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A Novel Genetic Study of Chinese Families with Autosomal Recessive Retinitis Pigmentosa

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Summary

Autosomal recessive retinitis pigmentosa (arRP) is the commonest form of RP worldwide. To date 22 loci have been implicated in the pathogenesis of this disease; however none of these loci independently account for a significant proportion of recessive RP. Linkage studies of arRP in consanguineous families have been mainly based on homozygosity mapping, but this strategy cannot be applied in the case of non-consanguineous families. Therefore, we implemented a systematic approach for identifying the disease locus in three non-consanguineous Chinese families with arRP. Initially, linkage analysis using SNPs/microsatellite markers or mutation screening of known arRP genes excluded all loci/genes except *RP25* on chromosome 6. Subsequently a whole genome scan for the three families using the 10K GeneChip Mapping Array was performed, in order to identify the possible disease locus. To the best of our knowledge this is the first report on the utilisation of the 10K GeneChip to study linkage in non-consanguineous Chinese arRP. This analysis indicates that the studied families are probably linked to the *RP25* locus, a well defined arRP locus in other populations. The identification of another ethnic group linked to *RP25* is highly suggestive that this represents a major locus for arRP.

Keywords: retinitis pigmentosa, linkage analysis, genetic diseases, locus

Introduction

Retinitis pigmentosa (RP: OMIM 268000) comprises a clinically and genetically heterogeneous group of retinal degenerations that result in severe visual impairment. RP can present with different modes of inheritance including autosomal dominant (ad), autosomal recessive (ar), X-linked (xl) and digenic. It has an incidence of $\sim$1/3500 with autosomal recessive RP (arRP) the most common form of RP worldwide (Weleber, 1994), interestingly accounting for the majority of cases in China (Hu, 1987).

Hitherto 22 loci have been implicated in the pathogenesis of arRP (http://www.sph.uth.tmc.edu/retnet/). The genes for five of these loci (*RP22*, *RP25*, *RP28*, *RP29* and *RP32*) still remain to be identified (Gu *et al.* 1999; Hameed *et al.* 2001; Finckh *et al.* 1998; Ruiz *et al.* 1998; Zhang *et al.* 2005). Due to this high number of loci it is more challenging to identify the gene mutation responsible for arRP in newly ascertained patients. Moreover, it is anticipated that many genes are yet to be identified, since known loci cannot explain all cases of recessive RP. Based on a prevalence of only 1-5% for each of the arRP genes found so far (Tuson *et al.* 2004), identifying a novel gene that may account for a significant proportion of recessive families

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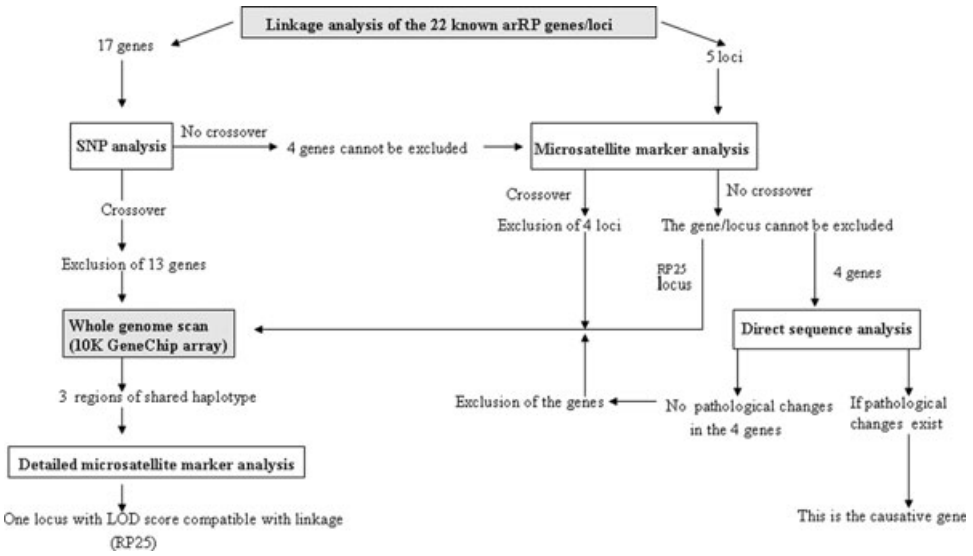

Figure 1 A flow chart describing a strategy for identifying the disease locus in autosomal recessive nuclear non-consanguineous families. The highlighted boxes denote the two main approaches required for mapping disease loci in cases of genetically heterogeneous disorders.

will be a major achievement in retinal degeneration research.

Traditionally, homozygosity mapping has been the basis for genetic mapping of recessive traits, using either a set of 300–400 microsatellite markers or high-density single nucleotide polymorphisms (SNPs) (Botstein & Risch, 2003; Daly *et al.* 2001), and more recently the 10K GeneChip Array (Affymetrix, Inc., Santa Clara, CA). This method has proven to be effective in cases of consanguineous families where the regions adjacent to the disease loci are likely to be homozygous by descent (Lander & Botstein, 1987; Sheffield *et al.* 1998). However, this is not the ideal approach for targeting the mutated gene in non-consanguineous families, since the possibility of compound heterozygosity exists (Kondo *et al.* 2004). Additionally, mutation screening of previously identified genes in such families, particularly in genetically heterogeneous disorders, is time consuming and less cost effective.

Therefore, in this study a systematic method for identifying the disease locus in small non-consanguineous arRP families was implemented (Figure 1). Initially, SNP microsatellite marker analyses or a mutation screening approach were used for linkage testing of the known recessive RP genes/loci. This was followed by a whole genome scan in all families, using the 10K GeneChip Mapping Array in order to identify common regions of shared haplotype. Whenever more than one region of shared haplotype was observed in all families, we undertook detailed microsatellite marker analysis and pooled the data. Pooling of the linkage data was based on the fact that all families originated from the same geographical region in China. This was followed by an evaluation of suitable candidate genes within the regions of shared haplotype. For example, the visual system homeobox 1 (*VSX1*) gene lies in the vicinity of an interval that showed evidence of linkage in our families, and is expressed in the bipolar cells of the retinal inner nuclear layer (Hayashi *et al.* 2000). Hence, it was mandatory to screen this gene for mutations in the families studied.

This proposed approach could be a useful tool for genetic mapping in cases of rare and genetically heterogeneous recessive traits. Subsequently, this should help to reveal the underlying genetic defect in such disorders.

