Colorectal and Endometrial Cancer Risk and Age at Diagnosis in *BLM*^{Ash} Mutation Carriers

Hagit Schayek PhD[1], Yael Laitman MSc[1], Lior H. Katz MD[3], Elon Pras MD[2,4], Liat Ries-Levavi PhD[2], Frida Barak MD[1] and Eitan Friedman MD PhD[1,4]

[1]Gertner Oncogenetics Unit, [2]Institute of Human Genetics and [3]Institute of Gastroenterology, Sheba Medical Center, Tel Hashomer, Israel
[4]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

ABSTRACT: **Background:** Biallelic *BLM* gene mutation carriers are at an increased risk for cancer, including colorectal cancer (CRC). Whether heterozygous *BLM* gene mutations confer an increased cancer risk remains controversial.

Objectives: To evaluate CRC and endometrial cancer risk in *BLM* heterozygous mutation carriers.

Methods: Ashkenazi Jews at high risk for colon or endometrial cancer, and endometrial cancer cases unselected for family history, were genotyped for the *BLM*^{Ash} predominant mutation.

Results: Overall, 243 high-risk individuals were included: 97 men with CRC (55.12 ± 12.3 years of age at diagnosis), 109 women with CRC (56.5 ± 13.7 years), 32 women with endometrial cancer (58.25 ± 13.4 years) and 5 women with both CRC and endometrial cancer. In addition, 120 unselected Ashkenazi women with endometrial cancer (64.2 ± 11.58 years) were genotyped. The *BLM*^{Ash} mutation was present in 4/243 (1.65%) of high-risk patients; 2/208 with CRC (0.97%) 2/35 with endometrial cancer (5.4%), and 1/120 unselected endometrial cancer patients (0.84%). Notably, in high-risk cases, *BLM*^{Ash} mutation carriers were diagnosed at a younger age (for CRC 47.5 ± 7.8 years, $P = 0.32$; endometrial cancer 49.5 ± 7.7 years, $P = 0.36$) compared with non-carriers.

Conclusions: Ashkenazi Jews at high risk for CRC and endometrial cancer, and women with endometrial cancer have a higher rate of *BLM*^{Ash} heterozygous mutation compared with the general population. *BLM*^{Ash} heterozygous mutation carriers are diagnosed with CRC and endometrial cancer at a younger age compared to non-carriers. These observations should be validated and the possible clinical implications assessed.

IMAJ 2017; 19: 365–367

KEY WORDS: Bloom Syndrome, Ashkenazi Jews, colon cancer, endometrial cancer, inherited cancer predisposition

Bloom syndrome (BS; MIM# 210900) is an autosomal recessive disorder characterized by chromosomal instability with an increased risk for developing various types of cancer, including colonic adenomatous polyps and colorectal cancer (CRC) [1-3]. BS is more common in individuals of Ashkenazi Jewish descent than in any other population, and approximately 1% of individuals from Ashkenazi backgrounds (1:111 in Israel) carry a single founder mutation in the *BLM* gene (c.2207_2212delATCTGAinsTAGATTC–*BLM*^{Ash}) [4,5]. Given the increased risk for developing cancer in homozygous *BLM* gene mutation carriers, the notion that heterozygous mutation carriers are also at an increased risk for cancer (especially CRC) has been postulated. The results of past studies that focused on the risk for developing CRC in *BLM* gene heterozygous mutation carriers are inconsistent and conflicting, with some showing an increased CRC risk [6] while other studies failed to show such an increase [7]. A recent study by de Voer and co-authors [8] identified a pathogenic *BLM* mutation in three of 185 (1.6%) CRC patients (≤ 50 years of age) and 1 of 532 control individuals (0.2%), suggesting that these mutations are enriched in early-onset CRC patients. Based on these observations and given the known phenotypic association between early onset CRC and endometrial cancer in the context of Lynch Syndrome [9], it seemed reasonable to explore this possible association between CRC and endometrial cancer and *BLM* heterozygous carrier state in Ashkenazi Jews.

