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ARTICLE

NRL S50T mutation and the importance of 'founder effects' in inherited retinal dystrophies

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The aim of this work was to identify *NRL* mutations in a panel of 200 autosomal dominant retinitis pigmentosa (adRP) families. All samples were subjected to heteroduplex analysis of the three exons of the *NRL* gene, and *Hph*I restriction digest analysis of exon 2 (to identify the S50T mutation). Families found to have the S50T mutation, and six additional larger pedigrees (which had previously been excluded from the other nine adRP loci) underwent linkage analysis using polymorphic markers located in the region of 14q11. *Hph*I restriction analysis followed by direct sequencing of the amplified *NRL* exon 2 product demonstrated the presence of the *NRL* S50T sequence change in three adRP families. Comparison of marker haplotypes in affected individuals from these families with those of affected members of the original 14q11 linked family revealed a common disease haplotype for markers within the adRP locus. Recombination events observed in these families define an adRP critical interval of 14.9 cM between D13S72 and D14S1041. Linkage analysis enabled all six of the larger adRP pedigrees to be excluded from the 14q11 locus. The *NRL* S50T mutation represents another example of a 'founder effect' in a dominantly inherited retinal dystrophy. Identification of such 'founder effects' may greatly simplify diagnostic genetic screening and lead to better prognostic counselling. The exclusion of several adRP families from all ten adRP loci indicates that at least one further adRP locus remains to be found. *European Journal of Human Genetics* (2000) **8**, 783–787.

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Introduction

Retinitis pigmentosa (RP) (MIM 268000, Online Mendelian Inheritance in Man: http:www.ncbi.nlm.nih.gov/omim) is the term applied to a clinically and genetically heterogeneous group of retinal degenerations, which primarily affect the rod photoreceptors and have an overall prevalence of about 1:3000.[1] RP is characterised by progressive loss of vision, initially manifesting a night blindness and reduction in the peripheral visual field, and later involving loss of central vision.[2] Ophthalmoscopic examination typically reveals pigmentary disturbances of the mid-peripheral retina. RP may be inherited as an autosomal recessive, autosomal dominant, digenic, or X-linked trait. Autosomal dominant RP (adRP) accounts for 20–25% of all cases of RP.[3]

We recently reported an S50T mutation in the *NRL* gene, located at chromosome 14q11, as the cause of adRP in a three-generation British family (designated RP251) linked to the marker D14S64 (Z_{max} = 5.72 at θ = 0.00).[4] adRP causing mutations have been identified in three other genes: *rhodopsin* (3q21–q25);[5] *peripherin-RDS* (6p21),[6] and *RPI* (8cen).[7,8] In addition, six mapped adRP loci in which the responsible genetic mutation remains unknown are located at 1cen, 7p, 7q, 17p, 17q, and 19q (Retnet: http://www.sph.uth.tmc.edu/Retnet/disease.ht).

We subsequently set out to screen a large panel of smaller adRP families for *NRL* mutations.

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Patients and methods

Two hundred apparently unrelated adRP families were identified from the Moorfields Eye Hospital retinal dystrophy register. The vast majority of these were British, originating from all over the country, but with the greatest number derived from the south and east due to the location of the hospital. After gaining informed consent, genomic DNA was prepared from peripheral blood obtained from members of these families. In order to identify *NRL* mutations all DNA samples were subjected to heteroduplex analysis and *Hph*I restriction digest analysis. In addition, six larger adRP pedigrees, which had previously been excluded from the other nine adRP loci, were analysed for linkage to markers within the RP251 critical region.

To aid heteroduplex detection new primers were synthesised from the genomic sequence of the gene,[9] which produced a much smaller product from exon 1, and amplified both exon 2 and exon 3 in two parts (exon 1F - GATGACCTCAGAGAGCTGGC; 1R - GTTCTAGGTGAGCGGCCTGAC (216 bp); exon 2.1F - GGCCTCCATGTGCTCCAGAC; 2.1R - GGCCTGGCCGGGTGCCCTCG (270 bp); exon 2.2F - CCTTCAGTGAACCAGGCATGGT; 2.2R - CTCAGGCCAGCTTGCTGACC (256 bp); exon 3.1F - CGGGTGCGACCTGGCGCTGAC; 3.1R - GCCAGGCGGGCCACCTCGGC (314 bp); exon 3.2F - GCCTGGCCGCCCAGCTGGAC; 3.2R - CCACTACACCACAAGGTGCTC (166 bp)). The resulting product was allowed to cool slowly to room temperature to maximise the formation of heteroduplexes,[10] and electrophoresed overnight at 1600–2000 Vh on MDE (Flowgen, Lichfield, Staffordshire, UK) polyacrylamide gels.

