The APC E1317Q and I1307K polymorphisms in non-colorectal cancers

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Abstract

Mutation of the adenomatous polyposis coli (APC) gene is an important initiating factor in the early stages of the multi-step colorectal cancer (CRC) carcinogenesis. APC E1317Q and I1307K variants have been linked to CRC. The aim of this study was to examine the association of these variants with non-colorectal cancers. Mutation screening was performed using real-time PCR. The APC E1317Q variant was detected in 1.25% individuals undergoing testing. Among 2076 patients that were analyzed for this mutation, 404 had cancer outside of the colon. None of the non-colorectal cancer patients was a carrier of the E1317Q polymorphism. The I1307K variant was found in 32 subjects with non-CRC cancer, while it is not found in cancers outside of the colon. The prevalence of the more common I1307K variant is similar to that of CRC.

1. Introduction

Colorectal cancer (CRC) is the second most common malignancy in the Western world [1]. Although 15–20% of CRC cases occur in the context of a family history, the specific genetic changes in most familial and sporadic colorectal carcinomas are not known [2].

Somatic mutations of the adenomatous polyposis coli (APC) gene have been found in a large portion of CR adenomas and cancers (>80%) [3]. Furthermore, the APC mutation, mostly leading to truncated proteins, is considered to be the earliest common event in CRC [4].

Previous studies have identified a novel APC gene polymorphism [5]. A specific germ-line missence mutation, involving transition of T to A at nucleotide 3920 of the APC gene causes the substitution of lysine for isoleucine at codon 1307. The I1307K mutation was found to be predominant in the Ashkenazi Jewish population and generates a polyadenine sequence, which creates a hypermutable region that may be prone to somatic mutations. Several additional genetic changes on the affected allele have been identified, particularly: an A insertion in the 1306–9 region; a G deletion at 1314; a G to T change at codon 1309, as well as several other point mutations [6]; and wild-type allele loss of heterozygosity (LOH) [7]. The APC I1307K mutation has been suggested to predispose the development of CRC. However, a previous study from our center showed that CRC risk in Ashkenazi Jews is only mildly elevated (OR1.4) [8].

Recently, an additional polymorphism which may contribute to CRC was identified [9,10]. The APC E1317Q variant is a substitution of glutamic acid for glutamine at codon 1317. This mutation is uncommon and its clinical effect on hypermutability and the interaction of risk for CRC are not clear as yet.

There is not much data regarding the prevalence of these polymorphisms in cancers outside of the colon. In this study we evaluated 404 patients for the APC E1317Q and I1307K variants, respectively. We found that none of the non-CRC

Abbreviations: CRC, colorectal cancer; APC, adenomatous polyposis coli.

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patients carried the APC E1317Q polymorphism, whereas the prevalence of the I1307K polymorphism was similar (7.9%) to that of CRC.

2. Materials and methods

2.1. Subjects

All patients completed a questionnaire and provided a blood sample. The Institutional Review Board (IRB) of the Tel Aviv Sourasky Medical Center and the Israeli Ministry of Health approved this study. Written informed consent was obtained from all participants, allowing anonymous use of their DNA for research purposes.

2.2. DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood lymphocytes and amplified by standard PCR. Mutation screening was performed using real-time PCR on a LightCycler. About 200 ng of genomic DNA from each sample was used for all reactions. The APC variants were identified using primers designed to detect I1307K and E1317Q as follows.

2.3. Determination of the I1307K polymorphism in the APC gene

The I1307 polymorphism is a substitution of isoleucine (I) (common allele) with lysine (K) (rare allele) at position 1307 of GenBank Accession No. NP_000029.2; SEQ ID NO: 8; which results from the T to A substitution at nucleotide 3977 of NM_000038.3; SEQ ID NO: 7. Genomic DNA was PCR amplified using the following primers: 5'-GAAATAG GATGTAATCAGACG-3' (forward) and 5'-AGTCTGCTGG ATTTGGTTCTA-3' (reverse). For real time PCR a sensor primer was designed according to the wild-type allele and downstream to it an anchor primer. For the detection of the specific polymorphic nucleotide (T/A at position 3977 of SEQ ID NO: 7) the anchor primer was: LC-Red 640-TTTGCAGGGTATTAGCAGAATCTGCTTCCTGTG-ph (SEQ ID NO: 9) and sensor primer was: CCAATCTTTT CTTTTTTTCTGC-FL (SEQ ID NO: 10).

