RESEARCH LETTER

A novel frameshift mutation in exon 12 of the adenomatous polyposis coli gene in an Italian family with familial adenomatous polyposis and desmoid tumour

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The adenomatous polyposis coli (APC) gene, localized on chromosome 5, consists of 15 exons. The largest is the exon 15, which comprises more than 75% of the coding sequence of the gene and is the most common target for both germline and somatic mutations (Groden et al, 1993). The germline mutations in the APC gene are in charge of the familial adenomatous polyposis (FAP), a rare autosomal inherited disease (Hegde and Roa, 2006). These mutations predispose to develop colonic polyps and other extraintestinal neoplasms, including desmoid tumour in subjects between 15 and 60 years. The estimated risk of developing a desmoid tumour in patients with FAP is between 4 and 20 percent. This risk increases about 2 years after colectomy (Groen et al, 2008).

We studied a woman (56 years old) affected with FAP and desmoid tumour and her three children (two daughters and one son). This study was carried out in accordance with the World Medical Association Helsinki Declaration, adopted in 1964 and amended in 1975, 1983, 1989, 1996 and 2000 **(**World Medical Association, 1998**)**. Informed consents were obtained from all subjects, and the study was approved and conducted according to the ethical guidelines of the Second School of Naples. The pedigree was generated by the genetic counsellor and the case-histories of the subjects were collected.

The pedigree and the main clinical data are summarised in Figure 1. The family originated from the Campania, a region of southern Italy. The proband affected with FAP had surgical treatment with restorative proctocolectomy and ileal J-pouch at the age of 54 years. Two years after the pouch procedure the proband developed desmoid tumour, consistent with previous reports (for example, Groen et al, 2008).

Out of three children of proband, a daughter and a son were affected with FAP, diagnosed at the ages of 28 year and 18 year, respectively. Both siblings received ileal J-pouch procedure. The eldest daughter (33 years old) did not display the disease phenotype.

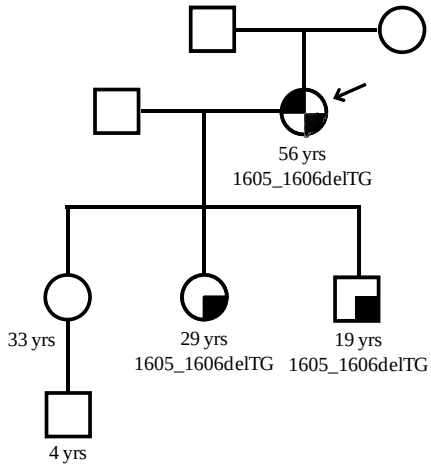

Figure 1. Pedigree of family with APC mutation identified. The arrow indicates the proband. Open symbols, clinically unaffected subjects; right inferior quadrate blackened symbols, patients affected with FAP; left superior quadrate blackened symbols, patients affected with desmoid tumours.

Peripheral blood from subjects was collected using standard procedures and all 15 exons of the APC gene and the flanking splice site regions were amplified using a set of 28 primer pairs (Table 1). One set of primers was used to amplify each exon and the adjacent intronic region except for exon 15, which was amplified as 14 separate PCR fragments due to it large size.

PCR products were sequenced using the ABI PRISM di-Deoxy Terminator Cycle sequencing kit in an ABI 9700 Thermal Cyclers and ABI PRISM 310 Genetic Analyzer. Deviations from the wild-type sequence were identified using Mutation Surveyor, sequence analysis and assembly software.

The analysis of the APC gene in the DNA sample from proband revealed the presence of the novel deletion, 1605_1606delTG. This frameshift mutation is located in exon 12 of APC gene and is characterized by a deletion of two nucleotides, generating a premature stop codon between codons 537 and 538 (Figure 2). The presence of the mutation was confirmed by resequencing a second DNA sample from the patient.

The genetic analysis conducted in family members showed the same mutation in the daughter and the son affected with FAP, while the healthy daughter did not have this mutation (Figure 1).

In this study, we identified in the proband, affected with FAP and desmoid tumour the novel mutation 1605_1606delTG. The proband passed on the mutation to one of her daughters and the son resulting in FAP phenotype. The different onset age of FAP in proband, daughter and son (54, 28 and 18 years, respectively) indicates intrafamilial variability. In the FAP, the sites of the mutation determine the presence or the absence of extracolonic manifestation of the disease, as desmoid tumours. The identified frameshift mutation is located in exon 12 at codon 535 in the 5'end of APC gene, a region of APC gene not usually associated with desmoid tumor. Indeed, the previous studies show that in patients affected with FAP and desmoid tumour the mutations of APC gene are localized predominantly in exon 15 (Lips et al, 2009). It is possible that the site of the mutation may also influence the severity of desmoid phenotype (Couture et al, 2000). It has been previously reported that the most severe desmoid phenotype (penetrance=100%) presents in families which carry mutations in the 3' end of the APC gene (Eccles et al, 2001).

