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Mapping of the Gene for X-linked Amelogenesis Imperfecta by Linkage Analysis

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Summary

X-linked Amelogenesis imperfecta (AI) is a genetic disorder affecting the formation of enamel. In the present study two families, one with X-linked dominant and one with X-linked recessive AI, were studied by linkage analysis. Eleven cloned RFLP markers of known regional location were used. Evidence was obtained for linkage between the AI locus and the marker p782, defining the locus DXS85 at Xp22, by using two-point analysis. No recombination was scored between these two loci in 15 informative meioses, and a peak lod score (Z_{max}) of 4.45 was calculated at zero recombination fraction. Recombination was observed between the more distal locus DXS89 and AI, giving a peak lod score of 3.41 at a recombination fraction of .09. Recombination was also observed between the AI locus and the more proximal loci DXS43 and DXS41 (Z_{max} = 0.09 at θ_{max} = 0.31 and Z_{max} = 0.61 at θ_{max} = 0.28, respectively). Absence of linkage was observed between the AI locus and seven other loci, located proximal to DXS41 or on the long arm of the X chromosome. On the basis of two-point linkage analysis and analysis of crossover events, we propose the following order of loci at Xp22: DXS89–(AI, DXS85)–DXS43–DXS41–Xcen.

Introduction

Enamel is almost entirely composed of two protein classes, of which amelogenin is the most predominant (Eastoe 1965; Termine et al. 1980). Amelogenesis imperfecta (AI) is a collection of genetic disorders affecting the formation of enamel. In males the X-linked hypoplastic type of AI results in the failure of the enamel to develop to normal thickness in primary and permanent teeth, and the main clinical feature of the hypomineralization form is an enamel which is softer than normal (Witkop and Rao 1971; Bäckman and Holmgren 1988). Females, who are heterozygous for the mutant gene, show vertically arranged alternating bands of abnormal and normal enamel, caused by random X-chromosome inactivation (Berkman and Singer 1971). In the general population AI occurs with a prevalence of about 1:14,000 (Witkop 1958), and the disorder is genetically heterogeneous, since X-linked dominant, X-linked recessive, autosomal dominant, and autosomal recessive inheritance has been reported (Witkop and Sauk 1976; Bäckman and Holmgren 1988). A partial cDNA sequence of mouse amelogenin has recently been cloned (Snead et al. 1983), and by using the mouse probe, human amelogenin sequences were mapped to the p21.1-p22.3 region of the X chromosome and near the centromere of the Y chromosome by hybridization to DNA from somatic cell hybrids containing different regions of the human X and Y chromosomes (Lau et al. 1989).

In the present study we have performed a linkage study of two X-linked AI families by using 11 polymorphic X chromosome–specific DNA probes of known regional location. One family showed a dominant mode of inheritance while the other family was classified as recessive, according to the definition of Witkop and Rao (1971) and McKusick (1988). Our results show that the defective gene in these two families is closely linked to the locus DXS85 at Xp22, supporting the hypothesis

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that X-linked AI is caused by mutations in the amelogenin gene.

Material and Methods

Subjects

The study comprises two Swedish families described elsewhere (Bäckman and Holmgren 1988). Family A has the hypoplastic type of AI, while family B has the hypomineralization form of AI. An X-linked mode of inheritance was established in both families on the basis of the segregation pattern of the disease in 3 generations.

Affected males showed an absence of normal enamel formation, and the diagnosis was confirmed by dental examination. Carrier females in family A showed a partial manifestation of the disease, with islands of normal and abnormal enamel indicating a dominant mode of inheritance, while carrier females in family B showed vertically ridged teeth, as is seen in the recessive form (Witkop and Rao 1971; McKusick 1988).

DNA Analysis

Total genomic DNA was prepared from peripheral blood collected from AI patients and their relatives. Ten micrograms of DNA were digested with the appropriate restriction endonucleases. The DNA was subjected to electrophoresis through 0.9% agarose gels and analyzed by Southern blot hybridizations (Southern 1975) using nylon membranes (Pall Biodyne, BioSupport Division, New York) and probes radiolabeled by random primed synthesis (Amersham multiprime kit). Eleven cloned DNA probes mapping between Xpter and Xq28 were used in the present study (table 1).

Linkage Analysis

Two-point linkage analysis was performed, using the computer program LINKAGE V3.5 (Lathrop and Lalouel 1984). A gene frequency of .0001 was assumed for the mutant allele. Penetrance was taken as complete for both males with the mutant allele, as well as for female heterozygotes.

