

## Screening for homozygosity by descent in families with autosomal recessive retinitis pigmentosa

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### Abstract

Retinitis pigmentosa (RP) is a genetically heterogeneous disease and an important cause of blindness in the state of Andhra Pradesh in India. In an attempt to identify the disease locus in families with the recessive form of the disease, we used the approach of screening for homozygosity by descent in offspring of consanguineous and nonconsanguineous families with RP. Microsatellite markers closely flanking 21 known candidate genes for RP were genotyped in parents and affected offspring to determine whether there was homozygosity at these loci that was shared by affected individuals of a family. This screening approach may be a rapid preliminary method to test known loci for possible cosegregation with disease.

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### Introduction

Retinitis pigmentosa (RP) is a group of hereditary retinal degenerations leading to progressive loss of vision and blindness. The first symptoms of RP are night blindness, loss of the mid-peripheral visual field and decline in electroretinographic (ERG) responses. The disease is progressive, and by adulthood constriction and gradual loss of the visual field may eventually lead to blindness. RP is extremely heterogeneous both genetically and phenotypically and is inherited as a highly penetrant, autosomal dominant, autosomal recessive or X-linked disease. RP is one of the major causes of childhood blindness in South India, accounting for about 16% of childhood blindness (Dandona *et al.* 1998). Population-based studies of blindness in both the city of Hyderabad and the state of Andhra Pradesh in India show that the prevalence of blindness due to RP is 0.1% (1 in 1000) and that RP accounts for

5–10% of the total blindness in the population (Dandona *et al.* 2001). The relative proportions of the autosomal dominant, autosomal recessive and X-linked forms of RP may vary between different regions of India, although the recessive form probably predominates in the South owing to a high prevalence of consanguinity.

About 38 different loci have been mapped for different forms of RP (RetNet, <http://www.sph.uth.tmc.edu/RetNet/>). Of these, 27 genes have been identified for autosomal dominant and recessive RP. The broad functional categories to which known RP gene products belong are: (a) components of the signal transduction pathway in the photoreceptor, (b) structural and membrane proteins of retinal cells, (c) proteins required for metabolism of retinoids, (d) transcription factors important for photoreceptors, (e) proteins that have ubiquitous functions, and (f) other proteins whose function in the human retina is inferred or partly understood.

Rod cell death in RP is followed by cone cell degeneration. The reason for death of neighbouring cones is not

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understood, although it is consistently observed in all types of RP. There are many hypotheses for this phenomenon, including release of toxic byproducts from the dying rods (Bird 1992), and loss of trophic factors normally derived from the rods (Huang *et al.* 1993; Steinberg 1994).

There have been few studies on the molecular-genetic basis of RP in the Indian population (Kumaramanickavel *et al.* 1994; Gu *et al.* 1997; Maw *et al.* 1997; Finckh *et al.* 1998). The types of mutations that occur, as well as their frequencies, could vary between populations. Knowledge of the precise genetic defect is potentially useful in genetic counselling, in developing new therapeutic approaches, and in applying specific therapies that may become available to a subset of patients with a specific type of defect.

We undertook to study autosomal recessive RP (arRP) to identify genes causing the disease. Since a high proportion of recessive RP in South India occurs in offspring of consanguineous marriages, we screened candidate gene loci for homozygosity by descent in affected individuals. The principle of this approach (Lander and Botstein 1987; Sheffield *et al.* 1995) is as follows: In an offspring of a consanguineous marriage, a region of several centimorgans (cM) surrounding the disease gene is homozygous by descent since it is derived from a common ancestor. Regions that are unlinked to the disease gene are also homozygous on a random basis and the proportion of the genome showing such homozygosity varies according to the coefficient of inbreeding (1/16 for offspring of a first-cousin marriage, 1/8 for offspring of an uncle–niece marriage, etc.). However, regions surrounding the disease gene are likely to be homozygous with a probability of almost 1 whereas a random unlinked locus is likely to be homozygous with a probability of 1/16, 1/8, etc. We therefore reasoned that if regions linked to known RP genes are homozygous in affected members of a family, there is a possibility that the said locus is segregating with the disease. Screening of the gene in question can then be undertaken to determine whether it is indeed the cause of disease. On the other hand, if no homozygosity is observed consistently in affecteds of a family in regions linked to a known candidate gene, that locus can be excluded from consideration as segregating with disease. There are caveats to these interpretations since both false positives (due to random homozygosity) and false negatives (due to recombination) can occur although they would be relatively infrequent.

