Am. J. Hum. Genet. 52:749–753, 1993

Linkage Analysis with Markers on 17q in 29 Swedish Breast Cancer Families

Annika Lindblom,* Sam Rotstein,† Magnus Nordenskjöld,* and Catharina Larsson*

*Department of Clinical Genetics, Karolinska Hospital, Stockholm; and †Department of Oncology, Danderyds Hospital, Danderyd, Sweden

Summary

Recently, a putative breast cancer gene was localized to the long arm of chromosome 17. A collaboration study was undertaken to confirm linkage, to further map the gene, and to determine to what extent breast cancer families are linked to this locus. The Swedish material consisted of 29 breast cancer families in which 68 affected members were studied. Linkage analysis of breast cancer susceptibility was performed with a number of markers on 17q. In this material a weakly positive LOD score in favor of linkage was observed in a subgroup of families with early onset, while no such linkage was observed in a subgroup of families with late onset.

Introduction

Breast cancer is most often a sporadic disease but also has long been known to be segregating in families as an autosomal dominant disease. In order to localize breast cancer–predisposing genes, a number of linkage analyses have been performed (King et al. 1983; Skolnick et al. 1984; Goldstein et al. 1989; Hall et al. 1989; Haile et al. 1990). Hall et al. (1990) reported linkage of breast cancer susceptibility, in families with early onset of the disease, to a marker on the long arm of chromosome 17. Linkage to the same marker was also demonstrated in a few families with breast cancer and ovarian cancer (Narod et al. 1991). These findings prompted an extensive collaborative study in order to further localize and confirm the linkage to the putative breast cancer gene on 17q. An extensive family study would also be able to determine the extent of heterogeneity among the breast cancer families (i.e., how many cases are due to a mutation on 17q) and to put clinical characteristics in relation to different susceptibility genes. In this report the Swedish family material submitted to the collaborative study is described in greater detail, with special emphasis on the linkage results.

Received June 15, 1992; revision received September 29, 1992.

Address for correspondence and reprints: Dr. Annika Lindblom, Department of Clinical Genetics, Karolinska Hospital, S-104 01 Stockholm, Sweden.

0002-9297/93/5204-0013$02.00

Patients and Methods

Patients

The families were identified through a questionnaire sent to 2,694 breast cancer patients in the Stockholm area. They represented all living patients who had been diagnosed in the breast cancer clinics of Karolinska Hospital and Danderyds Hospital between 1976 and 1988. The patients were asked to report their family history with regard to relatives with breast cancer or any other type of cancer. They were also asked to state the age at diagnosis and the age at death for all reported family members.

We obtained information from 1,976 families, 29 of which were available for linkage analysis (fig. 1). These families were selected because two or more family members were affected with breast cancer and were willing to participate in this study. Twenty-two families were of a type where breast cancer was the most common malignancy, with two to six cases of breast cancer in each family, while seven families were of a type with more cases with other types of cancer than cases with breast cancer. In five of the latter seven families, there were two cases of breast cancer in each family, and, in the other two families, there were three cases of breast cancer in each family, possibly part of a hereditary syndrome including breast cancer and other types of cancer. No cases with ovarian cancer were reported. For breast cancer, 9 of the 29 families had a mean age at onset less than 50 years of age, and 20 families had a

