The NOD2 3020insC Mutation and the Risk of Colorectal Cancer

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Abstract

Several predispositions to colorectal cancer have been identified, but little is known about genetic susceptibilities to disease in older persons. Colorectal cancer is a risk in Crohn’s disease and is believed to be associated with an inappropriate inflammatory response. Recently, the NOD2 gene has been associated with Crohn’s disease, which further strengthens the notion that the inflammatory response plays a crucial role in this disease. Several mutations have been identified in the NOD2 gene, which appear with significantly higher frequency in patients with the disease. One such mutation (3020insC) is believed to be clearly causative because it results in a prematurely truncated protein with a predicted reduction in functional efficiency. In this report, we have examined the frequency of the 3020insC mutation in a series of 856 individuals including 556 patients with colorectal cancer. The frequency of the 3020insC mutation in a consecutive series of 250 non-hereditary nonpolyposis colorectal cancer patients >50 years of age was significantly elevated compared with the control population (odds ratio, 2.23; P = 0.0046). The results indicate that NOD2 may be a predisposing factor to colorectal cancer characterized by an older average age of disease onset in persons who do not harbor any other genetic predisposition to disease.

Introduction

Individuals diagnosed with Crohn’s disease (CD) have been shown to have an increased risk of colorectal cancer (CRC; Ref. 1). How this is mediated remains undefined, but it is thought to involve genes that are involved with inflammatory response (such as IFN-γ and tumor necrosis factor-α) that result in the activation of neutrophils, which generate significant amounts of reactive oxygen species (2). The continuous exposure of the gastrointestinal epithelium to reactive oxygen species is believed to be associated with an increased mutation rate, which results in an increased likelihood of tumor development (2). Little is known about the risk of developing gastrointestinal cancers in CD because most studies focus on CD and ulcerative colitis. It has been estimated that somewhere between 1 and 2% of all colorectal cancer patients have been diagnosed with either CD or ulcerative colitis, and up to 15% of all patients with CD or ulcerative colitis will develop CRC (1).

The genetic basis of CD remains undefined, but epidemiological studies consistently suggest an inherited basis to the disease, and more recently, a genetic locus was identified on chromosome 16 (3), which harbored a gene known as NOD2/CARD15. The NOD2 gene comprises 12 exons and encodes a protein of 1040 amino acids, the exact function of which remains unknown (4). The predicted motifs encoded by the NOD2 gene suggest that it is involved in the dysregulation of immune function by either affecting a change in the detection or binding of bacterial proteins and/or impaired nuclear factor-κB signaling (5).

Recently, three common variants have been identified that have been associated with an increased likelihood of CD, two of which are missense changes (Arg702Trp and Gly908Arg), and one is an insertion mutation (3020insC; Refs. 4, 6). The insertion mutation 3020insC has been shown, when inherited in the homozygous state, to be associated with an almost 20-fold risk of developing CD, whereas if present in the heterozygous state, it increases risk by ~3-fold (7). The exact relationship between the 3020insC mutation and cancer risk has not been accurately assessed.

Because the 3020insC mutation is the only common variant that can unequivocally be associated with a deleterious change in the NOD2 protein, it lends itself to further investigation in relation to determining its role in cancer risk. In this report, we have investigated the association between the occurrence of the 3020insC mutation in a series of consecutively collected CRC patients, as well as patients clinically defined as having hereditary nonpolyposis colorectal cancer, and patients with a familial aggregation of cancer but no recognizable family cancer syndrome.

Materials and Methods

Patients. Four groups of patients affected by colorectal adenocarcinomas were studied. Group 1 consisted of 250 consecutive CRC patients who underwent surgery in the clinical hospital SPSK-2 Szczecin, Poland; hereditary nonpolyposis colorectal cancer patients were excluded from this group but not patients with an undefined cancer family aggregation. All patients in this group were >50 years of age. Group 2 was similar to group 1, except all 50 patients were ≤50 years of age. Group 3 consisted of 156 patients matching the criteria of hereditary nonpolyposis colorectal cancer but without MSH2 or MLH1, constitutional mutations detectable by DNA sequencing derived from all regions of Poland. Group 4 consisted of 100 CRC patients from the genetic counseling unit (of the International Hereditary Cancer Centre in Szczecin) from families where there were at least two other malignancies diagnosed on the same side of the family that confirmed an undefined cancer family aggregation. Group 5 consisted of control subjects (300 consecutive newborns from the clinical hospitals of Szczecin). DNA samples were obtained from the peripheral blood of CRC patients or from umbilical cord blood of newborns, according to the method of Miller et al. (8).

Methods. The method described by Ogura et al. (4) was used to identify the 3020insC mutation. The sequences of the PCR products were confirmed by DNA sequencing.

Statistical Analysis. χ2 tests with Yates correction were used to determine the significance of the results, and only those with P < 0.05 were considered significant.
Results and Discussion

The presence of the mutant allele was examined in the total population of 856 individuals as described above. The incidence of the 3020insC mutation in consecutive cases \( \leq 50 \) years of age diagnosed with colorectal cancer was not statistically different from that of the control population (Table 1). Nor was there any significant difference in the frequency of this mutation in families adhering to the diagnostic criteria of hereditary nonpolyposis colorectal cancer but not harboring changes in either hMSH2 or hMLH1. No differences were observed in patients diagnosed with colorectal cancer who came from families where there was an aggregation of cancers that did not fit any known syndrome.

