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Refinement of the localisation of the X linked keratosis follicularis spinulosa decalvans (KFSD) gene in Xp22.13-p22.2

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Abstract

X-linked keratosis follicularis spinulosa decalvans (KFSD) is a rare disorder affecting the skin and eyes. The disease was previously mapped in an extended Dutch family to Xp21.2-p22.2 between DXS16 and DXS269. Using five DNA probes and 14 CA repeat polymorphisms spanning this region an extensive linkage study was performed in the same pedigree. The highest lod scores were 12·07 for DXS365 (pRX-314) at $\theta = 0$, 11·72 for DXS418 (P122) at $\theta = 0{\cdot}015$, and 10·93 for DXS989 (AFM135xe7) at $\theta = 0{\cdot}045$. Analysis of recombination events locates the gene for KFSD between AFM291wf5 and DXS1226 (AFM316yf5). This is region Xp22.13-p22.2, an area covering approximately 1 Mb. These data confirm and greatly refine the regional localisation of KFSD and greatly improve reliability of carrier detection.

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Keratosis follicularis spinulosa decalvans (KFSD) or Siemens-1 syndrome (MIM 308800) is a rare X chromosomal skin and eye disease of unknown aetiology.[1 2] Recently, descendants from an extended Dutch pedigree were clinically investigated[3] and RFLP linkage analysis was performed, localising the gene to Xp21.2-p22.2.[4]

The disorder is characterised by follicular hyperkeratosis of the skin, scarring alopecia of the scalp, and absence of eyebrows and sometimes eyelashes. There is severe photophobia in childhood and corneal dystrophy develops later on. The symptoms tend to diminish with age and the prognosis for vision is good. There is no mental retardation nor other non-ectodermal symptoms suggestive of a contiguous gene syndrome.[1–8]

KFSD appears to be clinically and genetically heterogeneous,[9] but most published families with KFSD are compatible with X linked inheritance.[2–7] About 50% of heterozygotes show mild clinical symptoms.[2 3 7 8]

Localisation of the gene on Xp21.2-p22.2 enabled carrier detection in large families. Further narrowing of the KFSD region will improve reliability of carrier detection and may facilitate characterisation of the gene and its mutations and elucidation of clinical and genetic heterogeneity. Here we report on refined linkage analysis using both conventional DNA markers and CA repeat polymorphisms spanning Xp21.2-p22.2.

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Materials and methods

SUBJECTS

The study was performed on 54 subjects including 21 affected males, all descendants of a large Dutch pedigree[1 3 4 8] (fig 1). Patient ascertainment and method of DNA extraction have been described previously.[4]

DNA POLYMORPHISMS

A total of five DNA markers and 14 CA repeat polymorphisms was tested in the family. Characteristics of these DNA polymorphisms are listed in physical order in table 1, by using data as assigned at the 5th chromosome X workshop[10] and by others.[11–16]

SOUTHERN ANALYSIS

DNA aliquots of 5 µg were digested with the restriction enzymes *Msp*I, *Pvu*II, and *Taq*I, according to the manufacturer's recommendations. The fragments were separated by 0·7% agarose gel electrophoresis and transferred to nylon filters (Hybond-N® Amersham) by means of alkaline blotting. Hybridisation with α^{32}P-dCTP labelled probes pD2 (DXS43), CRI-L1391 (DXS274), pQST1H3 (DXS257), and B24 (DXS67) was performed at 65°C overnight according to Church and Gilbert.[17] Filters were washed to a stringency of 1 × SSC/0·1% SDS, followed by autoradiography for one to three days using an intensifying screen.

