

The APC Gene I1307K Variant Is Rare in Norwegian Patients with Familial and Sporadic Colorectal or Breast Cancer¹

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Abstract

Recently, a T-to-A transversion creating an 8-base mononucleotide tract in the APC gene, resulting in substitution of lysine for isoleucine at codon 1307 (I1307K), was found in a subset of Ashkenazi Jews. This sequence variant was most frequent in colorectal cancer patients with a positive family history of colorectal cancer. To determine whether the I1307K variant plays a role in colorectal or breast cancer predisposition in the Norwegian population, we have analyzed blood samples from 210 colorectal cancer patients and 183 breast cancer patients by PCR and direct sequencing. Thirty-seven of the colorectal cancer patients had a positive family history of cancer. Among the breast cancer patients, 24 had a family history of colorectal cancer and 75 a family history of breast and/or ovarian cancer. Only one colorectal cancer patient who belonged to a Jewish family was found to carry the A variant. Our data show that the I1307K variant is rare in the Norwegian population and should not be viewed as a candidate for susceptibility testing for colorectal cancer.

Introduction

Colorectal cancer is a major cause of mortality in Western countries and develops through an adenoma-carcinoma sequence. Several risk factors predispose to this disease, and some remain unidentified. Genetic predisposition to colorectal cancer includes dominant hereditary diseases, such as familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer syndromes (1). Familial adenomatous polyposis is caused by germ-line mutations in the APC³ gene, which acts as a tumor suppressor gene and maps to chromosome band 5q21 (2, 3). The APC gene is also frequently mutated in sporadic cases of this disease. The mutation spectra of both familial and sporadic tumors identify a cluster of mutations in the last and large exon of the gene, and the mutations usually lead to truncation of the protein. A disease-associated mutation with lower penetrance has been identified recently in this gene. Laken *et al.* (4) suggested that a T-to-A transversion creating an 8-base mononucleotide tract in the APC gene indirectly causes cancer predisposition in some Ashkenazi Jews. The mutation identified results in an amino acid exchange, lysine for isoleucine at codon 1307, which does not alter the function of the encoded protein but rather creates a hypermutable region in the gene, which may lead to truncation of the APC protein. The mutation was found in 10% of Ashkenazi Jews with colorectal cancer and in 28% of those with a positive family history for colorectal cancer, whereas

only 6% of non-colorectal cancer controls exhibited the same mutation (4).

A significant subgroup of breast and ovarian cancers are hereditary, and germ-line mutations have been identified in two major susceptibility genes, BRCA1 and BRCA2. Individuals with germ-line mutations in one of these genes have a very high lifetime risk for developing breast cancer and ovarian cancer, although ovarian cancer is not dominant in BRCA2 carriers (5). In addition, other cancers are found in these families, including a 4-fold risk for colorectal cancer. Following the Laken study (4), Petrukhin *et al.* (6) reported a 7% frequency of the I1307K mutation among Ashkenazim with breast/breast-ovarian cancers. No mutations were found in individuals with colorectal cancer in these breast-ovarian cancer families, indicating that other factors than the I1307K mutation contribute to the colorectal cancer risk in these families.

In the initial study, the I1307K polymorphism was not found among 243 non-Jewish controls (4), suggesting that the polymorphism might be rare in other populations. In the present study, we have analyzed the frequency of I1307K in Norwegian colorectal cancer patients and Norwegian and Swedish breast cancer patients, with and without a family history of cancer.

Materials and Methods

Colorectal Cancer Patients. Written information on standardized questionnaires concerning cancer among the relatives has been obtained from the 210 colorectal cancer patients included in this study (7). The patient samples were collected from seven hospitals in the Oslo-Akerhus region in the Southeast part of Norway. Cases in the families diagnosed after 1952 were checked through the Norwegian Cancer Registry. Hereditary nonpolyposis colorectal cancer may be divided into two subcategories, Lynch syndrome I versus Lynch syndrome II, based on the absence versus presence of extracolonic cancers (8). In the present study, 37 were included as familial cases according to criteria described previously (7). Briefly, two families met the strict "Amsterdam criteria" (9), and two families fulfilled "reduced Amsterdam criteria," meaning one less affected patient. Ten individuals had a family history of cancer of the following type: four diseased in each family who are first- or second-degree relatives, counting colorectal cancer, upper gastro-intestinal tract cancer, and endometrial cancer as affected (10). Twenty-three more had a positive family history for cancer when the number affected in the last group was reduced by one.