The frequency of the 3020insC mutation in the consecutive series of colorectal cancer patients \( > 50 \) years of age was, however, significantly elevated compared with the control population. The presence of the 3020insC variant was further investigated and found to be associated with an increased odds ratio of colorectal cancer risk of \( 2.23 \), as shown in Table 1. There was no difference between the ages of diagnosis in the group of patients harboring the 3020insC mutation (average age, 65 years; range, 52–78 years) compared with the patients without the mutation (average age, 64 years; range, 51–92 years).

Further analysis of the study population revealed that there were differences between the four groups. There was a significant difference in the frequency of the 3020insC mutation between the group of consecutively collected CRC patients \( \leq 50 \) years of age compared with those \( > 50 \) years of age. There was also a significant difference between the colorectal cancer patients who came from families where there was an aggregation of other tumors and the consecutively collected CRC cases diagnosed who were \( > 50 \) years of age. Finally, no differences were observed between all of the other groups.

In the study reported herein, we focused on the 3020insC mutation, which results in the disruption of a leucine-rich region in the NOD2/CARD15 gene that is important for protein-protein interaction (9). Other missense variants have been identified in the NOD2/CARD15 gene, but they may not necessarily be associated with such an overt change in protein function.

The frequency of the 3020insC mutation in the general population has been estimated in a number of populations to be somewhere in the vicinity of 8% (10), whereas in the population under study here, the frequency was found to be close to 7%. In comparison with the overall frequency reported by Ogura et al. (4), there was no significant difference between the Polish population and that from North America (\( P = 0.641 \)).

It could be argued that the control population is unrepresentative of that of the older general population residing in the region in and around the city of Szczecin. Several points suggest that the observed frequency in the newborn population has not significantly changed: (a) the frequency of the change corresponds to that of other populations; (b) the population of Szczecin has not experienced a significant influx or outflux of individuals over the past 50 years; and (c) the rate of CD has not dramatically increased in recent years.

Gastrointestinal cancers have been recognized as a complicating factor in CD, but only a small percentage of all CD patients will develop a malignancy (1). In this report, we have investigated a series of CRC patients to determine whether NOD2 is a contributing factor to malignant disease development. The results of the study indicate that NOD2 is more frequently represented in the population of patients who are \( > 50 \) years of age. It is not known if any of these patients had been diagnosed previously with CD, notwithstanding that the increased frequency of the 3020insC mutation in the consecutively collected CRC patient group \( > 50 \) years of age, compared with the control population, is suggestive of a significant relationship. Indeed, the odds ratio of developing CRC was \( > 2.2 \) (confidence interval, 1.23–4.10) if the 3020insC mutation was present. It appeared in this study that the average age of disease diagnosis was no different in the 3020insC carriers compared with the non-carriers. Because NOD2 is associated with immune modulation, it cannot be ruled out that other genetic factors involved in immune response may also contribute to disease risk, especially genes that encode proteins that are necessary for the control of inflammatory response.

A significant difference was observed between the frequency of the 3020insC mutation in the consecutive CRC population \( > 50 \) years of age and the families where there was an aggregation of familial cancer

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**Table 1. Frequency of patients harboring the 3020insC mutation**

<table>
<thead>
<tr>
<th>Group</th>
<th>( n )</th>
<th>Mean age at diagnosis ± SD (range)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1: CRC, ( &gt; 50 ) years of age</td>
<td>250</td>
<td>65.2 ± 8.2 (51–92)</td>
<td>14.4</td>
</tr>
<tr>
<td>Group 2: CRC, ( \leq 50 ) years of age</td>
<td>50</td>
<td>44.9 ± 4.2 (30–50)</td>
<td>2</td>
</tr>
<tr>
<td>Group 3: HNPPC(^a) without mutations</td>
<td>156</td>
<td>51.9 ± 11.7 (28–75)</td>
<td>10.25</td>
</tr>
<tr>
<td>Group 4: CFA, all ages</td>
<td>100</td>
<td>55.1 ± 11.9 (29–75)</td>
<td>4</td>
</tr>
<tr>
<td>Group 5: Newborns</td>
<td>300</td>
<td>—</td>
<td>7</td>
</tr>
</tbody>
</table>

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\( ^a \) HNPPC, hereditary nonpolyposis colorectal cancer; CFA, cancer family aggregation; OR, odds ratio; CI, confidence interval.

\( ^b \) Boldface represents significant findings.

\( ^c \) \( \chi^2 \) test with Yates correction factor.

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1605
that could not be classified into any known syndrome. This result suggests that the 3020insC mutation is likely to be specific to colorectal cancer risk and not a generalized risk factor for cancer at all sites. This is supported by the tumor spectrum in CD, which is associated with an increased risk of gastrointestinal tumors and nothing else (1).

In conclusion, the presence of the 3020insC mutation appears to increase the risk of developing CRC at an older age. It was beyond the scope of this study to examine whether familial aggregations of later onset CRC could be associated with the 3020insC change. Even in the absence of the 3020insC mutation, alterations in genes associated with inflammatory response could account for a large number of CRC cases and therefore be an access point to effective intervention strategies.

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References

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