PATIENTS AND METHODS

STUDY PARTICIPANTS: IDENTIFICATION AND RECRUITMENT

Study participants were all Ashkenazi Jews counseled at the Oncogenetics Unit, the Institute of Human Genetics at the Sheba Medical Center, Tel Hashomer, Israel from January 1, 2008 to July 1, 2015, with a personal and family history of CRC or endometrial cancer (high-risk group). Personal and family data were collected from all participants in the course of genetic counseling. In addition, Ashkenazi Jewish women who were diagnosed with endometrial cancer during the same time period who were unselected for any family history of cancer were also recruited (unselected group). The study was approved by the ethics committee and each participant gave informed consent.

GENETIC STUDIES

Each participant was genotyped (on peripheral blood leukocyte derived DNA) and found not to harbor any pre-

dominant Jewish Ashkenazi mutations in *BRCA1* (185delAG, 5382InsC) *BRCA2* (6174delT), *MSH2* (1906G>C; A636P) and *MSH6* (c.3984_3987dupGTCA; c.3959_3962delCAAG). Genotyping for 185delAG, 5382InsC and 6174delT were carried out using NanoChip® technology (Savyon Diagnostics, Ashdod, Israel) and genotyping for the predominant *MSH2* and *MSH6* mutations were performed as previously described [10,11]. All participants were genotyped for the *BLM* c.2207_2212delATCTGAinsTAGATTC mutation (*BLM*^{Ash}) using a previously published protocol based on the fact that the predominant Ashkenazi mutation introduces a novel Bst NI restriction site into exon 10 of the *BLM* gene [12].

STATISTICAL ANALYSES

The relative risk (RR) was calculated from the frequencies of *BLM*^{Ash} mutation carriers among different subgroups to the frequencies of *BLM*^{Ash} mutation carriers in the general Jewish Ashkenazi population (1:111). The mean age at diagnosis of *BLM*^{Ash} carriers was compared with non-carriers using the *t*-test. The results are presented as differences between proportions, RR, 95% confidence interval (CI), and *P* value. A *P* value of 0.05 was considered statistically significant.

RESULTS

STUDY POPULATION

A total of 243 high-risk individuals were included in the study. They included 97 males affected with CRC who were 55.1 ± 12.36 years of age at diagnosis (median 56 years, range 30–78 years), 109 females with CRC who were 56.5 ± 13.72 years of age at diagnosis (median 57.5 years, range 16–85 years), 32 females with endometrial cancer 58.25 ± 13.37 years of age at diagnosis (median 61 years, range 34–82 years) and 5 females with both CRC and endometrial cancer. Overall, the study included 146 females. Of participants, 172 had a family history of cancer, with 83 (38.9%) having another first degree relative with CRC and/or endometrial cancer. Thirty-eight fulfilled the Amsterdam 2 criteria for Lynch Syndrome [9].

The unselected group included 120 Jewish Ashkenazi women with histopathological confirmed endometrial cancer (mean age at diagnosis 64.2 ± 11.6 years, median 65 years, range 37–89 years).

Table 1. Clinical data and family history of BLMAsh mutation carriers

Case	Gender	Cancer type (age at onset)	Familial history for cancer (age at onset)
3010	M	CRC (49 years)	N/A
6669	F	Endometrial carcinoma (44 years)	Maternal uncle: melanoma (35 years)
11251	F	CRC (42 years) Melanoma (43 years) PPC (59 years)	Mother: BC (70 years) Maternal aunt: bladder cancer (60 years), LC 75 (years) Paternal uncle: prostate cancer (70 years)
13269	F	Endometrial carcinoma (55 years)	Mother: CRC (62 years) Paternal aunt: CRC (60 years)

F = female, M = male, CRC = colorectal cancer, PPC = primary peritoneal cancer, LC = lung cancer, N/A = not available

BLM^{ASH} CARRIER RATES

High-risk group

Overall, 2 of 206 CRC high-risk CRC patients (0.97%) and 2 of 37 high-risk endometrial cancer patients (5.4%) were heterozygous carriers of the *BLM*^{Ash} mutation. The difference in rates for *BLM*^{Ash} carriership in the high-risk CRC cases were not statistically significant compared to those reported for the general Jewish Ashkenazi population (RR = 1.07, 95%CI, 0.098–11.7, *P* = 0.95). The rates among endometrial cancer cases were higher than those in the general population, but in a statistically non-significant manner (RR = 5. 7, 95%CI, 0.53–61.6, *P* = 0.14).

