The I1307K APC Mutation Does Not Predispose to Colorectal Cancer in Jewish Ashkenazi Breast and Breast-Ovarian Cancer Kindreds

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Abstract

An increased incidence of colorectal cancer has been observed in breast and breast-ovarian cancer syndrome families, including those of Ashkenazi origin. Recently, a germ-line missense mutation in the APC gene, I1307K, was identified that may indirectly cause colorectal cancer in Ashkenazi Jews. To determine whether the excess of colon cancer in some breast-ovarian cancer families is related to the I1307K mutation, we evaluated 264 Ashkenazi Jews from 158 families. Most of these individuals had either a personal or a family history of breast and/or ovarian cancer, and 19.3% (51 of 264) carried one of the recurrent BRCA1 (185delAG or 5382insC) or BRCA2 (6174delT) mutations. We detected the APC I1307K mutation in 7% (11 of 158) of the Ashkenazi Jewish families and in 4.5% (12 of 264) of the individuals participating in these studies. Of the families studied, 26.6% (42 of 158) had at least one case of colorectal cancer in a first-, second-, or third-degree relative of the proband. Significantly, of the 12 individuals who possessed the I1307K mutation, none was diagnosed with colorectal cancer and none had a known first-, second-, or third-degree relative diagnosed with colon cancer. The results suggest that factors other than the I1307K mutation contribute to the increased incidence of colon cancer in Ashkenazi breast-ovarian cancer families. Our results emphasize that only a subset of Ashkenazi Jewish individuals with a family history of colorectal cancer should be viewed as candidates for genetic susceptibility testing for the I1307K APC mutation.

Introduction

Approximately 10% of cases of breast and ovarian cancers are hereditary (1). BRCA1 and BRCA2 mutations are thought to account for approximately 80% of breast cancer in families with a high incidence of early-onset breast and ovarian cancer (2). BRCA1 germ-line mutation carriers harbor a lifetime risk for breast cancer of about 85% (2, 3) and a 40–66% risk for ovarian cancer (2, 3). BRCA2 germ-line mutation carriers have a lifetime risk of breast cancer of about 85%, but their risk for ovarian cancer is lower (10–20%; Refs. 2 and 3). In addition, male BRCA2 mutation carriers have an approximate 7% lifetime risk for breast cancer (4). Other cancers (e.g., carcinoma of the brain, esophagus, lung, pancreas, stomach, head and neck, and malignant melanoma) have been observed in carriers of BRCA1 and BRCA2 mutations (5, 6). For example, BRCA1 mutation carriers have a 3-fold increased risk for prostate cancer and a 4-fold increased risk for colon cancer (7).

Several inherited forms of colorectal carcinoma are well-characterized, including FAP, a premalignant disease inherited as an autosomal dominant trait that affects about 1 in 1000 individuals. FAP patients are characterized by the early appearance (in the second and third decades of life) of hundreds or thousands of colorectal adenomas or polyps, some of which will progress to cancer (8, 9). FAP is associated with germ-line mutations in the tumor suppressor gene APC located in 5q21–22 (10, 11). Inactivating mutations in APC are believed to occur in the early stages of tumor development in the majority of sporadic colorectal cancers (8). The vast majority (>90%) of APC mutations lead to truncation and inactivation of the resulting APC protein. Recently, Laken et al. (12) identified a sequence alteration in the APC gene that occurred exclusively to Ashkenazi Jews. This missense mutation (T to A at APC nucleotide 3920) apparently does not alter the function of the encoded protein, but it results in a small hypermutable region of the gene and is therefore thought to indirectly contribute to colorectal cancer development (12). The mutation, referred to as I1307K, was detected in 6% (47 of 766) of the Jews tested and was absent in 243 non-Ashkenazim. The occurrence of the mutation was substantially higher (28% (7 of 25)) in Ashkenazi Jews affected with colorectal cancer who also reported a family history of colorectal cancer. Interestingly, none of the 13 affected individuals with a negative family history of colon cancer possessed the mutation, indicating that this sequence alteration alone may not be sufficient to initiate cancer. To determine whether the I1307K mutation in APC is associated with an increased incidence of colon cancer in some breast and breast-ovarian cancer-prone kindreds, we evaluated 264 Ashkenazi Jews for this sequence alteration using a HMA. Our analysis indicates that the I1307K mutation does not contribute greatly to the risk of colon cancer in Ashkenazi breast-ovarian cancer families and that general screening in the absence of a personal and/or family history of colorectal cancer is likely to be excessive.

