Linkage of internal minisatellite loci on chromosome 1 and exclusion of autosomal dominant retinitis pigmentosa proximal to rhesus

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Abstract

We report the exclusion of a locus for autosomal dominant retinitis pigmentosa proximal to the rhesus locus in a single large pedigree. In addition, a previously unreported linkage is described between two chromosome 1 markers, which confirms that a highly variable minisatellite locus is placed internally on chromosome 1.

Retinitis pigmentosa is an inherited human disorder characterised by progressive degeneration of the retina, leading to loss of visual field, night blindness, and ultimately to complete loss of sight late in the disease process.¹ There are X linked, autosomal dominant, and autosomal recessive forms, with phenotypic heterogeneity in all categories. Families falling into the autosomal dominant retinitis pigmentosa (ADRP) category can be further subdivided on the basis of age of onset, penetrance, and persistence of a measurable rod electroretinogram.¹⁻⁴ Several reports have suggested that an ADRP locus is linked to rhesus on chromosome 1p, but with lod scores no higher than 1.89 at a recombination fraction of 0.2 in male meioses and 0.4 in female meioses.⁵⁻⁸ In one large Irish pedigree such a location has been excluded, consistent with the hypothesis that phenotypic variation in ADRP is the result of underlying genetic heterogeneity.⁹ We report linkage data using two

VNTR probes derived from chromosome 1 in a single large family in which regional ADRP¹ has been diagnosed. One of these probes, YNZ2, obtained from Yusuku Nakamura, detects a moderately variable locus, *DIS57*, with at least eight alleles¹⁰ approximately 11 cM proximal to rhesus on chromosome 1p.¹¹ The other, known as pMS1, was donated by Professor Alec Jeffreys and is a truly hypervariable sequence (at least 33 alleles, none of which attain a significant population frequency) detected by fingerprinting probe 33.15.¹² This probe has been assigned to 1p33-35 by *in situ* hybridisation,¹³ but has as yet not been genetically mapped.

Materials and methods

Genomic DNA prepared from lymphocytes of subjects in family ADRP5 was digested at 65°C with *TaqI*, size fractionated on a 1% agarose gel, and capillary blotted onto nylon filters (Hybond N, Amersham). Filters were prehybridised in 7% SDS and 0.5 mol/l sodium phosphate buffer at 68°C for 30 minutes, then hybridised in the same buffer overnight with ³²P labelled probes. Washes were at 65°C in 0.1×SSC and 0.1% SDS.

Results and discussion

Lymphocyte DNA from subjects in the pedigree shown in fig 1, known as ADRP5, were digested with *TaqI*, southern blotted, then probed first with pMS1 and then with YNZ2. The lod scores obtained from analysis of the results are shown in the table. When calculating the lod scores for linkage between each probe and ADRP, all subjects classed as unaffected, who were either under the age of 25 or had not been seen by an ophthalmologist, were excluded. Large negative lod scores between ADRP and YNZ2 exclude a locus for ADRP proximal to rhesus in this family. Further experiments are under way in this laboratory to test this family with a suitable probe distal to rhesus, and also to analyse four other large ADRP families, one cone-rod dystrophy pedigree, and one family with Sorsby's macular degeneration with these

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Figure 1 Family ADRP5, a four generation R type pedigree,¹ showing autosomal dominant segregation of retinitis pigmentosa.

Lod scores between probes pYNZ2, pMS1, and ADRP in this family.

Main locus	Marker locus	Recombination fraction (θ)							
		0.000	0.001	0.05	0.10	0.20	0.30	0.40	0.50
ADRP	pMS1	-16.067	-10.356	-2.885	-2.084	-0.843	0.300	0.078	0.000
ADRP	pYNZ2	-17.119	-10.572	-3.555	-2.175	-0.883	-0.325	-0.106	0.000
pMS1	pYNZ2		-3.199	4.243	4.631	3.786	2.968	0.757	0.000

probes. By carrying out linkage studies only on pedigrees sufficiently large to obtain a significant lod score without pooling data between families, we can overcome the problem of potential heterogeneity in this defect.

Using data from all subjects in the pedigree, it was possible to show linkage between the two VNTR loci used in this study. The maximum likelihood recombination fraction (θ) was 0.10 with a lod score of 4.63. Whether pMS1 lies proximal or distal to YNZ2 on chromosome 1 cannot be established from these data. If it lies distal to YNZ2 then pMS1 is probably close to rhesus, and may therefore provide another informative molecular marker in addition to this blood group polymorphism. Either location would place it a considerable genetic distance from the chromosome 1 telomere, as previously suggested by *in situ* data.¹³ The first hypervariable sequences characterised were generally found associated with genes, since these were the most closely studied DNA loci.¹⁴ A map based on such highly informative probes would be a great asset to those interested in mapping human genetic diseases. However, more recently it has been suggested that VNTRs may be clustered at telomeres in regions of high recombination and consequently regions where the genetic map expands greatly relative to the physical map.¹³ Data presented in this study would indicate that this is not the case, at least for these two loci. While pYNZ2 is only a moderately

Figure 2 *TaqI* digested DNA from subjects in ADRP5, probed with pMS1. New bands are arrowed.

variable locus, pMS1 is a classic minisatellite with one of the highest mutation rates reported.¹² In analysing 130 meiotic events (93 observed and 37 inferred) we observed four offspring with putative new alleles denoted by asterisks in fig 1. Paternity in two cases could be confirmed by blood group marker analysis, giving no exclusions in 16 marker systems. The branch of the pedigree containing these subjects is shown in fig 2, with their DNA *TaqI* digested then probed with pMS1. In the other two unconfirmed cases, signal with pYNZ2 was consistent with correct paternity. This would indicate a mutation rate of 0.03 consistent with previously reported data on this locus.¹² New variant subjects were disregarded in linkage calculations. Only two homozygous subjects were observed, suggesting a heterozygosity of approximately 97% at this locus, which is again consistent with the data of Wong *et al.*¹²

In conclusion, two internal VNTR loci have been shown to be linked to each other, and have been used to exclude an ADRP locus proximal to rhesus in one large pedigree. It is hoped that further experiments will determine whether probe pMS1 is a hyper-informative marker in addition to the rhesus blood group polymorphism, as well as establishing whether there is a locus for ADRP on chromosome 1p.

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