Bridging markers defining the map position of X linked hypophosphataemic rickets

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SUMMARY Hypophosphataemic rickets is commonly an X linked dominant hereditary disorder associated with a renal tubular defect in phosphate transport and bone deformities. The gene causing this disorder has been mapped to Xp22.31→p21.3 by using cloned human X chromosome sequences identifying restriction fragment length polymorphisms (RFLPs) in linkage studies of affected families. The hypophosphataemic rickets gene locus (*HPDR*) was previously mapped distal to the X linked polymorphic locus *DXS41* (99.6) but its position in relation to the distal loci *DXS43* (D2) and *DXS85* (782) was not established. In order to obtain a precise mapping of the disease locus in relation to these genetic loci, additional affected families informative for these X linked markers have been investigated. The combined results from the two studies have established linkage with the loci *DXS41* (99.6) and *DXS43* (D2); peak lod score for *DXS41* (99.6) = 7.35, $\theta = 0.09$, and peak lod score for *DXS43* (D2) = 4.77, $\theta = 0.16$. Multilocus linkage analysis mapped the hypophosphataemic rickets gene distal to the *DXS41* (99.6) locus and proximal to the *DXS43* (D2) locus, thereby revealing two bridging genetic markers for the disease.

Hypophosphataemic (vitamin D resistant) rickets (McKusick No 30780) is the commonest inherited form of rickets and X linked dominant inheritance has been established.¹⁻⁴ The disorder is clinically characterised by childhood rickets, which is unresponsive to physiological doses of vitamin D, growth retardation, and poor dental development. In addition, extraskeletal ossification, limitation of joint mobility, deafness, and occasionally spinal cord compression may develop.⁵ Affected subjects have hypophosphataemia owing to a renal tubular defect, decreased intestinal absorption of calcium, and an inappropriately low serum 1,25-dihydroxy vitamin D concentration; the circulating concentrations of calcium, parathyroid hormone, and 25-hydroxy vitamin D remain within normal limits. Treatment consists of large doses of vitamin D or its active metabolite, calcitriol, and oral phosphate supplements.

The primary biochemical defect for this disorder of mineral metabolism remains unknown and mapping of the hypophosphataemic rickets mutation is the first step towards defining the gene abnormality and in further elucidating the pathogenesis. In a previous study⁶ we mapped the hypophosphataemic rickets gene locus (*HPDR*) to the short arm of the human X chromosome by showing linkage with locus *DXS41* (peak lod score = 4.82 at 10% recombination), using the polymorphic probe 99.6. This probe has been localised to the chromosomal region Xp22.31→p21.3 by in situ hybridisation.⁷ This regional mapping of *HPDR* has also been reported in another study.⁸ Our previous work using multilocus linkage analysis further localised *HPDR* distal to and within 10 cM of the *DXS41* (99.6) locus, but the position in relation to the more distal loci *DXS43* (D2) and *DXS85* (782) remained unclear.⁶ We therefore investigated additional affected families informative for these X linked markers and undertook a further linkage study to

clarify the position of *HPDR* in relation to these distal loci, thereby establish two bridging genetic markers (that is, one marker on either side of the disease locus) which could be of use in genetic counselling.

Materials and methods

FAMILIES

Twenty-two British and North American families in whom hypophosphataemic rickets has been inherited in an X linked dominant manner in three or more generations were collected for linkage studies. The diagnosis of hypophosphataemic rickets had been confirmed clinically, biochemically, and radiologically. Unaffected relatives were of normal height and normophosphataemic. Sixteen families proved informative and have been studied further. Venous blood was obtained after informed consent from 189 family members, 95 of whom were affected (61 females, 34 males) and 94 of whom were unaffected (34 females, 60 males). The results from 11 of these families (63 affected and 68 unaffected members) have been previously reported.⁶ Of the additional five families (32 affected and 26 unaffected members) included in this study, four originated from the Shriners Hospital, St Louis, Missouri, USA, and one from the Children's Hospital, Toronto, Canada.

