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Autosomal Dominant Retinitis Pigmentosa: Absence of the Rhodopsin Proline→Histidine Substitution (codon 23) in Pedigrees from Europe

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Summary

In exon 1 at codon 23 of the rhodopsin gene, a mutation resulting in a proline-to-histidine substitution has previously been observed in approximately 12% of American autosomal dominant retinitis pigmentosa (ADRP) patients. The region around the site of this mutation in the rhodopsin gene has been amplified and analyzed in affected individuals from 91 European ADRP pedigrees. The codon 23 mutation has been found to be absent in all cases, including a large Irish pedigree in which the disease gene has previously been shown to be closely linked to the rhodopsin locus. This indicates the presence of either allelic or nonallelic heterogeneity in ADRP.

Introduction

The group of inherited retinopathies termed retinitis pigmentosa (RP) contributes significantly to visual dysfunction in human populations, with prevalence estimates ranging from 1/3,000 to 1/7,000 (Bundey and Crews 1984; Bunker et al. 1984). Clinical features initially include reduced night and midperipheral vision, typically leading to the eventual loss of far-peripheral and central visual fields (Heckenlively 1988). The primary pathological change in RP is the degeneration of the photoreceptors and the outer nuclear layers of the retina (Flannery et al. 1989). Funduscopic changes including retinal vessel attenuation, pigmentary deposits, depigmentation and atrophy of the retinal pigment epithelium, and optic-disk pallor are frequently observed.

RP is both clinically and genetically heterogeneous and can be divided into X linked (XLRP), autosomal dominant (ADRP), and autosomal recessive (ARRP) groups based on pattern of inheritance. However, further genetic heterogeneity exists within these categories. The presence of two loci on the X chromosome separated by approximately 20 cM has been firmly established using combined linkage data from eight research groups (Ott et al. 1990). In the case of ADRP the disease gene segregating in a large Irish pedigree has been mapped close to the DNA marker D3S47 on 3q (McWilliam et al. 1989). More recently ADRP genes present in a number of other pedigrees have been excluded from this region of 3q, hence providing strong evidence for the presence of genetic heterogeneity within ADRP (Inglehearn et al. 1990; Farrar et al., in press; Jiménez et al., in press). Furthermore, initial evidence for a second ADRP gene on 3q approximately 10 cM from D3S47 has been reported (Olsson et al. 1990). The genetically heterogeneous nature of this group of inherited retinopathies is beginning to emerge.

The gene coding for rhodopsin, a rod photoreceptor–specific gene product, also maps to 3q. In a recent study a C→A transversion at codon 23 of the rhodop-

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sin gene, resulting in a proline→histidine substitution, has been observed in approximately 12% of ADRP patients (Dryja et al. 1990) and was not observed in a control group of unaffected individuals. Moreover, the proline residue at codon 23 is highly conserved in many receptor and G-proteins, hence implicating this mutation as causative in ADRP. It is interesting that the majority of patients showing this mutation were of British origin. In view of these data we have analyzed either by direct sequencing or by allele-specific oligonucleotide hybridization the region around codon 23 of the rhodopsin gene in patients from 91 ADRP pedigrees from Europe to investigate whether the same mutation is present. Included in this group is the ADRP family in which the disease gene was first mapped on 3q (McWilliam et al. 1989) and in which the disease gene has since been shown to be closely linked to the rhodopsin locus at .00 recombination (Farrar et al., in press).

