Molecular genetics of autosomal dominant retinitis pigmentosa (ADRP): a comprehensive study of 43 Italian families

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Retinitis pigmentosa is the most common form of retinal degeneration and is heterogeneous both clinically and genetically. The autosomal dominant forms (ADRP) can be caused by mutations in 12 different genes. This report describes the first simultaneous mutation analysis of all the known ADRP genes in the same population, represented by 43 Italian families. This analysis allowed the identification of causative mutations in 12 of the families (28% of the total). Seven different mutations were identified, two of which are novel (458delC and 6901C→T [P2301S]), in the CRX and PRPF8 genes, respectively. Several novel polymorphisms leading to amino acid changes in the FSCN2, NRII, IMPDH1, and RP1 genes were also identified. Analysis of gene prevalences indicates that the relative involvement of the RHOD and the RDS genes in the pathogenesis of ADRP is less in Italy than in US and UK populations. As causative mutations were not found in over 70% of the families analysed, this study suggests the presence of further novel genes or sequence elements involved in the pathogenesis of ADRP.

METHODS

Patients

ADRP families were recruited from northern, central, and southern Italy. Informed consent was obtained from all adult subjects enrolled in the study. The six ophthalmological centres that participated in the study used the same protocols for the clinical diagnosis and the classification of patients. Patients showing a well defined autosomal pattern of inheritance were selected (n = 43)—in particular those characterised by the presence of at least two affected generations and male to male transmission. The ophthalmological examination included the best corrected visual acuity with the Snellen visual chart, slit lamp biomicroscopy, fundus examination, Goldmann kinetic visual field examination, and electroretinography. The ERG was recorded by means of corneal contact lens electrodes with a Ganzfeld stimulator according to international clinical standards.

Mutation analyses

Genomic DNA was extracted from blood samples employing standard techniques and amplified by polymerase chain reaction (PCR) using oligonucleotide primer pairs that amplify the coding exons as well as the intron–exon junctions of the ADRP genes selected for this study. The sequences of the primers and the PCR conditions were as previously described and are summarised in supplementary table 1, which can be viewed on the JMG website (http://www.jmedgenet.com).

Abbreviations: ADRP, autosomal dominant retinitis pigmentosa; dHPLC, denaturing high performance liquid chromatography
We carried out mutation analysis in the selected families on Mutation analyses genes in the pathogenesis of ADRP in Italy. To assess whether or not they segregated with the retinitis patients recruited in this study assume that the incidence of retinitis pigmentosa in Italy is 1/17% of the entire population of people with the disease. If we estimate that the prevalence of ADRP in Italy constitutes approximately pigmentosa in Italy (unpublished data), we estimated that the incidence of retinitis pigmentosa (403C→T at the nucleotide level) represents the most frequent mutation in our set, as it was found in four families (9% of the total and 57% of all RHO mutations). Two other mutations were found in more than one family, namely the RHO P347L and the RP1 R677X (1040C→T and 2029C→T, respectively, at the nucleotide level).

Two new mutations were identified in this study, one in CRX and one in PRPF8. The novel CRX mutation was represented by the deletion of a cytosine at nucleotide position 458 (458delC) which leads to a premature truncation of the CRX protein at position 153 (P153fs). Patients harbouring this mutation have retinitis pigmentosa with macular dystrophy and extinguished ERG, which is consistent with the previously described ADRP phenotypes caused by CRX mutations. On the other hand, the novel PRPF8 mutation was a substitution of a cysteine with a thymidine at nucleotide position 6901 (6901C→T) which leads to the substitution of the proline in position 2301 of the PRPF8 predicted protein with a serine (P2301S). This mutation represents the seventh missense mutation identified in the PRPF8 gene so far. This suggests that the nucleotide at position 6901 of the PRPF8 coding sequence is a sensitive site for mutations, as it was already found to be the target of an ADRP missense mutation identified by McKie et al (namely 6901C→A, leading to P2301T).

As expected, the severity of the clinical phenotype in the families carrying the identified mutations could be related to the mutated gene. For example, in all the patients with RHO mutations, we observed a more severe phenotype. The patients had early onset of disease, retained central visual acuity until the second decade of life, had concentric isoptere shrinkage of up to 10° in the centre at the Goldmann kinetic visual field examination, and had extinguished electroretinograms in the early stages of the disease. On the other hand—as also previously described—the clinical phenotype

