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ARTICLE

Refinement of the RP17 locus for autosomal dominant retinitis pigmentosa, construction of a YAC contig and investigation of the candidate gene retinal fascin

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The RP17 locus for autosomal dominant retinitis pigmentosa has previously been mapped to chromosome 17q by linkage analysis. Two unrelated South African families are linked to this locus and the identification of key recombination events assigned the RP17 locus to a 10 cM interval on 17q22. The work reported here refines the mapping of the locus from a 10 cM to a 1 cM interval between the microsatellite markers D17S1604 and D17S948. A physical map of this interval was constructed using information from the Whitehead/MIT YAC contig WC 17.8. Sequence-tagged site (STS) content mapping of seven overlapping YACs from this contig was employed in order to build the map. A BAC library was screened to cover a gap in the YAC contig and two positive BACs were identified. Intragenic polymorphisms in the retinal fascin gene provided evidence for the exclusion of this candidate as the RP17 disease gene.

Keywords: retinitis pigmentosa; RP17; fine mapping; YAC contig; retinal fascin

Introduction

Retinitis pigmentosa (RP) is a generic name for a group of hereditary disorders that cause progressive retinal degeneration, usually leading to blindness in later life. Patients with RP characteristically experience night blindness, loss of peripheral vision and a decrease in contrast sensitivity. RP segregates as an autosomal dominant (adRP), autosomal recessive or an X-linked trait. The disorder may also occur in association with certain syndromic disorders, *eg* Usher and Refsum syndromes.[1] Nine distinct loci have been mapped for adRP, including the *rhodopsin* and *peripherin-RDS* genes and anonymous loci designated RP1, RP9, RP10, RP11, RP13, RP17 and RP18.[2–4]

The RP17 locus was first reported in 1995 in a South African family of German descent.[3] A second South African adRP family was later shown to be linked to this locus.[5] The two families share a common haplotype that is associated with the disease phenotype, thereby suggesting descent of the disease gene from a common ancestor. By combining the linkage data and using haplotype analysis of recombinant chromosomes in 17 newly recruited family members, the RP17 critical interval was reduced.

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In order to assemble a physical map of the RP17 critical interval, the Whitehead Institute/MIT Center for Genome Research (http://www.genome.wi.mit.edu/) physical map data of YAC contig WC 17.8 was used.[6] Sequence-tagged site (STS) content mapping was performed using CEPH mega-YACs, and STSs and expressed sequence tags (ESTs) from the RP17 region. The refined localisation of the RP17 locus, in conjunction with an overlapping YAC-based contig coverage of the critical interval, should facilitate the identification of this disease gene.

In combination with positional cloning approaches, candidate gene screening has also been undertaken to identify the RP17 gene. The retinal fascin gene was examined as a candidate because it is expressed exclusively in photoreceptor cells[7] and has been mapped to 17q (Tubb *et al*, in preparation).

Materials and Methods

Patient Material and DNA Analysis

Blood was collected from 44 members for family RPD8, and from 25 individuals from family RPD19, and DNA was extracted for linkage analysis using standard techniques. Microsatellite markers were typed on all the DNA samples by PCR with random incorporation of [α^{32}P]dCTP during the reaction. The amplified products (alleles) were resolved by size fractionation on 6% polyacrylamide denaturing gels and visualised by autoradiography. The pedigree relationships in both families were confirmed by the appropriate segregation of all the tested microsatellite markers.

YAC-based STS-content Mapping

Primers for 9 STSs and 3 ESTs were used to screen the CEPH Mega YAC Library. The YACs were selected from the Whitehead/MIT YAC contig WC 17.8. Colony PCR was performed on YACs grown on selective (URA-, TRP-) media, and the PCR products were resolved on 2% agarose gels and detected with ethidium bromide staining.

BAC-based STS-content Mapping

The Human Bacterial Artificial Chromosome DNA Pools Release III (Research Genetics, Inc., Huntsville, AL, USA) were screened according to the manufacturer's instructions. PCR products were electrophoresed on 1% agarose gels.