MICROSATELLITE MARKER ANALYSIS

Amplification of microsatellites (table 1) was performed in a Perkin Elmer Cetus thermal cycler. The reaction volume was 15 µl containing 1·0 mmol/l $MgCl_2$, 10 mmol/l Tris-HCl, pH 9·0; 50 mmol/l KCl; 0·01% gelatine; 0·1% Triton X-100; 0·6 U Amplitaq® (Cetus Inc); 0·2 mmol/l each of dATP, dGTP, and dTTP; 0·025 mmol/l dCTP; 0·27 pmol α^{32}P-dCTP (at 3000 Ci/mmol); 5 pmol of each oligonucleotide primer, and 200 ng of genomic DNA template. An initial denaturation step of five minutes at 94°C was followed by 25 to 30 cycles of one minute at 94°C, two minutes at

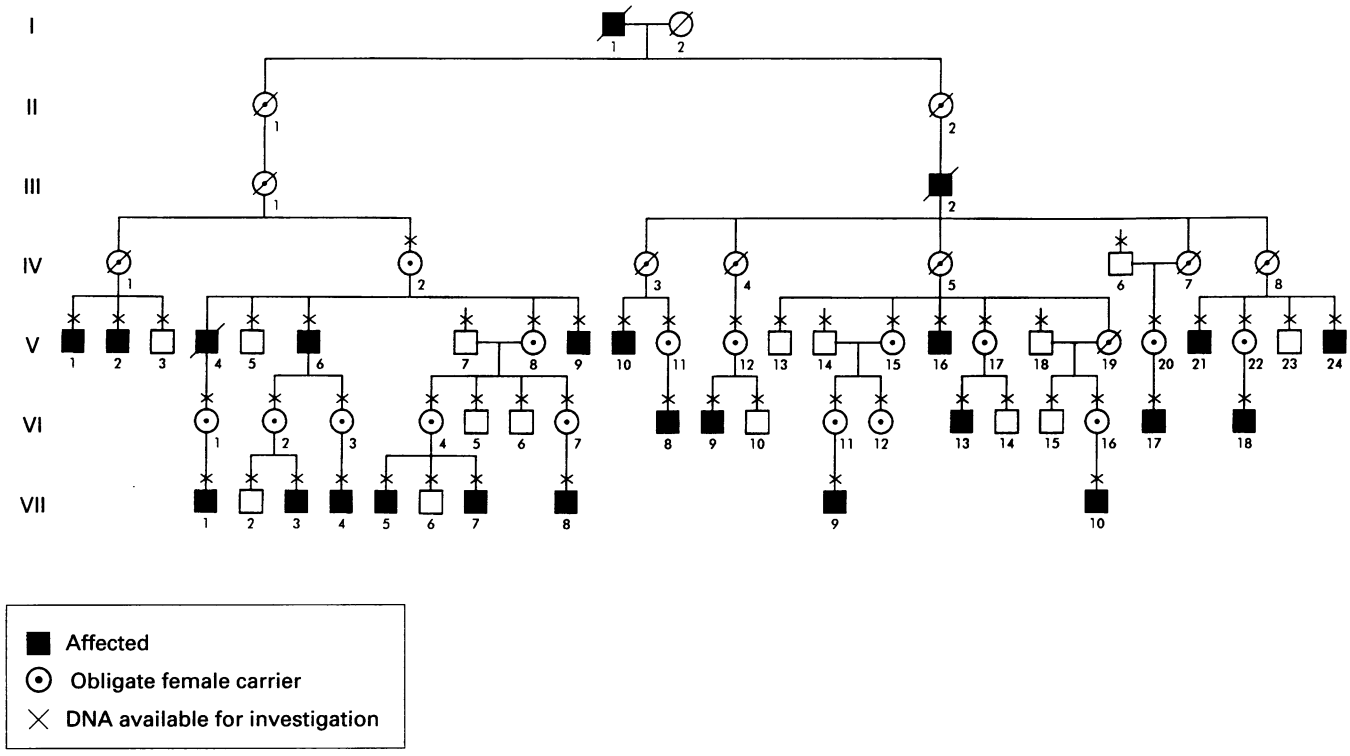

Figure 1 Pedigree of KFSD family investigated.

50–60°C, and one minute at 72°C, and a final step of 10 minutes at 72°C.