2.4. Determination of the E1317Q polymorphism in the APC gene

The E1317Q polymorphism is a substitution of Glutamic acid (E) (common allele) with Glutamine (Q) (rare allele) at position 1317 of GenBank Accession No. NP_000029.2; SEQ ID NO: 8; which results from the G to C substitution at nucleotide 4006 of NM_000038.3; SEQ ID NO: 7. Genomic DNA was PCR amplified using the following primers: 5'-GAAATAG GATGTAATCAGACG-3' (forward) and 5'-CACCTTTGGAGGGAGA-3' (reverse). Primers and detection of the specific polymorphic nucleotide (G/C at position 4006 of SQ ID NO: 7) was by real-time PCR using the anchor primer: TGCTGTGACACTGCTGGAACTTCGC-FL (SEQ ID NO: 11) and sensor primer: ph-LC-Red705-CACAG GATCTTGAGCTGACCTAG (SEQ ID NO: 12).

2.5. Statistical analysis

Database management and all statistical analyses were performed with SPSS software. The 95% confidence interval (CI) and odds ratio (OR) were estimated by using the SE of a logistic regression coefficient for each variable [11]. The prevalence of the APC variants and the incidence and characteristics of CR neoplasia were calculated using the Chi-Square test.

3. Results

The APC E1317Q variant was not found in any of the 404 individuals screened with cancers in all sites outside the colon and rectum (Table 1). However, the variant was found in 26 out of 2076 (1.25%) individuals within the whole dataset of healthy and diseased subjects. Thus, we would have expected to detect an average of 5 patients with cancers at sites other than the colon and rectum, with a statistical power of at least 0.95. Twenty five percent of the cases were randomly repeated and yielded identical results. Fig. 1 represents a typical case of E1317Q polymorphism in a subject with CRC. The APC I1307K variant was found in 32 individuals (7.9%) out of the 404 patients with cancers outside of the colon and rectum. This result matches to that in the overall population where 266 out of 3283 (8.1%) individuals undergoing testing displayed this genetic variant. It should be noticed that all carriers had only one polymorph allele, and no homozygous variant was noted. A representative APC I1307K polymorphism is depicted in Fig. 2. The distribution of I1307K carriers among these cases is shown in Table 1. In patients with pancreatic cancers, a high carrier rate of 12% was observed (7 out of 58). In the small intestine, liver and the esophagus no carriers were observed among 18, 9 and 12 individuals, respectively. These numbers are too small to draw any major conclusions, since only 2–3 cases were expected to be positive in this group.

Table 1: Distribution of I1307K carriers in non-CRC patients

<table>
<thead>
<tr>
<th>Site of cancer</th>
<th>Carriers</th>
<th>% of I1307K carriers</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas</td>
<td>7/58</td>
<td>12.0</td>
<td>0.432 (NS)</td>
</tr>
<tr>
<td>Liver</td>
<td>0/9</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Esophagus</td>
<td>0/12</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>4/68</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>Small intestine</td>
<td>0/18</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Urinary tract</td>
<td>1/6</td>
<td>16.7</td>
<td></td>
</tr>
<tr>
<td>Gynecological</td>
<td>4/31</td>
<td>12.9</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>7/85</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>2/44</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>3/34</td>
<td>8.8</td>
<td></td>
</tr>
<tr>
<td>Leukemia/lymphoma</td>
<td>1/7</td>
<td>14.3</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>3/14</td>
<td>21.4</td>
<td>0.432 (NS)</td>
</tr>
<tr>
<td>Brain</td>
<td>0/2</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Endocrine</td>
<td>0/16</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