Materials and Methods

Families and Clinical Data

Three non-consanguineous Chinese families were included in the study (Figure 2). Informed consent was obtained from all participants for clinical and molecular genetic studies. The study conformed to the tenets of the declaration of Helsinki. Each family had a pedigree structure consistent with a pattern of autosomal recessive inheritance. Clinical diagnosis was based on visual

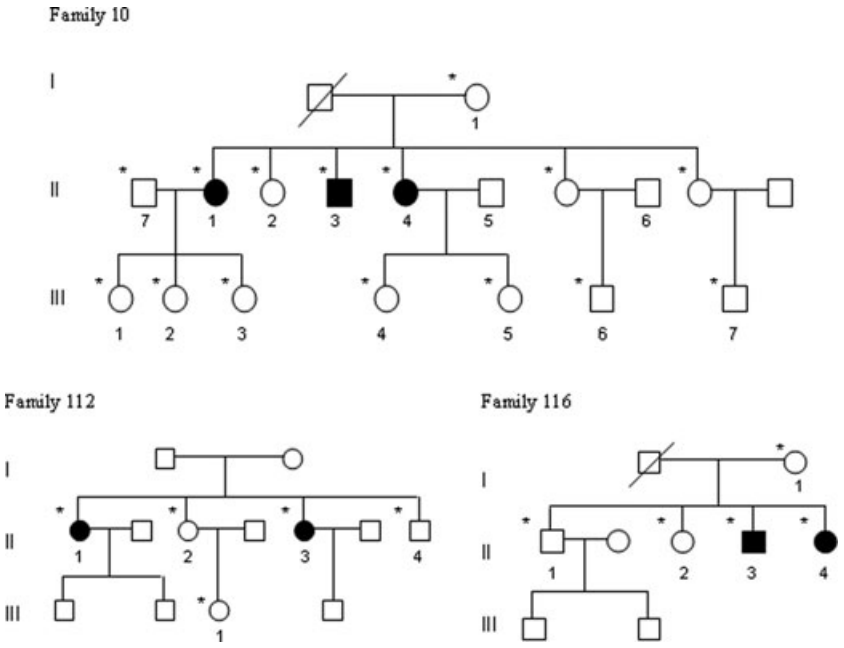

Figure 2 Pedigrees of the families participating in the study. Open and closed symbols denote unaffected and affected individuals, respectively. Deceased family members are denoted by diagonal slashes and asterisks indicate individuals examined both clinically and genetically.

acuity, fundus picture, and electroretinography (ERG) findings.

Methods

Blood samples were obtained from all patients and their relatives. Genomic DNA was extracted from peripheral blood lymphocytes according to the standard protocols. Molecular genetic studies were performed on 17 DNA samples, of which 7 came from affecteds.

SNPs selection, PCR amplification and sequence analysis: On average three SNPs were selected for each of the studied genes, ensuring coverage of the entire length of the gene (Table 1). The selection process was based on the SNP heterozygosity pattern which is available in the SNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/); the higher the heterozygosity of the SNP the more likely it was to be informative for linkage/exclusion. Fifty-four pairs of primers were designed using the Primer 3 Out put program (http://frodo.wi.mit.edu/cgibin/primer3/primer3_www.cgi), in order to screen the heterozygosity of the selected SNPs in all family members (primer information is available on request). Full PCR conditions and the sequencing protocol were as described previously (Abd El-Aziz *et al.* 2005).

Microsatellite markers and genotyping: In total 79 microsatellite markers were used, of which 56 were from the ABI PRISM® Linkage Mapping Sets V2.5. The remaining 43 markers were synthesized commercially according to the information obtained from the Genome Database (Table 1). The data for heterozygosity and the order of the markers used were obtained from the Marshfield Medical Research Foundation web site.

Multiplex microsatellite genotyping was performed using 4-6 markers per reaction using QPCR mix (ABgene, UK). Data collection and allele identification were performed using GeneScan and Genotyper software (Applied Biosystems). Alleles were assigned to individuals and genotypic data used to calculate the LOD scores using the programs Cyrillic and MLINK. The phenotype was analysed as an autosomal recessive trait with complete penetrance and a frequency of 0.0001 for the affected allele.

Mutation screening: Fifty one pairs of primers were designed for four genes, *CERKL*, *LRAT*, *SAG* and *MERTK*, in order to search for mutations in the families where both SNPs and microsatellite markers were

Table 1 SNP/microsatellite markers (msm_s) used for studying the known arRP loci in families 10 (I-1 and II:1-II-6), 112 (II-1:II-4) and 116 (I-1 and II:1-II-4). Affected members in each family are underlined and in bold. Cells marked with diagonal slashes denote the genes and the corresponding families that could not be excluded by this method. Alleles that are different across the affected individuals are underlined

