The single nucleotide substitution c.645+32C>T in the *APC* gene is a nonpathogenic polymorphism occuring in about 16% of the Czech population

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Abstract

Introduction: Familial adenomatous polyposis is an autosomal dominant disease characterised by predisposition to colon polyposis and colorectal cancer and caused by germline mutations in the *APC* gene. The aim of the study was to establish the frequency of c.645+32C>T substitution in intron 5 of the *APC* gene in patients with multiple colon polyposis and in the general population and to determine if this substitution is a nonpathogenic polymorphism or a pathogenic mutation associated with multiple polyposis coli.

Methods and results: The frequency of c.645+32C>T substitution in the *APC* gene was established in 170 patients with the clinical phenotype of familial adenomatous polyposis or its attenuated form using denaturating gradient gel electrophoresis and direct sequencing. We tested a population of 200 non-cancer persons using allelic specific polymerase chain reaction.

The c.645+32C>T substitution was detected in 27 of 170 patients with multiple colon polyposis (i.e. 15,9%). The substitution was found in 32 of 200 control persons, i.e. in 16%. The difference between patients with polyposis and the control group was not statistically significant (p=0.979; chi-square test).

Conclusion: Our results suggest that the c.645+32C>T substitution is a nonpathogenic single nucleotide polymorphism appearing in about 16% of the Czech population.

Keywords: familial adenomatous polyposis, *APC* gene, mutation, polymorphism

Familial adenomatous polyposis is an autosomal dominant disease characterised by predisposition to hundreds to thousands of colon and rectal polyps developing from the young age and to colorectal cancer. It is caused by germline mutations in the *APC* gene. Mutations in the first 5 exons, exon 9 and the 3´end of the *APC* gene are associated with the phenotype of attenuated familial adenomatous polyposis. This form is characterized by a lower number of polyps (usually less than one hundred) and later age of onset, the risk of colon cancer being also high (1). We have found a single nucleotide substitution c.645+32C>T in intron 5 of the *APC* gene in several patients with the clinical phenotype of attenuated familial adenomatous polyposis. The aim of the study was to establish the frequency of this single nucleotide substitution in patients with familial adenomatous polyposis or its attenuated form and in the general population and to determine if this substitution is a nonpathogenic polymorphism (a population variant) or a pathogenic mutation associated with multiple polyposis coli.

Patients and methods

One hundred and seventy patients with the clinical phenotype of familial adenomatous polyposis or attenuated familial adenomatous polyposis were included into the study. Informed consent was obtained from the patients. The c.645+32C>T substitution was detected using denaturating gradient gel electrophoresis (DGGE) and/or direct sequencing of the exon 5 and contiguous intronic sequences as previously described (2, 3). The sequences of the primers for DGGE were following: forward primer with the clamp: 5´-CCCCGCCCGGCCC GCCCCGCCCCCCGCCCCCCCCTCCCGGCCCGCCCCCCTGGCGCC CCGCTACAAGAT ATTGATACTTTTTTA-3´; revers primer, 5´- TGT AAT TCA TTT TAT TCC TAA TAG CTC-3´. The sequences of the primers for direct sequencing were following: forward primer, 5´-TAC AAG ATA TTG ATA CTT TTT TA-3´; revers primer, 5´-TGT AAT TCA TTT TAT TCC TAA TAG CTC-3´. PCR conditions were following: 95°C 5 min (95°C 60 s, 52°C 90s, 72°C 90s)$_{30}$ 72°C 7 min. DGGE gradient was 20% : 70% and electrophoresis duration 2 hours. The substitution was also detected in anonymous DNA samples of 200 non-cancer persons. We have designed an allelic specific polymerase chain reaction (PCR) method for its detection. The sequences of the primers were following: the normal forward primer, 5‘-GTT TCT AAG TGA TAA AAC AAC-3‘; the mutated forward primer, 5‘-GTT TCT AAG TGA TAA AAC AAT-3‘; the common revers primer, 5‘-TTG CTC AGC AGC CAT GAT AA -3‘ (Figure 1). As a control of a correct amplification, a set of primers used for the G20210A mutation in the protrombin gene were used (forward primer,

5´-TCTAGAAACAGTTGCCTGGC-3´; revers primer, 5´-ATAGCACTGGGAGCATTGAAGC-3´). PCR conditions were following: 94°C 5 min (94°C 40 s, 55°C 40s, 72°C 40s)$_{35}$ 72°C 7 min. Electorophoresis was performed on a 5% nondenaturation polyacrylamid gel. The fragments were vizualized using silver staining. In samples without the substitution, a fragment was present only in the tube with the normal forward primer. If the patient was heterozygous for the substitution (i.e. one allele of the gene is normal and one with the substitution), there were fragments present in both the tube with the normal and mutated forward primers. The fragment for prothrombin, i.e. internal control of amplification, was present in each sample (Figure 2).

