Heterozygosity for the \( BLM^{Ash} \) Mutation and Cancer Risk\(^1 \)

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Abstract

Bloom syndrome is an autosomal recessive disorder whose characteristics include an increased risk for many types of cancers. In contrast to the homozygous mutations of Bloom syndrome, heterozygous carriers of \( BLM \) mutations may be at increased risk for developing colorectal cancer. We have screened 2333 Jewish individuals, including 497 individuals with colorectal cancer, 125 with adenomatous polyps, 767 with noncolorectal cancers and 944 controls for the truncating \( BLM^{Ash} \) founder mutation. The \( BLM^{Ash} \) mutation was carried by 0.80% of individuals with colorectal neoplasia, 0.87% of those with any type of cancer and 0.85% of controls. In addition to case-control data, we found no evidence to support a significant relationship between increased cancer risk and heterozygous \( BLM^{Ash} \) mutations with respect to age of cancer diagnosis, tumor multiplicity or family cancer history.

Introduction

Bloom syndrome is a rare autosomal recessive disorder characterized by immunodeficiency, growth retardation, male sterility, and a predisposition to many types of cancers (1). The gene responsible for Bloom syndrome, \( BLM \),\(^3 \) encodes a homologue of recQ helicase (2). Loss of helicase activity in the cells of individuals with Bloom syndrome leads to genomic instability, which is characterized by increased rates of somatic recombination, chromosomal breakage, and gene mutation (3–5). Multiple types of cancer, including colorectal cancer and premalignant adenomatous polyps, have been observed in individuals with Bloom syndrome (1, 6). Approximately one-third of individuals with Bloom syndrome are of Jewish descent and carry the founder \( BLM^{Ash} \) mutation, a frameshift mutation in exon 10 (2281 delATC TGA insTAG ATT C; Ref. 7). In contrast to Bloom syndrome, which occurs in homozygous \( BLM \) mutation carriers, there are some data supporting the hypothesis that \( BLM \) heterozygotes are at increased risk of cancer. Increased rates of somatic recombination have been observed in cells from individuals who carry a single mutant \( BLM \) allele (BLM heterozygotes; Ref. 8), and transfection of wild-type \( BLM \) only partially corrects excessive sister chromatid exchange in \( BLM \)-deficient cells (9). Recently, Gruber et al. (10) studied Jewish populations from Israel and New York and reported that heterozygosity for \( BLM^{Ash} \) is associated with a 2.3–2.8-fold increase in the risk of colorectal cancer. Despite the obligate carrier status of parents of individuals with Bloom syndrome, this increased risk of cancer does not appear to have been previously appreciated. Confirmation of an increased risk of colorectal cancer is of obvious clinical importance to the ~1% of Ashkenazi Jewish individuals who carry the \( BLM^{Ash} \) mutation, as well as heterozygous \( BLM \) carriers from other ethnic groups. In this study, we have screened Jewish individuals with and without a personal history of cancer for the \( BLM^{Ash} \) mutation to assess the association of this allele with colorectal and other cancers.

Materials and Methods

Patient Populations. Study subjects were recruited from a variety of sources, including patients referred for colonoscopy or for cancer treatment at one of several teaching hospitals in Toronto or through the genetic epidemiology repository at the Centre for Research in Women’s Health in Toronto. Control subjects for this study were Jewish individuals without a personal history of cancer who had either undergone colonoscopy without any neoplastic mass identified or had been referred for \( BRCA1 \) or \( BRCA2 \) germ-line testing (i.e., at risk testing) but were found to not harbor mutations in these genes. A portion of tissue analyzed for this study was obtained from a previously described cancer DNA repository linked to an anonymous clinical database (11). The remaining DNA samples were used after individual informed consent was obtained. The use of DNA samples from human subjects for this study received institutional approval.