After PCR, 1 volume of formamide sample buffer (95% formamide, 20 mmol/l EDTA, 0·05% bromophenol blue, 0·05% xylene cyanol) was added to the samples. The samples were denaturated (five minutes at 94°C) and loaded on a DNA sequencing gel (6% acrylamide/bisacrylamide 19:1, 7 mol/l urea, 0·35% APS, 0·07% TEMED in 1 × TBE). Electrophoresis was performed for two to three hours at 40 mA and 65 W. Before loading, the upper buffer compartment was filled with 0·5 × TBE, the lower buffer compartment with 5/6 × TBE/0·5 mol/l NaAc, and the gel was prerun for 0·5 hours. After electrophoresis the gel was fixed for 30 minutes in 5% methanol/5% acetic acid and dried, followed by autoradiography overnight.

LINKAGE ANALYSIS

Two point linkage analyses to determine the maximum likelihood recombination distances (θmax) and the lod scores at θmax (Zmax) between the disease locus (KFSD) and the DNA probe loci were carried out according to guidelines[18 19] using the MLINK from the LINKAGE package 5·03.[20] The gene frequency of KFSD in the general population was taken to be 0·0001. Spontaneous mutation rate was taken as zero and penetrance in males was assumed to be 100%.

Results

Of the 19 polymorphisms tested, four conventional DNA markers and 12 CA repeats proved informative (table 2). No deletions were detected in affected patients using these 16 polymorphisms.

Table 1 X chromosome polymorphisms located at Xp22.2-p22.1 used for linkage analysis

Locus	*Probe*	*Polymorphism CA_n/enzyme*	*Allele number*	*Hetero-zygosity†*	*Allele size*
DXS987	AFM120xa9	CA_n	10	83%	224–206 bp
DXS43	pD2	*Pvu*II	2	0·50	6·6, 6·0 kb
DXS1195*	AFM207zd6	CA_n	3	61%	239–235 bp
DXS418	P122	CA_n	11	83%	158–140 bp
DXS999	AFM234yf12	CA_n	6	73%	276–260 bp
DXS257	pQST1H3	*Taq*I	2	40%	5·5, 4·5 kb
	AFM291wf5	CA_n	7	77%	254–240 bp
DXS443	pRX-324	CA_n	7	60%	210–204 bp
DXS1229*	AFM337wd5	CA_n	4	33%	230–202 bp
DXS365	pRX-314	CA_n	9	57%	217–201 bp
DXS1226	AFM316yf5	CA_n	10	85%	223–201 bp
DXS1052	AFM163yh2	CA_n	5	70%	143–159 bp
DXS274	CRI-L1391	*Msp*I	2	48%	11·0, 8·5 kb
DXS989	AFM135xe7	CA_n	9	80%	199–173 bp
DXS451	kQST80H1	CA_n	9	80%	204–182 bp
DXS67	B24	*Msp*I	2	0·08	1·6, 1·4 kb
DXS92*	pXG.16	*Taq*I	3	58%	7·1, 3·8, 3·5 kb
DXS1235	STR50	CA_n	6	70%	251–233 bp
DXS1236	STR49	CA_n	19	91%	257–227 bp

Based on references 10, 11, 12, 13, 14, 15, and 16.
* Not informative in this pedigree.
† Observed heterozygosity (%) or PIC value in published reports.