*Hph*I restriction digests were performed directly on 20 µl of exon 2 PCR product by overnight incubation at 37°C. Automated fluorescent sequencing (ABI Biosystems, Perkin-Elmer Ltd, Beaconsfield, Buckinghamshire, (UK model 373)) of exon 2 of *NRL* was carried out using the above primers.

Those families found on sequencing to have an *NRL* S50T mutation underwent haplotype analysis using polymorphic markers in the region of the disease critical interval on 14q11 (Table 1). After PCR the amplified products were separated by electrophoresis on 6–8% non-denaturing polyacrylamide gels (Protogel, National Diagnostics, Atlanta, GA, USA/Hessle, Hull, UK).

All agarose and polyacrylamide gels were stained with ethidium bromide.

Results

The *NRL* S50T mutation is caused by a T to A change at nucleotide 1942 which abolishes a recognition site for *Hph*I restriction enzyme. *Hph*I restriction analysis followed by direct sequencing of the amplified *NRL* exon 2 product was therefore used to demonstrate the presence of this sequence change in the *NRL* gene in three adRP patients from families RP57, RP357, and RP3097 (Figure 1). No other sequence changes were detected in any exon of the *NRL* gene in any patient sample.

Comparison of marker haplotypes in affected individuals from families RP57 and RP357 with those of affected members of the original RP251 family revealed a common disease haplotype for markers within the adRP locus on 14q11 (Table 1). The same disease haplotype can be inferred from the alleles present in individual number 1727 from family RP3097, but since only one member of this family was analysed this cannot be confirmed. This disease-associated haplotype was not observed on any of the 16 'control' chromosomes, belonging to individuals who were related by marriage to these families (RP57, RP251, RP357 and RP3097), which underwent haplotyping. Rare disease-associated alleles of both D14S990 (allele 3) and D14S64 (allele 5) were found to occur at a significantly higher frequency in the affected individuals from these families. χ^2: $P = 0.003$ for each marker).

Recombination events in affected individuals from the original family RP251 define a 17 cM critical genetic interval for this locus between the markers D14S261 and D14S1041 (Figure 2). A recombination event observed in subject number 87, from family RP357, reduces the adRP critical interval to 14.9 cM and defines D14S72 as the new centromeric boundary (Table 1).

Table 1 Comparison of the suggested haplotypes of polymorphic markers in the critical region on 14q11 between RP251 and three additional families with the codon 50 Ser to Thr mutation in the *NRL* gene identified by restriction digest analysis. The presence of the same variant allele for the centromeric marker, D14S261, in all additional cases may indicate a recombination event in the founder of RP251. Subject No. 87 has a recombination involving D14S 72 which refines the adRP critical interval to 14.9cM. Marker order obtained from Dib *et al*[19]

	RP251	*RP57*		*RP3097*		*RP357*		
Marker	*Haplotype*	*No. 2503*	*No. 2504*	*No. 1727*		*No. 2399*		*No. 87*
D14S 261	2	4 1	4 5	4 4		4 4		4 5
D14S 72	2	2 1	2 4	2 4		2 3		4 5
D14S 990	3	3 2	3 2	3 4		3 1		3 3
D14S 64[a]	5	5 3	5 4	5 1		5 2		5 3
D14S 1041	2	2 1	2 4	2 4		2 1		2 4
D14S 80	3	3 3	3 4	3 5		3 3		3 3

[a]Location of NRL; Farjo *et al*[9]

