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

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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.

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

Figure 1 Identification of a 1 bp deletion at codon 441 of the APC gene. (A) Heteroduplex-SSCP analysis of PCR products from 17 FAP patients in a 10% (2.5% cross linked) polyacrylamide gel supplemented with 7.5% urea and stained with ethidium bromide. In lane 11 the PCR amplified DNA of one index patient shows aberrant single (upper bands) and double stranded (lower bands) conformational polymorphisms. Homoduplex (solid arrow) and heteroduplex (broken arrow) bands observed in the double stranded DNA are indicated on the right side of the gel. (B) Direct DNA sequencing of the patient showing the aberrant bands in lane 11.

Mutations in the adenomatous polyposis coli (APC) gene cause the autosomal dominant disease familial adenomatous polyposis (FAP).¹⁻⁴ Congenital hypertrophy of the retinal pigment epithelium (CHRPE) is clinically the most common and most reliable extracolonic manifestation of FAP,^{5,6} and has been shown to be present in patients with mutations in codons 413-437 have an intermediate CHRPE score.⁷⁻⁹ 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,¹⁰ PCR amplified according to Miyoshi *et al.*,¹¹ 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.*¹² Segregation analysis was subsequently performed using genomic DNA ex-

- 5 Lewis RA, Crowder WE, Eierman LA, Nussbaum RL, Ferrell RE. The Gardner syndrome: significance of ocular features. *Ophthalmology* 1984;91:916-25.
- 6 Morton DG, Gibson J, Macdonald F, *et al.* Role of congenital hypertrophy of the retinal pigment epithelium in the predictive diagnosis of familial adenomatous polyposis. *Br J Surg* 1992;79:689-93.
- 7 Olschwang S, Turet A, Laurent-Puig P, Muleris M, Parc R, Thomas G. Restriction of ocular fundus lesions to a specific subgroup of APC mutations in adenomatous polyposis coli patients. *Cell* 1993;75:959-68.
- 8 Wallis YL, Macdonald F, Hultén M, *et al.* Genotype-phenotype correlation of constitutional APC gene mutation and CHRPE expression in familial adenomatous polyposis. *Hum Genet* 1994;94:543-8.
- 9 Caspari R, Olschwang S, Friedl W, *et al.* Familial adenomatous polyposis: desmoid tumors and lack of ophthalmic lesions (CHRPE) associated with APC mutations beyond codon 1444. *Hum Mol Genet* 1995;4:337-40.
- 10 Grobelaar JJ, Oosthuizen CJJ, Madden MV, Bailey SE, Retief AE, Kotze MJ. The use of DNA markers in the pre-clinical diagnosis of familial adenomatous polyposis in families in South Africa. *S Afr Med J* 1995;85:269-71.
- 11 Miyoshi Y, Ando H, Nagase H, *et al.* Germ-line mutations of the APC gene in 53 familial adenomatous polyposis patients. *Proc Natl Acad Sci USA* 1992;89:4452-6.
- 12 Kotze MJ, Theart L, Callis M, Peeters AV, Thiart R, Langenhoven E. Nonradioactive multiplex PCR screening strategy for the simultaneous detection of multiple low density lipoprotein receptor gene mutations. *PCR Meth Appl* 1995;4:352-6.
- 13 Grobelaar JJ, Oosthuizen CJJ, Kotze MJ. Screening South African familial adenomatous polyposis families for the five-nucleotide deletion at codon 1309 of the APC gene. *Mol Cell Probes* 1995;9:49-52.
- 14 Kotze MJ, Grobelaar JJ, Madden MV. Identification of a novel single base insertion in the adenomatous polyposis coli gene. *Hum Mutat* 1996;7:68-9.