Gene name	Gene size (Kb)/ no. of exons/ position (Mb)	SNPs/ msm_s	SNPs/ msm_s Position (Mb)	I-1 /10	**II-1** /10	II-2 /10	**II-3** /10	**II-4** /10	II-5 /10	II-6 /10	**II-1** /112	II-2 /112	**II-3** /112	II-4 /112	I-1 /116	II-1 /116	II-2 /116	**II-3** /116	**II-4** /116
1. *ABCA4*	128.30/50/	D1S2868	93.10	4/4	3/4	4/4	4/4	3/4	3/4	3/4	3/4	3/4	4/4	3/4	2/2	2/3	2/2	2/2	2/3
	94.23-94.35	D1S2849	93.30	2/4	4/4	2/2	2/4	2/4	2/4	2/4	2/4	2/2	2/2	2/4	1/2	2/3	1/2	1/2	1/3
		rs3945204	94.20	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/2	1/1	1/1	1/1	1/1	1/1	1/1	1/1
		rs1191231	94.28	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
		rs2151848	94.31	1/2	1/1	1/2	1/1	1/2	1/1	1/2	1/2	1/1	1/2	1/1	1/2	1/1	2/2	2/2	1/2
		D1S2819	95.30	2/3	2/3	2/4	2/3	2/2	3/3	2/2	2/2	2/2	1/2	2/2	1/2	1/1	2/2	2/2	2/2
2. *CRB1*	210.18/12/	D1S2816	194.9	1/3	1/1	1/3	1/3	1/1	1/3	1/3	1/4	2/4	2/4	1/4	1/3	1/3	1/4	1/3	1/4
	195.5-195.7	rs3790380	195.5	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/2	1/2	1/2	1/1	1/2
		rs949571	195.6	1/2	1/1	1/1	2/2	1/1	1/1	1/1	1/1	1/1	1/2	1/2	1/1	1/1	1/1	1/1	1/1
		rs1345529	195.7	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/2	1/2	1/2	1/2	1/1	1/2	1/2	1/2	1/2
		D1S413	196.9	1/2	1/1	1/4	1/2	1/1	1/4	1/4	1/3	1/3	2/2	2/2	1/5	1/5	4/5	1/5	1/4
3. *CNGA1*	16.28/8/	rs1012844	47.64	1/2	1/2	1/1	1/2	1/1	1/1	1/2	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
	47.63-47.67	rs1440224	47.65	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/2	1/1	1/1	1/1	1/1	1/1	1/1
		rs1371729	47.65	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/2	1/1	1/2	1/2	1/1
		rs1371732	47.66	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/2	1/1	1/2	1/2	2/2
		rs4695318	47.67	1/1	1/2	1/2	1/2	1/1	1/2	1/1	1/1	1/1	1/2	1/1	1/1	1/1	1/2	1/1	1/1
		D4S3002	48.3	2/4	2/3	2/4	2/3	2/2	2/2	2/3	2/2	2/2	2/3	2/2	1/3	1/1	1/1	1/3	1/1
4. *CERKL*	118.82/13/	D2S2310	181.87	3/4	3/3	3/3	3/3	3/4	3/4	3/4	1/2	1/2	1/2	1/2	2/2	2/3	2/3	2/3	2/3
	182.1-182.23	rs1047307	182.11	1/1	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/1	1/2	1/2	1/2	1/1	1/1
		rs763860	182.13	1/1	1/1	1/1	1/1	1/2	1/2	1/1	1/2	1/2	1/2	1/2	1/1	1/2	1/1	1/2	1/2
		rs6433926	182.18	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/2	1/2	1/2
		rs1372119	182.20	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/2	1/1	1/2	1/2	1/2
		rs1473295	182.22	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/2	1/2	1/2	2/2	2/2	1/2	1/2	1/2	1/2
		D2S364	182.74	1/4	1/2	1/2	1/2	1/4	4/4	1/2	2/4	4/5	2/4	2/2	2/2	1/2	2/4	2/4	2/4
5. *CNGB1*	87.49/33/	D16S3140	54.80	2/4	2/3	2/2	2/3	2/2	2/3	2/3	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2
	56.47-56.56	D16S3057	56.08	3/3	3/3	2/3	3/3	3/3	2/3	3/3	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2
		rs2033249	56.47	1/2	1/2	1/2	1/2	1/2	1/2	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
		rs376270	56.50	1/2	1/2	1/2	1/2	1/2	1/2	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
		rs691897	56.51	1/2	1/1	1/1	1/1	1/1	1/1	1/2	1/2	1/1	1/1	1/2	1/1	1/1	1/1	1/1	1/2
		rs2241771	56.57	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/2	1/1	1/1	1/2	1/2	2/2	1/1	1/2	1/1
		rs8055820	56.58	1/2	1/2	1/1	1/2	1/2	1/1	2/2	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
		D16S3038	56.97	2/3	2/3	2/3	2/3	3/3	2/3	2/3	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2

Table 1 (Continued).

Gene name	Gene size (Kb)/ no. of exons/ position (Mb)	SNPs/ msm_s	SNPs/ msm_s Position (Mb)	I-1 /10	**II-1** /10	II-2 /10	**II-3** /10	**II-4** /10	II-5 /10	II-6 /10	**II-1** /112	II-2 /112	**II-3** /112	II-4 /112	I-1 /116	II-1 /116	II-2 /116	**II-3** /116	**II-4** /116
6. *LRAT*	10.2/3/ 155.88-155.89	D4S3049	154.99	5/9	5/9	5/9	5/5	3/5	3/9	5/9	3/3	3/8	3/3	3/8	4/6	4/5	4/5	3/6	3/6
		D4S3021	155.15	3/4	3/4	3/4	3/4	3/3	3/3	3/4	1/1	1/4	1/1	1/4	1/2	1/2	1/2	1/2	1/2
		D4S2883	155.71	1/2	1/2	1/1	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2
		D4S1285	155.78	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2
		rs1061100	155.884	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
		rs201825	155.886	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/2	1/2	1/2	1/2
		rs201823	155.888	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
		rs201822	155.889	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/2	1/2	2/2	2/2	2/2
		rs12507608	155.891	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/2	1/1	1/2	1/2	1/2
		D4S2967	156.01	5/6	5/6	5/6	5/5	5/6	5/6	5/6	4/5	3/5	4/5	3/5	4/5	4/4	4/5	4/4	4/4
7. *MERTK*	130.69/19/ 112.37-112.50	rs1400323	112.38	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/2	1/2	1/2	1/2	1/1	1/2	1/1	1/1	1/1
		D2S1896	112.40	7/7	7/8	7/7	7/8	7/8	7/7	7/7	2/7	2/2	2/2	2/2	1/6	4/6	6/7	1/7	6/7
		rs1113418	112.42	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/1	1/2	1/1	1/1	1/1
		rs1554215	112.46	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/2	1/2	1/2	1/2	1/1	1/2	1/1	1/1	1/1
		rs6710591	112.48	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/1	1/2	1/2	1/2	1/2	1/2	1/2
		D2S2269	112.67	2/3	2/3	2/5	2/3	2/3	3/5	2/5	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2
		D2S160	112.71	2/3	2/3	2/5	2/3	2/3	3/5	2/5	5/5	5/5	5/5	3/5	3/4	3/3	3/3	3/4	3/3
8. *NR2E3*	7.7 /9/ 69.88-69.89	D15S131	68.97	2/3	2/4	2/2	2/3	2/4	2/2	2/2	1/3	1/1	1/1	1/1	1/6	1/3	1/1	3/6	1/1
		rs2723343	69.895	1/2	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/2	1/2	1/2	1/2	1/2
		D15S204	70.08	2/6	2/5	2/2	2/2	2/5	2/6	2/6	1/4	1/4	2/4	2/4	2/6	2/3	2/3	2/3	2/5
9. *PDE6A*	84.13/22/ 149.22-149.30	rs10515637	149.22	1/2	1/2	1/1	1/1	1/2	1/1	1/2	2/2	1/2	1/2	2/2	1/1	1/1	1/1	1/1	1/1
		rs10515637	14922	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/2	1/1	1/1	1/2	1/1
		rs251346	149.26	1/2	1/2	1/2	1/2	1/1	1/2	1/2	1/1	1/1	2/2	1/1	1/1	1/1	1/1	1/1	1/1
		rs152951	149.26	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/2	1/1	1/2	1/1	1/2
		rs30826	149.27	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
		rs2277926	149.30	1/2	1/2	1/1	1/1	1/2	1/1	1/2	1/2	1/1	1/1	1/2	1/2	1/1	1/2	1/1	1/1
10. *PDE6B*	44.52/22/ 0.609-0.653	D4S3360	0.105	3/3	3/4	3/3	3/4	3/4	3/4	3/3	3/3	3/3	3/3	3/5	3/3	3/5	3/5	3/3	3/5
		D4S3038	0.108	4/5	1/4	2/4	1/4	1/5	1/4	2/4	1/2	1/2	1/4	1/2	2/2	1/2	1/2	2/3	1/2
		D4S412	0.335	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/3	1/2	1/2	1/3	1/2	1/2	1/2	1/2	1/2
		rs1135375	0.637	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
		D4S2936	0.682	2/5	2/6	2/2	2/6	2/6	2/6	2/2	3/3	3/3	3/5	3/3	3/3	2/3	2/3	2/3	3/3

Table 1 (Continued).