Results

Two families, one with X-linked AI of a dominant form and the other with X-linked AI recessive form, were analyzed by using 11 markers which are distributed along the X chromosome (table 1). The results revealed linkage between the AI locus and markers in the Xp22 region. The segregation patterns for the four probes that showed linkage to the AI locus are present in figure 1, and the calculated peak lod score (Z_{max}) for each pattern is given in table 2. No recombination was recorded in 15 informative meioses between the AI locus and the marker p782, defining the locus DXS85 at Xp22. Z_{max} of 4.45 was calculated at a peak recombination fraction (θ_{max}) of zero (table 2). Linkage was also observed between AI and the more distal locus DXS89, giving a peak Z_{max} of 3.41 at θ_{max} = .09. The loci DXS43 and DXS41, which are located on the proximal side of DXS85 at calculated distances of 19 and 30 cM, respectively (Drayna and White 1985), gave Z_{max} values of .09 (θ_{max} = .31) and .61 (θ_{max} = .28), respectively.

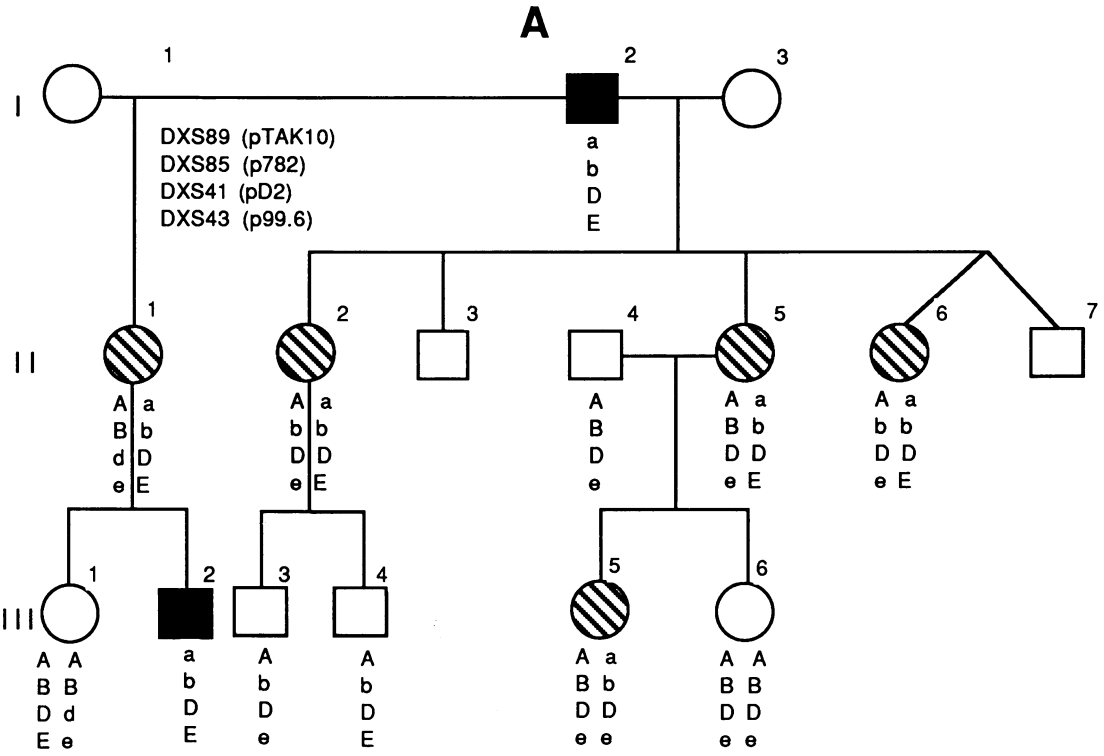
No linkage was observed between the AI locus and the seven remaining polymorphic markers (table 1) which are distributed along the X chromosome.

Additional information regarding the location of the

Table 1

Marker Loci Defined by X-Chromosomal DNA Probes Used in the Linkage Analysis of X-linked AI

Locus (Probe)	Location	Reference
DXS89 (pTAK10)	Xp22.3-22.2	T. A. Kruse, personal communication
DXS85 (p782)	Xp22.3-22.2	Hofker et al. 1985
DXS43 (pD2)	Xp22.2	Aldridge et al. 1984
DXS41 (p99.6)	Xp22.1	Aldridge et al. 1984
DXS84 (p754)	Xp21.2-21.1	Bakker et al. 1985
OTC (pOTC)	Xp21.1	Davies et al. 1985
DXS164 (pERT87.1)	Xp21	Kunkel et al. 1985
DXS14 (p58.1)	Xp11-cen	Aldridge et al. 1984
DXYS1 (pDp34)	Xq13-q21.2	Page et al. 1984
DXS42 (p7F1)	Xq24-26	Reilly et al. 1988
DXS52 (St14)	Xq28	Oberlé et al. 1985



AI locus relative to the markers used was obtained by analyzing crossover events. In this analysis it was assumed that the order of the loci at Xp22 is Xpter–DXS89–DXS85–DXS43–DXS41 (Drayna and White 1985; Davies et al. 1987). In family B (fig. 1) the unaffected female III:7 had inherited the a and e alleles defined by the probes pTAK10 (DXS89) and p99.6 (DXS41) from her carrier mother (II:9). The same alleles were inherited by her carrier sister III:9 and by her two affected brothers III:6 and III:8. On the other hand, individual III:7 had inherited the B allele defined by probe p782 (DXS85), unlike her affected siblings, who all had inherited the b allele. As DXS85 is located between DXS89 (pTAK10) and DXS41 (p99.6) (Drayna and White 1985; Davies et al. 1987), this finding suggests that two crossover events had occurred in the Xp22 region during the meiosis leading to III:7.