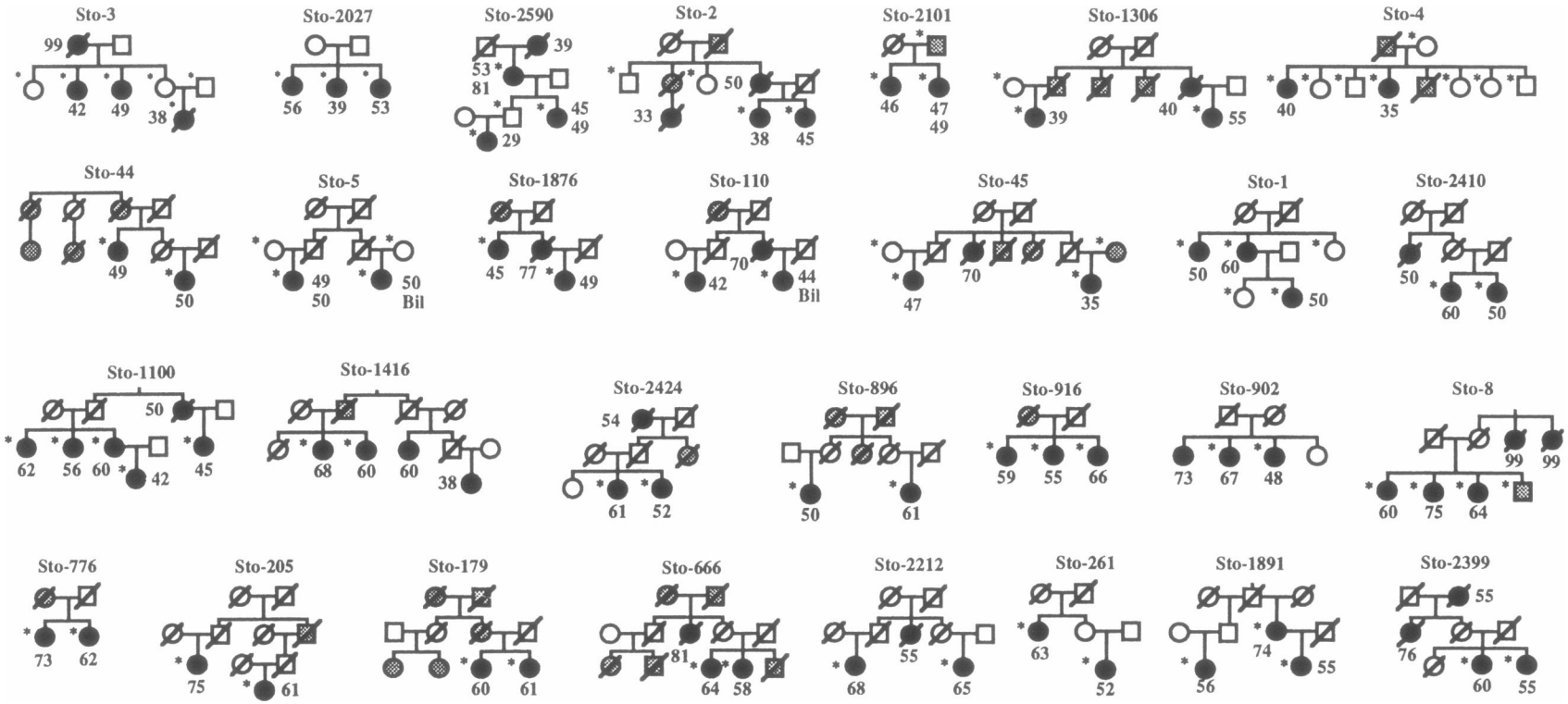

Figure 1 Pedigrees of 29 breast cancer families. Blackened symbols indicate members affected with breast cancer, and stippled symbols indicate members with other cancers. The age at diagnosis of breast cancer is given, and the members analyzed are marked by an asterisk. Age "99" implies age at onset unknown.

mean age of onset greater than 50 years of age. The linkage analysis included a total of 89 individuals in these 29 families.

DNA Analysis

Isolation of high-molecular-weight DNA from peripheral leukocytes, cleavage with restriction enzymes, Southern blot analysis, and hybridization to radioactively labeled DNA probes were performed as described elsewhere (Bergerheim et al. 1989; Larsson et al. 1990). Constitutional genotypes were compared by RFLP at the chromosomal loci CMM86 (D17S74), pEW101 (D17S40), pEW102 (D17S41), MPO (pMP503), and HOX2 (HH250/pGEM3H2) (Kidd et al. 1989). The following microsatellite markers were studied: 46E2 and 42D6, provided through the consortium by Mark Skolnick; GH (Polymeropoulos et al. 1991); NM23, provided through the consortium by Helen Solomon and Donny Black; mfd188 (D17S79), provided through the consortium; and mfd15 (D17S250) (Kidd et al. 1989). Standard PCR was carried out in a 10-μl volume containing 20–40 ng of genomic DNA template, 1 pmol of each oligodeoxynucleotide primer (one of which was end-labeled with ^{32}P), 100 μM nucleotides (25 μM dGTP, 25 μM dATP, 25 μM TTP, and 25 μM CTP), PCR buffer (15 mM $MgCl_2$, 500 mM KCl, and 100 mM Tris [pH 8.3]), and 0.2 U of *Taq* polymerase (AmpliTaq; Perkin Elmer Cetus). Samples were overlaid with mineral oil, heated for 4 min to 95°C and were processed through 25 temperature cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, with a final step of 72°C for 4 min. Aliquots of the amplified DNA were denatured with formamide and were electrophoresed on standard denaturing 6% polyacrylamide DNA sequencing gels. Gels were then fixed, dried, and subjected to autoradiography for about 24 h.

