

High Prevalence of the 999del5 Mutation in Icelandic Breast and Ovarian Cancer Patients¹

Gudrun Johannesdottir,² Julius Gudmundsson,² Jon T. Bergthorsson, Adalgeir Arason, Bjarni A. Agnarsson, Gudny Eiriksdottir, Oskar T. Johannsson, Ake Borg, Sigurdur Ingvarsson, Douglas F. Easton, Valgardur Egilsson, and Rosa Björk Barkardottir³

Laboratory of Cell Biology [G. J., J. G., J. T. B., A. A., B. A. A. G. E., S. I., V. E., R. B. B.], Department of Pathology, University Hospital of Iceland, House 14, Landspítalinn, 101 Reykjavik, Iceland; Institute of Oncology, University Hospital, S-221 85 Lund, Sweden; [O. T. J., A. B.]; and CRC Genetic Epidemiology Unit, Department of Community Medicine, Institute of Public Health, University of Cambridge, United Kingdom [D. F. E.]

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'-ATGGATAAGGGGGGACTA-3' (forward) and 5'-AAACTGAGATCACGGGTG-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- μ l volumes containing 0.3 units Dynazyme polymerase (Finnzyme Oy), the reaction buffer provided with the polymerase, 200 μ M 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 as 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'-ATGAAAGTCTGAAGAAAATGAT-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 $P_k = 2pF_k/g_k$, 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.

Received 6/10/96; accepted 7/11/96.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This work was supported by the Nordic Cancer Union, Science Fund of Iceland, and Memorial Fund of Berghthora Magnusdottir and Jakob B. Bjarnason.

² These authors contributed equally to this study.

³ To whom requests for reprints should be addressed. Phone: (354) 560 1906; Fax: (354) 560 1519; E-mail:rosa@rsp.is.

Table 1 Frequency of the 999del5 BRCA mutation in five Icelandic groups^a

Group	No. tested	Carriers	%
Breast cancer patients	459	39	8.5
Ovarian cancer patients	38	3	7.9
Prostate cancer patients diagnosed at age ≤65	75	2	2.7
Patients diagnosed with other cancers ^b	395	4	1.0
Controls	499	2	0.4

^a The different groups consist of unselected patients, except in the case of prostate cancer patients, where the age of diagnosis was confined to 65 years and younger.

^b All available DNA samples from patients diagnosed with cancer types other than those of the breast, ovaries, and prostate. These include a total of 29 different cancer types. Cancer in nine organs was represented by more than 10 individuals: colon ($n = 87$), lung ($n = 56$), kidneys ($n = 43$), stomach ($n = 36$), testis ($n = 27$), endometrium ($n = 20$), connective tissue ($n = 18$), thyroid ($n = 16$), and rectum ($n = 15$). The patients found to carry the 995del5 mutation had been diagnosed with cancer of the rectum, esophagus, stomach, and kidney at the age of 56, 66, 78, and 80 years, respectively.

Table 2 Proportion of the 999del5 BRCA2 mutation according to age at diagnosis of breast cancer^a

Age groups (yr)	No. tested	Mutation positive	% with mutation	Estimated penetrance ^b
<30	4	0	0	0
30–39	37	10	27.0	0.18
40–49	111	14	12.6	0.44
50–59	99	6	6.1	0.64
60–69	114	5	4.4	0.80
70–79	64	3	4.7	1.00
≥80	30	1	3.3	
Total	459	39	8.5	

^a The mean age at diagnosis of breast cancer in women carrying the mutation is 49.6 years. This is about 10 years lower than the mean age at diagnosis of the remaining breast cancer patients included in this study (58.9 years), and female breast cancer patients diagnosed in Iceland over a period of 30 years (59.4 years; Ref. 18).

^b Estimated penetrance relative to the penetrance by the age of 80 years.

Results and Discussion

We determined the frequency of the 999del5 BRCA2 mutation in an Icelandic control population and four groups of patients diagnosed with breast cancer, ovarian cancer, prostate cancer, and any other cancer (Table 1). We based the analysis on unselected patients, except for the prostate cancer patients who were at or below 65 years at diagnosis. Compared to the control group, the prevalence of the mutation was significantly higher in the groups of patients diagnosed with breast cancer ($P < 0.0001$) and ovarian cancer ($P = 0.003$), but not in the prostate cancer group or in the group of patients diagnosed with cancer in other organs. Relative risks for the groups described above to develop tumors compared to the control group were 23.1 (95% CI⁴, 7.0–141.9), 21.0 (45% CI, 3.4–162.2), 6.6 (95% CI, 0.81–56.9), and 2.5 (95% CI, 0.49–18.4), respectively. The proportions of mutation carriers in different age groups of breast cancer patients and estimates of age-specific cumulative penetrance relative to the penetrance by age 80 years are shown in Table 2. The estimated proportion of breast cancer cases carrying the mutation reduces from 24% below age 40 years to 4% above age 60 years. Based on these estimates, 44% of the cases in carriers occurring by age 80 years will occur below age 50 years.

