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Frequent Occurrence of BRCA2 Linkage in Icelandic Breast Cancer Families and Segregation of a Common BRCA2 Haplotype

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Summary

Cloning of a breast cancer–predisposing gene (BRCA2) on chromosome 13q12-14 has been reported recently. We analyzed seven large Icelandic breast cancer families with markers from the BRCA2 region. Five families showed strong evidence of linkage. The maximum two-point LOD scores for the five BRCA2-linked families ranged from 1.06 to 3.19. Haplotype analyses revealed a region with identical allele sizes between the families, suggesting that they have inherited the mutation from a common ancestor. Cancer types other than breast cancer occur in individuals, segregating the affected haplotype within these families. This suggests that mutations in the gene may also confer some risk of other malignancies in both males and females.

Introduction

Epidemiological studies have shown a significantly increased risk of breast cancer in the relatives of breast cancer patients (e.g., Claus et al. 1991 and references therein). Most segregation analyses have found that familial clustering of breast cancer is best explained by an autosomal dominant gene defect (Williams and Anderson 1984; Bishop et al. 1988; Claus et al. 1991). Genetic linkage analyses of high-risk breast cancer families have suggested that for the majority of the families the disease is probably due to a mutation in either of the two genes BRCA1 or BRCA2. BRCA1 is located on chromosome 17q and was recently identified by mutation analysis of affected families (Miki et al. 1994). The results from linkage analysis on 214 breast cancer families suggest that it accounts for most families with multiple cases of both breast and ovarian cancer and ~45% of families with breast cancer only (Easton et al. 1993). The average risk of developing breast or ovarian cancer by age 70 years for women carrying a defective BRCA1 gene is 85% and 63%, respectively, but the risk varies between families (Easton et al. 1995). Gene carriers are also at a fourfold risk of developing colon cancer and a threefold risk of developing prostate cancer (Ford et al. 1994). A report on 15 high-risk breast cancer families located the BRCA2 gene on chromosome 13q by linkage analysis. The results suggested that the gene may contribute to breast cancer in high-risk families to a similar degree as the BRCA1 gene. It was also suggested that BRCA2 mutations could be responsible in most of the families with male breast cancer cases (Wooster et al. 1994). Loss of heterozygosity (LOH) occurs very frequently at the BRCA2 locus in tumors of BRCA2 carriers. In each case, the defective chromosome is retained in the tumor cells. This strongly suggests that the BRCA2 gene, like the BRCA1 gene, is a tumor-suppressor gene (Collins et al. 1995; Gudmundsson et al. 1995).

In this paper, we show that in Iceland, a country with a population derived from a few relatively small groups of settlers, the majority of hereditary breast cancer is probably due to mutations in BRCA2 rather than BRCA1. Moreover, a proportion of other cancer types in these families may also result from BRCA2 mutations.

Subjects and Methods

High-Risk Breast Cancer Families

Included in this study are seven families that did not show linkage to the BRCA1 locus of eight families analyzed (Barkardóttir et al. 1995). These eight families were selected for linkage analysis, after pedigrees and clinical information from >200 families of Icelandic breast cancer patients were viewed.

DNA Analysis

Processing of peripheral blood and archive material and PCR amplification of DNA has been described elsewhere (Arason et al. 1993; Barkardottir et al. 1995). The markers used were D13S263, D13S219, D13S220, D13S267, D13S171, D13S260, D13S290, and D13S217 (Gyapay et al. 1994) and D13S1293,

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Table 1

Two-Point LOD Scores at Recombination Fractions of .00 and .05, for 13q Markers in Breast Cancer Families

	Chromosome 13q Markers						
	D13S1246		D13S260		D13S267		
Family	.00	.05	.00	.05	.00	.05	LOD Score at *BRCA1*[a]
2	1.09	.95	1.89	1.69	1.70	1.51	−.65
3	−.04	−.03	.13	.12	−.05	−.04	−.13
4	.52	.47	.21	.18	1.81	1.64	−1.89
5	.32	.37	1.35	1.33	3.19	2.88	−.42
6	−.54	.24	1.80	1.75	2.31	2.12	−.96
7[b]	.98	.86	.46	.39	1.06	.94	.57
8	−1.23	−.13	−1.38	−.75	−1.31	−.23	−.89

[a] LOD scores are derived from two-point linkage analyses using D17S250-BRCA1, except for families 4 and 6 (three-point; D17S250-BRCA1-D17S579) and family 8 (two-point; D17S855-BRCA1).
[b] LOD-score calculations were restricted to branch A in family 7.

D13S1246, and D13S1226 (Dib et al., in press). The PCR products were separated on 6.5 % acrylamide sequencing gels. Genotypes were scored by a nonradioactive procedure (Vignal et al. 1993), with the modification of employing (N-[2-hydroxyethyl]piperazine-N'-[2-ethane-sulfonic acid]) (HEPES) buffer (40 mM potassium HEPES, pH 7.2, with 1 mM $CoCl_2$) instead of cacodylate for tailing oligomers with terminal transferase for probe preparation (Barkardottir et al. 1995).

