXS14; B = PGK1P1; C = DXS339; D = PGK1; E = DXS72; F = DXYS1; and * = not typed.

sister with no phenotypic signs of EDA, and a mother with missing maxillary and mandibular lateral incisors, conical shaped central incisors, and absent nipples. The affected brothers had multiple missing and conical teeth, and as young children they were judged to have fine, sparse scalp and body hair. No sweat pore counts were performed; there was a family history of heat intolerance, but there were no documented episodes of hyperthermia. The condition was originally diagnosed as EDA when the affected brothers were less than 4 years of age. The family was reinterviewed, and current photographs were obtained. The scalp hair of the affected males, now 12 and 6 years of age, did not appear sparse, and they had no symptoms of heat intolerance. Therefore, we believe that, on the basis of their phenotype, the original diagnosis of X-linked EDA may have been incorrect, and the family was excluded from subsequent pairwise and multipoint analyses.

Pairwise and Multipoint Analyses

Pairwise linkage results between EDA and the 10 marker loci are given in table 4. As expected, all displayed significant evidence for close linkage to EDA. The highest lod score ($\hat{Z}$ = 28.19) was obtained with DXS339 at $\hat{\theta}$ = 0.00. A reanalysis of the linkage data, in which females with phenotypic manifestations were also designated with an unknown affection status (data not shown), yielded results comparable to the analyses with manifesting females designated as affected, shown in table 4.

There was no evidence for significant linkage disequilibrium between EDA and any of the five closest loci (DXS159, DXS339, DXS348, DXS732, and DXS453). This might be expected, since EDA produces significant mortality in early childhood, which would reduce the reproductive fitness (Clarke et al. 1987). The relative order of EDA, DXS339, DXS348, DXS159, and DXS732 could not be determined, since there were no observable recombinants either with EDA or between the markers. One phase-known recombinant was observed, with recombination between the DXS453 locus and both the DXS732 and EDA loci. Two recombinants were observed between the PGK1 locus and EDA, and two recombinants were identified between the PGK1P1 locus and EDA. Multipoint ILINK analyses using the two bracketing loci (DXS14 and DXSYX1) suggested that the PGK1 and DXS453 loci are distal to the EDA locus, while the PGK1P1 locus is proximal to it (table 5).

Discussion

High Resolution Mapping of the EDA Locus

Our previous genetic mapping of the EDA locus, utilizing 36 families and nine X-chromosome marker loci, localized EDA to the Xq11-21.1 region (Zonana et al. 1988*a*). Two of the tested marker loci (DXS159 and PGK1) showed no recombination with the EDA locus, except in one individual whose status we have subsequently changed to unknown (see Subjects, Material, and Methods). Therefore, the order of these marker loci and EDA could not be determined. However, physical mapping of a human/rodent somatic cell hybrid (AnLy) established from a female with EDA and a balanced 46,X,t(X;9)(q13.1;p24) translo-

Table 4

Pairwise Linkage Analysis of EDA vs. Marker Loci

Marker	Lod Score at θ =						θ_{max} (Z_{max})	Confidence Interval[a]
	.00	.01	.05	.10	.20	.30		
DXS14	−∞	11.04	11.79	10.94	8.17	4.87	.038 (11.85)	.007–.11
PGK1P1	−∞	11.73	13.04	12.35	9.60	6.19	.046 (13.04)	.015–.11
DXS159	18.93	18.58	17.15	15.32	11.50	7.55	.000 (18.92)	.000–.03
DXS339	28.19	27.84	25.79	22.71	16.69	10.54	.000 (28.19)	.000–.02
DXS732	21.20	20.78	19.10	16.94	12.44	7.77	.000 (21.20)	.000–.03
DXS348	9.84	9.65	8.87	7.83	5.64	3.38	.000 (9.84)	.000–.05
DXS453	−∞	24.32	22.94	20.56	15.26	9.65	.009 (24.33)	.001–.04
PGK1	−∞	13.95	14.02	12.91	9.94	6.58	.026 (14.25)	.004–.09
DXS72	−∞	9.34	9.69	8.95	6.83	4.45	.033 (9.77)	.004–.11
DXYS1X ...	−∞	6.87	9.73	9.80	7.98	5.30	.075 (9.93)	.028–.16

[a] Confidence interval of −1 lod unit.

