Polymorphisms in the adenomatous polyposis coli gene in Slovak families suspected of FAP

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Abstract

OBJECTIVES: Polymorphism in the *adenomatous polyposis coli* (*APC*) gene was analyzed in 33 families suspected of familial adenomatous polyposis (FAP) without identified *APC* gene mutation. Screening of 104 members of mentioned families for polymorphism in the *APC* gene, was performed using single strand conformation polymorphism (SSCP) and DNA sequencing.
RESULTS: Twelve different types of polymorphism were found in the cohort of the families analyzed. Nine polymorphisms were located within exon 15, one within exon 6, one within exon 11 and one within exon 13. Of the 12 polymorphisms, 11 were silent substitution and only one was responsible for the amino acid change – D1822V, which was identified in 60% of the families analyzed.
CONCLUSION: The most frequently detected polymorphism D1822V is potentially associated with the risk of colorectal cancer. Three detected polymorphisms - Y486Y, T1493T and S1756S – also seem to be associated with colon cancer risk. All these polymorphisms may be used as markers for diagnosis of colorectal cancer. The importance of other detected polymorphisms remains still unclear, but their involvement is being continuously observed.

INTRODUCTION

Mutations in the adenomatous polyposis coli (*APC*) gene are well known to predispose for colorectal cancer (CRC) in familial adenomatous polyposis (FAP). FAP is an autosomal dominant disorder, clinically manifested by the development of multiple benign adenomatous polyps (>100) in the colon and rectum. The *APC gene* is a tumor suppressor gene, located at 5q21-q22. It contains 15 exons and encodes a large protein with multiple functional domains. The APC protein plays a major role in tumor suppression by antagonizing the WNT signaling pathway. Inappropriate activation of this pathway through loss of APC function contributes to cancer progression. The *APC* gene plays a role in cell migration, adhesion, chromosome segregation, spindle assembly, apoptosis, and neuronal differentiation (Hanson & Miller 2005).

Germline mutations in *APC* have been demonstrated in the majority of FAP patients (Nagase & Nakamura 1993). The majority (95%) of these are

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nonsense or frameshift mutations that result in a truncated protein product with abnormal function. Mutations span through all *APC* exons, but a great number of all germ-line mutations are clustered in a relatively small region (also called the Mutation Cluster Region, MCR) between codons 1286-1513 in the 5´prime of the largest exon 15 (Beroud & Soussi, 1996).

Missense mutations are very rare, but two missense germline mutations of the *APC* gene have been frequently reported. Well known is the I1307K, which has been associated with an increased risk for colon cancer and adenomas in people of Ashkenazi descent (Gryfe *et al.* 1999). The second is E1317Q, which has a low frequency and does not appear to confer an increased risk for colorectal neoplasia in the general population (Hahnloser *et al.* 2003). In contrast, some studies concluded that this allele contributes to a predisposition to colorectal adenomas and carcinoma, but with low and variable penetrance (White *et al.* 1996; Frayling *et al.* 1998).

Besides these two variants, the most common one is D1822V with a variant allele frequency of ~ 22% (Slattery *et al.* 2001). The functional significance of the D1822V substitution is unknown, although this amino acid change is located in the center of a β-catenin down-regulation domain (Mihalatos *et al.* 2003). Another *APC* variant, G2502S has allele frequency of 10% in Caucasians and is not associated with a risk of colorectal cancer or adenomas (Tranah *et al.* 2005; Cleary *et al.* 2008).

The aim of this study was to investigate the *APC* polymorphisms in FAP suspected families without identification of *APC* mutation.

PATIENTS AND METHODS

Patients. Blood samples of 104 members of 33 families suspected of adenomatous polyposis were examined for mutations in the *APC* gene. Samples were collected in collaboration with the National Cancer Institute in Bratislava and several local hospitals all over Slovakia. Written informed consent for genetic testing was obtained.

DNA isolation and PCR amplification. Genomic DNA was extracted from peripheral blood lymphocytes using QIAamp^R DNA blood Kit (Qiagen). DNA samples were amplified using PCR mostly in the program: 5min at 94 °C, once; 1min at 94 °C, 1min at annealing temperature from 58 to 63 °C, 1min at 72 °C, 30 times; and 7 min at 72 °C, once.

Single-strand conformation polymorphism (SSCP). PCRs for SSCP were performed from approximately 150–200ng of genomic DNA, 80 mM dNTP, 1mM 10x PCR buffer (Qiagen), 0.5 U of Taq polymerase (Qiagen), 10 pmol of each primer, to a total volume of PCR mixture of 25μl. The sequences of the primers used for the *APC* gene were described by Groden *et al.* (1991). The samples were denaturated for 5 min. at 95 °C and then placed on ice for 5 min. in order to prevent reannealing, loaded onto a 6% polyacrylamide gel and electrophoresed overnight in a cold room at 55V. The gel was silver-stained as described (Kirchhoff *et al.* 1997).

Direct DNA sequencing. Amplicons were purified by solid-phase extraction and bidirectionally sequenced with the PE Applied Biosystems Big Dye Terminator Sequencing Kit according to the manufacturer's instructions. Sequencing extensive products were analyzed on a PE Applied Biosystems ABI-PRISM 310 sequencer (Zajac *et al.* 2007).

Table 1. Summary of *APC* polymorphisms identified in Slovak FAP suspected patients.