\( BLM^{Ash} \) Mutation Analysis. DNA was extracted from either peripheral blood lymphocytes or from nonneoplastic paraffin-embedded tissues. The DNA samples were screened for the \( BLM^{Ash} \) mutation by either SSCP analysis of a 75-bp PCR product (forward primer: 5’-CTTTATATCTAGATTCCTCGAC-3’; reverse primer: 5’-TGAGTATAATTTGTA-3’; PCR and SSCP conditions available upon request) or digestion of a 310-bp PCR product (forward primer: 5’-GATATTGACTAATAAAATA-3’; reverse primer: 5’-ATTCTTGGCAGCTGATAC-3’; PCR conditions available upon request) with the restriction enzyme Hpy188 I. Cases observed to be positive for the \( BLM^{Ash} \) mutation were confirmed by direct sequencing.

\( APC^{1307K} \) Polymorphism Analysis. Genomic DNA samples were screened for \( APC^{1307K} \) by SSCP (forward primer: 5’-GATTTCTGCTAATACCCGTGC-3’, reverse primer: 5’-GAACCTGCACACGAGAT-3’) as described previously (11). Cases observed to be positive for the \( APC^{1307K} \) polymorphism were confirmed by direct sequencing.

Statistical Analysis. ORs were calculated from proportions of \( BLM^{Ash} \) carriers among cases and controls and compared using Fisher’s exact test. Clinical features of \( BLM^{Ash} \) carriers were compared with noncarriers using Mann-Whitney nonparametric testing. PAR% was calculated as follows:

\[
\text{PAR\%} = \frac{[\text{genotype prevalence} \times (\text{OR} - 1)]}{[1 + (\text{genotype prevalence} \times (\text{OR} - 1))]} 
\]

Results

Among 2333 Jewish individual tested, we identified 21 (0.90%) heterozygous \( BLM^{Ash} \) carriers (Fig. 1). No individual was found to be homozygous for the \( BLM^{Ash} \) mutation. We observed no significant difference in the frequency of the \( BLM^{Ash} \) allele in Jewish individuals with either a personal history of cancer or controls without such a history (Table 1). Our carrier rate of 0.85% in 944 controls nearly identically mirrored a carrier rate of 0.86% in 8260 Jewish individuals.
analyzed in multiple previous studies (12–15). In our series, the BLM<sup>Ash</sup> mutation was present in only 0.80% of the 617 patients with colorectal neoplasms, a frequency not significantly different from that observed in 9204 controls from this series and others (OR/H11005 0.94; 95% CI, 0.38–2.32; Refs. 12–15).

Similarly, the risk for any type of cancer did not appear to be increased in BLM<sup>Ash</sup> carriers (OR/H11005 1.01; 95% CI, 0.54–1.91). Interestingly, when our colorectal neoplasia data were combined with those previously published, the OR for BLM<sup>Ash</sup> remained significant but fell to a modest 1.79 (95% CI, 1.17–2.74; P = 0.009).

Among the five heterozygous BLM<sup>Ash</sup> carriers with colorectal neoplasia, four had a single adenoma or cancer, and one individual had a cancer and two adenomatous polyps. The five BLM<sup>Ash</sup> carriers with colorectal cancer or adenomas were diagnosed at a median age of 74 years (range, 59–89 years), not significantly different from the median age of diagnosis of 71 years (range, 25–100 years) observed in 568 noncarriers with colorectal neoplasia. Among 375 individuals (ascertained through colonoscopy referral) where family history data were available, 2 of 4 BLM<sup>Ash</sup> carriers had a history of colorectal cancer in at least one first- or second-degree relative, and similarly, 164 of 371 (44%) noncarriers had such a history. DNA samples from 866 individuals in this series, including all BLM<sup>Ash</sup> carriers with colorectal neoplasia, were tested for the APC<sup>I1307K</sup> polymorphism, and this variant was observed in 61 of 582 (10.5%) Jewish patients with colorectal tumors, compared with 15 of 284 (5.3%) unaffected controls (OR/H11005 2.10; 95% CI, 1.17–3.77; P = 0.01). The only BLM<sup>Ash</sup> carrier found to carry the APC<sup>I1307K</sup> allele had no history of colorectal cancer or adenomatous polyps.