The carrier-female MZ twins II:8 and II:9 have inherited the same DXS43 (pD2) allele from the mother as have their healthy siblings, suggesting that a crossover event has taken place between the AI locus and DXS43. On the other hand, the AI locus cosegregated with DXS89 in this meiosis, suggesting that the AI locus is distal to DXS43.

Analysis of the female carrier III:2 in family B gives some additional clues as to the relative position of the AI locus. Her mother is informative for all four markers. III:2 has inherited the haplotype abdE, whereas her carrier sister has inherited the haplotype abde. This suggests that the AI locus is distal to DXS41, as it cosegregates with three markers all of which are located on the distal side of DXS41. The same conclusion can be drawn from an analysis of the haplotypes of individuals III:4 and III:5 in family A.

Furthermore, an analysis of individual III:5 in family B allows the conclusion that the AI locus is likely to be proximal to DXS89. It is clear that the haplotype ab is associated with the disease gene in the MZ twins II:8 and II:9. Still, III:5 has inherited the haplotype Ab, suggesting that a crossover event has occurred distal to the AI locus and proximal to DXS89 in III:5 (fig. 1). On the basis of our linkage data and these observed crossover events, we propose that the AI gene is located between the loci DXS89 and DXS43 at Xp22. The

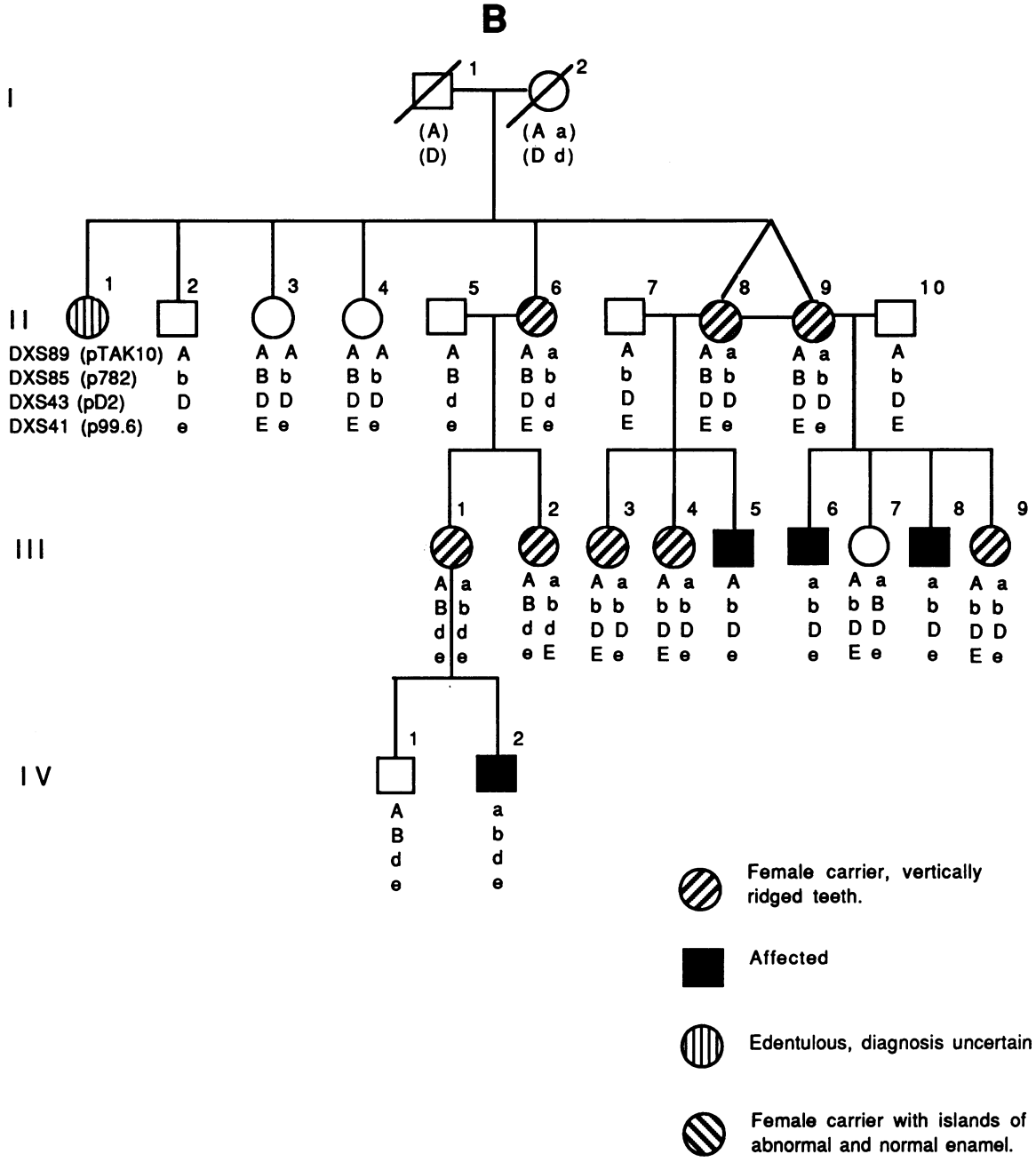


Figure 1 Pedigrees for families A and B. The results of the segregation analysis of the four most tightly linked probes are shown in the figure. Predicted haplotypes are marked with parentheses. Family A was defined as having a dominant form of X-linked AI, while family B showed a recessive form (Witkop and Rao 1971; McKusick 1988).

relative positions of AI and DXS85 cannot be established, as no recombinants have yet been identified between these two loci. We suggest the following order of loci at Xp22: Xpter–DXS89–(AI, DXS85)–DXS43–DXS41–Xcen.

Discussion

In the present investigation we have mapped the AI locus by using a series of polymorphic markers defined by cloned DNA segments covering the X chromosome. The results demonstrate close linkage between DXS85

Table 2

Z_{max} Values for AI and Four Marker Loci

Locus (probe) and Family	Recombination Fraction (θ)								
	.00	.01	.05	.10	.20	.30	.40	θ_{max}	Z_{max}
DXS89 (pTAK10):									
A	1.81	1.78	1.67	1.53	1.22	.88	.48	.00	1.81
B	$-\infty$	.45	1.60	1.87	1.76	1.31	.67	.13	1.90