Table 1: Summary of autosomal dominant retinitis pigmentosa mutations identified

<table>
<thead>
<tr>
<th>Gene</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Type of mutation</th>
<th>Exon</th>
<th>Number of families</th>
<th>Relative frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>RP1</td>
<td>2029C→T</td>
<td>R677X</td>
<td>Nonsense</td>
<td>4</td>
<td>2</td>
<td>4.6%</td>
</tr>
<tr>
<td>RHO</td>
<td>403C→T</td>
<td>R135W</td>
<td>Missense</td>
<td>2</td>
<td>4</td>
<td>9.3%</td>
</tr>
<tr>
<td>RHO</td>
<td>1040C→T</td>
<td>P347L</td>
<td>Missense</td>
<td>5</td>
<td>2</td>
<td>4.6%</td>
</tr>
<tr>
<td>PRPF8</td>
<td>6901C→T</td>
<td>P2301S</td>
<td>Missense</td>
<td>42</td>
<td>1</td>
<td>2.3%</td>
</tr>
</tbody>
</table>

Table 2: Novel single nucleotide polymorphisms leading to amino acid variations identified in autosomal dominant retinitis pigmentosa genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMPDH1</td>
<td>1142A→G</td>
<td>H381R</td>
</tr>
<tr>
<td>NRI</td>
<td>199C→T</td>
<td>P67S</td>
</tr>
<tr>
<td>FSCN2</td>
<td>412C→T</td>
<td>H138Y</td>
</tr>
<tr>
<td>RP1</td>
<td>5448C→A</td>
<td>C1816X</td>
</tr>
<tr>
<td>RP1</td>
<td>1380G→C</td>
<td>K460N</td>
</tr>
<tr>
<td>RP1</td>
<td>4735T→G</td>
<td>L1579V</td>
</tr>
<tr>
<td>RP1</td>
<td>1705A→G</td>
<td>T569A</td>
</tr>
</tbody>
</table>
associated with RPI gene mutations was less severe with a late onset of disease, after the third decade of life. The patients retained good visual acuity and recordable rod and cone ERG signals until the start of the fourth decade.

**Identification of novel polymorphisms**

We also found novel single nucleotide polymorphisms in some of the genes analysed (RPI, IMPDH1, NRL, and FSCN2) that caused amino acid substitutions (table 2). In particular, the RPI gene has a significantly high frequency of missense variations with no pathogenic significance. Interestingly, one of these sequence variants, 5448C→A, which determines a premature truncation of the RPI protein at amino acid position 1816 (C1816X), was found in the homozygous state in an unaffected relative of an ADRP patient. It was previously reported that premature truncations of the RPI protein in the C-terminal part (R1933X) were not involved in the retinitis pigmentosa pathogenesis. Our data not only extend to the amino acid position 1816 the N-terminal border for non-pathogenic sequence variations in RPI but also suggest that most of the 340 C-terminal amino acids of this protein are not endowed with an important function, because their complete loss in homozygosity is apparently well tolerated and not associated with any abnormal phenotype. This hypothesis is also confirmed by the low conservation across evolution of this portion of the RPI protein. The most common RHO mutation identified was the R135W (present in four families) followed by the P347L (in two families). On the other hand, the P23H mutation, which is the most common one found in the USA, was not detected in our sample, and this is consistent with previous studies carried out in other European populations.

Two families were mutated in the RPI gene, which therefore represents, after RHO, the gene most commonly responsible for ADRP in Italy. The other genes mutated in ADRP families were CRX, NRL, and PRPF8 (table 1). No mutations were detected in RDS, IMPDH1, and PRPF31, which together account for about 15% of ADRP in non-Italian populations (RETNET, http://www.sph.uth.tmc.edu/Retnet/disease.htm). Altogether, these findings confirm that retinitis pigmentosa pathogenesis is very variable in different populations.

We did not identify causative mutations in any of the 12 ADRP genes screened in over 70% of the families analysed (n = 31; fig 1). Thus the pathogenesis of the majority of ADRP families in Italy is not accounted for by mutations in the coding exons of the retinitis pigmentosa genes tested. We cannot exclude the possibility that the genes analysed in this report may nevertheless underlie retinitis pigmentosa pathogenesis in a fraction of these 31 Italian families owing to mutations residing in either unidentified exons or in regulatory elements of the analysed genes. However, our results strongly suggest the presence of additional unidentified genes that are involved in the pathogenesis of ADRP in these families, and extensive linkage analysis will be needed to discriminate between these two hypotheses.

We believe that this study, which is the first simultaneous and comprehensive clinical/molecular analysis of all the currently known ADRP genes in a well characterised set of families, will improve genetic counselling and the prognostic evaluation of retinitis pigmentosa in Italian patients, and shed further light on the molecular mechanisms underlying this complex group of disorders.

**ACKNOWLEDGEMENTS**

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**Figure 1** Classification of the families with autosomal dominant retinitis pigmentosa, based on the results of the mutation analyses.
REFERENCES