Results

The c.645+32C>T substitution of the *APC* gene was found in 27 of 170 patients with familial adenomatous polyposis or its attenuated form (i.e. 15,9%). The substitution was found in 32 of 200 non-cancer persons, i.e. in 16%. The difference between patients with polyposis and the control group was not statistically significant (p=0,979; chí-square test).

Discussion

The single nucleotide substitution c.645+32C>T in intron 5 of the *APC* gene is relatively frequently detected in patients with familial adenomatous polyposis or its attenuated form. Its pathogenic significance is not known, it has not been dealed with in the literature. Results of our study suggest that it appears in about 16% of the Czech population, i.e. in the same frequency as in patients with colon polyposis. With respect to the prevalence of familial adenomatous polyposis and its attenuated form in the population of 1 in 5000 to 7500 persons (1) we can conclude that the c.645+32C>T substitution in intron 5 of the *APC* gene is a nonpathogenic polymorphism, i.e. a population variant. Its carriership is not associated with an increased risk of colon polyposis.

List of used abbreviations

APC, adenomatous polyposis coli; PCR, polymerase chain reaction; DGGE, denaturating gradient gel electrophoresis

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Figure legends

Figure No 1. The c.645+32C>T substitution in intron 5 of the *APC* gene, normal and mutated sequence. NF, normal forward primer; CR, common revers primer; MF, mutated forward primer; arrows, direction of amplification.

Figure No 2. The c.645+32C>T substitution detection using allelic specific polymerase chain reaction, 5% polyacrylamid gel after electrophoresis. N, normal allele; M, mutated allele; sample 1, heterozygot for c.645+32C>T substitution (amplified both the normal and the mutated allele); samples 2 and 3, without c.645+32C>T substitution (amplified only the normal allele).

Figure No 1.

normal sequence:

exon 4

5‘-TTTTCCTTACAAACAGATATGACCAGAAGGCAATTGGAATATGAAGCAAGGCAAATCAGA

GTTGCGATGGAAGAACAACTAGGTACCTGCCAGGATATGGAAAAACGAGCACAG

intron 5

gtaagttacttgtttctaagtgataaaacag**C**gaagagctattaggaataaaatgaatta

(NF) 5´-**gtttctaagtgataaaacaac-3´**

cagctctgttaatattgattaaattttattaaagacataaggctgtgtttattttggctc

tatttcaaaataagatttatcatggctgctgagcaacataatcaatattcacatagttgt

3‘-**atagtaccgacgactcgtt-5´ (CR)**

gtctttaccatattcatttcccctggtactgttctgttctgccttggaattataagggag

agacagagttagatggtggtcttccggtagctaatgactagcttcagttctcctttgaaa-3´

mutated sequence:

exon 4

5‘-TTTTCCTTACAAACAGATATGACCAGAAGGCAATTGGAATATGAAGCAAGGCAAATCAGA

GTTGCGATGGAAGAACAACTAGGTACCTGCCAGGATATGGAAAAACGAGCACAG

intron 5

gtaagttacttgtttctaagtgataaaacag**T**gaagagctattaggaataaaatgaatta

(MF) 5´-**gtttctaagtgataaaacaat-3´**

cagctctgttaatattgattaaattttattaaagacataaggctgtgtttattttggctc

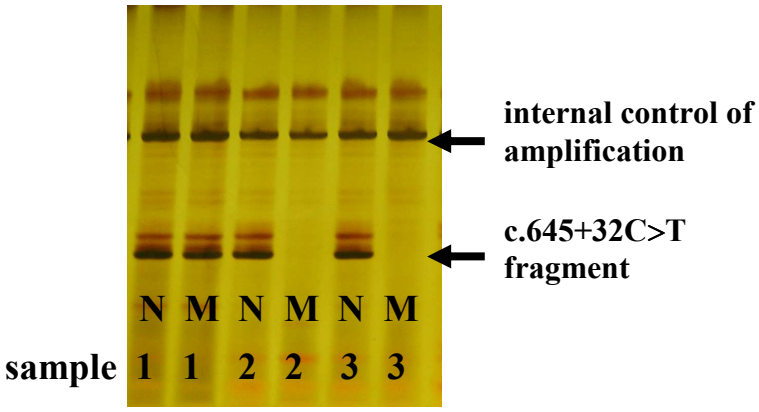
tatttcaaaataagatttatcatggctgctgagcaacataatcaatattcacatagttgt

3‘-**atagtaccgacgactcgtt-5´ (CR)**

gtctttaccatattcatttcccctggtactgttctgttctgccttggaattataagggag

agacagagttagatggtggtcttccggtagctaatgactagcttcagttctcctttgaaa-3´

Figure No 2.


internal control of amplification
c.645+32C>T fragment
N M N M N M
sample 1 1 2 2 3 3