Epidemiological studies have suggested that 5–10% of the breast cancer cases are due to inheritance of predisposing breast cancer alleles (15). If that is the case in Iceland, our value of 8.5% frequency of 999del5 BRCA2 mutation carriers in the unselected breast cancer group strongly suggests that the majority of inherited breast cancer in Iceland can be explained by this mutation. The observed frequency of heterozygous carriers of the 999del5 mutation in the Icelandic population is high (0.4%) and is comparable to the unusually high carrier frequency (1%) of the 185delAG BRCA1 allele in the Ashkenazi Jewish population (16).

⁴ The abbreviation used is: CI, confidence interval.

This study also provides an estimate of the age-incidence distribution of breast cancer in carriers of this mutation by comparing the prevalence of the mutation 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 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.

Acknowledgments

We thank Professor J. Hallgrímsson and our colleagues at the Department of Pathology for providing tissue samples, Dr. V. Gudnason for access to control samples, and Dr. M. Stratton for BRCA2 primers. We thank Ö. Arnarson for help with the statistical analysis.

References

- Tulinius, H., Sigvaldason, H., Olafsdottir, G., and Tryggvadottir, L. Epidemiology of breast cancer in families in Iceland. *J. Med. Genet.*, 29: 158–164, 1992.
- Colditz, G. A., Willett, W. C., Hunter, D. J., Stampfer, M. J., Manson, J. E., Hennekens, C. H., Rosner, B. A., and Speizer, F. E. Family history, age, and risk of breast cancer: prospective data from the nurses' health study (Erratum). *J. Am. Med. Assoc.*, 270: 1548, 1993). *J. Am. Med. Assoc.*, 270: 338–343, 1993.
- Slattery, M. L., and Kerber, R. A. A comprehensive evaluation of family history and breast cancer risk: the Utah Population Database. *J. Am. Med. Assoc.*, 270: 1563–1568, 1993.
- Easton, D. F., Bishop, D. T., Ford, D., Crockford, G. P., and The Breast Cancer Linkage Consortium. Genetic linkage analysis in familial breast and ovarian cancer: results from 214 families. *Am. J. Hum. Genet.*, 52: 678–701, 1993.
- Miki, Y., Swensen, J., Shattuck-Eidens, D., Futreal, P. A., Harshman, K., Tavtigian, S., Liu, Q., Cochran, C., Bennett, L. M., Ding, W., Bell, R., Rosenthal, J., Hussey, C., Tran, T., McClure, M., Frye, C., Hattier, T., Phelps, R., Haugen-Strano, A., Katcher, H., Yakumo, K., Gholami, Z., Shaffer, D., Stone, S., Bayer, S., Wray, C., Bogden, R., Dayananth, P., Ward, J., Tonin, P., Narod, S., Bristow, P. K., Norris, F. H., Helvering, L., Morrison, P., Rostek, P., Lai, M., Barrett, J. C., Lewis, C., Neuhausen, S., Cannon-Albright, L., Goldgar, D., Wiseman, R., Kamb, A., and Skolnick, M. H. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* (Washington DC), 266: 66–71, 1994.
- Wooster, R., Bignell, G., Lancaster, J., Swift, S., Seal, S., Mangion, J., Collins, N., Gregory, S., Gumbs, C., Micklem, G., Barfoot, R., Hamoudi, R., Patel, S., Rice, C., Biggs, P., Hashim, Y., Smith, A., Connor, F., Arason, A., Gudmundsson, J., Ficenec, D., Kelsell, D., Ford, D., Tonin, P., Bishop, D. T., Spurr, N. K., Ponder, B. A. J., Eeles, R., Peto, J., Devilee, P., Cornelisse, C., Lynch, H., Narod, S., Lenoir, G., Egilsson, V., Barkardottir, R. B., Easton, D. F., Bentley, D. R., Futreal, P. A., Ashworth, A., and Stratton, M. R. Identification of the breast cancer susceptibility gene BRCA2. *Nature* (Lond.), 378: 789–792, 1995.
- Langstone, A. A., Malone, K. E., Thompson, J. D., Daling, J. R., and Ostrander, E. A. BRCA1 mutations in a population-based sample of young women with breast cancer. *N. Engl. J. Med.*, 334: 137–142, 1996.
- FitzGerald, M. G., MacDonald, D. J., Krainer, M., Hoover, I., O'Neil, E., Unsal, H., Silva-Arrieto, S., Finkelstein, D. M., Beer-Romero, P., Englert, C., Sgroi, D. C.,

