High Frequency of Germ-Line BRCA2 Mutations among Hungarian Male Breast Cancer Patients without Family History1

Bela Csokay, Nora Udvarhelyi, Zoltan Sulyok, Istvan Besznyak, Susan Ramus, Bruce Ponder, and Edith Olah2

Department of Molecular Biology, National Institute of Oncology, H-1525 Budapest, Hungary [B. C., N. U., Z. S., I. B., E. O.], and Strangeways Research Laboratories, Cambridge CB1 8RN, United Kingdom [S. R., B. P.]

Abstract

To determine the contribution of BRCA1 and BRCA2 mutations to the pathogenesis of male breast cancer in Hungary, the country with the highest male breast cancer mortality rates in continental Europe, a series of 18 male breast cancer patients and three patients with gynecomastia was analyzed for germ-line mutations in both BRCA1 and BRCA2. Although no germ-line BRCA1 mutation was observed, 6 of the 18 male breast cancer cases (33%) carried truncating mutations in the BRCA2 gene. Unexpectedly, none of them reported a family history for breast/ovarian cancer. Four of six truncating mutations were novel, and two mutations were recurrent. Four patients (22%) had a family history of breast/ovarian cancer in at least one first- or second-degree relative; however, no BRCA2 mutation was identified among them. No mutation was identified in either of the genes in the gynecomastias. These results provide evidence for a strong genetic component of male breast cancer in Hungary.

Introduction

Male breast cancer is a rare disease, comprising <1% of all malignancies in men in the western world; however, in some developing areas, it is more frequent, accounting for 6–15% of all malignancies in men in the western world; however, in some

Materials and Methods

Patients. Blood samples were collected from all consenting patients with male breast cancer or gynecomastia at the National Institute of Oncology (Budapest, Hungary) in the period of 1996–1998. The histopathological typing of the cases was completed by board-certified pathologists. This panel represents consecutive cases who were not selected for family history of breast/ovarian cancer; however, information on family history was available for all patients. The patients with gynecomastia did not have a medical history of Klinefelter syndrome.

BRCA1 and BRCA2 Mutation Screening. Genomic DNA was extracted by standard methods from peripheral lymphocytes from all patients. PCR amplification of 160–550-bp DNA fragments covering the entire BRCA2 coding region and splice junctions was carried out by using a set of 44 primer pairs (5, 11). Similarly, all BRCA1 exons with the exception of exon 11 were amplified with a set of 23 primer pairs; exon 11 was amplified in three overlapping protein truncation test fragments as reported previously (5). Mutational screening was performed in germ-line DNA by combined multiple heteroduplex analysis and/or by single-strand conformation analysis. Mutation screening in both genes was performed irrespective of identification of a mutation in one gene, because it is possible that mutations of both BRCA1 and BRCA2 may be segregating in a family, as we reported in a Hungarian breast/ovarian cancer patient (17). PCR products from variant conformers were purified using Wizard PCR Prep DNA Purification System (Promega Corp.) and sequenced using the Thermosequenase Cyclo Sequencing kit (USB-Amersham).

Results

The entire coding regions and intronic splice sites of both BRCA1 and BRCA2 were screened using multiple heteroduplex analysis/single-strand conformation analysis and protein truncation test, followed by direct sequencing of abnormalities. Six of the 18 male breast cancer cases (33%) were observed to carry truncating mutations in the BRCA2 gene (Table 1). Four of the six truncating mutations were novel; the other two alterations (277delAC and 9326insA) were reported earlier (5, 18, 19). A review was conducted of the clinical and family history of the mutation carriers.
None of them reported a family history for breast/ovarian cancer. Two patients (11%) had a family history of breast/ovarian cancer in at least one first-degree relative and another two (11%) in at least one second-degree relative in this unscreened study; however, no BRCA2 mutation was observed among them.

No germ-line BRCA1 mutations were detected in the male breast cancer cases, nor was a mutation identified in either BRCA1 or BRCA2 in the three cases of gynecomastia; however, one patient (G2) from this group carried an unclassified BRCA2 variant allele with unclear significance (Table 2). There was no difference between mutation carriers and noncarriers with respect to clinicopathological features nor age at diagnosis (Table 3). The average age at diagnosis was 62 years; there was only an insignificant difference between the mean age of onset of carriers (58 years) and noncarriers (64 years).