Total	$-\infty$	2.23	3.27	3.40	2.98	2.19	1.15	.09	3.41
DXS85 (p782):									
A	1.20	1.19	1.12	1.02	.82	.58	.32	.00	1.20
B	3.25	3.20	3.00	2.75	2.20	1.57	.84	.00	3.25
Total	4.45	4.39	4.12	3.77	3.02	2.15	1.16	.00	4.45
DXS43 (pD2):									
A	$-\infty$	−1.93	−1.21	−.88	−.52	−.30	−.14	.50	.00
B	$-\infty$	−.22	.37	.53	.53	.39	.19	.14	.56
Total	$-\infty$	−2.15	−.84	−.35	.01	.09	.05	.31	.09
DXS41 (p99.6):									
A	$-\infty$	−2.36	−1.02	−.51	−.09	.06	.08	.37	.08
B	$-\infty$	−1.41	−.13	.31	.57	.54	.34	.23	.59
Total	$-\infty$	−3.77	−1.15	−.20	.48	.60	.42	.28	.61

and the AI locus, with cumulative $Z_{max} = 4.45$ at $\theta_{max} = 0$ (table 2). In our study two different subtypes of X-linked AI were included, namely, the hypoplastic dominant (family A) and the hypomineralization recessive (family B) forms. In spite of the different clinical manifestations of the two subtypes, both showed linkage to DXS85 and DXS89 (table 2). For family A a higher Z_{max} was obtained with DXS89 than with DXS85 (table 2), apparently because of the scoring of a higher number of informative meioses with the probe pTAK10 in this family. In the absence of crossover events, the location of the AI locus with respect to DXS85 could not be determined. The estimated distance between AI and DXS89 did not allow any more definite conclusions, since the distance between DXS89 and DXS85 is poorly defined (Davies et al. 1987).

A genetic map of the X chromosome has been established from linkage relationships for a number of probes (Drayna and White 1985; Davies et al. 1987). It was thus possible to position the AI locus relative to these markers. On the basis of these linkage relationships and the observed recombination events and linkage data from the present study, we suggest the following order of loci: Xpter–DXS89–(AI, DXS85)–DXS43–DXS41.

While this work was in progress, Lau et al. (1989) reported the mapping of the human amelogenin gene Xp22.1–p22.3 region of the human X chromosome by using a mouse cDNA probe and rodent-human somatic cell hybrids. Our linkage study positions the AI gene in the region where the amelogenin gene was mapped, suggesting that it is a defect in this gene that causes the X-linked form of the disease. There must, however, also exist other genes that give rise to a similar phenotype, since AI with autosomal inheritance has been observed (Witkop and Rao 1971; Bäckman and Holmgren 1988).

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