At the population level, the significance of a specific genotype on disease prevalence may be appreciated by calculating the PAR%. Using our observed data for APC<sup>I1307K</sup> (control prevalence = 5.3%; OR = 2.10), the PAR% for colorectal neoplasia was estimated to be 5.5%.

Our data could not be used to calculate a meaningful PAR% for BLM<sup>Ash</sup> as our OR was 1. However, when our data were combined with those previously published (control prevalence = 0.84%; OR = 1.79), the PAR% for BLM<sup>Ash</sup> was estimated to be 0.60% (10, 12–15).

### Table 1  Heterozygote BLM<sup>Ash</sup> carrier frequency in Jewish individuals with and without a personal history of cancer

<table>
<thead>
<tr>
<th>Group</th>
<th>BLM&lt;sup&gt;Ash&lt;/sup&gt;</th>
<th>BLM&lt;sup&gt;WT&lt;/sup&gt;</th>
<th>Total</th>
<th>Carrier frequency</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Colorectal neoplasia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenoma</td>
<td>2</td>
<td>123</td>
<td>125</td>
<td>1/63</td>
<td>1.90 (0.40–9.06)</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>3</td>
<td>494</td>
<td>497</td>
<td>1/166</td>
<td>0.71 (0.19–2.69)</td>
</tr>
<tr>
<td>Total, colorectal neoplasia</td>
<td>5</td>
<td>617</td>
<td>622</td>
<td>1/124</td>
<td>0.95 (0.31–2.91)</td>
</tr>
<tr>
<td><strong>Cancer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorectal</td>
<td>3</td>
<td>494</td>
<td>497</td>
<td>1/166</td>
<td>0.71 (0.19–2.69)</td>
</tr>
<tr>
<td>Breast</td>
<td>4</td>
<td>290</td>
<td>294</td>
<td>1/74</td>
<td>1.61 (0.48–5.40)</td>
</tr>
<tr>
<td>Ovary</td>
<td>2</td>
<td>254</td>
<td>256</td>
<td>1/128</td>
<td>0.92 (0.19–4.37)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0</td>
<td>109</td>
<td>109</td>
<td>0.50 (0.29–8.78)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
<td>106</td>
<td>108</td>
<td>1/54</td>
<td>2.21 (0.46–10.5)</td>
</tr>
<tr>
<td>Total, cancer</td>
<td>11</td>
<td>1253</td>
<td>1264</td>
<td>1/115</td>
<td>1.03 (0.41–2.56)</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>This study</td>
<td>8</td>
<td>936</td>
<td>944</td>
<td>1/118</td>
<td>1.01 (0.49–2.11)</td>
</tr>
<tr>
<td>Historical controls&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71</td>
<td>8189</td>
<td>8260</td>
<td>1/116</td>
<td></td>
</tr>
<tr>
<td>Total, Controls</td>
<td>79</td>
<td>9125</td>
<td>9204</td>
<td>1/117</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Historical controls taken from Refs. 12–15.
Discussion

Bloom syndrome is associated with increased risk of cancer, including leukemia and lymphoma in younger patients and carcinomas of larynx, lung, esophagus, colon, breast, and cervix in adults (1). It has recently been reported that heterozygous \textit{BLM}~\textsuperscript{Ash} mutations were carried by 1 of 54 (1.85%) Jewish individuals with colorectal cancer (10). This heterozygous carrier frequency was predicted to confer a 2.3–2.8-fold increased risk for colorectal cancer. To confirm this increased risk of colorectal cancer risk and examine whether this risk association is observed with other cancer types, we screened 2333 Jewish individuals with and without a personal history of cancer for the \textit{BLM}~\textsuperscript{Ash} founder mutation. The frequency of \textit{BLM}~\textsuperscript{Ash} heterozygous mutations in our series of individuals with colorectal and other cancers was nearly identical to our own control population and those reported previously (12–15). In fact, the frequency of \textit{BLM}~\textsuperscript{Ash} heterozygosity in 622 Jewish individuals with a personal history of colorectal neoplasia was slightly lower than unselected controls in our series. When our population is combined with near identical historical control study estimates (12–15), the power to rule out the unadjusted OR of 2.45 previously reported by Gruber et al. (10) was 63%. To rule out an OR of 1.3 (the lower 95% CI of the previous OR estimate) with 80% power (assuming $\alpha = 0.05$), a staggering 25,737 cases and 25,737 controls would have been required for analysis (10).

