

Genetic mapping of the Kallmann syndrome and X linked ocular albinism gene loci

Y Zhang, R McMahon, S J Charles, J S Green, A T Moore, D E Barton, J R W Yates

Abstract

The X linked form of Kallmann syndrome (KAL) and X linked ocular albinism (OA1) have both been mapped to Xp22.3. We have used a dinucleotide repeat polymorphism at the Kallmann locus to type 17 X linked ocular albinism families which had previously been typed for the Xg blood group (XG) and the DNA markers DXS237 (GMGX9), DXS143 (dic56), and DXS85 (782). Close linkage was found between KAL and OA1 with a maximum lod score (Zmax) of 30.14 at a recombination fraction (θmax) of 0.06 (confidence interval for θ: 0.03-0.10). KAL was also closely linked to DXS237 (Zmax = 15.32; θmax = 0.05; CI 0.02-0.12) and DXS143 (Zmax = 14.57; θmax = 0.05; CI 0.02-0.13). There was looser linkage to the Xg blood group (XG) and to DXS85 (782). Multipoint linkage analysis gave the map: Xpter-XG-0.13-DXS237-0.025-KAL-0.025-DXS143-0.015-OA1-0.09-DXS85-Xcen. Placement of OA1 proximal to DXS143 was supported by odds of 2300:1 compared to other orders. This confirms our previous localisation of OA1 and improves the genetic mapping of both disease loci.

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X linked ocular albinism is an hereditary eye disorder characterised by nystagmus and reduced visual acuity in affected males associated with iris translucency, hypopigmentation of the retina, and foveal hypoplasia.¹ Carrier females have normal vision but ophthalmic examination shows a characteristic mud splattered fundus appearance in over 90% of cases.² The X linked ocular albinism gene locus (OA1) has been mapped to Xp22.3 by the demonstration of linkage to the Xg blood group and the DNA markers DXS237, DXS452, DXS143, and DXS85.³⁻⁶ The studies of Schnur *et al*³ and Charles *et al*⁶ placed OA1 proximal to DXS143 whereas Bergen *et*

*al*⁴ reported a recombinant mapping OA1 distal to this marker.

Kallmann syndrome is an inherited disorder defined by the association of hypogonadotropic hypogonadism and deficiency of the sense of smell (anosmia) resulting from a neuronal migration defect. The X linked Kallmann syndrome gene (KAL) has also been mapped to Xp22.3 by physical and genetic mapping.⁷⁻⁹

We have typed 17 large families segregating for X linked ocular albinism with a highly polymorphic dinucleotide repeat polymorphism at the Kallmann syndrome locus.¹⁰ These families had previously been typed for the Xg blood group and the DNA markers DXS237, DXS143, and DXS85.^{5,6} This has allowed the fine scale genetic mapping of the Kallmann syndrome gene and improved the localisation of X linked ocular albinism.

Patients and methods

A large Newfoundland kindred and 16 British families with X linked ocular albinism were studied. The clinical evaluation of the families and results of previous linkage studies have been described elsewhere.^{5,6} Samples were available from 307 subjects including 77 affected males and 72 obligate carrier females.

The KAL primers were as described by Bouloux *et al*.¹⁰ Approximately 300 ng of total genomic DNA was subjected to 30 cycles of PCR amplification (one minute at 92°C, one minute at 54°C, and one minute at 72°C). All reactions were performed in a volume of 25 µl containing 50 mmol/l KCl, 10 mmol/l Tris HCl, pH 8.3, 1.5 mmol/l MgCl₂, 2.5 pmol/l dNTPs, 60 ng of each primer, and 1 unit *Taq* polymerase. The double stranded products from the 307 subjects were separated on fan cooled, 10 cm, 10% polyacrylamide gels for five to six hours at 18 V/cm and visualised using ethidium bromide staining. In order to size the alleles accurately, up to four subjects from each family were subjected to PCR as described above but with the addition of approximately 12 ng of reverse primer end labelled with ³²P. The products were denatured at 95°C separated on standard 30 cm/6% polyacrylamide sequencing gels containing 6 mol/l urea, and visualised by autoradiography overnight on Kodak XAR-5 film.

Linkage data were analysed using the computer programme LIPED¹¹ to calculate two point scores and the LINKMAP option of LINKAGE¹² for the multipoint analysis. Confidence limits were obtained by taking values of the recombination fraction at lod scores one unit below the maximum.¹³ For the multipoint

Molecular Genetics Laboratory, Addenbrooke's Hospital, Cambridge, UK.

Y Zhang
R McMahon
D E Barton

Royal Eye Hospital, Manchester, UK.
S J Charles

Memorial University of Newfoundland, St John's, Newfoundland, Canada.
J S Green

Department of Ophthalmology, Addenbrooke's Hospital, Cambridge, UK.

