High Prevalence of the 999del5 Mutation in Icelandic Breast and Ovarian Cancer Patients

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Abstract

Studies on Icelandic breast cancer families have shown that most of them segregate a 999del5 BRCA2 mutation. Here, we report the frequency of the 999del5 BRCA2 mutation in an Icelandic control population and four different groups of cancer patients diagnosed with (a) breast cancer; (b) ovarian cancer; (c) prostate cancer (patients younger than 65 years); and (d) other cancer types. The proportions of individuals carrying the mutation were 0.4% in the control population and in the patient groups 8.5%, 7.9%, 2.7%, and 1.0%, respectively. Our results indicate that BRCA2 confers a very high risk of breast cancer and is responsible for a substantial fraction of breast and ovarian cancer in Iceland, but only a small proportion of other cancers.

Introduction

About 6–19% of breast cancer patients have one or more close relatives that have been diagnosed with the disease (1–3). Two genes, BRCA1 and BRCA2, account for the disease in the large majority of breast cancer families (4–6). Much less, however, is known about the contribution of BRCA1 and especially BRCA2 to overall breast cancer incidence (7, 8). Studies on 29 Icelandic high-risk breast cancer families have previously identified 21 with positive BRCA2 linkage and only 1 linked to BRCA1 (9–11). The BRCA2 families were all found to carry a 999del5 BRCA2 mutation and to segregate a common BRCA2 haplotype (10, 11). Inspection of the BRCA2-linked families suggested that there might also be an elevated risk of cancer types other than breast cancer in the BRCA2 carriers (11, 12). In this study, we have determined the frequency of the 999del5 BRCA2 mutation in a group of patients diagnosed with breast cancer, in groups of patients diagnosed with other cancer types, and in an Icelandic control group.

Materials and Methods

Subjects. Ascertainment of the cancer patients included in this study was different depending on the cancer types. A fresh biopsy from breast tumors is routinely sent to our laboratory for estrogen and progesterone receptor assessment, and since 1987 we have collected nuclear pellets from these samples for DNA extraction. All available DNA samples isolated from nuclear pellets from patients diagnosed in the years 1989–1994 were included in this study. Samples from the prostate cancer patients were collected in 1993. It included all those patients diagnosed with prostate cancer, during the period 1983–1992, who were at or below the age of 65 years from whom archival normal and tumor tissue could be found at the Department of Pathology, University Hospital of Iceland. DNA from patients diagnosed with cancer other than breast and prostate cancer was obtained from a consecutive tumor bank that was established in our laboratory in 1991.

The control group consisted of randomly selected DNA samples from participants in the Icelandic National Diet Survey (13). All subjects came from the southwest part of Iceland, where well over 50% of the population lives. All individual identifiers were removed from the control samples prior to analysis, and investigators were thus blinded to the identification of samples which can no longer be traced to specific individuals.

The research plan is approved by the ethical committee at the University Hospital of Iceland and by the Icelandic Data Protection Committee.

Mutation Detection. We used the following primers for mutation detection based on allele size difference (186-bp normal and 181-bp mutant allele): 5'-ATGAGATAAGGGGAGACTA-3' (forward) and 5'-AAACTGAGATCAGGCGGTG-3' (reverse). DNA from tumor tissues was used in the screening process of cancer patients, and mutation detection was confirmed by using DNA from the normal tissue of the patient whenever possible. Thermal cycling (PCR) was carried out in 25-µl volumes containing 0.3 units Dynazyme polymerase (Finnzyme Oy), the reaction buffer provided with the polymerase, 200 µM of each deoxynucleotide triphosphate, 30 mg of genomic DNA, and 50 ng of each primer. Cycling conditions were 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 40 s. The PCR products were denatured in formamide buffer, loaded onto acrylamide gel (mutation detection enhancement solution; FMC Bioproducts), and subjected to electrophoresis at 40 W for 3 to 4 h and then transferred to a nylon membrane (Hybond N+; Amersham) and fixed at 80°C for 3 h. Hybridization and visualization of the PCR products were described by Barkardottir et al. (14), using a nonradioactive procedure (ECL system; Amersham).

Sequencing. All the samples shown to carry the mutated allele in the screening process were sequenced to verify the mutation. Solid-phase sequencing was carried out on exon 9 using the same reverse primer as in the mutation screening and the forward primer was 5'-ATGAGATCTGGAAGAAAAATGAT-3'. The reverse primer was 5'-biotinylated. The PCR products were immobilized on solid support using streptavidin beads (M-280; Dynal) and denatured with alkaline. The single-stranded biotinylated DNA template was sequenced using Sequenase and [α-32P]dATP (Amersham).

Statistical Analysis. Significance levels for comparing mutation frequencies in cases and controls were based on Fisher’s exact test. Confidence limits for the relative risk were based on the exact hypergeometric distribution because of the small number of positive cases. The incidence rates in BRCA2 carriers were estimated by noting that the proportion of breast cancer cases in age group k with a mutation is given using the formula \( P_k = 2pF_k x_k \), where \( F_k \) is the probability that a gene carrier develops breast cancer in age group k, and \( g_k \) is the corresponding probability for the Icelandic female population based on Icelandic breast cancer incidence rates from 1970 to 1984, and \( p \) is the population gene frequency. Since \( p \) cannot be accurately estimated, the penetrances in BRCA2 carriers have been expressed relative to the cumulative risk by age 80 years.
This study also provides an estimate of the age-incidence distribution of breast cancer in carriers of this mutation by comparing the prevalence of breast cancer in each age group with the overall age-specific incidence of the disease in Iceland. The absolute risk of developing cancer cannot be estimated reliably because this would require an accurate estimate of the gene frequency, and our estimate is based on only two mutations in controls. Our estimated gene frequency of 0.2% would, however, imply a lifetime risk of close to 100%.

The frequency of the 999del5 mutation in the ovarian cancer patients is similar to that in the breast cancer patients. This is based on a low number of ovarian cancer cases but the results are supported by the observation that one or more ovarian cancer cases occur in 11 of the 21 Icelandic families found to carry the BRCA2 mutation (10, 11). The increase in risk of ovarian cancer is not restricted to the 999del5 mutation, since ovarian cancer occurs in more than one third of the breast cancer families that have been reported to carry a BRCA2 mutation (6, 17). The frequency of the BRCA2 mutation observed in the prostate cancer group and in the group of patients diagnosed with breast cancer types other than breast, ovarian, and prostate was not significantly higher than in the control group. However, moderate increased risks of other cancers in mutation carriers cannot be ruled out.

The results presented in this article suggest that the 999del5 mutation found to segregate in 21 breast cancer families is also found among a large number of breast and ovarian cancer cases outside of these families. This finding gives rise to the possibility of additional studies of gene carriers to examine penetrance and the effects on risk due to other genetic or environmental factors. Affected and unaffected mutation carriers could also form an important group for examining possible preventative strategies.

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References