Table 5

Multipoint Analyses Using ILINK

Order and Distance	Lod Score	Relative Likelihood
DXS14 - - - EDA - - - DXS453 - - - DXYS1	48.69	213
.042 .011 .060		
DXS14 - - - DXS453 - - - EDA - - - DXYS1	46.36	1
.051 .012 .076		
DXS14 - - - EDA - - - PGK1 - - - DXYS1	39.13	182
.034 .025 .045		
DXS14 - - - PGK1 - - - EDA - - - DXYS1	36.87	1
.024 .024 .076		
DXS14 - - - PGK1P1 - - - EDA - - - DXYS1	37.00	617
.021 .042 .073		
DXS14 - - - EDA - - - PGK1P1 - - - DXYS1	34.21	1
.032 .033 .098		

cation suggested an order of cen–DXS159–EDA–PGK1–DXS72 (Cremers et al. 1988; Zonana et al. 1988*b*). Close linkage of EDA to the DXS159 locus has been confirmed (Hanauer et al. 1988), and two additional females with EDA and X-autosome balanced translocations, t(X;12) and t(X;1) have been described with breakpoints also in the region of Xq12-13.1 (Turleau et al. 1989; Limon et al. 1991).

In an attempt to confirm, by linkage analysis, the deduced order of the DXS159, PGK1, and EDA loci, we studied seven new families with EDA, including one large five-generation family. The new families contributed an additional 24 potentially informative phase-known and 19 phase-unknown, meioses. Among the new meioses, two recombinants were observed between EDA and the PGK1 locus, which on LINKMAP analysis clearly placed PGK1 distal to EDA, consistent with the previous ordering based on physical mapping. The physical mapping of the DXS159 locus proximal to EDA still could not be confirmed since there were no recombinants observed.

Linkage studies were conducted with two additional RFLP marker loci—DXS339 and DXS348—since they mapped to the Xq12-q13.1 region, and physical mapping placed DXS339 proximal and DXS348 distal to the AnLy breakpoint (N. S. T. Thomas, unpublished results). Neither locus showed recombination with EDA, and the ordering of these loci and EDA could only be inferred from human/rodent somatic cell hybrid analysis of the X;9 balanced translocations. The DXS732 locus was also inferred to be proximal to the EDA by its absence on physical mapping from the AnLy somatic cell hybrid line (Thomas et al. 1991) In addition, we have recently studied a male with EDA who was deleted for the DXS732 locus and no other close marker loci (Thomas et al. 1991). No linkage disequilibrium was observed between any of the marker loci, including the DXS732 locus, and the EDA locus.

On the basis of data from the linkage analyses, physical mapping of both the AnLy somatic cell hybrid line and X-deletion patient and from published maps of the pericentromeric region (Lafreniere et al. 1991), we suggest the following order of the marker loci and EDA pter–DXS14–PGK1P1–DXS159–DXS339–DXS732–EDA–(DXS348,DXS453)–PGK1–DXS72–DXYS1X–qter. On the basis of map-distance estimates obtained from the multipoint analyses presented in table 5, the 10 marker loci span a region of approximately 10–13 cM. There were no observable recombinants between any pair of five loci (DXS159, DXS339, DXS732, EDA, and DXS348). Therefore, appropriate map distance estimates between these markers are not available, and their order is based solely on physical mapping data. However, on the basis of the data in table 5, the following approximate distance relationships can be estimated from our data: DXS14–(2 cM)–PGK1P1–(2–5 cM)–DXS453–(1.5 cM)–PGK1–(4.5 cM)–DXYS1X.