Linkage Analysis

The model and the liability classes used in the linkage analysis of the families were as described by Wooster et al. (1994). Only breast and ovarian cancer cases were considered as affected. The frequencies of alleles were determined from 50 Icelandic control samples.

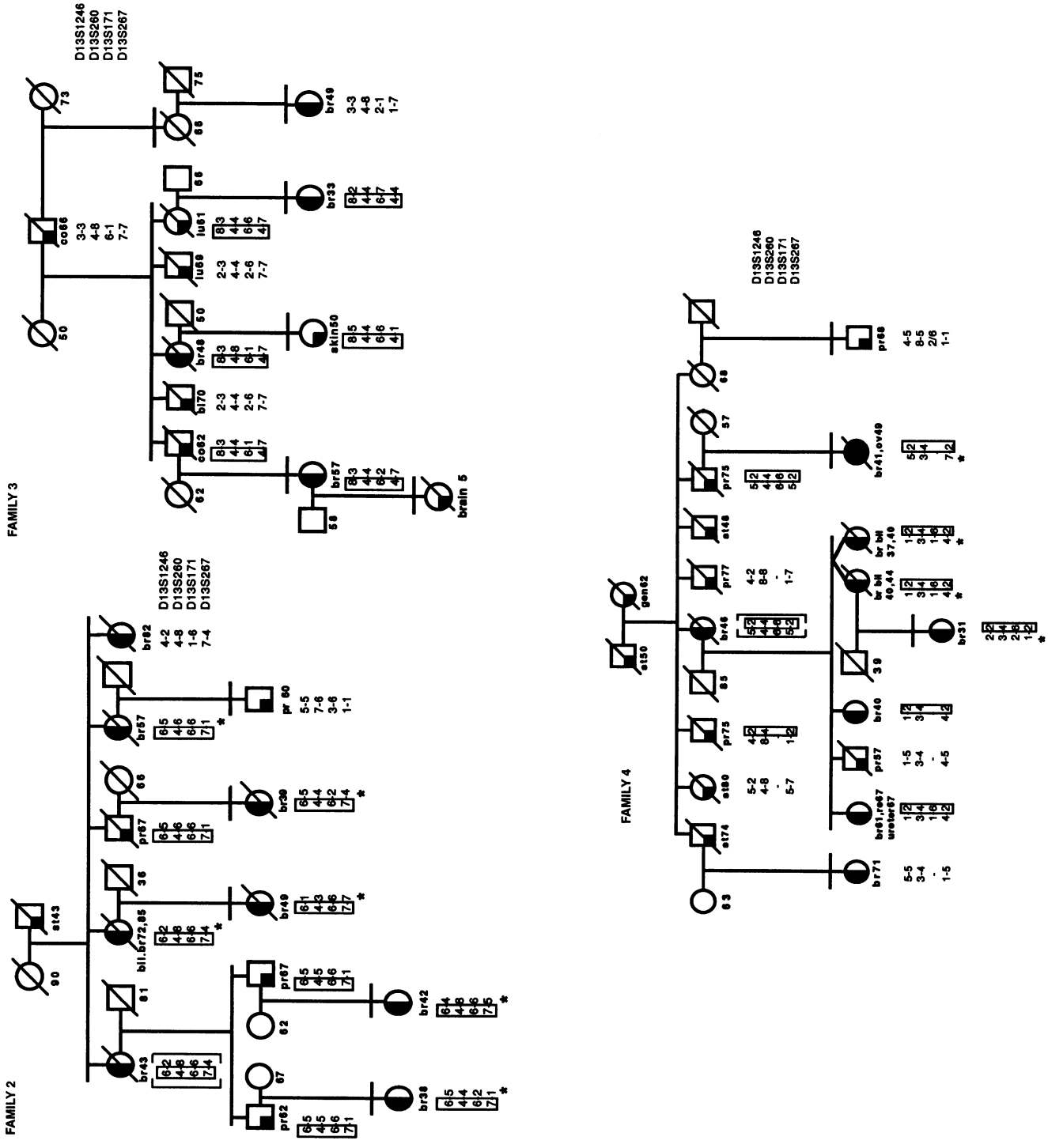
Results

Seven breast cancer families were typed with markers at chromosome 13q12-14. Two-point LOD scores for the markers D13S1246, D13S260, and D13S267 are presented in table 1. Families 2, 4, 5, 6, and 7 show strong evidence of linkage, maximum LOD scores for each family ranging from 1.06 to 3.19. Haplotype analysis and studies of LOH in breast tumors from carriers of the suspected haplotype added further evidence of chromosome 13q linkage in these families. Altogether, 43 breast cancer cases within these five families were typed, and 35 of them carried the suspected BRCA2 haplotype. Of 34 breast tumors analyzed from individuals carrying a suspected BRCA2 haplotype, 29 showed LOH, and in every case the suspected haplotype was retained in the tumors (fig. 1).