DNA HYBRIDISATION ANALYSIS

Venous blood samples were collected in tubes containing EDTA and kept frozen at -70°C . Leucocyte DNA was prepared by standard methods⁹ and 5 μg DNA was digested to completion with a four fold excess of one of the following restriction enzymes: *Taq*I, *Pst*II, *Pvu*II, *Eco*RI, or *Eco*RV (Boehringer or Pharmacia). The resulting fragments were separated by 0.8% agarose gel electrophoresis and transferred to a nylon membrane (Hybond-N, Amersham) by Southern blotting.¹⁰ DNA probes were labelled by nick translation,¹¹ using $\alpha^{32}\text{P}$ dCTP and a commercial kit from Amersham, or by oligonucleotide primed synthesis.¹² The following cloned DNA

probes from the short arm of the X chromosome were used in linkage studies: 782, D2, 99-6, C7, 754, pERT87, and L1-28. These polymorphic probes define the loci *DXS85*, *DXS43*, *DXS41*, *DXS28*, *DXS84*, *DXS164*, and *DXS7* respectively. The details of these probes and references are given in Goodfellow *et al.*¹³ The Southern blot was hybridised as described by Davies *et al.*¹⁴ Autoradiography with dual intensifying screens and preflashed Fuji medical x ray films was performed at -70°C for one to five days.

LINKAGE ANALYSIS

The data from the present study and the previously reported study⁶ were pooled for linkage analysis. Conventional two point lod scores for linkage between hypophosphataemic rickets and each marker were computed using the programmes MLINK and ILINK,¹⁵ and multipoint location scores were computed using the programme LINKMAP.¹⁶ The genetic map of Drayna and White¹⁷ regarding X chromosome markers was used to deduce the fixed framework of markers required for multilocus linkage analysis. A maximum of five loci (hypophosphataemic rickets and four markers) could be analysed simultaneously with LINKMAP on a 512K IBM PC-AT microcomputer, to calculate the location score curve. The probability of the most likely gene order and the relative likelihoods of other gene orders were then ascertained from this location curve. If, for a location score curve with marker framework A—B—C, the peak location scores x and y are obtained for the intervals A—B and B—C respectively, then the relative likelihood that the disease gene maps in the interval A—B as opposed to B—C is given by $e^{0.5(x-y)}$.

Results

The results of two point linkage analysis of all the 16 families are shown in table 1. Linkage between *HPDR* and the *DXS41* (99-6) locus was established with a peak lod score = 7.35, recombination fraction (θ) = 0.09, 95% confidence interval = (0.001, 0.20).

TABLE 1 Lod scores for linkage of X linked markers and *HPDR*.

Locus	Probe	Peak θ	Lod scores Z(θ)							
			Z(θ)	Z(0.001)	Z(0.05)	Z(0.10)	Z(0.15)	Z(0.20)	Z(0.30)	Z(0.40)
<i>DXS85</i>	782	0.05	-2.98	-17.31	-2.98	-0.94	-0.02	0.42	0.60	0.30
<i>DXS43</i>	D2	0.156	4.77	-10.42	2.86	4.40	4.77	4.62	3.51	1.81
<i>DXS41</i>	99-6	0.089	7.35	0.66	7.06	7.34	6.97	6.31	4.47	2.24
<i>DXS28</i>	C7	0.262	0.36	-3.62	-0.67	-0.07	0.20	0.32	0.35	0.22
<i>DXS164</i>	pERT87	0.333	0.29	-5.94	-1.71	-0.73	0.24	0.04	0.28	0.24
<i>DXS84</i>	754	0.05	-3.91	-17.87	-3.91	-1.19	0.14	0.86	1.32	0.98
<i>DXS7</i>	L1-28	0.05	-14.59	-43.18	-14.59	-8.87	-5.74	-3.71	-1.33	-0.24

Linkage was also established between *HPDR* and the *DXS43* (D2) locus, peak lod score = 4.77, recombination fraction (θ) = 0.16, 95% confidence interval = (0.05, 0.30). All the other X linked RFLP loci, *DXS85* (782), *DXS28* (C7), *DXS164* (pERT87), *DXS84* (754), and *DXS7* (L1.28) gave negative or low lod scores, although all are on the short arm of the X chromosome.

Analysis of the pedigree with genetic marker data shown in fig 1 further helped to localise the *HPDR* locus. This pedigree is informative for the X linked RFLP loci *DXS85* (782), *DXS43* (D2), *DXS41* (99.6), *DXS84* (754), and *DXS7* (L1.28) and multi-point crosses exist. There are no recombinants between the *HPDR* and *DXS41* (99.6) loci, though several recombinants occur with the other markers. Subject II.7 is an affected mother who is informative and phase known for *DXS85* (782), *DXS43* (D2),

DXS41 (99.6), *DXS84* (754), and *DXS7* (L1.28). Her affected son III.8 is recombinant for *HPDR* and the distal loci *DXS85* (782) and *DXS43* (D2), but not for the proximal loci *DXS84* (754) and *DXS7* (L1.28), while the unaffected daughter III.7 is recombinant for the proximal group of loci *DXS84* (754) and *DXS7* (L1.28) and the most distal locus *DXS85* (782), but non-recombinant for *DXS43* (D2). The minimum number of total recombinants in this pedigree is therefore obtained by locating *HPDR* between *DXS84* (754) and *DXS43* (D2).