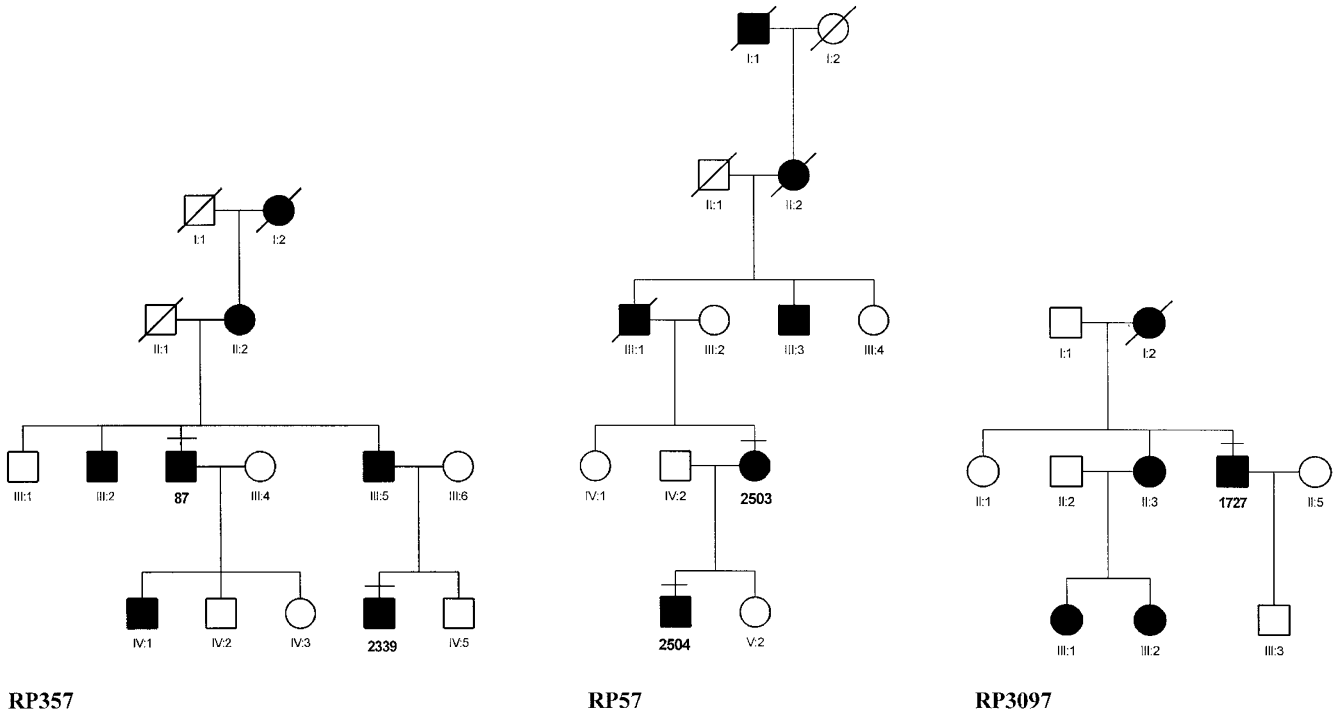

Figure 1 Pedigrees of additional families with the *NRL* S50T mutation. Patients whose DNA samples underwent mutation and haplotype analysis are denoted by a horizontal bar above the symbol representing that individual. DNA numbers correspond with those in Table 1 (affected individuals are denoted by solid symbols).