Essentially, microsatellite markers that are within 5–10 cM around known RP candidate genes were selected and were genotyped in parents and affected offspring. We looked for homozygosity in one affected offspring compared to parents initially, and, if present, other affected offspring in the family were tested for homozygosity of the same alleles as those detected in the proband (first affected offspring). Since there are a large number of genes identified for different forms of RP, we considered

that this approach would provide a relatively rapid preliminary screen for excluding known candidate RP loci. At least two markers were selected for each candidate gene locus. We report the results of this screening.

## Patients and methods

**Recruitment of patients and sample collection:** Families with multiple affected members with RP were identified from the L. V. Prasad Eye Institute's clinical records and contacted for this study. After obtaining informed consent, all subjects, both affected and unaffected, were evaluated clinically by a single observer (S. J.). All subjects underwent a comprehensive ophthalmic examination including fundus examination after dilatation using indirect ophthalmoscopy, electroretinography (LKC UTAS 2000), and, wherever possible, fundus photography and visual field testing (Humphrey field analyser, 30–2 and 60–2 programs). Family histories and pedigree data were recorded, and peripheral blood samples collected. All families fulfilled the criteria of the diagnosis of arRP. The study protocol was approved by the Institutional Review board.

**DNA isolation and genotyping:** Genomic DNA was isolated from peripheral blood leukocytes by standard procedures. Sequences of primers for amplifying each of the microsatellite markers were obtained from the Genome Database website (<http://gdbwww.gdb.org>). Markers were selected for each region based on proximity to the candidate gene (10 cM or less) and on a heterozygosity of 0.7 or greater. Genomic DNA was amplified by the polymerase chain reaction (PCR) using [ $\alpha$ - $^{32}$ P]dATP (Board of Radiation and Isotope Technology, Hyderabad; specific activity 4000 Ci/mmol). PCR amplifications were done using 0.2 mM dNTPs and 1  $\mu$ Ci [ $\alpha$ - $^{32}$ P]dATP, varying concentrations of magnesium chloride ranging from 1.5 mM to 5.0 mM to obtain optimal amplification, and 1 unit of *Taq* polymerase (MBI Fermentas, Vilnius, Lithuania).

Samples were mixed with equal volume of formamide, denatured at 95°C for 5 min, and loaded and run on 6% polyacrylamide gels with 7 M urea. Gels were dried and autoradiographed, and alleles were scored visually.

## Results

Ten families segregating for arRP were screened, of which seven were consanguineous and three nonconsanguineous. All families except one had two or three affected offspring (see table 1). A total of 41 markers flanking 21 candidate genes were tested. The genes are listed in table 2 with a brief description of their function. All genes selected for screening were either known to have mutations that give rise to one or more types of retinal dystrophies including autosomal dominant and recessive

retinitis pigmentosa and cone dystrophies, or are important for photoreceptor function. Markers that were selected for this study had high heterozygosity values, although the available data do not apply to the Indian population. However, all markers tested were actually polymorphic in the patient population studied, with the *minimum* number of alleles ranging from four to 12. We conclude that the number of alleles found in this study is

**Table 1.** Numbers of affected and unaffected offspring from the arRP families screened.

Family	No. affected	No. unaffected
RP101	1	1
RP102	3	0
RP105	3	0
RP106	2	2
RP107	3	1
RP108	4	0
RP109	3	1
RP110	2	1
RP111	2	0
RP112	3	1

the minimum number since the sample size was small and there may be more alleles which can be detected from an adequate sample size. An initial screen was conducted with DNA from parents and one affected offspring. There were 10 sets of genotypes (one set for each family) per marker. Seven markers did not give clearly interpretable results and were not taken into account. For the remaining 34 markers, 340 sets of genotypes were generated; 29 of these were not informative owing to homozygosity in either or both parents (data not shown). These were considered noninformative since it is not possible to conclude whether the locus is segregating with disease or not. In the initial screen, homozygosity in affected offspring was found in 27 cases (table 3; probands from all 10 families were homozygous at one or more of 16 marker loci). The remaining markers showed heterozygosity in the probands and these loci were tentatively excluded from consideration as segregating with disease. All 27 cases of homozygosity in the proband were genotyped in the remaining affected offspring.