MULTILOCUS LINKAGE ANALYSIS

Analysis of the pooled data from all the families using the LINKMAP programme yielded the location score curve shown in fig 2. There is a broad high peak distal to the *DXS41* (99.6) locus and proximal to the *DXS43* (D2) locus, maximum location score

FIG 1 Pedigree of family segregating for X linked hypophosphataemic rickets and short arm RFLP loci: *DXS85* (782) [Gg], *DXS43* (D2) [Jj], *DXS41* (99.6) [Pp], *DXS28* (C7) [Ee], *DXS84* (754) [Aa], and *DXS7* (L1.28) [Ff]. The loci are shown in the correct order but not the correct distances apart. In the females of known phase, the paternal X chromosome is shown on the left. * = recombinant.

TABLE 2 Order of genetic loci and their relative probabilities as calculated from the location score curve.

Locus order	Peak location score	Relative probability
Xpter-HPDR-DXS85-DXS43-DXS41-DXS84-Xcen	17.48	8.3×10^2
Xpter-DXS85-HPDR-DXS43-DXS41-DXS84-Xcen	4.04	1.0
Xpter-DXS85-DXS43-HPDR-DXS41-DXS84-Xcen	38.48	3.0×10^7
Xpter-DXS85-DXS43-DXS41-HPDR-DXS84-Xcen	16.53	5.2×10^2

FIG 2 Location scores of HPDR versus X chromosome short arm loci DXS85 (782), DXS43 (D2), DXS41 (99.6), and DXS84 (754). The horizontal axis is the genetic distance (w) from the DXS85 (782) locus. The right vertical axis is the odds ratio for the location of HPDR at a given distance compared to a location of HPDR at an infinite distance from the four fixed markers. The left vertical axis is the location score $l(w)$, defined as twice the natural logarithm of the odds ratio.

= 34.48, 0.11 Morgans distal to the DXS41 (99.6) locus. There are three subsidiary peaks between DXS41 (99.6) and DXS84 (754), between DXS43 (D2) and DXS85 (782), and between DXS85 (782) and Xpter. The location score for each peak is 16.53 at 0.03 Morgans proximal to DXS41 (99.6), 4.04 at 0.06 Morgans distal to DXS43 (D2), and 17.48 at 0.24 Morgans distal to DXS85 (782). The relative probability that the HPDR locus is in one of these segments is shown in table 2 and shows that the most likely order of genetic loci is Xpter-DXS85(782)-DXS43 (D2)-HPDR-DXS41 (99.6)-DXS84 (754)-Xcen.

Discussion

In our earlier study we showed that the HPDR locus was localised on the short arm of the X chromosome, distal to the DXS41 (99.6) locus, and in one of the

two positions predicted from a consideration of man-mouse homologies.⁶ Our new data confirm and extend these findings. By using the polymorphic probes D2 and 782 we have explored recombination in the distal part of the short arm of the X chromosome and have shown that HPDR maps between DXS41 (99.6) and DXS43 (D2).

The present data confirm linkage of the HPDR and DXS41 (99.6) loci, with the disease locus being the more distal, and also establishes linkage with the DXS43 (D2) locus but not the DXS85 (782) locus. Analysis of the pedigree shown in fig 1 indicates that HPDR is proximal to the DXS43 (D2) locus. Together, these two findings suggest that HPDR maps between the DXS41 (99.6) and DXS43 (D2) loci.

This is confirmed using the multilocus linkage analysis for all the families. The LINKMAP programme is able to use information from a number of multipoint crosses to calculate the most likely location for one unmapped gene in a framework of well mapped markers.¹⁶ Within the order Xpter-DXS85 (782)-DXS43 (D2)-DXS41 (99.6)-DXS84 (754)-Xcen, the location of HPDR between DXS43 (D2) and DXS41 (99.6) is well established. For example, the probability favouring the location of HPDR distal to DXS41 (99.6) is 58 000 to 1 and that favouring a location proximal to DXS43 (D2) is 30 million to 1. Thus the locus order

is demonstrated and reveals two bridging genetic markers for hypophosphataemic rickets. These bridging genetic markers will reduce the uncertainty in genetic counselling which arises from recombination between disease and a single genetic marker and the results of this study could be useful in the management of some patients. The precise mapping of hypophosphataemic rickets also enables a concentrated search for deletions and closer genetic markers to be carried out within this chromosomal segment, in order to elucidate further the gene

abnormality causing this disorder of phosphate metabolism.

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