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285 decreased binding and intracellular accumulation of β-catenin. Inactivation of the APC gene is ascribed to the development of cancers of many other tissues as well. Inactivation of the APC gene is accompanied by oncogenic mutations in the β-catenin gene itself and leads to decreased binding and intracellular accumulation of β-catenin. This signaling pathway, activated by downstream effectors of the Wnt pathway, promotes cell proliferation, cell-to-cell adhesion and inhibition of apoptosis. Vogelstein and coworkers have described the genetic changes contributing to the adenoma-carcinoma sequence of colorectal carcinogenesis [12, 13]. Much less is known for other cancers in the gastrointestinal (GI) tract. Previous studies have shown that small bowel carcinogenesis follow the same sequential events similar to those seen in CRC carcinogenesis [14,15]. At the same time, mutations in the APC gene do not play a major role in pancreatic, gastric, esophageal and/or esophageal adenocarcinomas.

We have recently demonstrated that CRC is significantly increased in E1317Q carriers [OR 3.73, 95% CI 1.44–11.4] (P = 0.0025); (paper submitted for publication). Thus, it was surprising not to find any mutation in the 18, 9 and 12 cases of small bowel, liver and esophageal adenocarcinomas, respectively. It was however relatively high in pancreatic adenocarcinomas (12%). Obviously, larger studies in these populations are required.

The rate of I1307K variant in high risk subjects and the OR of neoplasia in carriers are controversial. We have previously shown that there is only a slight increase in the risk of CRC among the I1307K variant carriers (OR 1.4, 95% CI, 0.89–2.3) [8]. Here we show that the I1307K polymorphism was found in 7.9% of evaluated patients. This is consistent with previous studies, including ours, which found that a similar proportion of Ashkenazim (6.1% [5], and 9.1% [8]) carried this alteration. Interestingly, no carriers were observed among patients with small intestine, liver or esophageal cancers at all. Although non-significant, the current study suggests that there are differences in the multi-step carcinogenesis process in the different organs in the gut even between the small and large bowel. APC loss of function was found to contribute to the carcinogenesis of the small intestine as a deletion in the long arm of chromosome 5 and was noted in three out of seventeen (17.6%) sporadic cases of small intestine adenocarcinomas [16]. FAP patients are known to have more tumors all along the alimentary tract. However, this frequency is still scarcer than in CRC and no truncating mutations were found, which ascribe for most of the events of APC loss of function.

It should also be noted that a higher number of carriers than expected was observed among patients with pancreatic cancer (12%, non-significant). This result proposes a familial predisposition to pancreatic cancer, especially while considering the study of Horii et al. [17], who showed that somatic mutations in the APC gene, leading to a truncated protein, are involved in the neoplastic changes of the pancreas.

APC is considered a classic tumor suppressor gene and as such, it requires dysfunction of both alleles for the development of CRC [7]. Alterations in the APC gene are the earliest common event in colorectal carcinogenesis and therefore, any germ-line mutation that predisposes to hyper mutability is of relevance.

Several studies have evaluated a potential role of APC I1307K mutation in other cancers. For example, an increase in prostate cancer risk associated with I1307K has been reported. However, the confidence limits were wide due to the small number of prostate cancers reported (OR 2.0, 95% CI 0.89–4.3). The in vivo study of Horii et al. [17] showed that somatic mutations in the APC gene are the earliest genetic alteration in the transformation from normal epithelium to adenoma is most likely the loss of function of the APC gene. Loss of function of the APC gene do not play a major role in pancreatic, gastric, esophageal and/or esophageal adenocarcinomas.

Fig. 1. Identification of the APC E1317Q mutation by real-time PCR: homozygous vs. heterozygous.

Fig. 2. Identification of the APC I1307K mutation by real-time PCR: homozygous vs. heterozygous.

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Interestingly, the I1307K variant was shown to modify the association between body mass index (BMI) and risk of prostate cancer [19] but, a recent study could not provide compelling evidence to support an association between prostate cancer and APC I1307K [20].

We evaluated herein the association between APC gene variants with non-colorectal cancers. We found that none of the non-colorectal cancer cases carried the APC E1317Q mutation whereas, 32 carriers of APC I1307K were found among 404 subjects (7.9%). APC I1307K may have a role in pancreatic cancer, and most probably no significance in the predisposition of small bowel and gastric cancers.

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References