In the proband, after three months of medical treatment with Toremifene, a selective oestrogen receptor modulator, the abdominal ultrasound showed no detectable hydronephrosis and the magnetic resonance imaging documented a downsizing of the mass. These data suggest that the mutations that are not in the 3' end of the APC gene may be associated with a less severe phenotype.

Both siblings affected with FAP and positive for APC gene mutation received colectomy nearly an year ago and at present are undergoing a clinical follow-up program. We propose that a longer follow-up could clarify the association between this novel mutation and the development of desmoid tumour. The identification of this novel mutation in APC gene and the outcome of the clinical follow-up of the subjects of this study are likely to be helpful in the FAP patient treatment and care strategies in the future.

ACKNOWLEDGMENTS

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COMPETING INTERESTS

None declared.

Gene	Exon	mRNA nt	Base change	Amino acid change	Designation	Mutation type
APC	12	1605-1606	delTG	STOP-codon 537-538	1605_1606delTG	frameshift

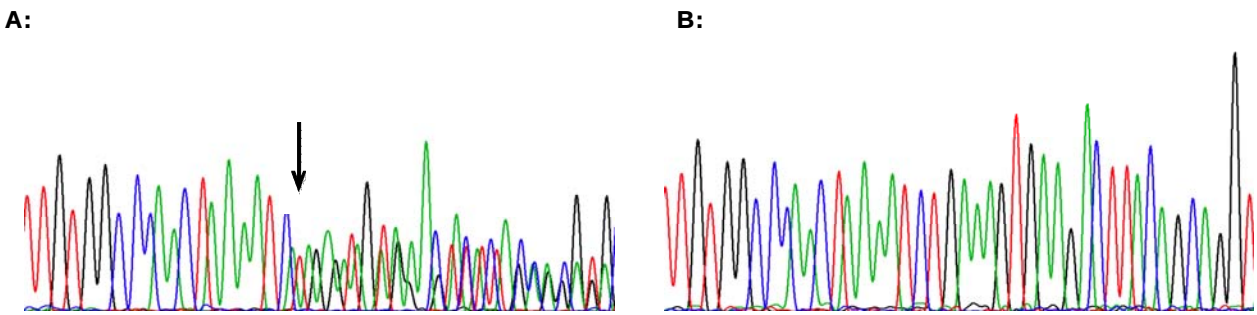

Figure 2. Carrier frameshift variant c.1605_1606delTG. Electropherogram for the sense strand of exon 12 of APC gene. DNA sequence reveals a heterozygous TG nucleotide deletion (**A**), resulting in frameshift and subsequent chain termination. The wide type sequence is shown in (**B**).

Table 1. APC Primer sequences (Amplification conditions: : 94°C for 30sec, 62°C for 60sec, 72°C for 60sec, 35 cycles).