Locus Heterogeneity

Current nosologic classification of the ectodermal dysplasias (EDs) number over 100 entities and include hypohidrotic forms with both autosomal recessive and

dominant inheritance (Freire-Maia and Pinheiro 1988). The opportunity for diagnostic misclassification exists because of the phenotypic and genetic heterogeneity of these disorders. Our previous study, utilizing a homogeneity analysis (HOMOG), failed to find evidence for locus heterogeneity in the 36 EDA families studied (Zonana et al. 1988*a*), and no evidence for heterogeneity was apparent in linkage studies reported by other investigators (Hanauer et al. 1988; Kruse et al. 1989). However, recently a family was described with a single affected male, a carrier mother and two carrier aunts, with the women's carrier status based on their minimally abnormal dental findings (Goodship et al. 1990). The linkage analysis of this family supported the possibility of either locus heterogeneity or misdiagnosis of the male proband or carrier females. The finding of at least one family in our analysis that does not appear to be linked to the EDA locus also raises the issue of nonallelic genetic heterogeneity versus misdiagnosis, and our family may be similar to the one reported by Goodship et al., since the male proband in their report had a normal sweat pore count. It is clear that there is phenotypic and genetic heterogeneity in the EDs, and atypical cases of EDA cannot be assumed to be at the Xq13.1 locus.

Clinical Application of Linked Marker Loci

Marker loci can be used in a linkage-based risk analysis for carrier detection, prenatal, and early postnatal diagnosis of EDA (Zonana et al. 1989, 1990). The expanded set of closely linked marker loci further improves our diagnostic capabilities, with no recombinants observed between four marker loci and EDA and with estimated genetic distances from the EDA locus of 0–3 cM (95% confidence interval). The DXS339 microsatellite polymorphism alone had a heterozygosity of 72%, and, when used in conjunction with the DXS159 and DXS732 loci, 92% of females in this study were heterozygous for at least one of the three loci located proximal to the EDA locus. The DXS453 microsatellite locus is a highly informative marker locus with an observed heterozygosity of 76%, which can be used, if necessary, in combination with the four distal RFLP loci, to provide a distal flanking marker. Currently, the most efficient method for carrier testing is to first analyze the microsatellite polymorphisms at the DXS339 and DXS453 loci. Fifty-two percent of the females in our families were doubly informative at these two loci, which flank the EDA locus.

Probable Homology between the Mouse Ta *and EDA Loci*

It has previously been suggested, on the basis of phenotype alone, that the *Ta* locus in the mouse and the human EDA locus are homologous (Blecher 1986). The *Ta* locus in the mouse is flanked proximally by the *Tfm* locus and distally by the phosphoglycerate kinase 1 (*Pgk1*) locus (Brockdorff et al. 1991*a*). Our linkage data provide support for a syntenic order in humans, with the placement of EDA in the interval between PGK1P1 and PGK1 loci, since the human androgen receptor locus and PGK1P1 are very closely linked and are the only loci deleted in a patient with testicular feminization (Lafreniere et al. 1991). The DXS732 locus, defined by the mouse genomic probe pcos169E/4, showed no recombination with the EDA locus, just as the mouse *DXCrc169* had no recombinants with the *Ta* locus in 100 meioses scored (Brockdorff et al. 1991*b*). The evidence for homology of the EDA and *Ta* loci is further strengthened by the finding of an affected male with EDA deleted at only the DXS732 locus and a radiation-induced cytogenetically nondetectable deletion in the mouse (Ta^{25H}), with a deletion of the *Tfm, DXCrc169,* and *Ta* loci (Brockdorff et al. 1991*a;* Thomas et al. 1991). Further investigation will be needed to determine whether the *DXCrc169* locus in the mouse and the DXS732 locus in humans contain candidate sequences for the *Ta* and EDA genes, respectively.