As shown in table 3, five homozygous genotypes found in the probands were also found in other affected siblings (two or more) of the family (indicated by arrows). These cases are from three families showing possible

**Table 2.** Candidate gene loci screened for involvement in arRP.

	Gene	Description
1	ATP-binding cassette (retinal) ( <i>ABCR</i> , <i>ABCA4</i> )	Member of ABC-transporter superfamily. Present in rod outer segments and cone cells
2	Arrestin ( <i>ARR</i> )	Functions in phototransduction by deactivation of rhodopsin
3	Cellular retinaldehyde-binding protein ( <i>CRALBP</i> )	Binds 11- <i>cis</i> -retinal in retinal pigment epithelium (RPE)
4	Cellular retinoid-binding protein ( <i>CRBP-1</i> )	Binds all- <i>trans</i> -retinol in the RPE
5	Cone-rod homeobox ( <i>CRX</i> )	Transcription factor essential for photoreceptor development
6	Guanylate cyclase activator 1A ( <i>GUCA1A</i> )	Activates guanylate cyclase and mediates recovery of rod cells after light-induced bleach
7	Interphotoreceptor retinoid-binding protein ( <i>IRBP</i> )	Present in interphotoreceptor matrix, transports retinoids from the rods to RPE
8	Myosin 7A ( <i>MYO7A</i> )	Unconventional myosin present in photoreceptors and hair cells of the inner ear
9	Neural retina leucine zipper ( <i>NRL</i> )	Photoreceptor-specific transcription factor
10	Phosphodiesterase 6 subunit alpha ( <i>PDE6A</i> )	Present in rod outer segment, hydrolyses cGMP in phototransduction cascade
11	Phosphodiesterase 6 subunit beta ( <i>PDE6B</i> )	Subunit beta of phosphodiesterase (see above)
12	Phosphodiesterase 6 subunit gamma ( <i>PDE6G</i> )	Inhibitory subunit of phosphodiesterase
13	Retinal degeneration slow/peripherin ( <i>RDS</i> )	Structural role in rod outer segment disks and cone lamelli
14	Retinal guanylate cyclase ( <i>RETGC</i> )	Synthesizes and restores cGMP levels during phototransduction in rods
15	Rhodopsin ( <i>RHO</i> )	Present in rod outer segment membrane, initiates phototransduction
16	Rod outer segment membrane protein 1 ( <i>ROM1</i> )	Structural role, forms heterodimers with RDS
17	RPE65, retinal pigment epithelial protein ( <i>RPE65</i> )	Functions in isomerization of vitamin A in RPE
18	Transducin	Activates cGMP phosphodiesterase in response to light
19	Tubby-like protein 1 ( <i>TULP1</i> )	Retina-specific, may function in rhodopsin transport
20	Rhodopsin kinase ( <i>RHOK</i> )	Phosphorylates rhodopsin and inactivates it
21	Cyclic nucleotide gated channel ( <i>CNGC</i> )	Mediates influx of Ca <sup>2+</sup> in response to cGMP

**Table 3.** Screening for homozygosity in affected members of RP families.

	Microsatellite marker	Candidate gene	Pedigree	Affected #1	Affected #2	Affected #3	
1	D1S406	<i>ABCR</i>	RP106	5,5	2,4	–	
2	D1S406	<i>ABCR</i>	RP109	4,4	4,4	4,4	←
3	D1S406	<i>ABCR</i>	RP111	2,2	2,2	–	←
4	D1S497	<i>ABCR</i>	RP107	3,3	3,6	3,3	
5	D1S497	<i>ABCR</i>	RP111	3,3	3,3	–	←
6	D1S219	<i>RPE65</i>	RP101	4,4	NA		
7	D1S219	<i>RPE65</i>	RP110	5,5	ND		
8	D2S206	<i>ARR</i>	RP106	3,3	3,5	–	
9	D2S331	<i>ARR</i>	RP105	4,4	4,9	4,9	
10	D3S1292	<i>CRBPI</i>	RP107	4,4	4,4	1,4	
11	D3S1290	<i>RHO</i>	RP102	2,2	2,4		
12	D4S3038	<i>PDE6B</i>	RP106	7,7	9,10	–	
13	D4S3038	<i>PDE6B</i>	RP109	1,1	7,8	1,7	
14	D5S436	<i>PDE6A</i>	RP107	3,3	3,3	6,7	
15	D5S436	<i>PDE6A</i>	RP109	3,3	3,6	3,4	
16	D6S1582	<i>RDS</i>	RP108	3,3	4,6	4,6	
17	D6S1582	<i>RDS</i>	RP109	6,6	3,4	6,6	
18	D6S1616	<i>GUCAIA</i>	RP107	3,3	3,3	3,3	←
19	D6S1616	<i>GUCAIA</i>	RP101	4,4	NA		
20	D6S1616	<i>GUCAIA</i>	RP109	1,1	1,4	1,1	
21	D11S4172	<i>MYO7A</i>	RP110	2,2	4,4	–	
22	D13S1315	<i>RHOK</i>	RP109	4,4	ND		
23	D15S1046	<i>CRALBP</i>	RP106	1,1	1,4	–	
24	D15S1046	<i>CRALBP</i>	RP111	1,1	1,1	–	←
25	D17S928	<i>PDE6G</i>	RP112	1,1	1,2	1,3	
26	C19S17	<i>CRX</i>	RP110	2,2	2,4	–	
27	C19S17	<i>CRX</i>	RP107	4,4	1,4	4,4	