Table 2 Two point linkage data for KFSD and 20 informative Xp22.2-p21.2 markers

Locus	*Recombination**	*0·0*	*0·001*	*0·005*	*0·01*	*0·05*	*0·1*	*0·2*	*0·3*	*Zmax*	*θmax*	*CI†*
DXS16‡	1/19	−∞	3·03	3·70	3·95	4·28	4·08	3·27	2·20	4·38	0·040	0·00–0·18
DXS987	2/25	−∞	3·48	4·83	5·36	6·19	6·06	5·08	3·67	6·21	0·055	0·01–0·18
DXS43	1/19	−∞	−1·22	0·83	1·68	3·32	3·63	3·26	2·41	3·65	0·090	0·03–0·28
DXS418	1/41	−∞	10·90	11·52	11·71	11·54	10·71	8·56	5·99	11·72	0·015	0·00–0·10
DXS999	0/14	5·31	5·30	5·28	5·25	4·97	4·55	3·58	2·49	5·31	0·000	0·00–0·11
DXS257	1/20	−∞	3·60	4·25	4·49	4·71	4·41	3·44	2·29	4·74	0·035	0·00–0·19
AFM291wf5	1/24	−∞	4·19	4·85	5·10	5·38	5·10	4·11	2·85	5·39	0·040	0·00–0·18
DXS443	0/18	5·84	5·83	5·80	5·75	5·39	4·92	3·88	2·71	5·84	0·000	0·00–0·11
DXS365	0/35	12·07	12·06	11·99	11·91	11·21	10·26	8·15	5·75	12·07	0·000	0·00–0·06
DXS1226	1/34	−∞	9·51	10·13	10·33	10·21	9·45	7·45	5·09	10·43	0·020	0·00–0·10
DXS1052	0/20	7·56	7·55	7·50	7·44	6·95	5·44	4·97	3·49	7·56	0·000	0·00–0·06
DXS274	1/26	−∞	4·98	5·64	5·88	6·12	5·79	4·68	3·27	6·15	0·045	0·00–0·13
DXS989	1/39	−∞	9·67	10·32	10·55	10·61	9·46	8·07	5·73	10·93	0·045	0·00–0·09
DXS451	1/19	−∞	3·75	4·41	4·67	4·97	4·75	3·91	2·81	4·99	0·065	0·00–0·19
DXS41‡	1/18	5·70	5·69	5·66	5·62	5·26	4·79	3·75	2·59	5·70	0·000	0·00–0·11
DXS67	0/10	4·25	4·24	4·22	4·19	3·96	3·42	2·91	2·04	4·25	0·000	0·00–0·11
DXS28‡	0/12	4·15	4·14	4·12	4·09	3·83	3·48	2·73	1·89	4·15	0·000	0·00–0·15
DXS1235	0/20	6·43	6·42	6·38	6·33	5·90	5·34	4·15	2·84	6·43	0·000	0·00–0·09
DXS1236	3/42	−∞	4·55	6·60	7·45	8·94	8·93	7·68	5·68	8·96	0·060	0·03–0·18
DXS269‡	2/21	−∞	0·33	1·26	2·25	3·32	3·48	3·10	2·35	3·48	0·100	0·01–0·28

* Number of recombinations encountered; the numerator is the number of recombinations, the denominator is the total number of informative meioses.
† Confidence interval as defined by θmax minus 1.
‡ Data from ref 4.

RECOMBINATION ANALYSIS

The segregation of the markers in this pedigree was compatible with the marker order as defined by the most recent X chromosome workshop[10] and confirmed for this region recently by Alitalo *et al*[16] and by Van de Vosse *et al.*[21] DXS999, DXS443, DXS365, DXS67, and DXS1235 showed no recombinants with KFSD. Several interesting recombinations were found between KFSD and the markers used. In fig 2 key recombinants are shown indicating that KFSD is located between DXS257 and DXS1226. The recombination in patient VI·13 between KFSD and DXS1226 (AFM316yf5) shows that this marker is the proximal border, and patient VII·10 with a recombination between KFSD and AFM291wf5 indicates that this is the distal border.

