PERGAMON

European Journal of Cancer 37 (2001) 499–502

European Journal of Cancer

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APC I1307K and E1317Q variants are rare or do not occur in Swedish colorectal cancer patients

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Received 5 June 2000; received in revised form 15 September 2000; accepted 1 November 2000

Abstract

Recently, a germ line mutation of the *APC* gene, I1307K, was discovered in a subset of Ashkenazi jews. The mutation involves an amino acid exchange and creates a tract consisting of eight contiguous adenosine residues believed to cause hypermutability in this region. Another germ line missense variant, E1317Q, not restricted to a certain ethnic population, could functionally alter the protein. These *APC* variants have been linked with increased colorectal cancer risk in several studies. However, they have not yet been investigated in Swedish colorectal cancer patients. Thus, our aim was to investigate the prevalence of I1307K and E1317Q in Swedish colorectal cancer patients in order to determine if these genetic variants are important predisposing factors to colorectal cancer in this population. To this end, sequence analysis was carried out of the *APC* gene in order to identify any I1307K and E1317Q variants in 106 unselected cases and 88 hereditary/familial colorectal cancer cases including 22 cases of hereditary non-polyposis colorectal cancer (HNPCC) fulfilling the Amsterdam criteria. Out of a total of 194 cases examined, we did not find any variants. It seems that these alterations are rare or absent in the Swedish population.

Keywords: *APC*; I1307K; E1317Q; Colorectal cancer

1. Introduction

The *APC* gene, located at 5q21-22, is a tumour suppressor gene whose germ line mutations are classically associated with familial adenomatous polyposis (FAP), a risk factor for colorectal cancer. In most sporadic colorectal cancers, inactivating mutations, spread between codons 168 and 1680, are believed to be critical in the early stages of tumour development [1]. Recently, less penetrant germ line variants have gained attention as predisposing for cancer. The polymorphism most thoroughly investigated is a transversion from T to A at codon 1307 (I1307K), which creates a poly A (A8) tract and results in an amino acid exchange, lysine for isoleucine. The variant is believed to increase cancer susceptibility since it gives rise to an unstable hypermutable region of the gene, leading to truncating somatic mutations at adjacent sequences, although a direct functional effect on the protein is also possible since it gives rise to a charge change in a critical part of the APC molecule. It has been linked with increased colorectal cancer risk with odds ratios (ORs) of 1.9 [2–4]. The variant was found in 28% of Ashkenazi Jews with a family history for colorectal cancer, in 10% of Ashkenazi Jews with sporadic colorectal cancer and in 6% of Ashkenazi Jews without colorectal cancer, but not in 243 non-Jewish individuals without colorectal cancer [2]. Several other groups have looked for this *APC* variant in non-Jewish populations to evaluate if the I1307K allele is unique to Ashkenazi Jews, but with negative results [5–7]. Only recently, Nathanson and colleagues [8] reported a non-Jewish woman of Italian descent, affected with breast and ovarian cancer and with a family history of colon cancer, who was heterozygotic for the I1307K allele. Another germ line missense variant in close proximity to I1307K (E1317Q) involves a mutation (G→C) which leads to a glutamic acid to glutamine substitution [5,6,9,10]. It has been suggested that this alteration contributes to a predisposition to colorectal adenoma

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0959-8049/01/$ - see front matter
PII: S0959-8049(00)00393-2

and carcinoma in both Ashkenazi and non-Jewish populations but with low and variable penetrance [6].

Since there is no information about I1307K and E1317Q in the Swedish population, the aim of the present study was to investigate the prevalence of I1307K and E1317Q in Swedish colorectal cancer patients in order to identify whether these alleles predispose to colorectal cancer. We also wanted to investigate whether there is a difference in the frequency of the mutations between the hereditary/familial and sporadic forms of the disease. The study was accomplished by sequence analysis of the *APC* gene for I1307K and E1317Q in 194 Swedish colorectal cancer patients, including some with hereditary and familial colorectal cancers.