Gene name	Gene size (Kb)/ no. of exons/ position (Mb)	SNPs/ msm_s	SNPs/ msm_s Position (Mb)	I-1 /10	**II-1** /10	II-2 /10	**II-3** /10	**II-4** /10	II-5 /10	II-6 /10	**II-1** /112	II-2 /112	**II-3** /112	II-4 /112	I-1 /116	II-1 /116	II-2 /116	**II-3** /116	**II-4** /116
11. *RGR*	14.09/7/	D10S1686	85.55	2/4	2/4	2/4	2/2	2/2	2/4	2/4	2/2	2/2	1/2	2/2	2/3	1/3	1/3	1/3	2/3
	85.99-86.00	rs738786	86.00	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/1	1/1	1/1	1/1	1/2	1/2	1/1	1/1	1/1
		D10S573	86.29	4/4	4/4	2/4	4/4	2/4	2/4	2/4	4/5	4/5	2/5	4/5	2/2	2/5	2/5	2/5	2/2
12. *RHO*	6.71/5/130.730-130.736	rs7984	130.730	1/1	1/1	1/2	1/2	1/2	1/2	1/2	1/1	1/2	1/2	1/1	1/1	1/1	1/2	1/2	1/1
13. *RLBP1*	11.82/9/	D15S979	86.63	2/2	2/4	2/6	2/6	2/4	2/4	2/6	4/4	2/4	2/4	2/4	3/6	3/5	3/5	5/6	5/6
	87.554-87.565	D15S1045	87.39	3/5	3/3	4/5	4/5	3/3	3/3	4/5	3/4	2/4	2/4	3/4	2/2	2/2	2/2	2/2	2/3
		rs2070780	87.56	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/1	1/1	1/1	1/1	1/2	1/2	1/2	1/2	1/2
		D15S202	87.80	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2
14. *RPE65*	21.2/14/	D1S198	66.78	5/7	7/8	5/7	5/5	7/8	7/8	7/8	3/4	1/4	1/3	4/4	4/5	4/4	5/5	4/5	4/4
	68.667-68.688	D1S410	67.91	1/8	1/7	8/8	7/8	1/7	1/7	1/7	1/7	7/7	1/7	7/7	1/7	1/6	1/6	6/7	7/8
		rs3125898	68.67	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/1	1/1	1/1	1/1	1/2	1/2	1/2	1/2	1/2
		D1S219	69.61	3/4	3/4	4/4	3/4	3/4	3/4	3/4	3/4	4/4	4/4	3/4	1/4	3/4	1/3	3/4	3/4
15. *SAG*	39.07/15/	D2S2344	233.152	1/5	1/4	1/4	1/4	1/5	4/5	1/5	2/2	2/3	2/2	3/3	2/3	2/3	3/3	2/3	2/3
	233.881-233.920	D2S206	233.416	3/4	3/3	3/3	3/3	3/4	4/4	3/4	4/4	1/3	4/4	1/4	3/4	3/5	4/5	4/5	3/5
		rs1978921	233.881	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
		rs12623795	233.888	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
		rs2304777	233.892	1/2	1/1	1/1	1/1	1/2	1/1	1/1	1/1	1/2	1/1	1/2	1/1	1/2	1/2	1/1	1/2
		rs11891546	233.897	1/2	1/1	1/1	1/1	2/2	1/1	1/2	1/1	1/1	1/1	1/2	1/1	1/2	1/2	1/2	1/2
		rs3792100	233.899	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/2	1/2	1/2	1/2	1/2	1/1
		rs3792097	233.903	1/2	1/2	1/2	1/2	1/1	1/2	1/2	1/1	1/2	1/1	1/2	1/2	1/2	1/2	1/2	1/2
		rs1000141	233.907	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/1	1/2	1/1	1/2	1/2	1/2	1/2	1/1	1/1
		rs1046974	233.920	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/2	1/2	1/2	1/2	2/2	2/2
16. *TULP1*	15.00/15/	D6S1611	35.48	1/2	1/2	1/2	1/2	2/2	1/2	1/2	1/2	1/2	2/2	1/2	1/3	3/3	3/4	3/4	3/3
	35.57-35.58	rs2273000	35.58	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
		D6S291	36.37	1/3	3/3	3/3	3/3	3/3	2/3	3/3	1/2	1/3	2/2	2/3	1/2	2/2	2/2	1/2	2/2
17. *USH2A*	249.44/21/	D1S2703	211.15	5/11	5/5	5/9	5/5	5/5	5/9	5/1	8/9	8/8	8/8	8/8	1/5	5/7	5/7	4/5	4/5
	213.86-214.66	D1S2827	214.20	3/5	3/3	2/3	3/5	3/3	2/3	3/5	2/5	3/4	3/4	3/5	3/5	2/5	2/5	2/5	3/5
		rs1324330	214.45	1/1	1/2	1/1	1/2	1/2	1/1	1/2	1/1	1/1	1/1	1/1	1/2	1/2	1/2	1/2	1/2
		D1S227	215.36	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/4	2/2	3/3	2/3	2/3	2/3	2/3
18. RP22	3.77/	D16S3041	19.30	6/2	2/3	6/3	6/4	2/4	2/3	6/4	4/6	4/6	4/4	4/6	2/3	3/3	2/3	2/3	3/3
	19.17-22.94	D16S3046	20.79	1/5	1/5	1/5	2/5	1/5	1/5	2/5	2/3	3/4	2/5	3/4	2/4	1/4	2/4	1/4	2/4
		D16S403	22.94	5/5	5/9	5/7	5/9	5/9	5/7	5/9	2/3	4/7	2/3	4/7	1/6	2/6	1/3	2/6	1/3
		D16S3068	25.47	1/2	1/3	1/2	2/3	1/3	1/1	2/3	1/2	2/2	1/2	2/2	1/2	1/1	2/3	1/1	2/3
		D16S3100	26.48	2/3	2/3	2/3	3/3	2/3	2/2	3/3	2/2	1/3	2/2	1/3	1/2	2/2	1/1	2/2	1/1

Table 1 (Continued).