A T Moore

Department of Clinical Genetics, Addenbrooke's Hospital, Cambridge CB2 2QQ, UK.
J R W Yates

Department of Pathology, University of Cambridge, UK.
J R W Yates

Correspondence to Dr Yates.

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Table 1 Allele frequencies for the Kallman syndrome dinucleotide repeat in normal subjects (spouses) and in subjects with ocular albinism (the topmost affected subject in each family).

Allele	Size (bp)	Normal chromosomes (n = 103)		OA1 chromosomes (n = 17)	
		No	Frequency	No	Frequency
A0	177	16	0.16	1	0.06
A1	179	28	0.27	1	0.06
A2	181	10	0.10	2	0.12
A3	183	7	0.07	9*	0.53
A4	185	26	0.25	4	0.24
A5	187	16	0.16	0	0.00

* The association between the OA1 disease allele and A3 is significant ($\chi^2 = 29.7$, $p < 0.001$).

linkage analysis the marker order and map distances described by Yates *et al*¹⁴ were used.

Results

Table 1 shows the allele frequencies for the Kallmann syndrome dinucleotide repeat polymorphism in 103 chromosomes carried by normal subjects marrying into the families. For alleles A1 to A5 the results were similar to those reported by Bouloux *et al*¹⁰ but a sixth allele of 177 bp (A0) was also present at modest frequency in both the British and Newfoundland populations. The heterozygosity in females (n=23) was 82%. The OA1 disease allele showed a significant association with the A3 allele (table 1). Nine families from central England were responsible for this association which presumably reflects a common and close ancestry. Given that several recombinants were observed between OA1 and KAL in this study, we would not expect to see true linkage disequilibrium as a population phenomenon.

All of the 17 families were informative for the Kallmann syndrome dinucleotide repeat. The results of the two point linkage analysis are shown in table 2. Close linkage was found between KAL and OA1 with a maximum lod score (Zmax) of 30.14 at a recombination

fraction (θ_{max}) of 0.06 (confidence interval for θ : 0.03–0.10). KAL was also closely linked to DXS237 (Zmax=15.32; θ_{max} =0.05; CI 0.02–0.12) and DXS143 (Zmax=14.57; θ_{max} =0.05; CI 0.02–0.13). There was looser linkage to the Xg blood group (XG) and to DXS85 (782).

For the multipoint analysis we used the well established marker order XG (distal), DXS237, DXS143, DXS85 with recombination fractions of 0.13, 0.05, and 0.10 respectively, taken from Yates *et al*.¹⁴ The LINKMAP analysis placed KAL exactly midway between DXS237 and DXS143 with a maximum location score of 116. This position was favoured by odds of 23:1 compared to a placement between DXS237 and XG and by odds exceeding 10 000:1 compared to all other locations. Placement of KAL between DXS237 and DXS143 is consistent with physical mapping data.^{7,8}

Table 3 shows the recombinants observed between OA1 and the other loci. Seven recombinants map OA1 proximal to KAL. Recombinant 10 maps OA1 proximal to DXS143 and has been reported previously.⁶ Using a marker map with the inclusion of KAL and the omission of XG, a LINKMAP analysis was carried out to position OA1. The maximum location

Table 2 Two point lod scores between the dinucleotide repeat polymorphism at the Kallmann syndrome locus (KAL) and adjacent loci.

Locus	Lod score Z at recombination fraction (θ)							Zmax	θ_{max}	Confidence interval for θ
	0	0.001	0.05	0.1	0.2	0.3	0.4			
XG	–∞	–5.60	2.33	3.16	3.13	2.28	1.11	3.30	0.14	0.05–0.30
DXS237	–∞	10.13	15.32	14.76	12.09	8.48	4.22	15.32	0.05	0.02–0.12
DXS143	–∞	9.25	14.57	14.12	11.64	8.25	4.25	14.57	0.05	0.02–0.13
OA1	–∞	18.31	30.14	29.22	24.16	17.25	9.08	30.14	0.06	0.03–0.10
DXS85	–∞	–4.07	8.87	10.12	9.53	7.24	3.88	10.22	0.12	0.06–0.20

Table 3 Individual recombinants between OA1 and other loci in distal Xp. Shading indicates regions of probable exclusion of OA1.