Breast Cancer Patients. The breast cancer patients were interviewed with respect to family history by standardized questionnaires. Family members to 141 of 183 index cases, diagnosed after 1952, were confirmed by the Norwegian Cancer Registry. Familial clustering was considered if one or more first- or second-degree relatives had suffered from breast cancer and/or ovarian cancer (7, 11, 12). Sixty-one of the breast cancer patients had at least one first-degree relative, and 14 had at least one second-degree relative with breast- and/or ovarian cancer. Twenty-four of the breast cancer patients had one or more colorectal cancers in the family. Seventeen of these were Swedish patients (13) and were included, because it is unlikely that they differ from Norwegians with respect to frequency of common polymorphisms.

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³ The abbreviation used is: APC, adenomatous polyposis coli.

DNA Analysis. DNA from peripheral blood leukocytes was extracted in an automated nucleic acid extractor (Applied Biosystems) using phenol/chloroform extraction, followed by ethanol precipitation.

To examine the sequence of the region surrounding the I1307K mutation, an 82-bp fragment from genomic DNA was amplified using the following primer set: 5'-GCAGATTCTGCTAATACCCTGC-3' and 5'-CTTCGCTCACAGGATCTTCAGC-3' (4). PCR products were directly sequenced using the forward primer, Taq DNA polymerase, and Dye Terminators, and the products were purified (Qiagen kit) and run on an Applied Biosystems 373 DNA sequencer.

Results and Discussion

Laken *et al.* (4) suggested that a T-to-A transversion in codon 1307 in the APC gene may predispose to colorectal cancer among Ashkenazi Jews, causing an approximate 2-fold increased risk. This polymorphism was particularly frequent in those with a positive family history of colorectal cancer. In the study by Petrukhin *et al.* (6), no I1307K carriers were found in Ashkenazi Jews with colorectal cancer belonging to breast/breast-ovarian cancer families. In addition, none of the identified carriers had a positive family history of colorectal cancer (6). In the present series of 393 Norwegian patients with colorectal cancer or breast cancer, only one colon cancer patient of Jewish origin was a carrier of this mutation. There are no indications for a positive family history of cancer for this patient, but limited information is available; therefore, the possibility cannot be excluded. The probability of identifying 1 Jew among 210 colorectal cancer cases drawn from the 1 million people, including ~1000 Jews in the region in question, is on average 20%. The majority of Jews in Norway are Ashkenazim, but such information is not available for the identified I1307K carrier. The tumor from this patient did not reveal microsatellite instability based on analyses of seven markers (7). Among 37 colorectal cancer patients with a family history of cancer, two fulfilled the clinical criteria for hereditary nonpolyposis colorectal cancer, and four others showed microsatellite instability in their tumors suggestive of a defective mismatch repair system (7). It has been speculated that the 8-base mononucleotide tract of the APC gene might be more easily mutated in the germ line as well as somatically in patients with a defective mismatch repair component (6). This hypothesis was not supported in the present series.

More than 10% of the Ashkenazim with colorectal cancer showed this base change in their germ line as compared with 6% of the Ashkenazim without colorectal cancer. Among the sporadic colorectal cancer cohort described (4), some familial cases might be included due to lack of information, and thus the expected carrier frequency of the variant among true sporadic cases might be <10%. Our series of sporadic cases is based on answered written questionnaires, and if the polymorphism in question exists with a similar frequency as in the Ashkenazi population, between 10 (6%) and 17 (10%) individuals of 173 tested should carry the A base. However, only one carrier of the rare allele (the A) was detected, and the carrier was of Jewish origin. The probability of finding 0 among 172 is 0.03 if the prevalence is at least 2%; therefore, we conclude that the true prevalence is <2% among Norwegian sporadic colorectal cancer cases. Stratification of the Ashkenazi patients according to age showed an increased fre-

quency for the rare allele in the younger patients (<66 year). Our series (7) was comparable with the Ashkenazi cohort (4) in age distribution.

The frequency of the rare allele of the polymorphism was shown to be the same in Ashkenazi Jews with breast or breast-ovarian cancer as in Ashkenazi Jews in general (7% versus 6%). Among the 183 familial and sporadic breast or breast-ovarian cancer cases in the present series, only the T variants were detected. An expectation of 6% carriers of the rare variant suggests 11 among 183 cases.

Laken *et al.* (4) analyzed 243 non-Jews for this polymorphism and found no carriers of the rare allele. Although they did not describe the population origin of the individuals analyzed, our results support the lack of this variant in our population, excluding one Jew with the rare allele. The probability of finding none among the 392 analyzed cases is 0.02 if the prevalence is 1% in our population. Thus, we conclude that the true prevalence of the A variant is <1% in the Norwegian population.

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