Gene name	Gene size (Kb)/ no. of exons/ position (Mb)	SNPs/ msm_s	SNPs/ msm_s Position (Mb)	I-1 /10	**II-1** /10	II-2 /10	**II-3** /10	**II-4** /10	II-5 /10	II-6 /10	**II-1** /112	II-2 /112	**II-3** /112	II-4 /112	I-1 /116	II-1 /116	II-2 /116	**II-3** /116	**II-4** /116
19. RP28	3.39/	D2S147	64.02	4/4	3/4	4/5	3/4	4/5	4/5	4/5	2/9	2/9	9/9	2/9	2/4	4/7	4/7	3/4	2/3
	62.67–66.06	D2S380	65.50	5/7	7/7	3/5	7/7	5/7	3/5	3/5	1/3	1/3	3/3	1/3	7/7	7/7	7/7	7/7	5/7
		D2S2293	65.80	3/4	4/5	1/3	4/5	3/5	1/3	1/3	4/4	3/4	3/4	4/4	1/3	3/4	3/4	3/4	1/4
20. RP29	7.1/	D4S1597	170.20	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/8	3/8	3/3	3/3	3/3
	173.31–180.41	D4S1595	174.55	5/7	7/7	5/7	5/7	6/7	6/7	5/7	6/7	6/7	5/7	6/7	5/6	5/6	5/5	5/5	5/7
		D4S1539	175.92	3/4	3/3	3/4	3/4	3/3	3/3	3/4	3/3	3/3	3/3	3/3	2/3	3/3	3/3	3/3	3/4
		D4S415	178.94	7/7	7/8	2/7	7/8	2/7	2/7	7/8	2/8	2/8	2/2	2/8	2/3	2/2	2/3	2/3	2/3
		D4S2920	184.70	3/5	3/4	3/5	4/5	3/5	4/5	3/4	2/5	4/4	2/4	4/5	3/5	2/5	4/5	4/5	2/5
21. RP32	8.94/	D1S206	101.46	1/3	3/6	3/3	1/3	3/6	1/6	3/6	4/8	4/9	4/9	4/8	2/2	2/6	2/2	2/2	2/6
	101.74–110.68	D1S495	102.33	4/4	3/4	4/4	4/4	3/4	3/4	3/4	7/8	7/10	7/10	7/8	3/7	3/7	3/7	3/7	3/3
		D1S2726	110.80	6/8	7/8	6/6	6/8	6/7	7/8	6/7	3/4	3/4	3/3	4/4	3/4	3/4	3/4	3/4	3/4

unable to exclude them as disease-causing genes (Table 1). The genes were amplified and sequenced as previously described (Abd El-Aziz *et al.* 2005).

GeneChip Mapping 10K Array: DNA samples from seven affected individuals and three unaffected carriers were genotyped by total genome-sampling analysis using the Affymetrix 10K GeneChip Mapping Array (version Xba142). The median physical distance between SNPs and their average heterozygosity were 105 kb and 0.37, respectively, predicting an average spacing of 3 fully informative markers per 1 Mb. Genotypes for 10204 SNPs were called by the GeneChip DNA Analysis Software (GDAS v3.0, Affymetrix). The detailed methodology for genotyping using the GeneChip array has been previously described (Sellick *et al.* 2004).

Analysis of the GeneChip Mapping 10K Array: The analysis of the data was performed using the ALOHOMRA software (Rüschendorf & Nürnberg, 2005). Mendelian errors and the correct relationships within the families were checked using the PedCheck and GRR programs, respectively (O'Connell & Weeks, 1998; Abecasis *et al.* 2001). Identification and deletion of non-Mendelian errors and unlikely genotypes was performed. Uninformative SNPs were removed from the data by Merlin (Abecasis *et al.* 2001). For parametric linkage analysis, the data were converted for Genehunter, where the analysis was done in subsets of markers in a non-overlapping moving window. The haplotyping data were then used from the Genehunter file. The software searched for regions of shared haplotypes between the affected members in each family; we then combined the data from the three families in order to identify the regions of shared haplotypes. Further investigations of these regions were based on two criteria: 1. 10 or more consecutive SNPs were shared between all families, ensuring that the parents were heterozygous. 2. the shared region spanned at least a 3 Mb interval.

Mutation screening of the VSX1 gene: Five pairs of primers were designed in order to screen the coding regions together with the splice sites (GT/AG) and the 5′UTR, of the *VSX1* gene (Table 2). The genes were amplified and sequenced as previously described (Abd El-Aziz *et al.* 2005).

Exon no.	Sequence (5′ . . . 3′)	Product size (bp)	$MgCl_2$	Temp.
Exon 1 F	GCGGAGTCACTGTCCCTTAC	940	0795 AB mix	58.5° C
Exon 1 R	GGGATTTAGGATGCAGCAAG			
Exon 2 F	AATAGCAGCAGCCATTTTGG	399	1.5 mM	57° C
Exon 2 R	CCGGGCCATAAATTCTCAG			
Exon 3 F	CATTCAGAGGTGGGGTGTTC	422	1.5 mM	57° C
Exon 3 R	AGCTCTTGTGGTGCCTTCAG			
Exon 4 F	CCTCGGGAGCTATTTCCTTC	432	1.5 mM	57° C
Exon 4 R	ACTGACGTTGCTTTGCTTTG			
Exon 5 F	CCAATGCCAATCACTGTGTC	696	1.5 mM	57° C
Exon 5 R	CCCTAGGTCACTCATCTGTCC			

Table 2 Primers designed for amplification of VSX1 gene

Results

Clinical Data

All affected subjects had clinical manifestations of RP, including variable grades of low visual acuity, waxy pallor of the optic disc, attenuation of the retinal blood vessels, bone spicule pigmentation and flat or absent ERG. The clinical data of the patients are described in Table 3.