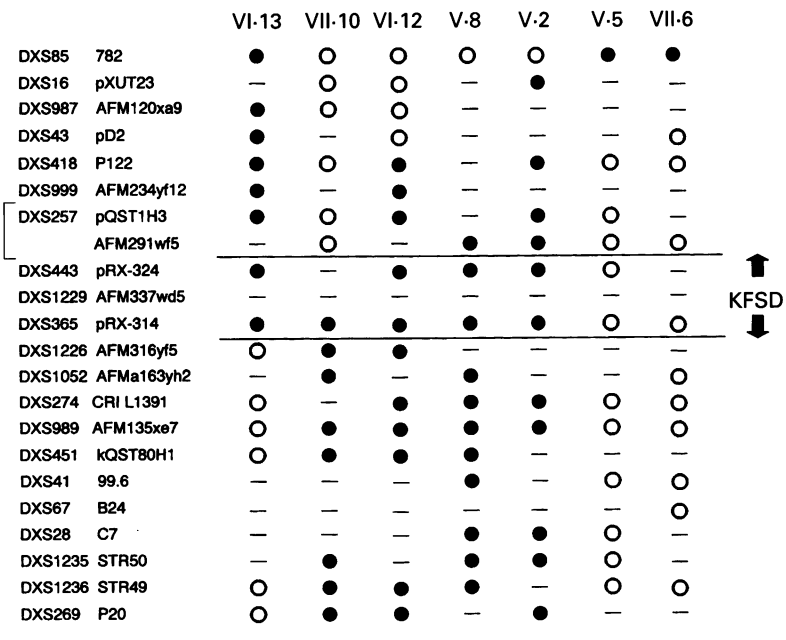

Figure 2 Key recombinants. Graphic representation of haplotypes and meiotic recombination events in maternally derived X chromosomes. The allele at risk is a black circle, the not at risk allele is an open circle. The order of loci is indicated on the left. Horizontal dashes mean maternal homozygosity or unknown phase.

LINKAGE ANALYSIS

Since the markers used map in the region between DXS16 and DXS269 they are all supposed to be linked to KFSD. In table 2 two point linkage data for KFSD and the 14 informative loci are summarised. It shows that the Z values are >2 for all informative markers. The highest Z values were 12·07 for DXS365 (pRX-314) at $\theta = 0{\cdot}00$, 11·72 for DXS418 (P122) at $\theta = 0{\cdot}015$, and 10·93 for DXS989 (AFM135xe7) at $\theta = 0{\cdot}045$.

Discussion

KFSD is a rare ectodermal disorder that is clinically and genetically heterogeneous.[3,9] It has previously been mapped to Xp22.2-21.2 by linkage analysis in a large Dutch family using conventional DNA probes. In this study we performed a linkage analysis in the same family with 19 highly polymorphic DNA markers spanning this region of the X chromosome, of which 16 proved informative.

Recombination events were recorded for 10 out of the 16 informative polymorphisms tested. These recombinations were in accordance with the marker order as suggested at the 5th X chromosome workshop[10] and by others,[11–15] and confirmed in detail for this specific region by Alitalo *et al*[16] and Van de Vosse *et al.*[21] Alitalo *et al*[16] placed DXS257 distal to DXS999, but with regard to recombination events in our family this discrepancy does not affect the definition of the KFSD region (fig 2). Key recombinants locate the gene for KFSD between AFM291wf5 distally and DXS1226 (AFM316yf5) proximally. DXS443 and DXS365, which are located in the KFSD region, show no recombinations. DXS1229, located between the latter two polymorphisms, was unfortunately not informative.

Two point linkage analyses show Z values of 3·48 and above for all informative markers used, which is statistical proof for close linkage of KFSD to all markers in this region. Zmax is 12·07 for DXS365 at $\theta = 0$, which is in the KFSD region mentioned above.

Given the small candidate region for KFSD and the lack of reliable genetic distance data

for the probes covering this region, we did not perform multipoint linkage analysis. We assume that physical mapping analysis, as published by Alitalo *et al*[16] and currently in progress in our laboratory using contigs spanning this region,[21] will eventually lead to location of the KFSD gene.

These results confirm and greatly refine our previous mapping efforts of X linked KFSD in this large Dutch pedigree. The KFSD region has been narrowed down to between AFM291wf5 and DXS1226 (AFM316yf5). This is in Xp22.13-p22.2, an area covering approximately 1 Mb.[21] KFSD is located just proximal to the retinoschisis gene (RS).[21 22] The KFSD region shows considerable overlap with the regions for Coffin-Lowry syndrome (CLS)[23] and hypophosphataemic rickets (HYP).[24] Since our data are based on one extended family the issue of genetic heterogeneity need not be addressed. We would, however, like to get in touch with colleagues who know of other KFSD families in order to confirm these linkage data.

The refined mapping of KFSD is useful for carrier detection in families with X linked KFSD. This is relevant for genetic counselling since only 50% of heterozygotes show clinical symptoms. Moreover we assume that finding the KFSD gene will be facilitated by the reduction of the candidate region to approximately 1 Mb. This may eventually enable elucidation of the clinical and genetic heterogeneity of the disorder.

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