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Bridging markers defining the map position of X linked hypophosphataemic rickets

R V THAKKER*, A P READ†, K E DAVIES‡, M P WHYTE§, R WEKSBERG‖, F GLORIEUX¶, M DAVIES**, R C MOUNTFORD†, R HARRIS†, A KING‡, G S KIM§, D FRASER‖, S W KOOH‖, AND J L H O'RIORDAN*

*From *the Department of Medicine, The Middlesex Hospital, London W1N 8AA; †the Department of Medical Genetics, St Mary's Hospital, Manchester M13 0JH; ‡the Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DU; §Shriners Hospital, St Louis, Missouri, USA; ‖ The Hospital for Sick Children, Toronto, Canada; ¶Shriners Hospital, Montreal, Canada; and **the Department of Medicine, Manchester Royal Infirmary, Manchester M13.*

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, $\theta = 0{\cdot}09$, and peak lod score for *DXS43* (D2) = 4·77, $\theta = 0{\cdot}16$. 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.

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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 study[6] 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 methods[9] and 5 μg DNA was digested to completion with a four fold excess of one of the following restriction enzymes: *Taq*I, *Pst*I, *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 α^{32}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 study[6] 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 White[17] 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*		*Lod scores Z(θ)*						
		$\hat{\theta}$	*Z($\hat{\theta}$)*	*Z(0·001)*	*Z(0·05)*	*Z(0·10)*	*Z(0·15)*	*Z(0·20)*	*Z(0·30)*	*Z(0·40)*
DXS85	782	0·05	−2·98	−17·31	−2·98	−0·94	−0·02	0·42	0·60	0·30
DXS43	D2	0·156	4·77	−10·42	2·86	4·40	4·77	4·62	3·51	1·81
DXS41	99·6	0·089	7·35	0·66	7·06	7·34	6·97	6·31	4·47	2·24
DXS28	C7	0·262	0·36	−3·62	−0·67	−0·07	0·20	0·32	0·35	0·22
DXS164	pERT87	0·333	0·29	−5·94	−1·71	−0·73	0·24	0·04	0·28	0·24
DXS84	754	0·05	−3·91	−17·87	−3·91	−1·19	0·14	0·86	1·32	0·98
DXS7	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 multipoint 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