Name	Primer sequence (5' to 3')	Product size
1-f	GCATATTAACACAATTCTTCTTAAACGTC	327
1-r	GAATACTGAATAAAAATGGATAAACTACAATTAAA	
2-f	GTCAAGAAATACAGAATCATGTCTTGAA	223
2-r	TCTACACACCTAAAGATGACAATTTGAG	
3-f	GACCCAAGTGGACTTTTCAGG	423
3-r	ACAATAAACTGGAGTACACAAGGCA	
4-f	TGCTCTTCTGCAGTCTTTATTAGCA	255
4-r	CCTGAATTTTAATGGATTACCTAGGTACT	
5-f	GCTTTTTTGCTTTTACTGATTAACGT	248
5-r	GAGCTGTAATTCATTTTATTCCTAATAGCTC	
6-f	TGATTTGACATAACCCTGAGCTTT	235
6-r	AACTACCTATTTTTATACCCACAAACAAGA	
7-f	AAGAAAGCCTACACCATTTTTGC	251
7-r	GGTAAGATTTATAGATCATTCTTAGAACCAT	
8-f	ACCTATAGTCTAAATTATACCATCTATAATGTGCT	184
8-r	GTCATGGCATTAGTGACCAGG	
9-f	AGTCGTAATTTTGTTTCTAAACTCATTTG	458
9-r	GCTTTGAAACATGCACTACGATG	
10-f	AAACATCATTGCTCTTCAAATAACAA	217
10-r	CTACCATGATTTAAAAATCCACCAGT	
11-f	TTAGATGATTGTCTTTTTCCTCTTGC	217
11-r	TGAGCTATCTTAAGAAATACATGTTATAAAAACA	
12-f	GGCTTCAAGTTGTCTTTTTAATGATC	186
12-r	AGACCCTGCCTCAAAGAAAAAG	
13-f	TTTCTATTCTTACTGCTAGCATTAAAAACA	307
13-r	AATACACAGGTAAGAAATTAGGAAATCTCAT	
14-f	ACTCTAATTAGATGACCCATATTCTGTTTC	316
14-r	CAATTAGGTCTTTTTGAGAGTATGAATTCT	
15a-f	GTTACTGCATACACATTGTGACCTTAATTT	453
15a-r	CGATGAGATGCCTTGGGACTT	
15b-f	GCGAAGTACAAGGATGCCAATATT	449
15b-r	GCAGTGGTGGAGATCTGCAAA	
15c-f	CAACTACCATCCAGCAACAGAAAAT	491
15c-r	TTGGTGTATCTAGTTCTCCATCATTATCAT	
15d-f	TCAATACCCAGCCGACCTAGC	527
15d-r	CCACATGACGTTTCTCTTCATTATATTTT	
15e-f	GTAAGCCAGTCTTTGTGTCAAGAAGAT	478
15e-r	CAGCTGATGACAAAGATGATAATGAAC	
15f-f	CTTGCAAAGTTTCTTCTATTAACCAAGA	454
15f-r	CGACTCTCAAAACTATCAAGTGAACTG	
15g-f	ACCTGAACACTATGTTCAGGAGACC	524
15g-r	CATTTGATTCTTTAGGCTGCTCTGATT	
15h-f	ACAGAAAGATGTGGAATTAAGAATAATGC	476
15h-r	CTCCTTCTCCAGCAGCTAACTCAT	
15i-f	ACATCTCTAAGTGATCTAACAATCGAATC	650
15i-r	TCATCATCATCAAAATCTAGAGAACTCA	
15l-f	CTATTGAAGGAACTCCTTACTGTTTTTC	669
15l-r	TGCCACCCATATTTCTGGG	
15m-f	GCCTTCAAGACTCAAGGGTGA	657
15m-r	GGAGAAGTGGTGGCTGTTTG	
15n-f	CCTTAAGACTCCAGCCTCCAA	656
15n-r	GTCTAGATGATGGAGAAAGAGATTCAA	
15o-f	CAACCTTAAGAAGAAAATTGGAGGA	664
15o-r	GATCTAGGATTGTTAATGGGACAGTC	
15p-f	TGCTGTTTCTAAAACAGAGGATGTT	663
15p-r	AGTTTCATTTGAAACAAAATGTCTATATAGC	

REFERENCES

Couture J, Mitri A, Lagace R, et al. 2000. A germline mutation at the extreme 3' end of the APC gene results in a severe desmoid phenotype and is associated with overexpression of beta-catenin in the desmoid tumor. Clin Genet, 57, 205-212.

Eccles D, Harvey J, Bateman A and Ross F. 2001. A novel 3' mutation in the APC gene in a family presenting with a desmoid tumour. J Med Genet, 38, 861-863.

Groden J, Gelbert L, Thliveris A, et al. 1993. Mutational analysis of patients with adenomatous polyposis: Identical inactivating mutations in unrelated individuals. Am J Hum Genet, 52, 263-272.

Groen EJ, Roos A, Muntinghe FL, et al. 2008. Extra-intestinal manifestations of familial adenomatous polyposis. Ann Surg Oncol, 15, 2439-2450.

Hegde MR and Roa BB. 2006. Detecting mutations in the APC gene in familial adenomatous polyposis (FAP). Curr Prot Hum Genet, 10, U10.8.

Lips DJ, Barker N, Clevers H and Hennipman A. 2009. The role of APC and beta-catenin in the aetiology of aggressive fibromatosis (desmoid tumors). Eur J Surg Oncol, 35, 3-10.

World Medical Association (WMA). 1998. The WMA declaration of Helsinki 1960 with recommendations on biomedical research on human subjects (modified in 1975, 1980 and 1989). Chirurgia (Bucur), 93, 138-140.