Classically, cancer-causing germ-line mutations have been associated with an observable family cancer history, a younger age of a cancer diagnosis, and an increased rate of synchronous and metachronous neoplastic lesions. In the current series, carriers of the \textit{BLM}~\textsuperscript{Ash} mutation did not appear to have a higher likelihood of a family history of colorectal cancer, nor did they appear to have a younger age of diagnosis of colorectal cancer, or an increased number of colorectal neoplasms per patient compared with noncarriers. Similarly, Gruber et al. did not find a younger age of diagnosis or stronger colon cancer family history in \textit{BLM}~\textsuperscript{Ash} carriers with colorectal cancer, compared with noncarriers (S. Gruber, personal communication).

In addition to human case-control data, \textit{BLM} heterozygosity has been implicated in colorectal tumorigenesis by observations that \textit{Apc}\textsuperscript{\textdash}\textit{\textit{B}lm}\textsuperscript{\textdash} mice develop increased number of adenomas compared with littermate multiple intestinal neoplasia \textit{Apc}\textsuperscript{\textdash}\textit{B}lm\textsuperscript{\textdash} mice (16, 17). However, spontaneous intestinal neoplasia was not observed in studies of two separate \textit{B}lm\textsuperscript{\textdash} mouse models (17, 18) and was rare in a third (16). Furthermore, the use of mouse modeling for \textit{BLM} heterozygosity is questionable as homozygous \textit{BLM} mutation causes the Bloom syndrome in humans but is embryonic lethal in the mouse (16–18). Nonetheless, it may be hypothesized from previous mouse studies that rather than providing a direct risk, heterozygous \textit{BLM} mutation may modify the risk of other cancer causing alleles. The \textit{APC}~\textsuperscript{1207K} polymorphism has been observed in >10% of Ashkenazi Jews with colorectal neoplasia and is estimated to approximately double the risk of colorectal cancer (11, 19). If \textit{BLM}~\textsuperscript{Ash} modifies the risk of cancer in individuals with other genetic predispositions to colorectal cancer, we might postulate that the \textit{BLM}~\textsuperscript{Ash}~\textit{APC}~\textsuperscript{1207K} genotype would be overrepresented in individuals with colorectal cancer. However, similar to our other results, the \textit{BLM}~\textsuperscript{Ash}~\textit{APC}~\textsuperscript{1207K} genotype was not observed in 581 Jewish individuals with colorectal neoplasia.

On the basis of this case-control data, cancer phenotype, and cancer family history analyses, it did not appear in our series that heterozygosity for the \textit{BLM}~\textsuperscript{Ash} allele was a significant risk factor for colorectal cancer or cancer from any site in general. Furthermore, it appears that if in fact \textit{BLM}~\textsuperscript{Ash} confers increased risk for colorectal neoplasia, this risk is more modest than originally estimated. Given only a modest individual risk in addition to the low prevalence of this allele, the PAR% for \textit{BLM}~\textsuperscript{Ash} heterozygosity was just 0.60%. Thus, although our data cannot fully rule out subtle increases in the risk for colorectal or other cancers because of \textit{BLM}~\textsuperscript{Ash}, if present, these differences are not likely to appreciably alter individual patient care, nor are they likely to impact significantly on the Ashkenazi Jewish population.

References

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