LETTER TO JMG

Novel association of RP1 gene mutations with autosomal recessive retinitis pigmentosa

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RP1 gene mutations are the second most common cause of autosomal dominant retinitis pigmentosa (RP).

A homozygous T373I mutation (ACA to ATA) was found to segregate with the disease in two autosomal recessive (ar) RP families from Pakistan.

A 4 bp insertion at nucleotide 1461 adds a termination codon after codon 487 in another arRP family. This results in the production of a severely truncated protein of 487 instead of 2156 amino acids.

A second missense A669T substitution (GCC to ACC) was found in a heterozygous state in one sample from a panel of 150 RP patients.

These results suggest that the frequency of RP1 gene mutations is relatively low in Pakistan.

To our knowledge this study provides the first report of the involvement of mutations in the RP1 gene in the autosomal recessive RP phenotype.

For genetic analysis, peripheral blood samples were collected from all members of the families with informed consent. Control samples were also collected from 100 ethnically matched, unrelated, normal Pakistani individuals. Patients were examined at the retina clinic of one of the KRL General Hospital, Islamabad, the Al-Shifa Trust Eye Hospital, Rawalpindi, and the Christian Hospital, Taxila.

Linkage analysis

Genomic DNA was extracted from whole blood using the standard phenol/chloroform extraction procedure. DNA was amplified using specific primers for polymorphic microsatellite markers (version 8; Research Genetics) for known genes and loci associated with various types of retinal degeneration according to the conditions described previously.

Mutation detection

Exon specific primers were designed from the genomic sequence of RP1.7 PCR was performed in a 50 µl reaction volume using 1 µg of genomic DNA. Heteroduplex analysis was performed using an automated denaturing high performance liquid chromatography (DHPLC) instrument (WAVE DNA Fragment Analysis System, Transgenomic, Crewe, UK).8 Samples were prepared by denaturing and re-annealing of the unpurified PCR products. The temperature conditions required for the successful resolution of heteroduplexes were obtained from the Stanford University website (http://insertion.stanford.edu/melt.html).

Following heteroduplex analysis, DNA from all family members was sequenced in the forward and reverse directions using a commercially available kit (Big Dye, Applied Biosystems), and the products were analysed on an ABI...
Prism 377 automated DNA sequencer. To examine the possibility that the mutations are polymorphisms, a panel of 100 normal individuals was also analysed for RP1 gene mutations, initially by DHPLC followed by direct DNA sequencing.

RESULTS AND DISCUSSION

Exclusion studies of known RP loci were carried out for the three arRP families. Homozygosity was observed for markers D8S285 and D8S1815 for all the patients. This locus contains RP1, which has previously been reported to be associated with dominant RP.

As most of the mutations identified previously are clustered in a small region, the 5' region of exon 4 was screened. DHPLC analysis showed heteroduplex peaks for the unaffected parents and some of the siblings of the patients in both the 442RP and 452RP families. Samples of all affected individuals showed homoduplexes. All the family members were then sequenced to identify the change in exon 4. Sequencing analysis revealed that patients had a homozygous C→T substitution at nucleotide 1118 (fig 2A). Parents of the affected individuals were heterozygous for this change. This substitution causes a missense codon 373 that codes for isoleucine (ATA) instead of threonine (ACA) in the mutated protein product. The normal family members including their parents (carriers) who are heterozygous for the Thr373Ile change have normal vision with no signs of RP. Eukaryotic linear motif (ELM: http://elm.eu.org/) searches showed that the T373I missense mutation abolishes the glycogen synthase phosphorylation recognition site (GSK3), resulting in a conformational change in the mutated protein. To check if there was another mutation in this gene associated with the disease phenotype in both these families, the remaining five coding exons of the RP1 gene were also sequenced. No other disease associated change was found in any patient of the 442RP and 452RP families. None of the 100 normal controls carried the T373I change in the homozygous state, although some heterozygous individuals were observed. Payne et al10 have reported this change to cause adRP; however, Berson et al11 categorised this mutation as non-pathogenic because it did not cosegregate with the disease phenotype in one of their adRP families. They suggested that mutations in RP1 might be recessive, causing RP or some other retinal disease. Our data shows that the homozygous T373I change causes arRP in consanguineous families.

In the unaffected members of the third family (336RP), heteroduplex peaks were also observed for another fragment of exon 4. Sequencing analysis of all family members showed a homozygous 4 bp insertion (1461–1465insTGAA) in the patients (fig 2B). This insertion adds a stop codon immediately after nucleotide 1461, resulting in a severely truncated protein of 487 instead of 2156 amino acids. This 4 bp insertion was present in a heterozygous state in some unaffected members of the family, including the parents of the patients. Subsequently, the panel of normal controls was screened for this insertion. None of the 100 normal individuals carried this change.

A random panel of 150 RP patients was also screened for RP1 gene mutations. A heterozygous single base pair G→A substitution at nucleotide position 2005 was found in one patient (fig 2C). This substitution replaces alanine (GCC) with threonine (ACC) at codon 669. This change was not found in any of the 100 normal individuals.

In addition to these disease causing mutations, several sequence variants and polymorphisms were also found in this study (table 1). These results show that two polymorphisms, N985Y and P1205P, are prevalent in the Pakistani samples.

Previous studies have identified 21 different disease causing mutations that are responsible for 6–10% cases of adRP in ethnically diverse populations.5 7 11 None of these mutations were found in the Pakistani samples. Identification of only one mutation in the unrelated 150 RP patients and two mutations with recessive RP among a large number of patients and families shows that RP1 gene mutations are not a major cause of RP in Pakistan.

Dominant RP caused by mutations in the RP1 gene often shows late onset of the disease phenotype, usually by the third decade of life. Pierce et al9 observed that patients suffering from adRP who were heterozygous for the RP1 mutation had classic, less severe adRP phenotype with late onset of disease. In contrast, patients that were homozygous for the mutation in the same family had substantially more severe forms of the disease and the age of onset was variable.12 All three recessive Pakistani families presented here showed the severe form of RP with early onset.

It is well known that Mendelian disorders caused by the dysfunction of a single gene have a wide heterogeneity of disease phenotypes.13 RP is a heterogeneous group of retinal disorders and mutations within a single gene are known to cause different clinical phenotypes.14 15 The work presented here shows, for the first time, that arRP is also caused by (homozygous) mutations in the RP1 gene. This could be due...
to centuries of consanguineous marriages in this gene pool, altering the penetrance of the mutated gene in the heterozygous state.

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