Materials and Methods

Blood Samples. As part of a Fox Chase Cancer Center Institutional Review Board-approved protocol, peripheral blood samples were obtained from consenting affected and unaffected high-risk family members through the Margaret Dyson/Family Risk Assessment Program. Individuals participating in the Family Risk Assessment Program have agreed to allow their samples to be used for a wide range of research purposes, including screening for mutations in candidate predisposing genes.

Determination of Family History. Individuals from 158 families (66 breast cancer only, 5 ovarian cancer only, 44 breast-ovarian cancer, 21 "other" cancer kindreds, and 22 "sporadic" cancer) were evaluated in this study. “Other” refers to families in which three or more cancers other than breast, ovarian or colon cancer are observed in at least two successive generations. “Other” cancers include hematopoietic malignancies and tumors of the blad...
Confirmation of the I1307K Mutation by DNA Sequencing. The DNA isolated from mutant allele carriers was amplified by PCR, and the product was separated from primers using Wizard resin (Promega) according to the manufacturer’s specifications. The purified DNA was subjected to cycle sequencing using an automated fluorescence-based cycle sequencer (Model 377A Automated Sequencer; Applied Biosystems) and Taq dye terminator chemistry. Sequencing primers were the same as those used to amplify the template. To test the sensitivity of our assay, we sequenced 20 DNA samples that failed to produce a mobility shift. As expected, all 20 samples yielded wild-type sequence.

Results

Constitutional DNA isolated from 264 Jewish individuals (Table 1) was screened for the T to A transversion at nucleotide 3920 of APC by a HMA and by direct DNA sequencing. Genomic DNA isolated from peripheral blood was amplified using the primer set indicated in “Materials and Methods.” Twelve of the 264 (4.5%) DNA samples showed a mobility shift that was consistent with the I1307K-positive control (Fig. 1). The presence of the altered allele was confirmed first by PCR-HMA using another set of primers and then by direct sequencing (data not shown).

Evaluation of the pedigrees for the 12 I1307K-positive individuals found that all but 1 had a significant family history of breast and/or ovarian cancer and none had a personal or family history of colorectal cancer (Fig. 2). In 5 of the 11 I1307K mutant families (i.e., kindreds 1–5), a BRCA1 and/or BRCA2 mutation had previously been detected (Fig. 2A). For example, in family 1 (Fig. 2A), the affected proband (ovarian cancer at age 57 years) inherited the I1307K APC mutation paternally (unaffected father alive at age 85 years) and a 185delEAG BRCA1 mutation maternally (mother diagnosed with breast cancer at age 63 years and colon cancer at age 80 years). Further evaluation of this family found that the proband’s paternal first cousin, diagnosed with ovarian cancer at age 55 years, was negative for the I1307K APC mutation and positive for a 5382 insC BRCA1 mutation.

![Fig. 1. Detection of the I1307K and E1317Q mutations using a HMA/SSCP assay.](image-url)
THE 11307K APC MUTATION DOES NOT PREDISPOSE TO COLORECTAL CANCER
BRCA1 or BRCA2 mutant allele carriers are indicated. Arrow, the initial individual (proband) tested in the family.

An, brain cancer; Bo, bone cancer; Br, breast cancer; BS, basal squamous cancer; CAT, cervical cancer; £,v, esophageal cancer; Ho, Hodgkin's disease; Le, leukemia; Lu, lung cancer; or man is the age at diagnosis. Number in [Hirenthrxc's. the current age or age of death of the individual. Unk, unknown age of diagnosis; BCC, basal cell carcinoma; HI, bladder cancer; PSU, primary site unknown. Asterisks (*) identity confirmed I1307K mutant allele carriers. Neg, family members who do not have the I1307K mutation. Confirmed BRCA1 or BRCA2 mutant allele carriers are indicated. Arrow, the initial individual (proband) tested in the family.

Fig. 2. I1307K mutant kindreds. Symbols are defined as listed in the Fig. 2 legend.

During our screen, we detected a second sequence alteration in this region of APC. Two of the 264 (0.76%) individuals (Fig. 3) evaluated possessed a G to C transversion at nucleotide 3949 (Fig. 1). Sequence analysis revealed a GAA to CAA point mutation affecting codon 1317 of exon 15, resulting in a glutamic acid to glutamine amino acid substitution. This sequence alteration, referred to as E1317Q, has previously been observed in a cancer-prone family that does not result in FAP (14). This sequence alteration in APC did not completely cosegregate with cancer in this kindred and is therefore likely to be a relatively infrequent polymorphism in the Jewish population. However, it has not been determined if the E1317Q alteration is present in non-Ashkenazim as well. Our results suggest that inherited copies of either the I1307K or the E1317Q APC mutation are likely not to be associated with the classical FAP phenotype and may only marginally alter a person's risk of developing colorectal cancer in the absence of family history of that disease.