Vectorette End Fragment Isolation Method

End probes were generated from BAC 100E23 and BAC 229L19 insert DNA using a modified vectorette-PCR procedure.[8] The BAC DNA was digested with *Rsa*I, *Pvu*II and *EcoRV* and then ligated with the blunt-end vectorette cassette as described.[8] Using these three vectorette libraries as templates, PCR was performed with a universal vectorette primer (5' TCT CCC TTC TCg AAT CgT AAC CgT TCg TAC 3') and either primer T7 or SP6 to amplify the 5' or the 3' arm, respectively. The resulting PCR products obtained adjacent to the T7 vector end for BAC 100E23 and adjacent to the SP6 end for BAC 229L19, respectively, were gel-eluted using a QIAquick Gel Extraction Kit (Qiagen, Germany; purchased from: Southern Cross Biotechnology (Pty) Ltd., Cape Town, SA) and labelled with [α^{32}P]dCTP before being used as a probe. One microlitre of each of the seven YACs and the two BACs comprising the RP17 contig was dotted on to a Hybond-N$^+$ membrane (Amersham; purchased from AEC Amersham (Pty) Ltd., SA) and hybridised to the purified vectorette PCR product overnight. The blot was washed to a final stringency of 0.6 × SSC; 0.3% SDS at 65°C and exposed to X-ray film (Agfa Curix, Germany; purchased from: Agfa Division of Bayer (Pty) Ltd., SA) for 20 h.

SSCP Analysis and DNA Sequencing of Retinal Fascin

Two polymorphisms found in the 5' putative promoter region and an intragenic polymorphism found in exon 1 of the retinal fascin gene were genotyped in the two adRP families using standard SSCP techniques.[9] The allele frequencies observed for the two 5' polymorphisms are 0.84 and 0.16 for an A to G transversion 707 bp upstream of the initiation codon; and 0.96 and 0.04 for a C to G transition 622 bp upstream of the initiation codon (Tubb *et al*, in preparation). These two polymorphisms were amplified and detected in a single PCR product using the following primers, forward-5' gAC gTC gAC Agg CTg CAC ggC CAC TgT gT 3' and reverse-5' TgA TCC CCT TTT CCT CAT AgC 3'. The polymorphism in exon 1 is a T to C transition located 738 bp 3' of the initiation codon. The PCR primers used to amplify this polymorphism are: forward-5' gCA TgA ATT CgA gCC gAC ggT ACT gCC TCA AgT CC 3' and reverse-5' CgT AgT CgA CCT TgC CgC ACA gAg ACg TAg Cgg Tg 3'.

Primers were designed to PCR amplify the 5 exons of retinal fascin and the products were sequenced on an ABI PRISM 310 Genetic Analyser (Perkin Elmer, Norwalk, CT, USA).

Results

A total of 44 members from family RPD8 (Figure 1a) and 25 members from family RPD19 (Figure 1b) were genotyped with microsatellite markers from 17q22. The microsatellite markers used in the study and the genetic distances between them are illustrated in Figure 2.[10] The analysis of recombinant events in informative meioses allowed for the fine mapping of the RP17 locus from a 10 cM to a 1 cM critical interval. One individual, V-15 (Figure 1a) is recombinant for the markers D17S790 through to D17S1604. In this individual the maternally inherited chromosome had recombined. He is an unequivocally affected 55-year-old male and on dilated fundal examination, was found to have the typical features of retinitis pigmentosa (including waxy pallor of the optic nerve heads, retinal vascular attenuation and some retinal pigment epithelial atrophy). This recombinant provides evidence that the adRP disease gene maps distal to D17S1604.

