Somatic **BRAF-V600E** Mutations in Familial Colorectal Cancer

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**Abstract**

The **BRAF** gene is mutated in 4% to 12% of unselected colorectal cancers, particularly those with high microsatellite instability and in premalignant lesions, such as serrated adenomas and hyperplastic polyps. However, it has been shown that activating **BRAF** mutations are almost never found in tumors from hereditary nonpolyposis colorectal cancer patients. To evaluate the role of oncogenic **BRAF** mutations in non-hereditary nonpolyposis colorectal cancer/non-familial adenomatous polyposis familial colorectal cancer, we did a mutation screening of the most common **BRAF** mutation, the V600E mutation, in 194 colorectal tumors from patients with a positive family history of the disease. The **BRAF-V600E** mutation was identified in 100% (8 of 8) of microsatellite-unstable tumors and in 9.7% (18 of 186) of microsatellite-stable tumors. Interestingly, families with extracolonic tumors showed a much higher mutation frequency (17.5%) compared with families with colonic cancer only (3.5%; \( P = 0.009 \)). In addition, we studied colonoscopic results from 448 family members who had been under colonoscopic surveillance for several years. Subjects from families where the V600E mutation was identified had less adenomas compared with those from families where no **BRAF** mutation had been found (odds ratio, 8.5; 95% confidence interval, 1.1-64.6). These findings indicate that adenomas might be less important in the cancer development in the group of families with **BRAF-V600E** mutations and indirectly support a previous hypothesis that tumors might develop through the hyperplastic polyp-serrated adenoma pathway. In conclusion, our results suggest that **BRAF-V600E** mutations are mainly involved in colorectal cancer families characterized by an increased risk of other common malignancies.

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**Introduction**

Colorectal cancer is a multistep disease, and mutations in several genes are needed for colorectal cancer progression. Mutations in the **KRAS** gene, a member of the mitogen-activated protein kinase signaling pathway, are involved in colorectal carcinoma development. Recently, the **BRAF** gene, another member of this pathway, is mutated in colorectal tumors (1, 2). This gene codes for a cytoplasmic serine-threonine kinase of the Raf family. Oncogenic mutations in **BRAF** have been shown to destroy the kinase domain, which results in a constitutive activation of the mitogen-activated protein/extracellular signal-regulated kinase kinase/extracellular signal-regulated kinase and nuclear factor-κB signaling pathways. This domain contains a hotspot at a nucleotide position 1,796 where a T>A transversion causes an amino acid change of glutamate to valine at residue 600. This V600E mutation is frequent and accounts for up to 90% of all **BRAF** mutations in colorectal cancer (2, 3). Activating **BRAF** mutations have been identified in 4% to 12% of unselected colorectal tumors in all Dukes’ stages (2, 4-8) as well as in premalignant lesions, such as adenomas, hyperplastic polyps, and serrated adenomas (5, 6), and also in aberrant crypt foci, the earliest stages of colorectal cancer development (9). There is a strong association between **BRAF** mutations and deficient mismatch repair characterized by microsatellite instability (MSI; refs. 2, 3, 5). **BRAF** mutations are found in 30% to 75% of MSI-positive tumors (3, 5, 7), in contrast to **KRAS** mutations that are more prevalent in MSI-negative tumors. In fact, **KRAS** and **BRAF** mutations have been suggested to be mutually exclusive, indicating their similar role in colorectal carcinogenesis (2, 4, 7). MSI in sporadic tumors is mainly associated with an epigenetic inactivation of the mismatch repair gene **hMLH1**, and a strong association has been found in **BRAF**-mutated tumors with methylation of **hMLH1** (3, 4, 7, 10). In contrast, MSI tumors in hereditary nonpolyposis colorectal cancer (HNPPC) associated with germ-line mismatch repair gene mutations rarely show **BRAF** mutations (3, 7, 8, 11). Because the **BRAF** pathway is not involved in HNPPC, the question is whether this pathway is prevalent only in sporadic colorectal cancer or if there is also a familial equivalent. In a study by Domingo et al. (11), no **BRAF** mutations were found. However, only a limited number of MSI-negative familial tumors were used in this study. To further elucidate the role of the V600E mutation in familial colorectal cancer, we did a mutation screening for this mutation in 202 tumors from 194 families with familial non-HNPPC/familial adenomatous polyposis (FAP) colorectal cancer.

