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Identification of a Polymorphic Missense (G338D) and Silent (106V and 121L) Mutations within the Coding Region of the Peripherin/RDS Gene in a Patient with Retinitis Punctata Albescens

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Retinitis punctata albescens is a progressive retinal disorder which shows a variety of clinical manifestations. It is very similar to retinitis pigmentosa, but the affected individuals do not show intraretinal pigmentation. In this report we studied one sporadic type retinitis punctata albescens patient with the assumption that mutation in the peripherin/RDS gene could contribute to the disease phenotype. We have analyzed the sample from an affected individual by single-strand conformation polymorphism following polymerase chain reaction amplification of the exons of the peripherin/RDS gene. The amplified products were sequenced by the dideoxy chain termination method. DNA sequencing of the exon-1 revealed two silent mutations (106V and 121L) and of exon-3 one missense mutation (G338D) in the affected individual. These mutations were not found in unrelated individuals in certain normal population. The above data suggest that sporadic retinitis punctata albescens is not caused by mutations within the coding sequence of the peripherin/RDS gene.

Retinitis punctata albescens (RPA) is a clinically heterogeneous retinal disorder which usually affects at an early age. It is characterized by attenuated retinal vessels, subretinal white, yellow white or light grey dots, color vision impairment, decreased visual acuity, loss of visual field and night blindness (1). The disease is a progressive disorder which shows a variety of clinical manifestations. Its degenerative course and electroretinogram (ERG) are very similar to retinitis pigmentosa (RP) but the affected individuals do not show intraretinal pigmentation. The condition is inherited as an autosomal dominant, recessive and sporadic type. The biochemical basis of RPA is not understood. However, recently a null mutation in the peripherin/RDS gene was shown to be associated with the autosomal dominant type disorder (2). In this report we describe one patient with a sporadic type RPA and a screening of the peripherin/RDS gene with the assumption that mutations in this gene could contribute to the disease phenotype. We found a missense and two silent mutations in the third and first exons, respectively, of the peripherin/RDS gene.

MATERIALS AND METHODS

The family under study is shown in Fig 1 (panel A). The proband at the age of 14 complained about difficulty in reading. A careful ophthalmological examination showed a visual acuity of 20/600 OD and 2/400 OS. The patient had subretinal yellow dots and attenuated retinal vessels, but did not show intraretinal pigmentation. An electroretinogram (ERG) performed on the proband indicated a flat response whether stimulated with a bright flash blue, bright flash red or bright flash white light stimulus. A flickering white light at 30 cycles per second also resulted in a poor response. The tracing appeared quite symmetric when comparing the two eyes suggesting a disturbance in the rod and cone function of both eyes. The remaining members of the family were found to be clinically and electrophysiologically normal.

We collected venous blood from the affected patient (the other members of the family unfortunately were not available for this study) and 9 unrelated normal individuals. The high molecular weight DNA was extracted as described (3). We used a combination of the single strand conformation polymorphism (SSCP) method (4) and DNA sequencing to screen the coding region of the peripherin/RDS gene. The three exons of the gene were amplified by the polymerase chain reaction (PCR) by using the conditions and the primers described elsewhere (2,5,6). DNA fragments that showed an alteration in their mobility were sequenced by the standard dideoxy terminator technique to locate the specific nucleotide alteration.

RESULTS AND DISCUSSION

The results of screening for mutations in the peripherin/RDS gene by SSCP (Fig 1, panel B) and sequencing

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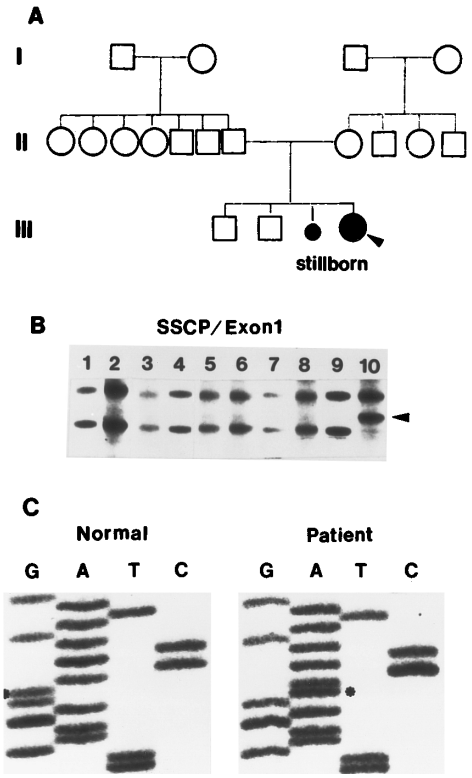