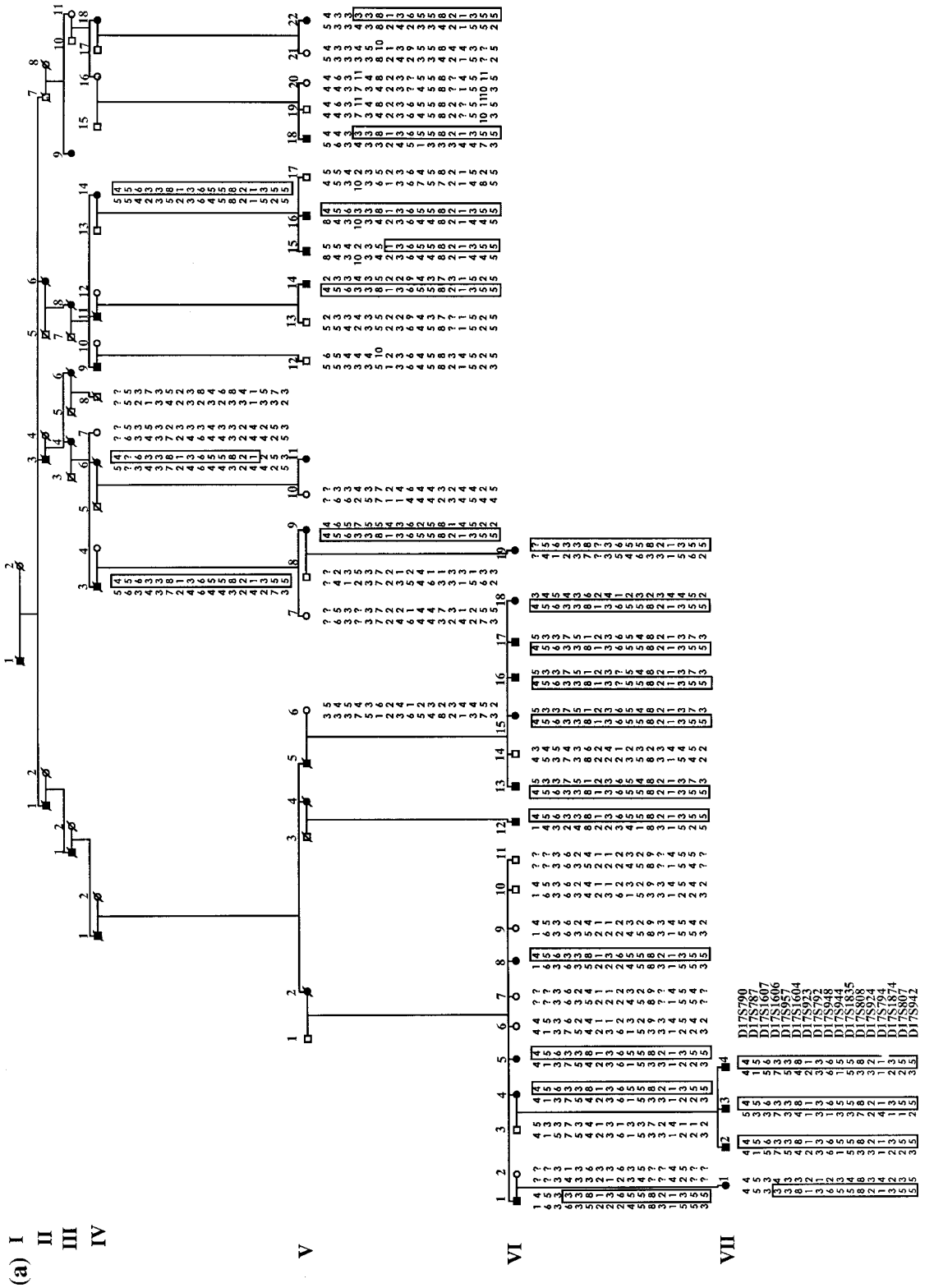

Figure 1 *Pedigrees of the two kindreds, (**a**) family RPD8 and (**b**) family RPD19 linked to the RP17 locus. The haplotype associated with the adRP disease phenotype is depicted in a box beneath each individual. In family RPD19, individuals III-8, IV-5, IV-6, and IV-7 inherit the disease-associated haplotype for markers D17S948 to D17S942 but they do not exhibit any manifestations of RP (see text)*