**Materials and Methods**

**Cases and Tumor Samples.** Patients with a suggested familial predisposition to colorectal cancer because of a family history or an early age of onset of colorectal cancer were enrolled through the Cancer Family Clinic at the Karolinska Hospital (Stockholm, Sweden). In total, 202 tumor samples from 194 non-HNPPC/non-FAP families were included in the study. Of these, 53 families had at least three first- or second-degree relatives with colorectal cancer, 98 families had at least two first- or second-degree relatives, 16 families had two cases of colorectal cancer and endometrial cancer, 1 family was diagnosed as a cancer family with many cancer cases, including 1 individual with colorectal cancer, 25 individuals were single cases of early onset colorectal cancer, and finally 1 case had several cancer diagnoses, including colorectal cancer. A cancer family history was available from all families. Colorectal cancer diagnoses were verified through medical records or death certificates. Other cancer diagnoses were not always verified, and therefore, age of onset was not available for all cases. FAP was excluded by colonoscopic examination, and HNPPC was excluded by the current clinical screening.

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colorectal polyps (hyperplastic polyps and serrated adenomas). Adenomas and polyps between families with a detected BRAF mutation and families without any detected mutation were tested. The numbers of variables, adenomas and polyps, were categorized into three classes: 0, 1-2, and >2. The Pearson χ² was used to compare differences in frequencies of polyps and adenomas between the two groups. A P < 0.05 was considered to be statistically significant. To evaluate the relationship between the BRAF-V600E mutation and each class of variables, a logistic regression analysis was used to calculate odds ratios and 95% confidence intervals. Odds ratios were adjusted for age.

Results

The Frequency of BRAF-V600E in Familial and Sporadic Colorectal Cancer. In the present study, we determined the frequency of the BRAF-V600E mutation in 202 colorectal tumors from 194 colorectal cancer families and in 179 sporadic colorectal cancer tumors. Overall, the BRAF-V600E mutation was detected in 13.4% (26 of 194) of tumors of patients with a positive family history of colorectal cancer and in 10.1% (18 of 179) of sporadic tumors (not significant, P < 0.4, Pearson χ² test). Among familial cases, all MSI tumors (8 of 8) were V600E positive, whereas the V600E mutation was identified in 42.9% (9 of 21) of sporadic MSI tumors. In the group of MSI-negative tumors, the V600E mutation was detected in 9.7% (18 of 186) and 5.7% (9 of 158) of familial and sporadic tumors, respectively. In both groups of familial and sporadic tumors, the difference between MSI-positive and MSI-negative groups was statistically significant (P < 0.001, Pearson χ² test). In addition, the frequency of the V600E mutation varied among familial cases depending on the presence of extracolonic tumors in the families. A significantly higher frequency of the BRAF-V600E mutation (17.5%) was identified in families afflicted with colonic and extracolonic tumors compared with families where only colonic tumors were seen (3.5%; P = 0.009, Pearson χ² test; Table 2). In seven families, more than one colorectal tumor was studied, and in two of these families, tumors showed discordant MSI status. In family 237, one tumor was MSI positive and one was MSI negative and both were BRAF mutation positive. In family 409, two tumors were MSI and BRAF positive and a third tumor was MSI and BRAF negative. Among the remaining five families, tumors were MSI negative and all except one were BRAF-V600E negative.