- Smith, B. L., Younger, J. W., Garber, J. E., Duda, R. B., Mayzel, K. A., Isselbacher, K. J., Friend, S. H., and Haber, D. A. Germ-line BRCA1 mutations in Jewish and non-Jewish women with early-onset breast cancer. *N. Engl. J. Med.*, *334*: 143–149, 1996.
9. Arason, A., Barkardottir, R. B., and Egilsson, V. Linkage analysis of chromosome 17q markers and breast-ovarian cancer in Icelandic families, and possible relationship to prostatic cancer. *Am. J. Hum. Genet.*, *52*: 711–717, 1993.
 10. Gudmundsson, J., Johannesdottir, G., Arason, A., Bergthorsson, J. T., Ingvarsson, S., Egilsson, V., and Barkardottir, R. B. Frequent occurrence of BRCA2 linkage in Icelandic breast cancer families and segregation of a common BRCA2 haplotype. *Am. J. Hum. Genet.*, *58*: 749–756, 1996.
 11. Thorlacius, S., Olafsdottir, G., Tryggvadottir, L., Neuhausen, S., Jonason, J. G., Tavtigan, S. V., Tulinius, H., Ögmundsdottir, H. M., and Eyfjörd, J. E. A single BRCA2 mutation in male and female breast cancer families from Iceland with varied cancer phenotypes. *Nat. Genet.*, *13*: 117–119, 1996.
 12. Gudmundsson, J., Johannesdottir, G., Bergthorsson, J. T., Arason, A., Ingvarsson, S., Egilsson, V., and Barkardottir, R. B. Different tumor types from BRCA2 carriers show wild-type chromosome deletions on 13q12–q13. *Cancer Res.*, *55*: 4830–4832, 1995.
 13. Gudnason, V., Sigurdsson, Jr. G., Steingrimsdottir, L., Humphries, S., and Sigurdsson, G. Association of apolipoprotein E polymorphism with plasma lipid levels of high density lipoprotein and Lp(a), and effect of diet in healthy men and women. *Nutr. Metab. Cardiovasc. Dis.*, *3*: 136–141, 1993.
 14. Barkardottir, R. B., Arason, A., Egilsson, V., Gudmundsson, J., Jonasdottir, A., and Johannesdottir, G. Chromosome 17q-linkage seems to be infrequent in Icelandic families at risk of breast cancer. *Acta Oncol.*, *34*: 657–662, 1995.
 15. Claus, E. B., Risch, N., and Thompson, D. Genetic analysis of breast cancer in the cancer ad steroid hormone study. *Am. J. Hum. Genet.*, *48*: 232–242, 1991.
 16. Stuewing, J. P., Abeliovich, D., Peretz, T., Avishai, N., Kaback, M. M., Collins, F. S., and Brody, L. C. The carrier frequency of the BRCA1 185delAG mutation is approximately 1 percent in Ashkenazi Jewish individuals. *Nat. Genet.*, *11*: 198–200, 1995.
 17. Tavtigan, S. V., Simard, J., Rommens, J., Couch, D., Shattuck-Eidens, D., Neuhausen, S., Merajver, S., Thorlacius, S., Offit, K., Stoppa-Lyonet, D., Belanger, C., Bell, R., Berry, S., Bogden, R., Chen, Q., Davis, T., Dumont, M., Frye, C., Hattier, T., Jammulapat, S., Janecki, T., Jiang, P., Kehrer, R., Leblanc, J.-F., Mitchell, J. T., McArthur-Morrison, J., Nguyen, K., Peng, Y., Samson, C., Schroeder, M., Snyder, S. C., Steele, L., Stringfellow, M., Stroup, C., Swedlund, B., Swensen, J., Teng, D., Thomas, A., Tran, T., Tranchant, M., Weaver-Feldhaus, J., Wong, A. K. C., Shizuya, H., Eyfjörd, J. E., Cannon-Albright, L., Labrie, F., Skolnick, M. H., Weber, B., Kamb, A., and Goldgar, D. E. The complete BRCA2 gene and mutations in chromosome 13q-linked kindreds. *Nat. Genet.*, *12*: 333–337, 1996.
 18. Tulinius, H., Bjarnason, O., Sigvaldason, H., Bjarnadottir, G., and Olafsdottir, G. Tumors in Iceland. Malignant tumors of the female breast. *Acta Pathol. Microbiol. Immunol. Scand.*, *96*: 229–238, 1988.