Linkage Analysis

The genetic model for the analyses is the same as that described in detail in the study by Easton et al. (1993) in this issue of the *Journal*. The analyses were performed by using the LINKAGE program package (Lathrop et al. 1984), under the assumptions of (*a*) a disease gene frequency of .003 and (*b*) breast cancer risks corresponding to the model of Claus et al. (1991) (with separate liability classes for affected and unaffected individuals). The analyses assume equal allele frequency for each marker. Members of the families with other types of cancer were considered as unaffected. Penetrance was assumed to be 67% at 70 years of age. Two-point analysis was performed for the markers that showed acceptable information and that were within an area most likely to harbor the gene according to the earliest multipoint analysis performed by the consortium. These markers were, in order from 17qter, GH, CMM86, NM23, 46D6, mfd188, and mfd15. The number of alleles assumed was 20 for GH and CMM86, 5 for NM23, 6 for 42D6, and 10 for mfd188 and mfd15.

Results and Discussion

The linkage study by Hall et al. (1990) showed linkage only to families with early age at onset, suggesting a separate and distinct clinical and genetic entity. We therefore divided our families into two groups—those with a mean age at onset less than 50 years of age and those with a mean age at onset of or greater than 50 years of age. The former group consisted of 9 families (Sto-3, Sto-2027, Sto-2590, Sto-2, Sto-2101, Sto-1306, Sto-4, Sto-44, and Sto-5), and the latter group consisted of 20 families (fig. 1).

Table 1 gives the LOD scores for these two groups, with all the markers tested at different recombination fractions. As shown, this family material gave no significant LOD score for any marker on 17q. However, the combined LOD scores for the early-onset families showed low positive values for all markers except NM23 and GH.

The evidence in favor of linkage in these families is weak. However, in the group of early-onset families, the linkage data are consistent with linkage to the long arm of chromosome 17, without recombination in five families (Sto-2, Sto-4, Sto-44, Sto-2027, and Sto-2590). In the group of late-onset families, a more heterogeneous pattern was found. In three families the affected members did not share alleles for any of the markers; in two families the affected persons shared only the marker for NM23; and in two other families the marker mfd188 was excluded from linkage (data not shown).

Through linkage analyses, the disease locus in early-onset breast cancer families has been linked to a locus on 17q (Hall et al. 1990). In the Swedish material alone no significant LOD scores were obtained. In this material the early-onset families were more likely to have a putative breast cancer gene on chromosome 17 segregating with the disease than were late-onset families.

It is generally believed that morbidity and mortality in neoplastic diseases may be reduced by prevention and early detection and by identifying individuals "at increased risk." By identifying breast cancer families through their family history, one can identify individuals with an increased risk of 20%–50% (Lynch et al.

Table 1

Pairwise LOD Scores for Linkage of Breast Cancer to Markers on 17q in Families with Early Onset (Less than 50 Years of Age) and Late Onset (50 or More than 50 Years of Age)

	LOD Score at Recombination Fraction of					
Marker and Status	.001	.01	.05	.1	.2	.3
mfd15:						
Early onset.....	.52	.51	.45	.39	.26	.15
Late onset.....	−.90	−.87	−.75	−.60	−.37	−.20
mfd188:						
Early onset.....	.56	.54	.46	.36	.19	.07
Late onset.....	−1.16	−1.05	−.74	−.52	−.27	−.14
42D6:						
Early onset.....	.30	.33	.39	.42	.41	.33
Late onset.....	−1.20	−1.09	−.76	−.52	−.24	−.09
NM23:						
Early onset	−.18	−.17	−.15	−.12	−.08	−.05
Late onset.....	.44	.44	.42	.38	.27	.15
CMM86:						
Early onset.....	1.32	1.30	1.18	1.04	.75	.47
Late onset.....	−1.62	−1.48	−1.07	−.76	−.36	−.15
GH:						
Early onset.....	−.33	−.27	−.07	.08	.20	.21
Late onset.....	−1.71	−1.57	−1.16	−.82	−.41	−.17

1990). Because of the heterogeneity in the disease, one would expect only some families to be linked to 17q. Therefore, to be able to differentiate between the carriers and the noncarriers of a gene in a specific family, each family must be large enough to provide a LOD score significant in itself. It is also necessary to study large families to get a LOD score suggestive for alternative chromosome locations of other breast cancer genes. Another approach to identifying breast cancer genes could be to study allele losses in hereditary tumors (Knudson 1987; Vogelstein et al. 1989; Devilee et al. 1991; Lindblom et al., in press). We would like to think that chromosomal regions frequently deleted in hereditary breast tumors harbor candidate breast cancer genes.