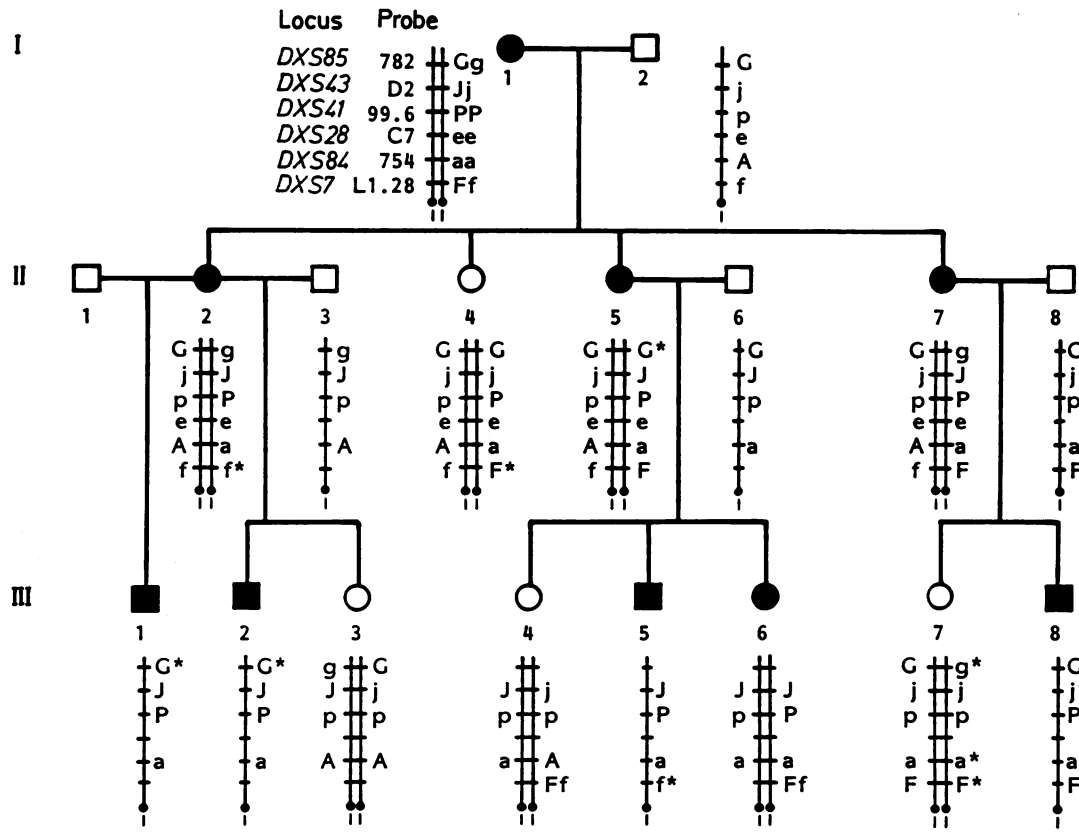


FIG 1 *Pedigree of family segregating for X linked hypophosphataemic rickets and short arm RFLP loci: DXS85 (782) [Gg], DXS43 (D2) [Jj], DXS41 (99·6) [Pp], DXS28 (C7) [Ee], DXS84 (754) [Aa], and DXS7 (L1·28) [Ff]. The loci are shown in the correct order but not the correct distances apart. In the females 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	$8·3 \times 10^2$
Xpter–*DXS85*–***HPDR***–*DXS43*–*DXS41*–*DXS84*–Xcen	4·04	1·0
Xpter–*DXS85*–*DXS43*–***HPDR***–*DXS41*–*DXS84*–Xcen	38·48	$3·0 \times 10^7$
Xpter–*DXS85*–*DXS43*–*DXS41*–***HPDR***–*DXS84*–Xcen	16·53	$5·2 \times 10^2$

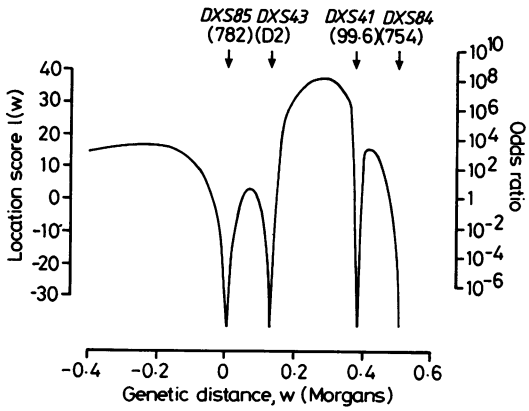


FIG 2 *Location scores of HPDR versus X chromosome short arm loci DXS85 (782), DXS43 (D2), DXS41 (99·6), and DXS84 (754). The horizontal axis is the genetic distance (w) from the DXS85 (782) locus. The right vertical axis is the odds ratio for the location of HPDR at a given distance compared to a location 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.*

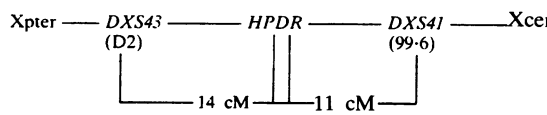
= 34·48, 0·11 Morgans distal to the *DXS41* (99·6) locus. There are three subsidiary peaks between *DXS41* (99·6) and *DXS84* (754), between *DXS43* (D2) and *DXS85* (782), and between *DXS85* (782) and Xpter. The location score for each peak is 16·53 at 0·03 Morgans proximal to *DXS41* (99·6), 4·04 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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Correspondence and requests for reprints to Professor J L H O'Riordan, Department of Medicine, The Middlesex Hospital, Mortimer Street, London W1N 8AA,