SNP/microsatellite marker analyses and mutation screening of the 17 known arRP genes

Intergenic SNP analysis enabled the exclusion of 13 out of 17 known genes, since non-segregation of the haplotypes within affected family members in a given gene ruled out its involvement in the pathogenesis of the disease (Table 1). However, four remaining genes (*CERKL* and *LRAT* in families 112 and 114, *SAG* in family 112, and *MERTK* in family 10) were not excluded by this approach, as all the SNPs screened were segregating with the disease phenotype. Consequently, microsatellite markers spanning these four genes were investigated. Although the markers were highly informative, the four genes could not be excluded. Thus, it was mandatory to screen these genes for mutations in their corresponding families by direct sequence analysis. Mutation screening of the coding regions, splice sites and the 5′UTR of these four genes revealed no pathological sequence alterations, and they were consequently excluded as disease causing for RP in our families. However, it is important to consider that the methodology used in this study cannot detect large heterozygous deletions or intronic mutations.

Microsatellite marker analysis of the remaining 5 known arRP loci: Microsatellite marker analysis excluded four of the remaining 5 arRP loci (*RP22*, *RP25*, *RP28*, *RP29* and *RP30*) by achieving LOD scores of minus infinity for the analysed markers. Only the *RP25* locus could not be excluded in the studied families, where LOD scores suggestive of linkage were obtained (Figure 3).

GeneChip Mapping 10K Array and further analysis: The GeneChip mapping data showed that the affected members from each family shared multiple regions of haplotype. By combining the data from the three families, three regions of tentative linkage were identified (Table 4). The largest region was at the 6p12.1-q12 (*RP25*), followed by regions at 20p11.21-q12 and 6q24.3-q25.3, corresponding to 16.1, 15.2 and 9.7 Mb intervals, respectively. Further investigation of these regions using highly informative microsatellite markers confirmed linkage to the *RP25* locus, a well defined locus for recessive RP. The density of markers in this interval was increased in order to achieve the maximum LOD score for each of the families studied and to identify new crossovers. All the studied markers in the vicinity of *RP25* generated positive LOD scores. We pooled the linkage data from all families, as previously stated. A maximum LOD score of 3.27 for the marker D6S1681 at $\theta = 0$ was generated (Table 5). Recombination events defined a critical interval spanning $\sim$10 cM interval on chromosome 6, between markers D6S294 and D6S460 (Figure 3), which was similar to a previous report in Spanish families with arRP (Ruiz *et al.* 1998), and the disease interval could not be further refined. However, in case of the 6q24.3-q25.3 and the

Table 3 Clinical data of the patients participating in the study

Family no	Patient no.	Sex	Age	Age of Onset	VA OD unaided	VA OS unaided	Visual filed	RP changes	Optic **disc**	Nyctalopia
10	II-1	F	49	42	$20/30^{-2}$	$20/30^{-1}$	Constricted	Diffuse	Mild **pallor**	+
10	II-3	M	45	36	20/50	20/40	Constricted	Diffuse	Mild **pallor**	+
10	II-4	F	35	20	$20/30^{-3}$	$20/30^{-3}$	Constricted	Diffuse	Mild **pallor**	+
112	II-1	F	52	10	$20/50^{-2}$	20/30	Constricted	Sectorial	-	+
112	II-3	F	46	11	20/30	20/30	Constricted	Sectorial	-	+
116	II-3	M	34	22	0.3+	0.3+	Constricted	Diffuse	-	+
116	II-4	F	41	19	20/100	20/100	Constricted	Diffuse	-	+

20p11.21-q12 loci, additional work ruled out linkage to 6q24.3-q25.3 and narrowed the interval of the 20p11.21-q12 region to 8 Mb with a maximum LOD score for all families of 1.48 at $\theta = 0$ for the marker D20S484 (Figure 4). It is interesting to note that there was no common allele between the studied families in the regions suggestive of linkage. Ultimately, this can rule out the possibility that the mutation in the causative gene will be due to a common founder effect.

Bioinformatic analysis of the regions with positive LOD scores: The work described had identified two regions of interest, one at chromosome 6p12.1-q12 and the other at 20p11.21-q12, for detailed computational analysis (Figures 3 and 4, respectively). The 8 Mb interval in 20p11.21-q12 contains approximately 100 genes and by studying their function no good candidates for RP were identified, apart from the *VSX1* gene that was previously reported to be expressed in the inner nuclear layer of the adult retina. Similarly, for *RP25* at 6q we are fully aware that there are at least 105 genes in this interval. None of these genes are retina specific but some are expressed in the retina, and until now we and others have excluded some of these genes as disease-causing for recessive RP (Abd El-Aziz *et al.* 2005, 2006; Marcos *et al.* 2000, 2002, 2003; Li *et al.* 2001; Barragan *et al.* 2005a, 2005b).

Mutation screening of the VSX1 gene: Mutation screening of the *VSX1* gene revealed no pathological changes in all 3 families studied. However, three SNPs were identified, two of which were novel. The novel SNPs were a heterozygous transition of C to T 13 bp upstream of exon 1, and a heterozygous transition of G to A at nucleotide position 700, depicting a change of amino acid from arginine to glutamine at codon 222. All changes were assigned a nucleotide number starting at the first translated base of the *VSX1* gene according to the Genbank entry NM_014588.

The identified SNPs segregated with the disease phenotype, and were detected with an allele frequency of 4.7 and 8.3% in 100 control chromosomes, respectively. Thus, based on these data it is highly likely that *RP25* could be the locus responsible for linkage in our families.