Discussion

Given that BRCA1 mutation carriers have a risk for colon cancer that is approximately 4-fold greater than that of the general population (7) and that the I1307K APC mutation may predispose to colorectal cancer in Ashkenazim (12), we evaluated 264 Jews, many from breast and breast-ovarian cancer syndrome families, for this APC mutation (Table 1). We found that 7% (11 of 158) of these Ashkenazi Jewish families carried the I1307K mutation. This is consistent with the report of Laken et al. (12), which found that a similar proportion of Ashkenazim [6.1% (47 of 766)] carried this alteration. In contrast to their study, we observed that none of the 12 individuals carrying the mutation have developed colorectal cancer, as have none of their relatives. The results of our evaluation suggest that inheritance of the I1307K mutation alone does not seem to contribute greatly to the risk of colon cancer, at least in these high-risk breast and ovarian cancer families.

There are several potential explanations for our findings. The simplest explanation that supports our results is that this missense mutation alone is not pathogenic. Although it occurs within the mutation cluster region, the preferred location for most acquired and many germ-line mutations, I1307K is not typical of the majority of reported APC mutations (15–19); the protein product is full length and involves only a single amino acid substitution. A second possibility is that the I1307K mutation contributes to colorectal cancer development, but only in individuals already predisposed to this disease. Because our patients differ substantially from the ones previously studied in that many were affected with breast and/or ovarian cancer and more than half reported a significant family history of these diseases, the risk for developing colorectal cancer would not be significantly elevated. A third possibility is that due to inherited predisposition by mutations in BRCA1, BRCA2 (i.e., families 1–5), and other unidentified breast-ovarian susceptibility genes, the individuals within these kindreds do not live long enough to develop colorectal cancers. Review of our pedigrees would suggest otherwise (Fig. 2). Many family members (especially males) who are obligate gene carriers live well into their 60s and 70s, the age at which Lanken et al. (12) observed a high incidence of the I1307K mutation in colorectal cancer patients (16% of patients <66 years of age versus 6.6% of patients >66 years of age carry the I1307K mutation).

A plausible explanation for Laken's and our observations is that the T to A transversion results in a hypermutable tract [AAATAAAAA to (A)₉ in APC; Ref. 12] which is thereby a target for further alteration in tumor cells already deficient in mismatch repair or other DNA replication/repair activities, such as in familial and sporadic colorectal cancer (8, 20–22). It has been postulated that the incidence of somatic APC mutations may be increased if there are alterations in other inherited non-FAP genes. For example, in HNPCC, mutations in one or more of the mismatch repair genes cause replication errors throughout the genome (8). This genetic instability is demonstrated by shifts in the electrophoretic mobility of microsatellite repeat fragments and may be observed within the APC sequence. In this way, inherited mutation in the mismatch repair genes or other related genes can cause mutation in APC either as a somatic event or in the germ line. It is not clear from the previous study of Laken et al. (12) whether the mutations observed within or near the (A)₉ tract actually contribute to the development of colorectal cancer, given that a wild-type APC allele was present in all but one of the tumor samples examined. Therefore, it would be interesting in future studies to compare the natural history and clinical course of HNPCC Jewish patients with and without the I1307K allele. Thus, the risk of developing colorectal cancer in an individual who carries the I1307K APC mutation and...
lacks a history of colorectal cancer may be considered to be quite low and no greater than that of the general population.

The available data indicate that detection of germ-line APC mutation in association with colorectal cancer may not always have an obvious single interpretation and that other factors or genes might also be involved. It is our opinion that the I1307K mutation is more susceptible to further genetic damage in individuals predisposed to colorectal cancer due to mutation in the known HNPPC genes or related, yet to be identified, genes that give rise to either type I or type II microsatellite instability. In this aspect, we have found that mononucleotide repeats, such as the one generated by the T to A transversion, are sensitive targets for type II microsatellite instability. This is consistent with the work presented by Laken et al. (12) that found that 28% of Jewish probands with colorectal cancer and a family history of disease carried the I1307K mutation as compared to 6.1% of Ashkenazi Jews (unselected for cancer) and 0% (0 of 13) of affected probands with no family history of colorectal cancer. Therefore, it can be inferred that in the absence of a family history of colorectal cancer, the I1307K alteration may be considered a benign polymorphism or only weakly tumorigenic. Our results suggest that not all carriers of this mutation are destined to develop colorectal cancer and that I1307K screening should be offered to only a subset of the Jewish population, namely those with a personal and/or family history of colon cancer.

Acknowledgments

We thank Drs. Lisa Henske and Amanda Prowse for critical review of the manuscript and Agnes Masny, Patti Barse, Ann Agro, and Christine Harrop for their help and contribution to this work.

References


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