FIG. 1. Pedigree and mutational analysis of peripherin/RDS gene. Panel A: Pedigree of a sporadic RPA family. White circles and squares denote unaffected females and males, respectively. The black circle denotes an affected female. The proband is shown by an arrow mark. Panel B: SSCP analysis of the peripherin/RDS gene exon-1. Lanes 1–9 represent amplified DNA from unaffected normal individuals and lane 10 is from an affected female in the pedigree. The variant strand is indicated by an arrowhead. Panel C: mutation analysis of the RPA patient by DNA sequencing. A transition mutation (G to A) is indicated by an asterisk.

(Fig 1, panel C) are shown. Of 10 individuals (9 were unrelated normal controls, lanes 1-9, panel B) only one SSCP alteration (the affected individual, lane 10, panel B) was observed. DNA sequencing of the exon-1, revealed a G to A transition (panel C) in codon 106 and a G to T transversion (data not shown) in codon 121 in the affected patient. Both of these changes correspond to silent mutations in the amino acids valine and leucine respectively. Similar analyses of exon-2 did not reveal any additional sequence alterations. However, the sequencing of amplified products of exon-3 revealed a homozygous missense mutation (Fig. 2) at codon 338 (G338D) in the affected individual. This mutation has changed the encoded amino acid glycine to aspartic acid and is not found in unrelated normal individuals.

Peripherin/RDS is a photoreceptor-specific protein, localized to the rim region of the disc membrane and implicated in some form of RP, RPA, sporadic RP and other forms of photoreceptor degeneration (7). Since several other sporadic type disorders such as sporadic aniridia (8) meningiomas (9) and Alzheimer's disease (10) show mutations in aniridia, neurofibromatosis type II, and APOE-epsilon 4 genes, respectively, it is logical to assume that the peripherin/RDS gene could be involved in a sporadic case of RPA. The data presented above indicate one silent mutation in exon-1 (second silent mutation is not shown) and one missense mutation in exon-3. The silent mutations have not been found in 9 unrelated normal controls (from our own laboratory) and 160 control subjects around the world (11). Similar silent mutations in rhodopsin (12), the β-subunit of rod phosphodiesterase (13) (these proteins are known to be associated with retinal degeneration) and cystic fibrosis transmembrane conductance regulator (CFTR) gene (14) have been described. However, none of these mutations have been found to be the cause for the disease. Therefore, it is unlikely that the silent mutations in exon-1 described in the present study can cause sporadic RPA. However, we cannot rule out at present the possibility that such silent mutations (G to A and G to T) in the gene could introduce a cryptic splice-site or could interfere in the translation of mRNA thereby contributing to the sporadic RPA phenotype. On the other hand, the missense mutation may be more deleterious to the protein. However, it is not possible at present to conclude that it is disease related simply because the same mutation has been described in certain normal population (5) although our normal controls did not show this variation. Hence, it may represent a polymorphic variation which does not produce pathological problems similar to the mutation described in the factor IX gene. Although we cannot completely exclude the peripherin/RDS gene as a candidate gene, the above data suggests that sporadic RPA is not caused by mutations within the coding sequence

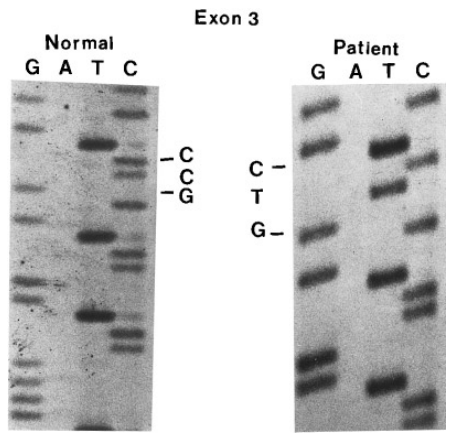

FIG. 2. Identification of a missense mutation in the third exon of the peripherin/RDS gene. The nucleotide sequence of the mutant part of the exon-3 are shown. The sequence change in the proband is G to A which results in the substitution of the amino acid glycine to aspartic acid. This mutation has not been seen in unrelated normal individuals.

of the peripherin/RDS gene. Secondly, the amino acid substitution in the C-terminal region of the protein appears to be highly tolerable for the function of the protein. Further experiments such as site-directed mutagenesis and transgenic mice are needed to understand the role of this mutation in the disease phenotype.

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