Table shows the markers for which homozygosity was observed in the screen of probands versus parents. Also shown are the candidate gene flanked by the marker, the pedigree in which the results were observed, and the genotypes of affected offspring. Arrows point to the cases where homozygosity was consistent in all affected offspring. NA indicates not available and ND indicates not done.

**Table 4.** Segregation with disease for markers showing homozygosity in two or more affected offspring.

Pedigree	Marker	Segregating with disease
RP109	D1S406	Yes
RP111	D1S406	Yes
RP111	D1S497	Yes
RP111	D15S1046	Yes
RP107	D6S1616	Yes

co-segregation with disease at four marker loci. Among them, pedigree RP111 has two affected offspring who are homozygous for two markers on chromosome 1 (D1S406, D1S497) that flank the same candidate gene locus, namely *ABCR* (table 4), which is associated with arRP as well as cone-rod dystrophy. Family RP109 showed homozygosity in three affecteds for one of the above markers (D1S406) but was uninformative for the second marker in this region. For the other two cases listed in table 4, i.e. homozygosity among affected individuals of

RP111 and RP107 at loci D15S1046 and D6S1616 respectively, the remaining flanking markers for these two loci (data not shown) did not show any homozygosity that was related to disease.

## Discussion

Retinitis pigmentosa shows an extreme degree of genetic heterogeneity, which renders identification of the disease genes very challenging. Apart from the large number of already known disease genes, many more loci remain to be identified, since the known loci do not account for a large proportion of RP cases (Inglehearn 1998). Mapping of a disease locus by genetic linkage analysis would require families of sufficient size to obtain significant evidence of linkage since pooling of small families will not be a feasible approach owing to the genetic heterogeneity. In case of recessively inherited disease present in association with consanguinity, it is possible in principle to map a disease locus with as few as three offspring (Farrall 1993), provided one has a suitable informative marker, although this may not be achieved in practice in

every case. Candidate gene screening may provide information if one studies a very large patient cohort since not more than 3–4% of patients may harbour mutations in a particular gene (McLaughlin *et al.* 1995; Dryja *et al.* 1999; Pierce *et al.* 1999). We have employed the approach of screening for homozygosity by descent since the regions linked to the disease gene are almost certain to be homozygous in affected individuals of a family, and be common to all such members of the same family. The five cases that showed homozygosity in our study will be pursued further with (a) genotyping additional markers in the respective regions to confirm or rule out homozygosity and (b) screening of relevant candidate genes for mutations. In those cases where we could not conclude segregation with disease due to homozygosity in the parents, we intend to look for additional markers that cosegregate with disease. The majority of loci screened for the 10 families with genotypes that were discordant with the occurrence of disease were eliminated from further screening since they are unlikely to be linked to the disease.

By this screening method we were able to cover a large number of loci and screen in a stepwise fashion so as to reduce the amount of genotyping required to test known loci. Further, the method enabled us to test even small families which would not be significant in themselves in a linkage analysis. While the power of this approach is increased by consanguinity it depends on the location of the markers and their frequencies and polymorphism in the population being studied. The latter two parameters are essentially unknown in the population from which our patient sample was obtained. It is possible to come up with false positives owing to random homozygosity, and to miss the true locus because the marker position is inaccurately known or because of recombination events. Despite these drawbacks, we consider this approach to be fast and to have the potential to indicate which loci should be screened further.

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