In the clinical context, new breast cancer families are usually of the small type represented in our material. Hence, in a particular family, it will be difficult to predict whether the disease is linked to a particular candidate gene. However, cloning of the different breast cancer gene(s) could make it possible to identify the predisposing mutation, even in smaller families.

Acknowledgments

We thank Dr. Clas Wadelius for valuable suggestions. This work was supported by the King Gustav V Jubilee Fund, the Swedish Cancer Society, the Axel and Margaret Axson Johnson Fund, the Swedish Medical Research Council and by the Bert von Kantzows, the Nilsson-Ehle, Magnus Bergwall, Søderberg, and Lars Hiertas memorial foundations.

References

Bergerheim U, Nordenskjöld M, Collins VP (1989) Deletion mapping in renal cell carcinoma. Cancer Res 49:1390–1396

Claus EB, Risch N, Thompson WD (1991) Genetic analysis of breast cancer in the Cancer and Steroid Hormone Study. Am J Hum Genet 48:232–242

Devilee P, van Vliet M, van Sloun P, Kuipers Dijkshoorn N, Hermans J, Pearson PL, Cornelisse CJ (1991) Allelotype of human breast carcinoma: a second major site for loss of heterozygosity is on chromosome 6q. Oncogene 6:1705–1711

Goldstein AM, Haile RW, Spence MA, Sparkes RS, Paganini-Hill A (1989) A genetic epidemiologic investigation of breast cancer in families with bilateral breast cancer. II. Linkage analysis. Clin Genet 36:100–106

Haile RW, Goldstein AM, Weeks DE, Sparkes RS, Paganini-Hill A (1990) Genetic epidemiology of bilateral breast cancer: a linkage analysis using the affected-pedigree-member method. Genet Epidemiol 7:47–55

Hall JM, Lee MK, Newman B, Morrow JE, Andersson LA, Huey B, King MC (1990) Linkage of early-onset familial

breast cancer to chromosome 17q21. Science 250:1684–1689

Hall JM, Zuppan PJ, Anderson LA, Huey B, Carter C, King M-C (1989) Oncogenes and human breast cancer. Am J Hum Genet 44:577–584

Kidd KK, Bowcock AM, Schmidtke J, Track RK, Ricciuti F, Hutchings G, Bale A, et al (1989) Human gene mapping 10. Cytogenet Cell Genet 52:622–947

King M-C, Go RCP, Lynch HT, Elston RC, Terasaki PI, Petrakis NL, Rodgers GC, et al (1983) Genetic epidemiology of breast cancer and associated cancers in high-risk families. II. Linkage analysis J Natl Cancer Inst 71:463–467

Knudson AG (1987) A two-mutation model for human cancer. Adv Virol Oncol 7:1–16

Larsson C, Byström C, Skoog L, Rotstein S, Nordenskjöld M (1990) Genomic alterations in human breast carcinomas. Genes Chromosom Cancer 2:191–197

Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA 81:3443–3446

Lindblom A, Skoog L, Ikdahl-Andersen T, Rotstein S, Nordenskjöld M, Larsson C. Four separate regions on chromosome 17 show loss of heterozygosity in familial breast carcinomas. Hum Genet (in press)

Lynch HT, Watson P, Conway TA, Lynch JF (1990) Clinical/genetic features in hereditary breast cancer. Breast Cancer Res Treat 15:67–71

Narod SA, Feunteun J, Lynch HT, Watson P, Conway T, Lynch J, Lenoir GM (1991) Familial breast-ovarian cancer locus on chromosome 17q12-q23. Lancet 338:82–83

Polymeropoulos MH, Rath DS, Xiao H, Merril CR (1991) A simple sequence repeat polymorphism at the human growth hormone locus. Nucleic Acids Res 19:689

Skolnick MH, Thompson EA, Bishop DT, Cannon LA (1984) Possible linkage of a breast cancer-susceptibility locus to the AB0 locus: sensitivity of LOD scores to a single new recombinant observation. Genet Epidemiol 1:363–373

Vogelstein B, Fearon ER, Kern SE, Hamilton SR, Preisinger AC, Nakamura Y, White R (1989) Allelotype of colorectal carcinomas. Science 244:207–211