Adenomas and Polyps in Family Members under Colonoscopic Surveillance. To determine phenotypic differences between families with and without a BRAF-V600E mutation, we studied colonoscopic data from 448 individuals from 194 colorectal cancer families under surveillance (Table 3). Based on the presence of the V600E mutation in at least one colorectal tumor from each family, 78 patients belonged to families where BRAF mutations were found and 370 were from families without detectable BRAF mutations. Each patient was categorized based on the number of precursor lesions into the following classes: with 0, 1-2, or >2 adenomas or polyps associated with the “serrated pathway” (hyperplastic polyps and serrated adenomas). Adenomas were found in 126 patients (range, 1-13 adenomas), whereas hyperplastic polyps or serrated adenomas were identified in 153 patients (range, 1 to >30 polyps). Overall, risk individuals in families with BRAF mutations seemed to have a lower number of adenomas (not significant, P < 0.07, Pearson χ² test). Logistic regression analysis adjusted for age was used to compare the proportion of subjects within the different classes of precursor lesions among families with and without a BRAF mutation. Families with BRAF mutations had significantly fewer family members with more than two adenomas (odds ratio, 8.5; 95% confidence interval, 1.1-64.5). There was no
Table 2. **BRAF-V600E** mutations in 194 familial colorectal tumors

<table>
<thead>
<tr>
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<th>Wild-type, n (%)</th>
<th>V600E, n (%)</th>
<th>P*</th>
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<tr>
<td>Families with colonic and</td>
<td>113 (82.5)</td>
<td>24 (17.5)</td>
<td>0.009</td>
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<tr>
<td>extracolonic tumors†</td>
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<tr>
<td>Families with colonic</td>
<td>55 (96.5)</td>
<td>2 (3.5)</td>
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</tr>
<tr>
<td>tumors only</td>
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*Pearson χ² test.
†Extracolonic tumors include breast, gastric, prostate, pancreatic, endometrial, bladder, gall bladder, kidney, cervix, liver, and lung cancers, sarcoma, melanoma, brain tumors, and leukemia.

Discussion

In our study of familial colorectal cancer, the **BRAF-V600E** mutation was identified in 13.4% of all tumors, which is slightly more than in sporadic tumors, 10.1% in our study, and in unselected colorectal cancers reported previously (4-12%; refs. 2-8). The overall higher frequency of the **BRAF-V600E** mutation in the group of familial colorectal cancer cases suggests a role of the **BRAF** gene in the development of familial colorectal tumors. Moreover, the significantly higher incidence of **BRAF** mutations seen among families afflicted with extracolonic cancers (17.5%) compared with families afflicted only with colon cancer (3.5%) suggests the existence of a new familial syndrome characterized by a high incidence of common malignancies and frequent **BRAF** mutations in colorectal cancers.

Genetic predisposition plays an important role in tumor development in up to one third of all colorectal cancer cases, and several syndromes, such as HNPCC and FAP, have been described. However, these syndromes account for <5% of all colorectal cancer cases. Thus, in most of the families with a pedigree suggesting a genetic predisposition, the cause of the disease remains to be elucidated. Recently, an inherited colorectal cancer syndrome associated with an increased risk of colorectal cancer and somatic **BRAF** mutations was suggested in families showing a discordant MSI status and a high frequency of hyperplastic polyps (16). The association between **BRAF** mutations and a family history of colorectal cancer was further supported by a second study (17). However, in this study, a positive correlation was found only for MSI-negative tumors. In our study, **BRAF-V600E** mutations were identified in both MSI-positive and MSI-negative familial colorectal tumors with a higher incidence among MSI-positive tumors. In this group, all eight tumors were found to harbor the **BRAF-V600E** mutation. Because somatic **BRAF** mutations are rarely, if ever, found in HNPCC tumors, these findings confirm that these tumors constitute non-HNPCC cases (3, 7, 8, 11). In three of these families, additional tumors were tested and found to be MSI negative. These results further exclude HNPCC in these families and support the findings published by Young et al. (16), indicating the existence of a colorectal cancer syndrome associated with somatic **BRAF** mutations and a variable MSI status. We studied only one tumor from most families, and therefore, there is a possibility of undetected families with MSI variable phenotype and **BRAF** mutations. In this study, we also found evidence of an increased risk of extracolonic tumors among families with **BRAF** mutations. The extracolonic spectrum included the most common tumors seen in general population, such as breast, gastric, prostate, pancreatic, endometrial, bladder, gall bladder, kidney, cervix, brain, and lung cancers as well as sarcoma, melanoma, and leukemia (Table 1). When studying the types of extracolonic tumors separately, all of them showed a higher frequency of **BRAF** mutations compared with the group with colorectal cancer only.