The construction of a high-resolution genetic map around the EDA locus, coupled with the construction of physical maps in the area, and the identification of at least four unique patients with EDA with either a balanced X;autosome translocation or deletion should greatly assist in the positional cloning of the EDA locus. The existence of an apparently homologous mouse mutation (*Ta*) will prove very useful in the eventual testing of putative candidate genes. Studies of candidate gene expression and function in normal and tabby mouse embryos, as well as correction of the *Ta* mutation by the insertion of the candidate gene into a transgenic (*Ta*/Y) mouse, should be feasible.

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References

Barker D, Dietz-Band J, Donaldson C, Andersen W, Turco A, Pole A, Willard H, et al (1989) Further isolation, characterization and physical localization of X-chromosome RFLP markers, comparing VNTR-directed and random isolation strategies. Cytogenet Cell Genet 51:958

Blecher S (1986) Anhidrosis and absence of sweat glands in mice hemizygous for the Tabby gene: supportive evidence for the hypothesis of homology between Tabby and human anhidrotic (hypohidrotic) ectodermal dysplasia. Invest Dermatol 87:720–722

Brockdorff N, Montague M, Smith S, Rastan S (1990) Construction and analysis of linking libraries from the mouse X chromosome. Genomics 7:573–578

Brockdorff N, Kay G, Cattanach B, Rastan S (1991*a*) Molecular genetic analysis of the Ta^{25H} deletion: evidence for additional deleted loci. Mammalian Genome 1:152–157

Brockdorff N, Kay G, Smith S, Keer JT, Hamvas RMJ, Brown SDM, Rastan S (1991*b*) High-density molecular map of the central span of the mouse X-chromosome. Genomics 10:17–22

Browne D, Zonana J, Litt M (1991) Dinucleotide repeat polymorphism at the PGK1 locus. Nucleic Acids Res 19: 1721

——— (1992) Dinucleotide repeat polymorphism at the PGK1P1 locus. Nucleic Acids Res 20:1169

Clarke A, Phillips D, Brown R, Harper P (1987) Clinical aspects of X-linked hypohidrotic ectodermal dysplasia. Arch Dis Child 62:989–996

Crawford PJM, Aldred MJ, Clarke A (1991) Clinical and radiographic dental findings in X linked hypohidrotic ectodermal dysplasia. J Med Genet 28:181–185

Cremers FPM, van de Pol TJR, Wieringa B, Hofker MH, Pearson PL, Pfeiffer RA, Mikkelsen M, et al (1988) Molecular analysis of male-viable deletions and duplications allows ordering of 52 DNA probes on proximal Xq. Am J Hum Genet 43:452–461

Don RH, Cox PT, Wainwright BJ, Baker K, Mattick JS (1991) "Touchdown" PCR to circumvent spurious priming during gene amplification. Nucleic Acids Res 19:4008

Freire-Maia N, Pinheiro M (1988) Ectodermal dysplasias: some recollections and a classification. Alan R Liss, New York

Goldgar D, Nguyen K, Fain P, Skolnick M, Barker D (1989) Genetic linkage map of 19 loci on chromosome Xq. Cytogenet Cell Genet 51:1006

Goodship J, Malcolm S, Clarke A, Pembrey MD (1990) Possible genetic heterogeneity in X linked hypohidrotic ectodermal dysplasia. J Med Genet 27:422–425

Hanauer A, Alembik Y, Arveiler B, Formiga L, Gilgenkrantz S, Mandel J (1988) Genetic mapping of anhidrotic ectodermal dysplasia: DXS159, a closely linked proximal marker. Hum Genet 80:177–180

Hodge SE, Anderson CE, Neiswanger K, Sparkes RS, Rimoin DL (1983) The search for heterogeneity in insulin-dependent diabetes mellitus (IDDM): linkage studies, two-locus models, and genetic heterogeneity. Am J Hum Genet 35:1139–1155