Material and Methods

ADRP Pedigrees

The 21 Irish pedigrees assessed for the codon 23 mutation showed a probable autosomal dominant mode of inheritance. Affected members exhibited classical changes characteristic of RP, including night blindness, reduced peripheral visual fields, intraretinal bone-corpuscle pigmentary deposits in 360° of the retinal midperiphery, waxy disk pallor, retinal vessel attenuation, and reduced or extinguished electroretinograms (ERGs). All patients on whom ERGs were obtained were tested by using Arden's short protocol (Arden et al. 1983). Responses were recorded using Medelec equipment. Wave responses were computer averaged. The ERG was considered extinguished if the b-wave response was less than 5 μV. The Irish pedigrees were ascertained either through the Royal Victoria Eye and Ear Hospital, Dublin, or the Royal Victoria Hospital, Belfast, the former being a referral center for ophthalmological conditions in the Republic of Ireland and the latter being a referral center for Northern Ireland. The pedigrees from Northern Ireland were ascertained from an RP register which contains almost all cases of RP in Northern Ireland (B. Page and R. Redmond, unpublished data), whereas those from the Republic of Ireland have been referred from ophthalmologists throughout the country. According to estimates of the prevalence of ADRP in the Republic which have been calculated from the number of probands and the ascertainment bias for the ADRP group, these ADRP pedigrees represent approximately 60% of the estimated population of ADRP patients in the Republic of Ireland, resulting in a total estimate (for the Republic of Ireland and Northern Ireland combined) of approximately 70%.

The 25 British families included in the present study were ascertained from the genetic register of one of us (M.J.). Clinical examinations in all of these families were conducted by Professors A. C. Bird and B. Jay (Moorefields Eye Hospital, London).

The German families were ascertained from an RP register of the German RP Association by H. Gusseck and Dr. K. Gerull. All patients were personally interviewed. Both family history and medical records available were suggestive for ADRP.

Six Swiss ADRP families were ascertained by one of us (A.S.). A detailed ophthalmological examination was carried out on at least one patient in each family.

DNA Extraction, Polymerase Chain Reaction (PCR) Amplification, and DNA Sequencing

PCR amplifications were performed in reactions containing 0.5–1.0 μg of genomic DNA, 50 mM KCl, 10 mM Tris-HCL pH 8.3, and 1.0 mM $MgCl_2$. Primers (at positions 192–211 and 391–410, respectively) were designed to amplify a 219-bp fragment of exon 1 of rhodopsin including codon 23 (Nathans and Hogness 1984). PCR-amplified DNA was chloroform extracted, treated with proteinase K, phenol 1 chloroform extracted, chloroform extracted, spun through a Sepharose CL-6B column, and then ethanol precipitated (Yandell and Dryja 1989).

The purified DNA was sequenced using a nested primer (at position 301–320) within the amplified fragment. End-labeled sequencing primer 1–2 pmol [p-32] was combined with 250–500 ng of the amplified, purified DNA template and was heat denatured at 94°C for 5 min. The T7 DNA Polymerase Sequencing System (Promega) was then followed, with the omission of the labeling step. Samples were heat denatured at 70°C for 5 min prior to being run on 6% polyacrylamide sequencing gels and then autoradiographed for 1–2 d at −70°C. Oligonucleotides specific for either the wild-type or the mutant sequence were used for dot-blot hybridization as described by Dryja et al. (1990) and Lester et al. (1990).

Results and Discussion

The region around the rhodopsin mutation at codon 23 has been analyzed in affected individuals from 91

