A novel deletion at codon 441 of the APC gene associated with ophthalmic lesions (CHRPE) in a South African family

Johanna J Grobbelaar, Ari Ziskind, Greetje de Jong, C J Joubert Oosthuizen, Maritha J Kotze

Abstract
A novel mutation at codon 441 in exon 10 of the adenomatous polyposis coli (APC) gene was identified in a South African family of mixed ancestry, using a convenient, non-radioactive, heteroduplex-SSCP screening assay. This single thymidine deletion after nucleotide position 1322 creates a frameshift resulting in a downstream stop codon at amino acid residue 453 of the APC gene. Genotypes of nine family members were subsequently correlated with the presence or absence of congenital hypertrophy of the retinal pigment epithelium (CHRPE), since expression of this common extracolonic manifestation of FAP is largely determined by the length of the truncated protein. CHRPE was absent in the five unaffected family members analysed, while four mutation positive subjects showed these ophthalmic lesions. Correlation between the molecular analysis and ophthalmic examinations, performed without knowledge of clinical and genetic status respectively, provided additional evidence in favour of the view that the range of phenotypic expression in FAP may result from different allelic manifestations of APC mutations.

Key words: familial adenomatous polyposis coli; ophthalmic lesions (CHRPE); frameshift mutation.

Mutations in the adenomatous polyposis coli (APC) gene cause the autosomal dominant disease familial adenomatous polyposis (FAP). Congenital hypertrophy of the retinal pigment epithelium (CHRPE) is clinically the most common and most reliable extracolonic manifestation of FAP and has been shown to be present in patients with mutations in codons 457–1387 of the APC gene. Patients with mutations in codons 136–302 and beyond codon 1444 regularly do not develop CHRPE, while patients with mutations in codons 413–437 have an intermediate CHRPE score. The expression of ophthalmic lesions can thus potentially be used to restrict mutation screening in a subset of clinically diagnosed FAP patients to a specific region of the APC gene. In this study we identified a novel mutation at codon 441 in exon 10 of the APC gene and showed that this allelic site is associated with the presence of CHRPE.

Methods
Genomic DNA was extracted from 17 apparently unrelated South African FAP patients, PCR amplified according to Miyoshi et al., and screened for mutations in exon 10 (codon 439–470) of the APC gene by combined heteroduplex-SSCP analysis, as described by Kotze et al. Segregation analysis was subsequently performed using genomic DNA ex-
A novel deletion at codon 441 of the APC gene

Figure 2 Pedigree of the index patient (marked by an arrow) with the 1 bp deletion. Dark shaded symbols represent clinically diagnosed FAP patients. Subjects who underwent colectomies, DNA screening, and indirect ophthalmoscopy are indicated with + for presence and − for absence of the respective parameters. NA = not analysed. CHRPE = congenital hypertrophy of the retinal pigment epithelium.

Ophthalmic lesions in CHRPE positive subjects. Large lesions were bigger than half optic disc diameter (DD), small lesions were smaller than one half DD

<table>
<thead>
<tr>
<th>Patients (n=4)</th>
<th>Lesions</th>
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<tbody>
<tr>
<td>At least 1 CHRPE</td>
<td>1</td>
</tr>
<tr>
<td>≥ 4 CHRPE</td>
<td></td>
</tr>
<tr>
<td>Large lesions present</td>
<td>2</td>
</tr>
<tr>
<td>Small lesions only</td>
<td>1</td>
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extracted from 15 members of one relatively large family (21 members). Direct DNA sequence analysis was performed by using a PCR preparation kit and T7 DNA Polymerase (Pharmacia Biotechnology), as previously described. Indirect ophthalmoscopy was performed in the index case and eight of his relatives following pupil dilatation. The criteria used to differentiate between a CHRPE positive and CHRPE negative status are presented in detail elsewhere.

Results

In one of the 17 FAP patients screened for mutations in exon 10 of the APC gene (fig 1A), a single thymidine deletion was identified after nucleotide position 1322 at codon 441 (fig 1B). Mutation screening of PCR amplified DNA obtained from 15 family members of the index patient (II-1) showed that six additional subjects inherited the single nucleotide deletion (fig 2). Indirect ophthalmoscopy performed on the index patient and eight family members showed that four subjects had CHRPE lesions (table). To date, the mutation/CHRPE positive subjects III-1 (10 years) and II-9 (6 years) have not shown any clinical symptoms. The five mutation negative subjects (II-10, II-3, II-6, III-7, and III-8) did not present with CHRPE lesions.

Discussion

A novel single nucleotide deletion (T) at codon 441 of the APC gene was identified in a South African family. This frameshift mutation creates a downstream stop codon at a position corresponding to amino acid residue 453. The truncated APC protein, predicted to result from this alteration, probably underlies the FAP phenotype in the index patient and his affected family members. Co-segregation of the mutation with the FAP phenotype was shown in three generations. The previously described relationship between the site of APC mutation and phenotypic expression of CHRPE, the most common extracolonic manifestation of FAP, was subsequently studied in the South African family. As expected, all the mutation positive subjects identified in this study, who could be traced and were subjected to ophthalmic examinations, showed the CHRPE phenotype. Those that did not inherit the deleted allele were CHRPE negative, thus confirming the intrafamilial CHRPE correlation.

The majority of FAP related mutations identified worldwide, including all the mutations identified to date in South Africa, are frame-shifts resulting in truncated proteins. Consistent findings that the expression of CHRPE is largely determined by the length of the truncated APC protein product, therefore, have important diagnostic implications with respect to facilitating the detection of FAP related mutations. The screening of the entire coding region of the APC gene for mutation detection in order to provide diagnostic reliability can now be narrowed down to specific gene regions in cases examined ophthalmoscopically.

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