Keith D, Singer-Sam J, Riggs A (1986) Active X chromosome DNA is unmethylated at eight CCGG sites clustered in a guanine-plus-cytosine-rich island at the 5′ end of the gene for phosphoglycerate kinase. Mol Cell Biol 6:4122–4125

Kidd K, Bowcock A, Schmidtke J, Track R, Ricciuti F, Hutchings G, Bale A, et al (1989) Report of the DNA committee and catalogs of cloned and mapped genes and DNA polymorphisms. Cytogenet Cell Genet 51:622–947

Kruse T, Kolvraa S, Bolund L, Kaitila I, Soderholm A, de la Chapelle A, MacDermot K (1989) X-linked anhidrotic ectodermal dysplasia (EDA): multipoint linkage analysis. Cytogenet Cell Genet 51:1026

Lafreniere RG, Brown CJ, Powers VE, Carrel L, Davies KE, Barker DF, Willard HF (1991) Physical mapping of 60 DNA markers in the p21.1 to q21.3 region of the human X chromosome. Genomics 11:352–363

Lathrop G, Lalouel J, Julier C, Ott J (1984) Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci 81:3443–3446

Limon J, Filipiuk J, Nedoszytko B, Mrozek K, Castren M, Larramendy M, Roszkiewicz J (1991) X-linked anhidrotic ectodermal dysplasia and de novo t(Z;1) in a female. Hum Genet 87:338–340

Luty JA, Guo Z, Willard HF, Ledbetter DH, Ledbetter S, Litt M (1990) Five polymorphic microsatellite VNTRs on the human X chromosome. Am J Hum Genet 46:776–783

Mahtani MM, Lafreniere RG, Kruse TA, Willard HF (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

Mérette C, Lehner T, Ott J (1991) Interpreting nonsignificant outcomes of heterogeneity tests in gene mapping. Am J Hum Genet 49:1381–1384

Nizetic D, Zehetner G, Monaco A, Gellen L, Young B, Lehrach H (1991) Construction, arraying, and high-density screening of large insert libraries of human chromosomes X and 21: their potential use as reference libraries. Proc Natl Acad Sci USA 88:3233–3237

Ott J (1983) Linkage analysis and family classification under heterogeneity. Ann Hum Genet 47:311–320

——— (1991) Analysis of human genetic linkage, rev ed. The Johns Hopkins University Press, London and Baltimore

Searle A, Peters J, Lyon M, Evans E, Edwards J, Buckle V (1987) Chromosome maps of man and mouse, III. Genomics 1:3–18

Thomas N, Davies K, Zonana J, Rastan S, Brockdorff N (1991) Molecular deletion analysis in X–linked hypohidrotic ectodermal dysplasia. Am J Hum Genetics 49 [Suppl]: A205

Turleau C, Niaudet P, Cabanis M-O, Plessis G, Cau D, de Grouchy J (1989) X-linked hypohidrotic ectodermal dysplasia and t(X;12) in a female. Clin Genet 35:462–466

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

Zonana J, Clarke A, Sarfarazi M, Thomas NST, Roberts K, Marymee K, Harper PS (1988*a*) X-linked hypohidrotic ectodermal dysplasia: localization within the region Xq11-21.1 by linkage analysis and implications for carrier detection and prenatal diagnosis. Am J Hum Genet 43:75–85

Zonana J, Roberts S, Thomas N, Harper P (1988*b*) Recognition and reanalysis 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

Zonana J, Sarfarazi M, Thomas N, Clarke A, Marymee K, Harper P (1989) Improved definition of carrier status in X-linked hypohidrotic ectodermal dysplasia by use of restriction fragment length polymorphism-based linkage analysis. J Pediatr 114:392–399

Zonana J, Schinzel A, Upadhyaya M, Thomas N, Anton-Lamprecht I, Harper P (1990) Prenatal diagnosis of X-linked hypohidrotic ectodermal dysplasia by linkage analysis. Am J Med Genet 35:132–135