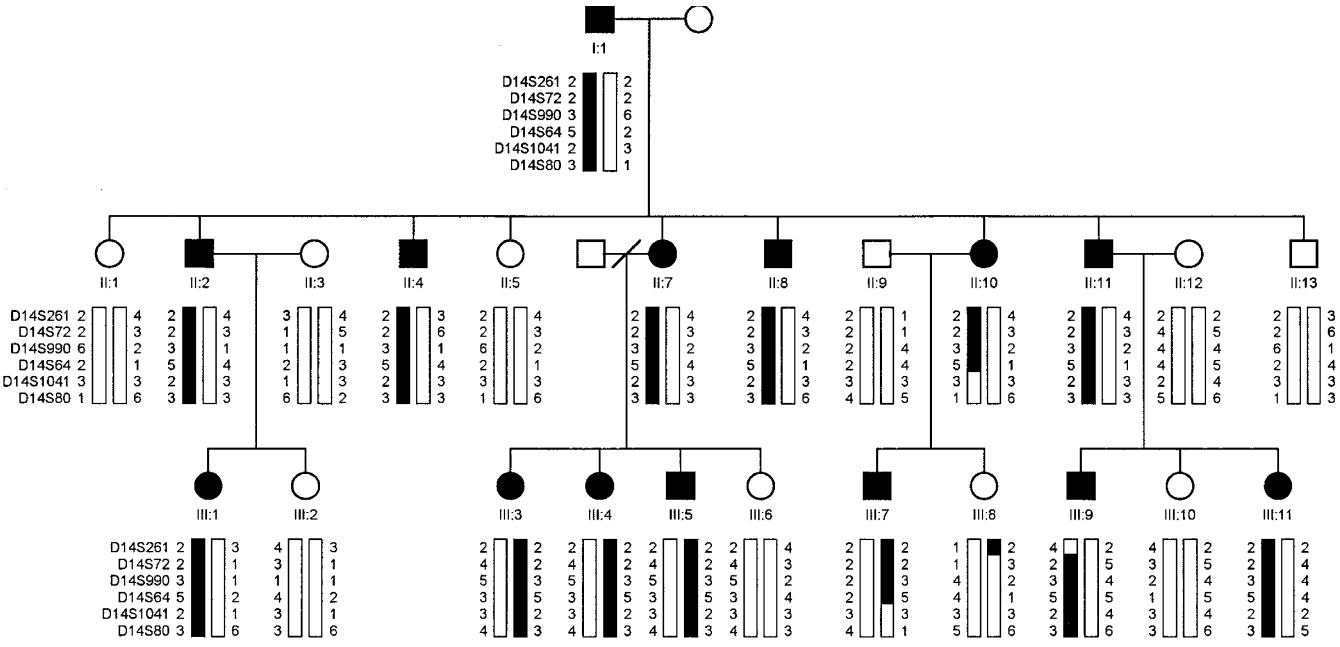

Figure 2 Pedigree of family RP251 showing haplotypes for the polymorphic markers in the centromeric region of chromosome 14q. Marker order was determined from the Généthon sex-averaged genetic map.[19] Centromeric recombination events in individuals III-8 and III-9, and a telomeric recombination in affected individual II-10 define D14S261 and D14S1041 as the flanking markers for the adRP locus. The affected haplotype is indicated by solid black in the bars situated between the marker alleles.

Linkage analysis enabled all six of the larger adRP pedigrees to be excluded from the 14q11 locus (data not shown).

Discussion

The identification of a tenth locus for adRP at 14q11 further highlights the genetic heterogeneity which is associated with this condition and with non-syndromic retinal dystrophies in general (RetNet). The exclusion of additional adRP families from all ten adRP loci indicates that at least one further adRP locus remains to be found.

Since affected individuals from these four families (RP57, RP251, RP357, and RP3097) share nearly 15 cM of chromosome 14q surrounding the *NRL* gene it seems likely that they are, in fact, closely related and that the *NRL* S50T mutation arose relatively recently. The earliest known affected family member from these pedigrees (from RP57) was born around 1850. These four families all originate from south-east England and genealogical studies are in progress to determine the exact relationship between them.

In all four pedigrees the RP phenotype is fully penetrant and exhibits only limited variation in expressivity. This is comparable with the phenotypes ascribed to specific *rhodopsin* mutations and in contrast to the variable expressivity and incomplete penetrance associated with the 7p, 8cen and 19q adRP loci respectively.[11–13] Detailed, clinical, electrophysiological and psychophysical studies of affected individuals from these four families suggest that there may be characteristic features of the 14q11 adRP phenotype (including early severe loss of rod function, whilst cone function is preserved and a very high incidence of macular oedema), which may aid in the identification of additional linked families (Bessant *et al*., manuscript in preparation).

Since we have only been able to link one adRP pedigree to the 14q11 locus, and since mutation screening of *NRL* in 200 additional adRP families revealed only three additional, related families it seems likely that mutations at this locus are a relatively rare cause of adRP. The fact that the additional three pedigrees are related to the family RP251 is further evidence of the importance of the 'founder effect' in autosomal dominant retinal dystrophies. There now exist several striking examples of this effect:

(1) the *rhodopsin* Pro23His mutation which accounts for around 10% of cases of dominant retinitis pigmentosa in the United States;[14]

(2) the *TIMP-3* Ser181Cys mutation in patients of British origin with Sorsby fundus dystrophy;[15]

(3) the *peripherin-RDS* Arg172Trp mutation in dominant macular dystrophy;[16]

(4) the many descendants of Jean Nougaret with congenital stationary night blindness due to a mutation in *GNAT1*;[17] and

(5) perhaps most spectacularly the 39 apparently unrelated pedigrees from several different countries with dominant drusen of the Doyne/Malattia leventinese type, all of whom were found to have an Arg345Trp mutation in the gene *EFEMP1*.[18]

Identification of 'founder effects' in local populations, or in some cases internationally, can greatly simplify genetic analysis of the relevant disease and may have important implications for the speed and cost-efficiency of diagnostic screening services. Associated clinical studies may provide accurate information about disease progression for the purpose of prognostic counselling. Large groups of individuals whose eye disorder is due to a single mutation are also ideal candidates for recruitment into trials of any potential future treatment for the inherited retinal dystrophies.

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