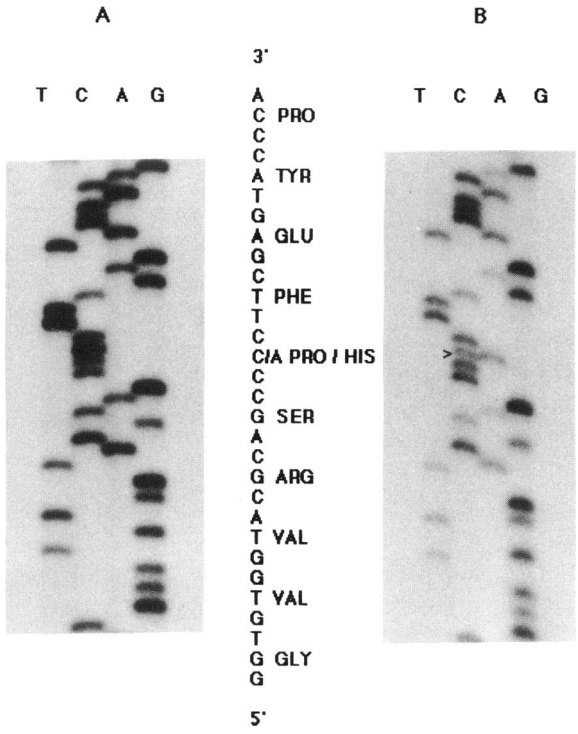

Figure 1 *A*, DNA sequence around codon 23 in exon 1 of the rhodopsin gene from an Irish ADRP patient. The mutation at codon 23 is clearly absent in this individual, as is also the case in all patients tested. *B*, DNA sequence from an ADRP patient (DNA donated by Dr. Ted Dryja and colleagues) who has the C→A transversion at codon 23.

European pedigrees. This mutation, previously observed in approximately 12% of American ADRP patients, most of whom were of British ancestry (Dryja et al. 1990), has not been found in our patient population (fig. 1). Hence, if the mutation in codon 23 of rhodopsin is present in this population it is extremely rare.

This ADRP sample includes pedigrees that are consistent with the clinical classifications of either type I and type II ADRP (Massof and Finkelstein 1981; Lyness et al. 1985; Kemp et al. 1988), some pedigrees showing early onset and diffuse loss of rod and cone sensitivity and others showing later onset with a regionalized loss of rod and cone sensitivity. It is interesting to note that the rhodopsin codon 23 mutation was not observed in either of these groups. However, it is our belief that, in view of the rapidly emerging genetic heterogeneity in ADRP, the validity of these clinical categories will need to be reassessed.

In view of the fact that the mutation at codon 23 has been observed in an American population largely of British origin, it will be of interest to note that it is not present in British and Irish ADRP families studied so far. Variations in the frequency of disease mutations between populations of different ethnic origins have been well documented; for example, Tay-Sachs disease and factor XI deficiency are more frequently found in Ashkenazi Jews (Myerowitz 1988; Asakai et al. 1989). Similarly, variability has been observed in the distribution of cystic fibrosis mutations in different European countries (De Arce et al., in press). Possibly the absence of the codon 23 mutation in the rhodopsin gene in this study may be explained in terms of variation in the frequency of the mutation. Alternatively, the presence of the codon 23 mutation in the American population may be the result of a founder effect. Such effects have been observed, for example, in the porphyria variegata population in South Africa (Dean 1971) and in the Huntington chorea population in Venezuela (Gusella et al. 1983).

In recent studies with TCDM1, the large Irish pedigree in which an ADRP gene was first mapped to 3q, the rhodospin gene has now been found to map into the middle of the linkage group on 3q between the markers D3S47 and D3S21 (Farrar et al., in press). All three of these loci—D3S47, rhodopsin, and D3S21—have been mapped close to the ADRP gene in TCDM1, each showing no recombination with the disease gene. Therefore the rhodospin gene, which is specifically expressed in rod photoreceptors and which maps to the center of the linkage group, must be considered as a prime candidate for ADRP in TCDM1. In this regard it is interesting that the codon 23 mutation has not been observed in the TCDM1 pedigree (Farrar et al., in press). Therefore the ADRP in TCDM1 must be either the result of a different mutation in rhodopsin, e.g., in that part of the molecule which interacts with transducin. Alternatively, there could be a mutation in a gene closely linked to rhodopsin. The genes for a number of other retinal specific products have also been mapped to chromosome 3, including the alpha subunit of GNAT1 (transducin), a guanine nucleotide-binding (G) protein gene expressed specifically in the rod photoreceptors (Blatt et al. 1988), and the cellular retinol binding proteins I and II (Demmer et al. 1987; Nilsson et al. 1988).

In conclusion, the rhodopsin codon 23 mutation has not been observed in the European ADRP population to date. However, further studies will be required both to assess the distribution and frequency of this mutation in an even larger patient population and to establish whether mutations at other positions in the rhodopsin gene may also result in RP-like retinopathies.

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