2. Patients and methods

2.1. Patients

The study included colorectal cancer patients from two separate groups. Fresh frozen normal colorectal tissue were received consecutively from 106 (55%) of unselected patients, whose family histories were unknown, diagnosed between the years 1984 to 1999 in the region of Linköping and Norrköping. Patients' sex, age, tumour site and Dukes' stage, were confirmed from surgical and pathological records. The grade of differentiation was scored by one of the authors as previously recommended [11–13]. There were 62 men (58%) and 44 women (42%), ranging from 47 to 89 years of age. Sixty-three tumours (59%) were from the colon and 42 (40%) from the rectum. Blood samples were collected from 88 patients diagnosed with colorectal cancer in the region of Stockholm between the years 1991 to 1998. There were 22 (11%) cases of hereditary non-polyposis colorectal cancer (HNPCC) who fulfilled the Amsterdam criteria, 19 cases (10%) with hereditary colon cancer not fulfilling the Amsterdam criteria, 23 cases (12%) with one close relative with colorectal cancer, 9 cases (5%) with early onset colorectal cancer (1: < 30, 4: < 35, 4: < 50 years) and 15 cases (8%) with family cancer histories of different origins (endometrial, breast, ventricular, gastric). One patient was taken from each kindred.

2.2. DNA extraction

Colorectal tissue was digested in a mixture of 8 mM Tris-HCl, 80 mM NaCl, 0.8mM EDTA, 2% sodium dodecyl sulphate (SDS) and proteinase K (Boeringer Mannheim, Germany). DNA was extracted with phenol, phenol/chloroform and chloroform, precipitated in 95% (v/v) ethanol, pelleted and resuspended in sterile double-distilled water. DNA from blood leucocytes had been extracted using the same phenol/chloroform method.

2.3. Polymerase chain reaction (PCR)

DNA was amplified from codons 1293–1364 of the *APC* gene in order to identify any I1307K and E1317Q variants using 30 cycles of denaturation, annealing and extension, with the forward primer 5′ CGACACAGGAAGCAGATTCT 3′ and the reverse primer 5′ CACTTTTGGAGGGAGATTTC 3′. The reaction was performed in a reaction mixture of 22 μl containing 1× polymerase buffer (75 mM Tris-HCl, 0.1% (v/v) Tween 20), 2mM $MgCl_2$, 0.2 mM dNTP, 1 μM of each primer, 0.5 U taq polymerase (Promega/SDS, Wisconsin). The PCR products were confirmed by electrophoresis on a 1% agarose gel stained with ethidium bromide and visualised by ultraviolet (UV) transillumination.

2.4. DNA sequencing

The PCR products were separated from the primers using Wizard™ PCR Preps DNA Purification System (Promega/SDS) before they were included in the sequencing reaction. Manual sequencing was performed with the Thermo sequenase radiolabelled terminator cycle sequencing kit (Amersham, Little Chalfont Buckinghamshire, UK). Terminating γ-ddNTP^{33} was incorporated during 30 cycles of amplification using the same conditions as the preceeding PCR reaction using the forward primer. The resultant DNA fragments were separated by electrophoresis on a denaturating polyacrylamide (6%) gel containing 8M urea. The DNA sequence is shown in Fig. 1.

3. Results

Out of totally 194 cases examined, including 106 unselected cases and 88 hereditary/familial colorectal cancer cases including 22 cases of HNPCC, we did not find the *APC* germ line mutations I1307K or E1317Q.