Discussion

In the first studies of BRCA2 analysis (4, 11), the subjects of scrutiny were mostly large families that sometimes also contained members with male breast cancer. In more recent reports (20–22), sets of population-based male breast cancer cases were studied for the frequency of germ-line BRCA2 carriers. Here we analyzed male breast cancer patients from a hospital-based study to determine the contribution of BRCA1 and BRCA2 mutations to the pathogenesis of male breast cancer in Hungary. Frequencies reported of BRCA2 mutations in male breast cancer vary between 4% (21) and 40% (23), the latter distribution of either germ-line BRCA2 carriers. Here we analyzed male breast cancer cases, 6 of 7 (85%) of whom had a family history of breast cancer. Similarly to our results, Haraldsson (21) found two mutation carriers in their panel, one of whom had a family history. Similarly to our results, Haraldsson et al. (22) reported recently that only one of seven BRCA2 mutation carriers had a positive family history of breast cancer.

No evidence for differences in clinicopathological features was seen between mutation carriers and noncarriers. Most patients had invasive ductal carcinoma (17 of 18; 95%), including all germ-line BRCA2 mutation carriers. In contrast with the results of Couch et al. (11), who identified germ-line BRCA2 mutations in 7 of 50 (14%) male breast cancer cases, 6 of 7 (85%) of whom had a family history of breast cancer. Friedman et al. (21) found two mutation carriers in their panel, one of whom had a family history. Similarly to our results, Haraldsson et al. (22) reported recently that only one of seven BRCA2 mutation carriers had a positive family history of breast cancer.

No evidence for differences in clinicopathological features was seen between mutation carriers and noncarriers. Most patients had invasive ductal carcinoma (17 of 18; 95%), including all germ-line BRCA2 mutation carriers (Table 3). This is consistent with literature data that invasive ductal carcinomas account for 84–93% of cases (25). The average age of men at diagnosis of breast cancer is close to 65, about 5 years older than the average age for women. In our studies, the average age at diagnosis in carriers was not significantly lower than that of noncarriers. Compared with other BRCA2 mutations, 9326insA appears to be linked with younger age of onset in female breast cancer (26) and possibly also in male breast cancer (22).

No mutation was identified in either of the genes in the three cases of gynecomastia; however, one patient (G2) from this group carried a missense BRCA2 variant allele (7081A→G) with unclear significance. This alteration was reported eight times in the Breast Cancer Information Core as an unclassified variant and lies in a conserved region of the gene in human, rat, and mouse; however, expression analysis would be required to elucidate the nature of this type of alteration.

4 Unpublished data.

5 E. Olah et al., manuscript in preparation.

The data presented here imply that germ-line mutations in the \textit{BRCA2} gene are involved in the development of one-third of male breast cancer cases in Hungary. There may be other genetic factors that are responsible for some of the male breast cancers that cannot be explained by mutations in the \textit{BRCA2} gene. There is no evidence for a male breast cancer-specific cluster region in the \textit{BRCA2} gene thus far, but because the majority of \textit{BRCA2} mutations found in male breast cancer are unique and not identified in female breast cancer cases, it is possible that at least some \textit{BRCA2} mutations may predispose to male rather than female breast cancer. On the other hand, certain mutations (e.g., 9326insA) result in both male and female breast cancers and can be associated with various phenotypes in different families. This could be explained by modifying factors in disease development different in males and females. The first evidence of a modifying genetic factor of \textit{BRCA2} in male breast cancer was provided recently (27), reporting that an interstitial tandem duplication on 9p increases the risk of breast cancer in male patients conferred by \textit{BRCA2}. The lack of a positive family history in all carriers suggests that at least some \textit{BRCA2} mutations may have a lower penetrance than others, and selection of putative \textit{BRCA2} mutation carriers among male breast cancer patients cannot be based on the family background of the disease. Our data provide further evidence that in certain populations there is a high genetic component in the development of this disease, and in these populations all patients with male breast cancer, regardless of their family history, should be screened for \textit{BRCA2} mutations.

\begin{table}
\centering
\caption{BRCA2 polymorphisms and unclassified variants}
\begin{tabular}{|l|l|l|l|l|}
\hline
Patient & Variant & Codon & Exon & Effect & Comment \\
\hline
MBC\textsuperscript{a}-10 & 5972 C/T & Thr1915Met & 11 & Missense & Reported 1 time (6\%) \\
MBC-14, 15 & IVS11 + 79del14\textsuperscript{b} & Intron 11 & None & Reported 1 time (28\%) \\
G2\textsuperscript{c} & 7081 A/G & Ile2285Val & 12 & Missense & Reported 8 times \\
\hline
\end{tabular}
\begin{flushright}
\textsuperscript{*}MBC, male breast cancer. \\
\textsuperscript{a}IVS, intronic variant. \\
\textsuperscript{c}G, Gynecomastia.
\end{flushright}
\end{table}