All four heterozygous *BLM*^{Ash} carriers were diagnosed with cancer before the age of 55. Mean age at diagnosis for CRC was 56.3 ± 12.5 years (males and females) in non-carriers compared with 47.5 ± 7.8 years in heterozygous *BLM*^{Ash} mutation carriers (95%CI, -26.25–8.6, *P* = 0.32). The mean age at diagnosis for endometrial cancer in non-carriers was 58.2 ± 13.4 years compared with 49.5 ± 7.7 years in heterozygous *BLM*^{Ash} carriers (95%CI, -28.25–10.7, *P* = 0.36). Analysis restricted to individuals diagnosed with CRC under age 55 years showed that the rates of *BLM*^{Ash} mutation carriers was 2.32%, a twofold rate increase compared to the general population (RR = 2.54, 95%CI, 0.23–27.6, *P* = 0.44).

The clinical data and family history of study participants in the high-risk group who were *BLM*^{Ash} mutation carriers are shown in Table 1.

In addition, 1 of 120 unselected endometrial cancer patients (0.84%) was a heterozygous carrier of the *BLM*^{Ash} mutation, a rate that does not show a statistically significance different from those reported for the general Jewish Ashkenazi population (RR = 0.94, 95%CI, 0.059–14.7, P = 0.96).

DISCUSSION

In the current study, the rate of heterozygous *BLM*^{Ash} mutation carriers among Jewish Ashkenazi high-risk CRC cases (0.97%) was not statistically different from the rate in the healthy, average-risk Ashkenazi population (0.9%) (*P* = 0.95). These results do not support the results of a previous study from Israel [6] that reported that 1 of 54 (1.85%) Jewish individuals with CRC was a heterozygous *BLM*^{Ash} mutation carrier, a rate predicted to confer a 2.3- to 2.8-fold increased risk for CRC. However, our results are in line with another study from Israel [13] and our own previously published data [14] showing that being a heterozygous *BLM*^{Ash} mutation carrier is not associated with a clinically significant risk for CRC in Jewish Ashkenazi individuals. An intriguing aspect is the seemingly early age at diagnosis of the two CRC cases

who are also *BLM*^{Ash} mutation carriers (47.5 ± 7.8 years) compared to a mean age of 56.3 ± 12.5 years in males and females who are not *BLM*^{Ash} mutation carriers. This difference in age at diagnosis of CRC is in line with a study by de Voer and co-authors [8] who identified pathogenic *BLM* mutations in 3/185 (1.6%) non-Jewish CRC patients diagnosed ≤ 50 years of age. In contrast, Gruber and colleagues [6] did not report a younger age of diagnosis of CRC in *BLM*^{Ash} carriers compared with non-carriers. These differences in rates and effect on age at diagnosis in the high-risk group may be attributed to patient selection and recruitment. The study by Gruber et al. [6] was a consecutive, population-based study, whereas the current study identified participants from within a high-risk clinic population. It may also be that the current findings are a chance occurrence due to the limited number of cases, compared with that of Gruber et al [6]. Yet, the fact that both the present study and the one by de Voer et al. [8] report that early onset at CRC diagnosis may be indicative that heterozygous germline *BLM* mutations may act as modifiers of age at diagnosis and not as a cancer predisposition gene per se.

Due to the syndromic association between CRC and endometrial cancer in the context of Lynch Syndrome, the possible effect of being a heterozygous *BLM*^{Ash} mutation carrier for endometrial cancer risk was assessed. Despite the seemingly impressive 5.6% rate and the putative effect on age at diagnosis of endometrial cancer in high-risk cases, these preliminary data that failed to reach statistical significance, should be interpreted cautiously due to the limited number of cases studied and the mode of recruitment. Moreover, it is clear that the rates in endometrial cancer cases unselected for family history or age at diagnosis, the rates are similar with that in the average risk, healthy population. Clearly more studies encompassing more cases are needed to better define the effect, if any, of heterozygous *BLM* mutations on endometrial cancer risk.