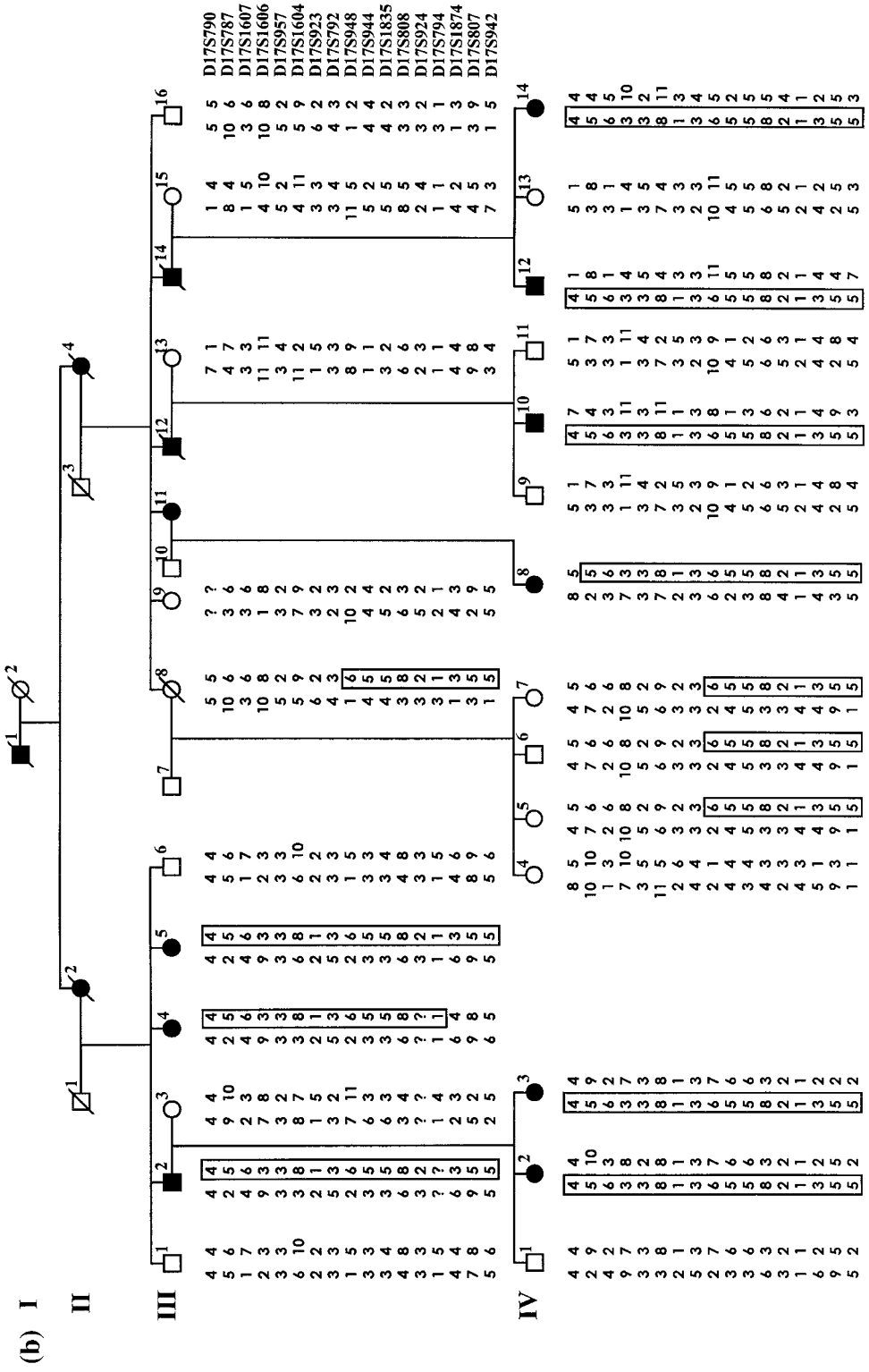

Figure 1 *Continued*

Similarly, individual III-8 (Figure 1b) is recombinant for the markers D17S948 through to D17S942. In this individual the maternally inherited chromosome had recombined. She died at age 73, and had evidently,