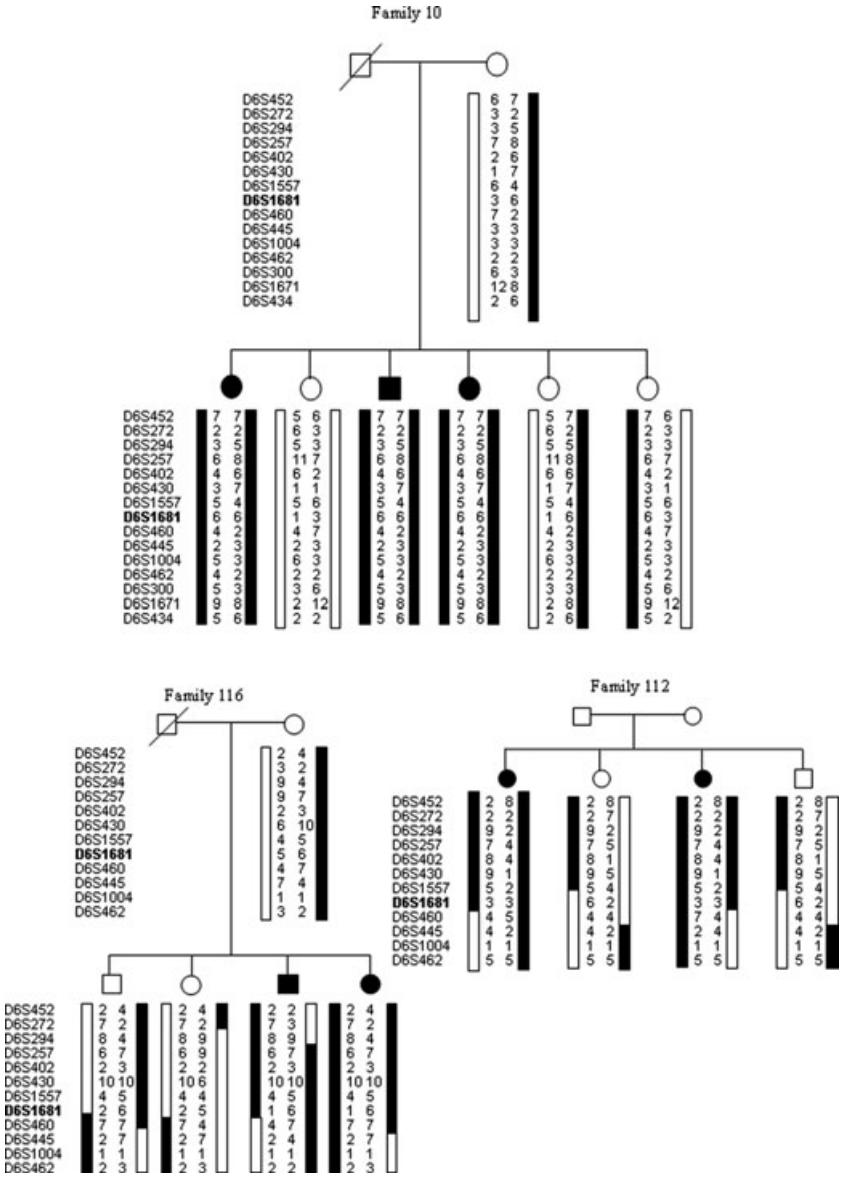

Figure 3 Haplotypes of families 10, 112 and 116 using microsatellite markers on 6p12.1-q12. Recombination events between markers D6S294 and D6S460 are shown in families 112 and 116. The maximum LOD score for D6S1681 is shown in bold.

Discussion

Herein we report the linkage of three non-consanguineous Chinese families with arRP to the *RP25* locus at chromosome 6p12.1-q12. In addition, we propose a systematic method for identifying disease loci in small non-consanguineous recessive families (Figure 1). Initially, a SNP analysis approach was undertaken to identify if any of the 17 known arRP genes showed linkage in the families studied. It is generally accepted that one crossover in 100 meioses relates to a genetic distance of 1 cM, and on average this equates to a physical distance of 1 Mb. For that reason, our selection of three or more SNPs per gene should be sufficient to exclude the gene if a crossover is observed, since the physical size of the 17 recessive RP genes ranged between 4-250 kb. The SNP approach proved to be effective in excluding these genes, particularly if more than two affected individuals were evaluated per family. Microsatellite markers were adopted on two occasions:

Table 4 Common regions of shared haplotype in each family and in all families using the 10K GeneChip array

Regions	Family 10	Family 112	Family 116	Minimum interval in all Families
6p12.1-q12 (RP25)				
Interval in cM	68	24.9	5.9	5.9
Interval in Mb	87.5	31.8	16.1	16.1
No. of SNPs	332	116	41	41
6q24.3-q25				
Interval in cM	33.8	26.4	16.9	16.9
Interval in Mb	19.2	15.9	9.7	9.7
No. of SNPs	102	85	50	50
20p11.21-q12				
Interval in cM	43.1	8.6	27.7	8.6
Interval in Mb	33.6	15.2	26.6	15.2
No. of SNPs	120	29	77	29

cM: genetic distance, Mb: physical distance, No. of SNPs: number of SNPs in each region

1) if the SNPs showed segregation of the haplotypes with the disease in any of the 17 genes; 2) for studying the 5 loci where the genetic interval was large. However, in this study neither the SNPs nor the microsatellite markers were able to exclude four of the genes studied where the haplotype within the families segregated with the disease phenotype. Hence, we performed direct sequence analysis of these genes in their corresponding families in order to search for mutations or to exclude these genes as being disease causative. This was both cost-and time-effective in comparison to sequencing all known recessive genes in all families. Subsequently, all families studied were subjected to a whole genome scan using the 10K GeneChip to search for regions of common haplotypes between the families studied.

To the best of our knowledge, this is the first application of the 10K array in non-consanguineous autosomal recessive RP families. It has been reported that the 10K SNP-chip has a >99% chance in detecting heterozygosity, compared to a 70% chance in the case of microsatellite markers, and consequently in exclusion of linkage (Woods *et al.* 2004). Thus the 10K GeneChip also has a potential role in homozygosity mapping. However, we are presented with a different scenario in the case of non-consanguineous families, where the mutation is probably compound heterozygous and so no regions of homozygosity are expected. Thus, in our study we used the 10K GeneChip in order to detect regions of shared haplotypes between the affected members in each family, which was followed by combining the data from different families.

The data obtained from the GeneChip mapping 10K array showed that there were three regions of shared haplotype, 6p12.1-q12, 20p11.21-q12 and 6q24.3-q25.3, between all 3 families across the whole genome. Further genetic analysis of these three loci with highly informative markers excluded the region of linkage at the telomeric end of chromosome 6, and reduced the interval on chromosome 20 to 8 an Mb region which contained a good candidate gene, *VSX1.* Even though this gene has been implicated in the pathogenesis of posterior polymorphous dystrophy and keratoconus, its expression is mainly in the retina and not in the cornea or the lens (Heon *et al.* 2002). However, our data showed no pathological changes in this gene and therefore it was excluded as disease-causing in our families. Therefore, combining the data obtained from both microsatellite markers and the 10K GeneChip suggests that our families are probably linked to the *RP25* locus.