The well-established adenoma-carcinoma sequence, typical for the majority of colorectal cancers, outlines the high risk of developing carcinomas in adenomas. Recently, a different carcinogenic pathway was suggested where polyps can progress over the so-called ‘‘serrated pathway’’ from hyperplastic polyps, over serrated adenomas or mixed polyps into MSI-positive tumors (18-22). To look for phenotypical differences about precursor lesions in families with this syndrome associated with **BRAF** mutations, we compared the frequency of polyps and adenomas in 448 individuals under surveillance from the 194 colorectal cancer families included in the **BRAF** mutation screening study. Overall, there was a trend among subjects from families with **BRAF** mutations to have fewer adenomas. A statistically significant difference was obtained for individuals with many adenomas, suggesting that among families with **BRAF** mutations there are fewer members with many adenomas. This finding is consistent with the idea that the major risk factor for disease in the **BRAF** families might be less dependent on the prevalence of adenomas and indirectly supports a previously suggested hypothesis of an alternative pathway of colorectal cancer development (19-22). To rule out that the differences in numbers of polyps were associated with a difference in the cancer risk, we compared the group with the highest risk (those with at least three relatives with colorectal cancer) to the group with a lower risk (those with only two close relatives with colorectal cancer). In our study, no difference in the numbers of hyperplastic polyps/serrated adenomas between the groups of families was seen. However, we have previously studied larger numbers of subjects under surveillance and found that subjects in non-HNPCC families develop more adenomas and hyperplastic polyps than subjects from HNPCC families and controls (23). Moreover, those in the lower-risk group had even more polyps than those in the high-risk group (23). Thus, there is no simple relation between the number of adenomas and hyperplastic polyps and a cancer risk. The cancer risk in any risk individual is likely to be related to the number of polyps, as well as to the types of polyps, but is also dependent on the predisposing genetic risk factors. As long as those risk factors are unknown (or empirical), it is impossible to know the risk of cancer in any separate polyp in an individual.

Table 3. The frequency of polyps and adenomas in risk individuals under surveillance

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<thead>
<tr>
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<th>No. polyps*</th>
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<tr>
<td></td>
<td>1-2</td>
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<tr>
<td>BRAF WT, n (%)</td>
<td>77 (20.8)</td>
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<tr>
<td>BRAF-V600E, n (%)</td>
<td>16 (22.9)</td>
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<tr>
<td>P</td>
<td>0.60</td>
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<td>OR (95% CI)</td>
<td>1.18 (0.64-2.18)</td>
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Abbreviations: WT, wild-type; OR, odds ratio; 95% CI, 95% confidence interval.
*The group of polyps included hyperplastic polyps and serrated adenomas.
In conclusion, our study is the first report describing the frequency of the BRAF-V600E mutation in familial non-HNPCC colorectal cancer. BRAF mutations were identified with a higher frequency than in unselected colorectal cancers and were strongly associated with MSI. Our results also support the existence of a familial syndrome associated with a variable MSI status and BRAF mutation-positive colorectal tumors as proposed by Young et al. (16). Moreover, our study suggests that in this syndrome there is also an increased risk of the most common extracolonic tumors. Finally, our results indicate that colorectal tumors in this syndrome develop through a pathway less dependent of adenomas as precursor lesions.

References
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