Exon	Codon	Alteration	Consequence	Family No.
6	235	TTA→TTG	Leu→Leu	25
11	486	TAT→TAC	Tyr→Tyr	35, 56, 81, 155, 161
13	545	GCG→GCA	Ala→Ala	155
15 E	1084	GAT→GAC	Asp→Asp	102, 117
15 G	1367	CAG→CAA	Glu→Glu	8
15 H	1493	ACG→ACA	Thr→Thr	4, 8, 12, 161
15 J	1678	GGG→GGA	Gly→Gly	4, 8, 12, 39, 160, 161
15 K	1756	TCT→TCG	Ser→Ser	4, 8, 39, 119, 160, 161
15 K	1769	AAA→AAG	Lys→Lys	39
15 L	1822	GAC→GTC	Asp→Val	19, 39, 45, 85, 87, 88, 100, 101, 109, 115, 117, 118, 125, 126, 127, 128, 129, 131, 134, 160
15 N	1960	CCG→CCA	Pro→Pro	4, 134, 161
15 S	2407	GGT→GGG	Gly→Gly	69

RESULTS

SSCP technique was used for the mutational screening of the *APC* gene, exons 1-15. Exon 15 was divided into 19 overlapping segments of approximately 350-500 bp. When visible extra bands were found in SSCP gel, we used direct sequencing for the exact definition of changes revealed by the SSCP. Exon 15 is the most common target for both germline and somatic mutations and therefore we used direct sequencing for analysis of this part of the *APC* gene in some families.

In the cohort of FAP suspected families analyzed, 12 different types of polymorphisms (***Table 1***) were found and there were no mutations. Nine polymorphisms were located within exon 15, one within exon 6, one within exon 11 and one within exon 13. Of the 12 polymorphisms, 11 were silent substitution and only one – D1822V – was responsible for the amino acid change. This polymorphism was identified in 20 families.

Polymorphisms G1678G and S1756S were found in six families (18%), Y486Y in five families (15%), T1493T in four families (13%), P1960P in three families (9%) and D1084D in two families (6%). In comparison, polymorphisms L235L, A545A, K1769K and G2407G were identified only in one family (3%).

DISCUSSION

The *APC* gene was analyzed for germline mutations in 113 familial adenomatous polyposis suspected families from all over Slovakia (Mátelová *et al.* 2008). Mutations in the *APC* gene were found in 39 (34.5%) Slovak families and 25 different pathogenic mutations throughout the *APC* gene were identified. The present study was aimed at identification of polymorphisms in 33 FAP suspected families without identification of *APC* mutation. Although a missense mutation represents more subtle and more common genetic changes, it may have a varying impact on protein stability, structure and function, leading to an increased or decreased risk of neoplastic transformation and progression. It may interact with environmental factors, thereby influencing the risk of developing CRC.

D1822V is the most common *APC* variant and we identified it in 60% of the families analyzed. The functional significance and clinical relevance of the D1822V substitution is unknown, the substitution changes the amino acid residue from a hydrophilic aspartate to a hydrophobic valine residue. This amino acid change is located in the center of a ß-catenin down-regulation domain. Specifically, it is 180 amino acids downstream of the fourth and 26 amino acids upstream of the fifth of the seven 20-amino acid repeats (Groden *et al.* 1991). Disregulation of the Wnt/ß-catenin signaling pathway leads to nuclear accumulation of ß-catenin, resulting in aberrant cellular proliferation in the upper crypt. Wnt/ß-catenin overactivation, often resulting from mutated *APC*, leads to imbalanced cellular proliferation and differentiation. It has been documented in tumors and implicated in human cancer. On the other hand, efficient ß-catenin down regulation requires a minimum of three of the seven binding repeats, suggesting that the D1822V polymorphism may not have an appreciable effect on ß-catenin degradation (Bright-Thomas & Hargest, 2003).

The clinical relevance of D1822V polymorphism has not been exactly characterized as yet. Previous work suggests that this polymorphism may either be a low-penetrance allele that increases the risk of developing CRC (Wallis *et al.* 1999) or a common polymorphism without clinical implication (Ruiz-Ponte *et al.* 2001). Slattery *et al.* (2001) reported that people who were homozygous for 1822V allele were at lower risk if they consumed a low-fat diet. Cleary *et al.* (2008) observed that this homozygous variant likely confers a protective effect against CRC. In our families studied only heterozygotes were found for this variant.

Chen *et al.* (2006) revealed significant differences at S1756S, Y486Y and T1493T between the healthy and patient group examined.. Further analysis indicated that these genotypes had high odd ratios for CRC and may therefore serve as markers predicting the development of CRC. The frequence of these polymorphisms was high in our cohort as well (18%, 15 % and 13%). These polymorphisms may in future serve as markers for CRC.

It is possible that *APC* variants associated with amino acid changes may alter the risk of colon cancer in the presence of environmental exposures, or they may independently alter colon cancer risk in subsets of the population. Nonetheless, the results of Cleary *et al.* (2008) imply that the majority of genetic risk accounting for familial and even seemingly sporadic CRC arise presumably from as yet uncharacterized genes, or what is even more likely, complex gene-gene (digenic) or gene-environment interactions may also be involved.

One of the candidates of gene-environment interactions are bacteria of the patient's intestinal tract. There is increasing evidence that the mucosa-associated bacteria may play important roles in the pathogenesis of inflammatory bowel disease, ulcerative colitis, Crohn's disease and colon cancer (Martin *et al.* 2004; Darfeuille-Michaud *et al.* 2004; Zajac *et al.* 2006). This evidence has been supported by our preliminary results, which suggest the important role of bacteria in cancer processes (data prepared for publication).

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