The data presented here imply that germ-line mutations in the \textit{BRCA2} gene are involved in the development of one-third of male breast cancer cases in Hungary. There may be other genetic factors that are responsible for some of the male breast cancers that cannot be explained by mutations in the \textit{BRCA2} gene. There is no evidence for a male breast cancer-specific cluster region in the \textit{BRCA2} gene thus far, but because the majority of \textit{BRCA2} mutations found in male breast cancer are unique and not identified in female breast cancer cases, it is possible that at least some \textit{BRCA2} mutations may predispose to male rather than female breast cancer. On the other hand, certain mutations (e.g., 9326insA) result in both male and female breast cancers and can be associated with various phenotypes in different families. This could be explained by modifying factors in disease development different in males and females. The first evidence of a modifying genetic factor of \textit{BRCA2} in male breast cancer was provided recently (27), reporting that an interstitial tandem duplication on 9p increases the risk of breast cancer in male patients conferred by \textit{BRCA2}. The lack of a positive family history in all carriers suggests that at least some \textit{BRCA2} mutations may have a lower penetrance than others, and selection of putative \textit{BRCA2} mutation carriers among male breast cancer patients cannot be based on the family background of the disease. Our data provide further evidence that in certain populations there is a high genetic component in the development of this disease, and in these populations all patients with male breast cancer, regardless of their family history, should be screened for \textit{BRCA2} mutations.

\begin{table}
\centering
\caption{Clinicopathological characteristics of patients tested in this study}
\begin{tabular}{|l|l|l|l|}
\hline
Patient & \textit{BRCA2} mutation & Phenotype of individual & Age at diagnosis & Family history of cancer \\
\hline
MBC\textsuperscript{d}-1 & No & Inv. ductal carcinoma & 80 & Negative \\
MBC-2 & 4232insA & Inv. ductal carcinoma & 60 & Negative \\
MBC-3 & 277delAC & Inv. ductal carcinoma & 75 & Negative \\
MBC-5 & No & Inv. ductal carcinoma & 61 & Negative \\
MBC-6 & No & Inv. ductal carcinoma & 79 & Negative \\
MBC-7 & No & Inv. ductal carcinoma & 66 & Grandfather: stomach, prostate \\
MBC-8 & No & Inv. ductal carcinoma & 63 & Negative \\
MBC-9 & 3868insT & Inv. ductal carcinoma & 68 & Negative \\
MBC-10 & No & Tubular adenocarcinoma & 62 & Negative \\
MBC-12 & No & Inv. ductal carcinoma & 46 & Negative \\
MBC-13 & No & Inv. ductal carcinoma & 85 & Daughter: breast; Sister: ovary \\
MBC-14 & No & Inv. ductal carcinoma & 77 & Negative \\
MBC-15 & 7979delG & Inv. ductal carcinoma & 46 & Grandmother: lung \\
MBC-17 & No & Inv. ductal carcinoma & 49 & Aunt: breast; Cousin: brain \\
MBC-19 & 841insCTTA & Inv. ductal carcinoma & 52 & Father: stomach; Grandmother: lymphoma \\
MBC-20 & No & Inv. ductal carcinoma & 53 & Mother: breast; Aunt: ovary \\
MBC-21 & No & Inv. ductal carcinoma & 48 & Cousin: bilateral breast \\
MBC-22 & 9326insA & Inv. ductal carcinoma & 48 & Negative \\
G1 & No & Gynecomastia & 84 & Negative \\
G2 & 7081A \rightarrow G & Gynecomastia & 21 & Negative \\
G3 & No & Gynecomastia & 40 & Mother: uterus; Sister: colon; Aunt: colon \\
\hline
\end{tabular}
\begin{flushright}
\textsuperscript{d}MBC, male breast cancer; Inv., invasive.
\end{flushright}
\end{table}
Acknowledgments

We thank Janos Papp and Marco van der Looij for excellent technical help.

References

High Frequency of Germ-Line BRCA2 Mutations among Hungarian Male Breast Cancer Patients without Family History

Bela Csokay, Nora Udvarhelyi, Zoltan Sulyok, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/59/5/995

Cited articles
This article cites 25 articles, 5 of which you can access for free at:
http://cancerres.aacrjournals.org/content/59/5/995.full.html#ref-list-1

Citing articles
This article has been cited by 10 HighWire-hosted articles. Access the articles at:
/content/59/5/995.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.