CONCLUSIONS

In conclusion, the *BLM*^{Ash} mutation in the heterozygous state does not confer an overall CRC risk in Ashkenazi Jewish high-risk cases but seems to affect age at diagnosis in that population. The effect, if any, of the same mutation on endometrial cancer risk and age at diagnosis needs to be further explored.

Correspondence
Dr. E. Friedman
Gertner Oncogenetics Unit, Sheba Medical Center, Tel Hashomer 5265601, Israel
Phone: (972-3) 530-3173
Fax: (972-3) 535-7308
email: feitan@post.tau.ac.il, eitan.friedman@sheba.health.gov.il

References

1. Zauber NP, Sabbath-Solitare M, Marotta S, et al. Clinical and genetic findings in an Ashkenazi Jewish population with colorectal neoplasms. *Cancer* 2005; 104 (4): 719-29.
2. Amor-Guéret M. Bloom syndrome, genomic instability and cancer: the SOS-like hypothesis. *Cancer Lett* 2006; 236 (1): 1-12.
3. Arora H, Chacon AH, Choudhary S, et al. Bloom syndrome. *Int J Dermatol* 2015; 53 (7): 798-802.
4. Oddoux C, Clayton CM, Nelson HR, Ostrer H (1999) Prevalence of Bloom Syndrome heterozygotes among Ashkenazi Jews. *Am J Hum Genet* 1999; 64 (4): 1241-3.
5. Peleg L, Pesso R, Goldman B, et al. Bloom syndrome and Fanconi's anemia: rate and ethnic origin of mutation carriers in Israel. *IMAJ* 2002; 4 (2): 95-7.
6. Gruber SB, Ellis NA, Scott KK, et al. BLM heterozygosity and the risk of colorectal cancer. *Science* 2002; 297 (5589): 2013.
7. Cleary SP, Zhang W, Di Nicola N, et al. Heterozygosity for the BLM^{Ash} mutation and cancer risk. *Cancer Res* 2003; 63 (8): 1769-71.
8. de Voer RM, Hahn MM, Mensenkamp AR, et al. Deleterious germline BLM mutations and the risk for early onset colorectal cancer. *Sci Rep* 2015; 5: 14060.
9. Vasen HF, Watson P, Mecklin JP, Lynch HT. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative Group on HNPCC. *Gastroenterology* 1999; 116 (6): 1453-6.
10. Guillem JG, Rapaport BS, Kirchhoff T, et al. A636P is associated with early-onset colon cancer in Ashkenazi Jews. *J Am Coll Surg* 2003; 196: 222-5.
11. Laitman Y, Herskovitz L, Golan T, Kaufman B, Paluch SS, Friedman E. The founder Ashkenazi Jewish mutations in the MSH2 and MSH6 genes in Israeli patients with gastric and pancreatic cancer. *Fam Cancer* 2012; 2: 243-7.
12. Straughen JE, Johnson J, McLaren D, et al. A rapid method for detecting the predominant Ashkenazi Jewish mutation in the Bloom's syndrome gene. *Hum Mutat* 1998; 11 (2): 175-8.
13. Baris HN, Kedar I, Halpern GJ, et al. Prevalence of breast and colorectal cancer in Ashkenazi Jewish carriers of Fanconi anemia and Bloom syndrome. *IMAJ* 2007; 9 (12): 847-50.
14. Laitman Y, Boker-Keinan L, Berkenstadt M, et al. The risk for developing cancer in Israeli ATM, BLM, and FANCC heterozygous mutation carriers. *Cancer Genet* 2016; 209 (3): 70-4.

Capsule

Targeting non-viral antigens in viral-driven cancer

Adoptive cell transfer harnesses a patient's own T cells to destroy cancer. The strategy can successfully treat epithelial tumors driven by human papillomavirus (HPV), but it remains unclear why only some patients respond. Stevanović and colleagues examined the anti-tumor T cell response associated with HPV+ cervical cancers that underwent complete regression. Unexpectedly, reactive T cells were not directed against virally associated antigens, but rather against cancer-germline antigens or neoantigens not previously recognized by the immune system. These findings counter the widely held belief that T cell responses against viral antigens are responsible for therapeutic effects in HPV-driven cancers.

Science 2017; 356: 200

Eitan Israeli