4. Discussion

The results imply that neither I1307K nor E1317Q severely affect the risk of developing colorectal cancer in the Swedish population. Interestingly, in a Norwegian study of 210 colorectal cancer patients, and 183 breast cancer patients with and without a family history of cancer, no patient exhibited the allele I1307 K except for a colorectal cancer patient of Jewish descent. The prevalence was estimated to be < 1% [7]. Our study supports their findings and it appears that the frequency of this polymorphism does not significantly differ between

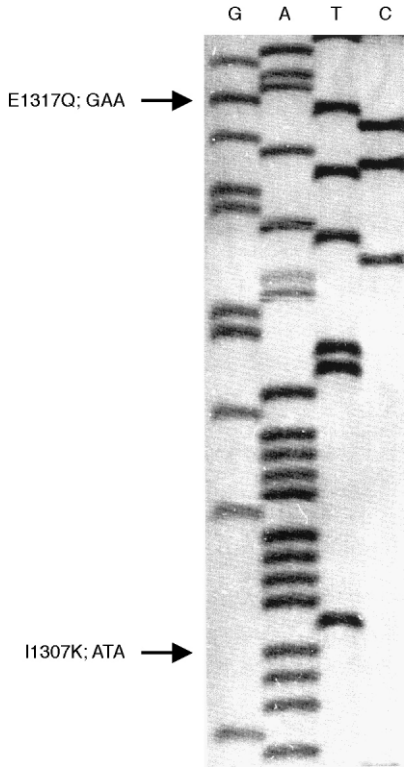

Fig. 1. Direct sequencing of polymerase chain reaction (PCR) amplified codons 1293–1364 of the *APC* gene in normal colorectal tissue showing the normal sequences of I1307K (ATA) and E1317Q (GAA).

the Swedish and Norwegian populations. The ethnic background was unknown for all of the patients in the present study. 0.2% of the Swedish people are estimated to be of Jewish descent. Thus, it is unlikely that any of the 194 patients in this study have a Jewish background.

I1307 K has been examined in several studies including both Jewish and non-Jewish patients [5–7]. In all the populations of non-Jewish descent including healthy black Americans and Italian, Finnish, Hawaiian-Japanese, Norwegian and British colorectal cancer patients, no case carrying the I1307K allele has been identified. Recently, Nathanson and colleagues [8] examined 176 non-Jewish individuals with breast or breast/ovarian cancer, 104 of whom had at least one relative with colon cancer. They found that one woman, who had breast and ovarian cancer and whose mother had colon cancer, carried the I1307K allele. Thus, it seems this polymorphism may play a significant role in Jewish populations, but not in non-Jewish populations.

The E1317 Q allele is not restricted to a certain ethnic population, it has been found both in Jewish and non-Jewish populations [5,6,9,10]. It was first found in a family with unknown ethnic background, where five of eight siblings developed cancer. Two of the four siblings affected with colorectal cancer possessed this germ line mutation and both had retained only the mutated allele in the tumours. Interestingly, this mutation was found clonally expanded as a somatic event in a sporadic colorectal adenoma, indicating that the mutation is pathogenic [9]. The variant was further found in four non-Ashkenazi British patients with or without a family history of colorectal cancer, out of 164 individuals affected with colorectal adenoma and/or carcinoma. Three of the variant cases possessed metaplastic polyps, including one case with a large number of metaplastic polyps [6]. Recently Popat and colleagues [14] discovered two carriers of E1317Q among 364 British colorectal cancer patients and two carriers among 290 healthy controls. None of the carriers had a family history of colorectal cancer. Thus, this *APC* variant might be associated with the development of multiple metaplastic polyps and further, with a moderately increased risk for colorectal cancer. At the molecular level, the E1317Q variant substitutes an uncharged hydrophilic amino acid for an acidic hydrophilic amino acid, which may be sufficient to affect the structure or function of the APC protein [6].

It is possible that common low risk alleles like I1307K will gain further attention in the future since they give us the opportunity to study the influence of dietary and other environmental factors in a certain population with a defined genetic cancer susceptibility.

To conclude, I1307K and E1317Q seem rare or absent in the Swedish population. Moreover, although a larger study is needed to truly appreciate the prevalence and penetrance of these *APC* variants, they will not be targeted by us for future genetic screening. Hence, our research will focus attention on other factors including common low risk alleles that may have more importance for susceptibility to cancer of the Swedish population.

Acknowledgements

Supported by grants from the Swedish Cancer Foundation and FORSS.

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