The linkage data reported here is similar to that previously reported in Spanish and Pakistani families with arRP (Ruiz *et al.* 1998; Khaliq *et al.* 1999), signifying that *RP25* might be a major locus for recessive RP. However, this will eventually be clarified when the *RP25* gene is cloned and additional mutations in a larger collection of arRP families and sporadic cases have been detected. So far, a number of genes have been excluded from RP25 but many genes remain to be screened (Abd

Theta (q) Markers/F	0.00	0.01	0.05	0.10	0.2	0.3	0.4
D6S452 (10)	1.57	1.54	1.4	1.2	0.83	0.44	0.12
(112)	−0.03	−0.013	−0.012	−0.010	−0.006	−0.002	−0.0007
(116)	−∞	−1.1	−0.54	−0.299	−0.11	−0.003	−0.008
D6S272 (10)	1.57	1.54	1.4	1.2	0.83	0.44	0.12
(112)	0.63	0.61	0.54	0.45	0.29	0.14	0.03
(116)	−∞	−1.3	−0.65	−0.17	−0.06	−0.06	−0.013
D6S294 (10)	0.97	0.93	0.78	0.59	0.26	0.05	0.00
(112)	−0.013	−0.013	−0.012	−0.010	−0.006	−0.002	−0.0007
(116)	−∞	−1.53	−0.82	−0.59	−0.22	−0.08	−0.02
D6S257 (10)	1.57	1.54	1.4	1.2	0.83	0.44	0.12
(112)	0.65	0.63	0.56	0.48	0.31	0.15	0.04
(116)	0.15	0.15	0.15	0.14	0.10	0.05	0.014
D6S402 (10)	1.57	1.54	1.4	1.2	0.83	0.44	0.12
(112)	0.65	0.63	0.56	0.48	0.31	0.15	0.04
(116)	0.24	0.24	0.2	0.15	0.08	0.03	0.007
D6S430 (10)	1.57	1.54	1.4	1.2	0.83	0.44	0.12
(112)	0.65	0.63	0.56	0.48	0.31	0.15	0.04
(116)	0.15	0.15	0.15	0.14	0.1	0.05	0.014
D6S1557 (10)	0.64	0.64	0.59	0.53	0.38	0.21	0.06
(112)	0.65	0.63	0.56	0.48	0.3	0.15	0.04
(116)	0.24	0.24	0.2	0.15	0.08	0.03	0.007
D6S1681 (10)	**1.57**	**1.54**	**1.4**	**1.2**	**0.83**	**0.44**	**0.12**
(112)	**0.85**	**0.83**	**0.74**	**0.63**	**0.40**	**0.20**	**0.05**
(116)	**0.85**	**0.83**	**0.74**	**0.62**	**0.4**	**0.20**	**0.05**
D6S460 (10)	0.64	0.64	0.002	0.53	0.38	0.21	0.06
(112)	−∞	−2.6	−1.3	−0.81	−0.34	−0.13	−0.03
(116)	−∞	−1.4	−0.74	−0.4	−0.19	−0.07	−0.017
D6S445 (10)	0.002	0.002	0.6	0.002	0.001	0.0006	0.0001
(112)	0.013	−0.013	−0.012	−0.01	−0.006	−0.002	−0.0007
(116)	−∞	−1.5	−0.82	−0.51	−0.22	−0.08	−0.02
D6S1004 (10)	0.67	0.66	0.6	0.53	0.37	0.22	0.06
(112)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(116)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D6S462 (10)	0.67	0.66	1.4	0.53	0.37	0.22	0.06
(112)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(116)	−∞	−1.18	−0.5	−0.2	−0.10	−0.03	−0.008
D6S300 (10)	1.57	1.54	1.4	1.2	0.83	0.44	0.12
D6S1671 (10)	1.57	1.54	1.4	1.2	0.83	0.44	0.12
D6S434 (10)	1.57	1.54	1.4	1.2	0.83	0.44	0.12

(F) Families (10, 112 and 116)

Table 5 LOD score for the families studied (10, 112 and 116) in the RP25 interval. The maximum Lod score at the D6S1681 marker is shown in bold

El-Aziz *et al.* 2005, 2006; Marcos *et al.* 2000, 2002, 2003; Li *et al.* 2001; Barragan *et al.* 2005a, 2005b).

In summary, this is the first comprehensive genetic study in the Chinese population with arRP. We have proposed an effective strategy for mapping disease loci in nuclear non-consanguineous recessive families. The three families studied are likely linked to the *RP25* locus previously reported in Spanish and Pakistani populations. Therefore, the *RP25* locus might be considered as a major locus for recessive RP. Mutation screening of genes in the *RP25* region is underway, and we are applying our genetic mapping strategy to other nuclear non-consanguineous recessive families to further investigate its applicability.

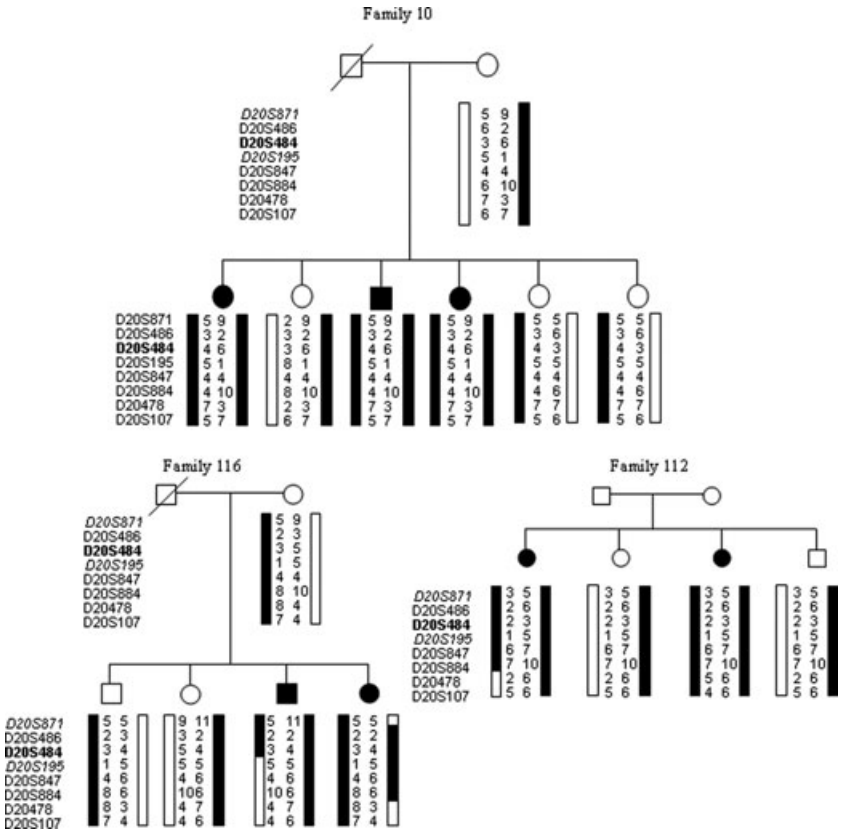

Figure 4 Microsatellite marker analysis of chromosome 20 in families 10, 112 and 116. D20S871 and D20S195 are in italics and define the minimal critical interval in family 116. D20S484, shown in bold, is where the maximum LOD score was achieved in all families.

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