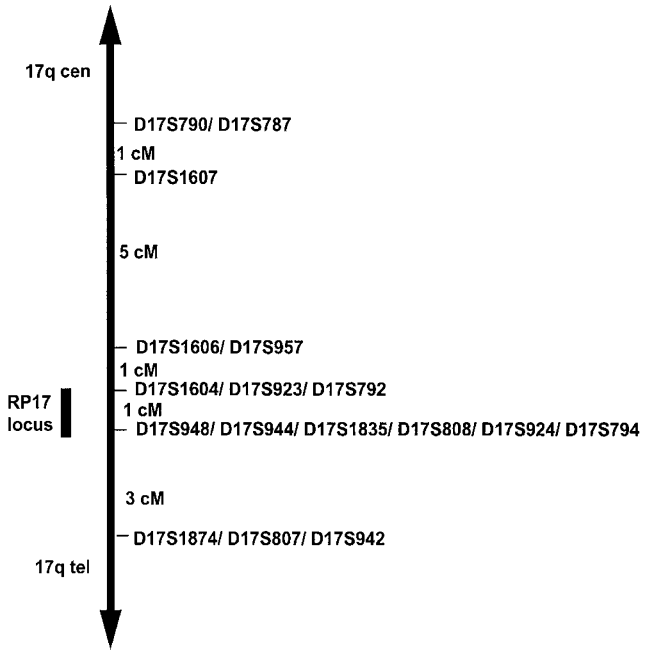

Figure 2 *Schematic diagram depicting the positions of the microsatellite markers on 17q22 used in the study as well as the genetic distances calculated between adjacent loci. Markers separated by a slash (/) could be separated genetically using the CEPH reference families*

except for the presence of cataracts, not shown any signs of visual problems up to her death. The construction of haplotypes indicates that three of her four offspring (individuals IV-5, IV-6 and IV-7) had inherited the recombinant allele from their mother. They are all over 50 years of age (the mean age of onset of RP in the family is between 20 and 30 years of age) and were subsequently referred to an ophthalmologist for examination. No abnormality suggestive of retinitis pigmentosa was detected. The haplotype data presented here therefore provides evidence that the adRP disease gene maps proximal to D17S948.

By combining the haplotype data obtained from the two families, the minimal co-segregating region for RP17 was refined to a 1 cM interval between markers D17S1604 and D17S948. A physical map of this 1 cM interval was constructed using the Whitehead/MIT database and the CEPH Mega YAC library. STS-content analysis was employed to construct a contig of seven overlapping YACs as illustrated in Figure 3. A total of 9 STSs and 3 ESTs from the RP17 critical interval were used and the YACs were ordered according to their marker content. Upon further investigation, it was revealed that there was a gap in the YAC contig between markers WI-6805 and CHLC.GATA11C11 and an attempt was made to cover this interval by screening a BAC library. Two positive clones were