Recombinant No	Family	Subject	Sex	Status	XG	DXS237	KAL	DXS143	DXS85	DXS43
1	2948	II.2	M	A	X	X	X	O	–	–
2	3239	IV.18	M	A	–	X	X	O	O	nt
3	4220	III.6	M	N	X	X	O	–	–	nt
4	4222	II.18	F	N	–	X	X	–	O	nt
5	4222	III.3	F	C	–	X	X	–	O	nt
6	4503	II.4	F	OC	–	–	X	–	–	nt
7	4574	III.2	M	A	X	X	O	–	–	O
8	4896	III.4	F	OC	–	X	–	–	–	nt
9	4896	V.48	F	N	–	–	X	O	O	nt
10	4896	VII.6	F	C	–	–	X	X	O	O
11	3126	III.2	F	C	–	–	–	–	X	–
12	4216	IV.3	M	A	–	–	O	O	X	X
13	4222	III.14	M	A	–	O	O	–	X	X
14	4896	VI.36	M	A	–	–	O	O	X	X
15	4896	IV.8	M	A	–	–	–	–	X	–
16	4896	VI.35	M	N	–	–	–	O	X	X
17	4896	V.51	M	N	–	–	O	O	X	nt

Key

M = Male	OC = Obligate carrier female
F = Female	X = Recombinant
N = Normal	O = Non-recombinant
A = Affected	– = Not informative
C = Carrier female based on clinical examination	nt = Not typed

score was 229 with OA1 proximal to DXS143 at a distance of 1.5 cM (figure). This placement was supported by odds of 2300:1 compared to a position for OA1 between DXS143 and KAL, and by odds exceeding 10^{12} :1 compared to other possible orders.

Discussion

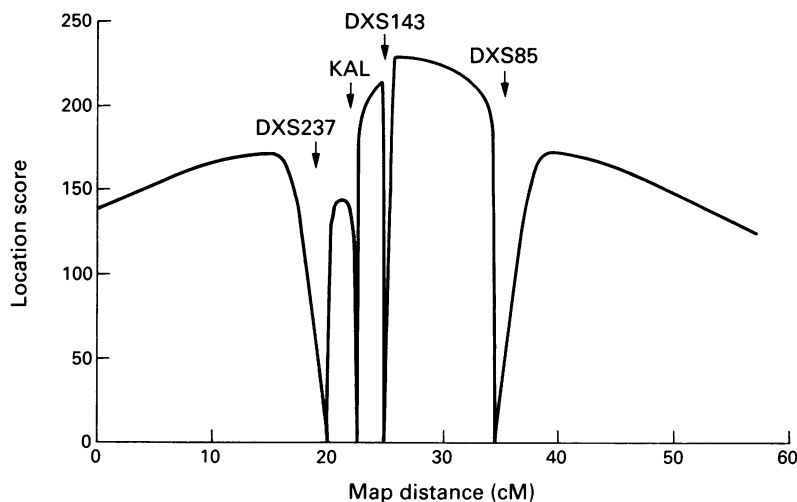
The gene for the X linked form of Kallmann syndrome has been localised between DXS237 (GMGX9) and DXS143 (dic56) by physical mapping in patients with deletions and rearrangements of Xp22.3^{7,8} and confirmed by the cloning of the Kallmann syndrome gene from this interval.¹⁵⁻¹⁷ Previous family studies have shown linkage between Kallmann syndrome and the marker DXS278 (CRI-S232) which maps to Xp22.3.⁹ In this study we have used a dinucleotide repeat polymorphism located 3 kb upstream of the 3' end of the Kallmann syndrome gene¹⁵ to refine the genetic mapping of this locus to the midpoint of the interval between DXS237 and DXS143 and also to establish the genetic relationship between KAL and the gene for X linked ocular albinism. Our results agree with available linkage data for this marker.¹⁸

Our previous linkage studies in these X linked ocular albinism families have shown that the OA1 locus is close to DXS143 and probably proximal to this marker.^{5,6} The additional data presented here confirm this localisation. These results are consistent with the genetic mapping results of Schnur *et al*³ in five families from North America and Canada. The only conflicting data is a recombinant reported by Bergen *et al*⁴ 1991 which would place OA1 telomeric to DXS143. As we have discussed previously,^{5,6} provided that the clinical assessment and DNA typing are correct, this result

can only be explained on the basis of a double recombination event or locus heterogeneity. However, there is no evidence of locus heterogeneity in our families.

The dinucleotide repeat polymorphism at the Kallmann syndrome locus proved to be highly informative and the recombination fraction with OA1 was 0.06 (confidence interval 0.03-0.10). This marker permits rapid non-isotopic tracking of the OA1 gene and would be useful for carrier diagnosis in X linked ocular albinism families, particularly in combination with a proximal flanking marker.

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Multipoint analysis using LINKMAP to position the X linked ocular albinism locus (OA1) with respect to loci in Xp22.3. XG was omitted so that the remaining loci could be accommodated in a single analysis.

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