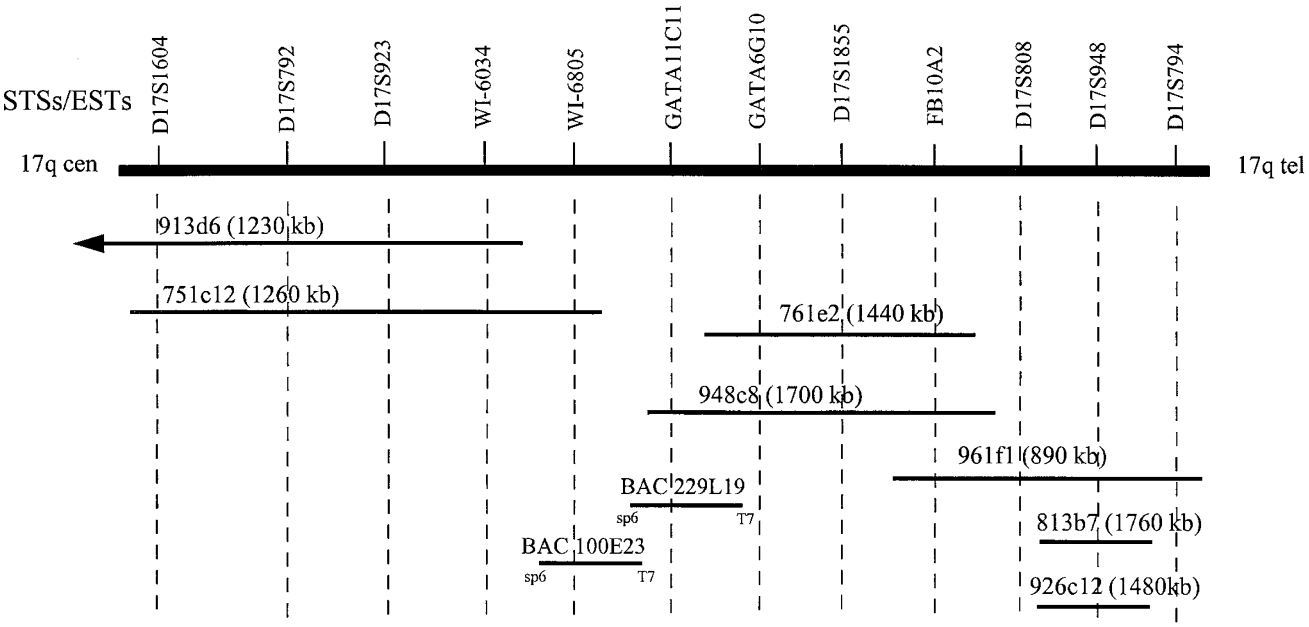

Figure 3 *A diagram illustrating a YAC contig and STS marker map spanning the RP17 critical interval. A gap in the contig is evident between markers WI-6805 and CHLC.GATA11C11. Investigations to cover this gap identified the two BAC clones 100E23 and 229L19*

identified: BAC 100E23 (positive for WI-6805) and BAC 229L19 (positive for CHLC.GATA11C11). Using the modified vectorette end fragment isolation method,[8] an end-clone of BAC 100E23 insert DNA was produced. Southern blot analysis indicated that this 500 bp end-clone hybridised to BAC 229L19 DNA. Similarly, an end probe derived from BAC 229L19 was shown to hybridise to BAC 100E23. These results therefore provide strong evidence that the two BACs overlap and, assuming that none of the YACs have an internal deletion, thereby ensure complete and overlapping clone coverage of the RP17 critical interval.

Retinal fascin was considered a good candidate for the RP17 disease gene, however, the detection of multiple recombination events in both families using novel intragenic polymorphisms, provided evidence for the exclusion of this gene. Furthermore, DNA sequencing did not reveal any pathogenic mutations in the five exons of the gene.

Discussion

Since the initial mapping of the RP17 locus to a 10 cM interval, DNA from an additional 17 family members from family RPD8 and family RPD19 has been collected for study. Based on recombination analysis in the two families, RP17 has been refined to an interval of approximately 1 cM between D17S1604 and D17S948 on 17q22 (Figure 2).

The newly constructed RP17 YAC/BAC contig spanning the interval lays the groundwork for the construction of a gene map for this region. Although 1 cM may be small by genetic standards, this interval may contain more than 50 separate genes (given an average gene density of five genes per 100 kb for the human genome). The rate-limiting step in positional cloning approaches is the identification and subsequent characterisation of genes in the critical interval. In an attempt to isolate the RP17 gene, the cDNA selection approach[11] will be used to create a transcription map of the candidate region.

Our complementary approach to genetic and physical mapping involves the testing of candidate genes suspected to lie within the critical interval. One such candidate is the retinal fascin gene which was shown to not be the adRP gene in the two 17q-linked families. The information presented here on the three novel polymorphisms in the retinal fascin gene should facilitate investigations involving this gene in other retinopathies localised to 17q.

Also under investigation are other positional candidates including the EST FB10A2 and the T-Box 2 gene *TBX2*.[12,13] FB10A2 maps within the RP17 YAC contig and has been found in retinal-specific cDNA libraries. The transcriptional regulator TBX2 has been mapped to a YAC from this contig, clone 961f1[12] and exhibits strong sequence homology, within the T-box domain, to the *Drosophila* optomotor blind (*omb*) gene.[13] Mutations in two other T-box genes *TBX5* and *TBX3* have been shown to be responsible for Holt-Oram syndrome[14] and ulnar-mammary syndrome,[15] respectively.

In conclusion, these studies significantly refine the RP17 critical interval and provide the cloned genomic DNA necessary for positional cloning of the gene causative of this specific form of retinitis pigmentosa.

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