Family 8 shows negative LOD scores with all markers tested (table 1), and, according to haplotype analysis, the family is unlikely to be linked to chromosome 13q (data not shown). The results from family 3 are inconclusive. The family shows low positive LOD scores for marker D13S260 but negative LOD scores for the other two markers (table 1). Haplotype analyses revealed that three of four breast cancer cases in the family share a haplotype that is apparently from their mother who died unaffected at the age of 50 years. The breast cancer case that does not carry the suspected haplotype is not related to this woman but is a grandchild of her husband from his second marriage. No reliable information about LOH in breast tumors is available from members of family 3.

Comparison of the suspected haplotypes from the families showing strong evidence of BRCA2 linkage revealed that all of them had identical alleles for two or more markers within and surrounding the BRCA2 region (table 2). The suspected haplotypes from family 4 and 7 share the same allele size at all 10 markers that were tested between D13S217 and D13S219, and family 5 and 6 at all 9 markers from D13S290 to D13S220. These four families share identical alleles at all six markers tested from D13S290 to D13S267. Population frequencies of the alleles segregated by this haplotype differ and are as low as 6% at marker D12S267 (table 2). Family 2 and 3 share the same alleles with the other four families at markers D13S260 and D13S171. The allele frequencies for these two alleles are 39% and 45%, respectively.

Within the five BRCA2 linked families, 94 members have been diagnosed with a total of 108 cancers of various types (fig. 1). DNA was available from 74 of the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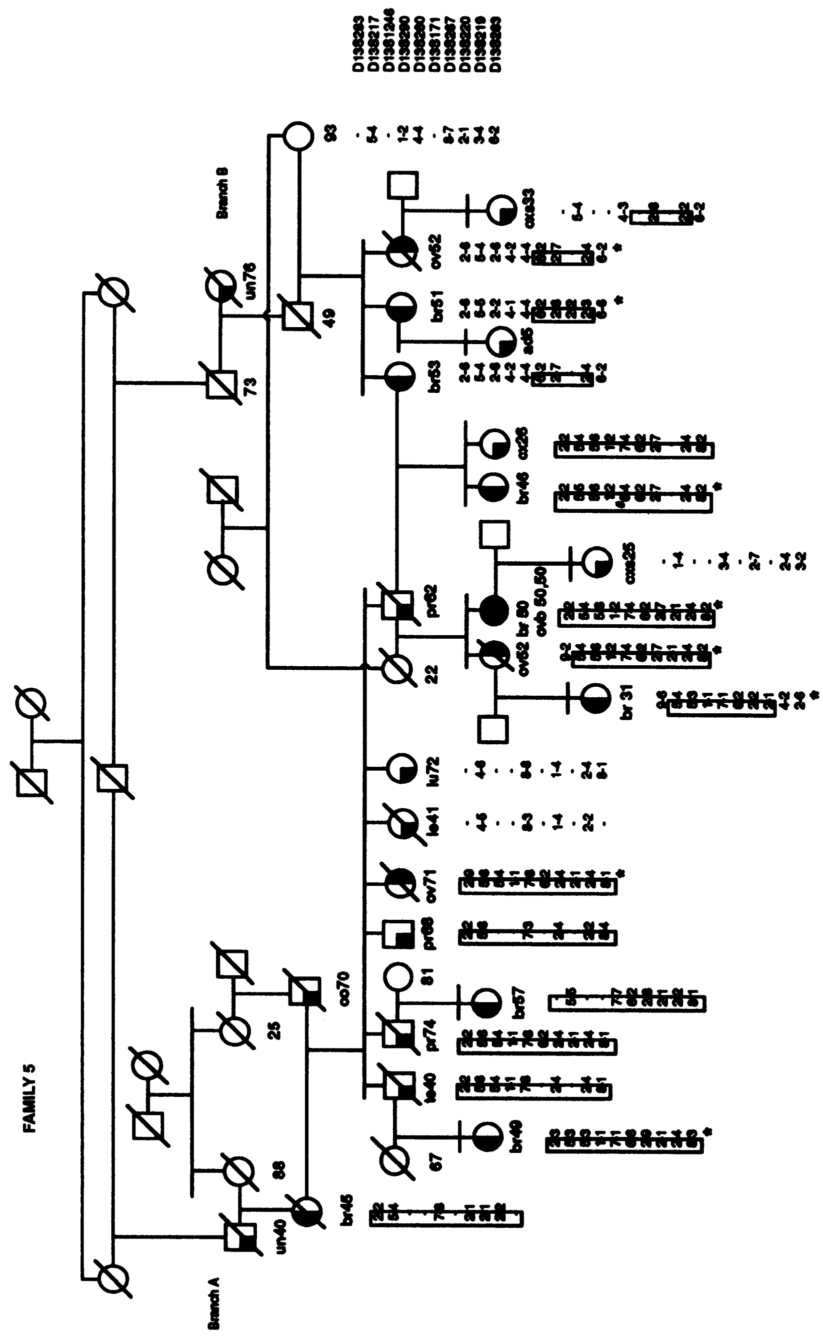
FAMILY 3
FAMILY 4
D13S1246
D13S260
D13S171
D13S267


FAMILY 5
Branch A
Branch B

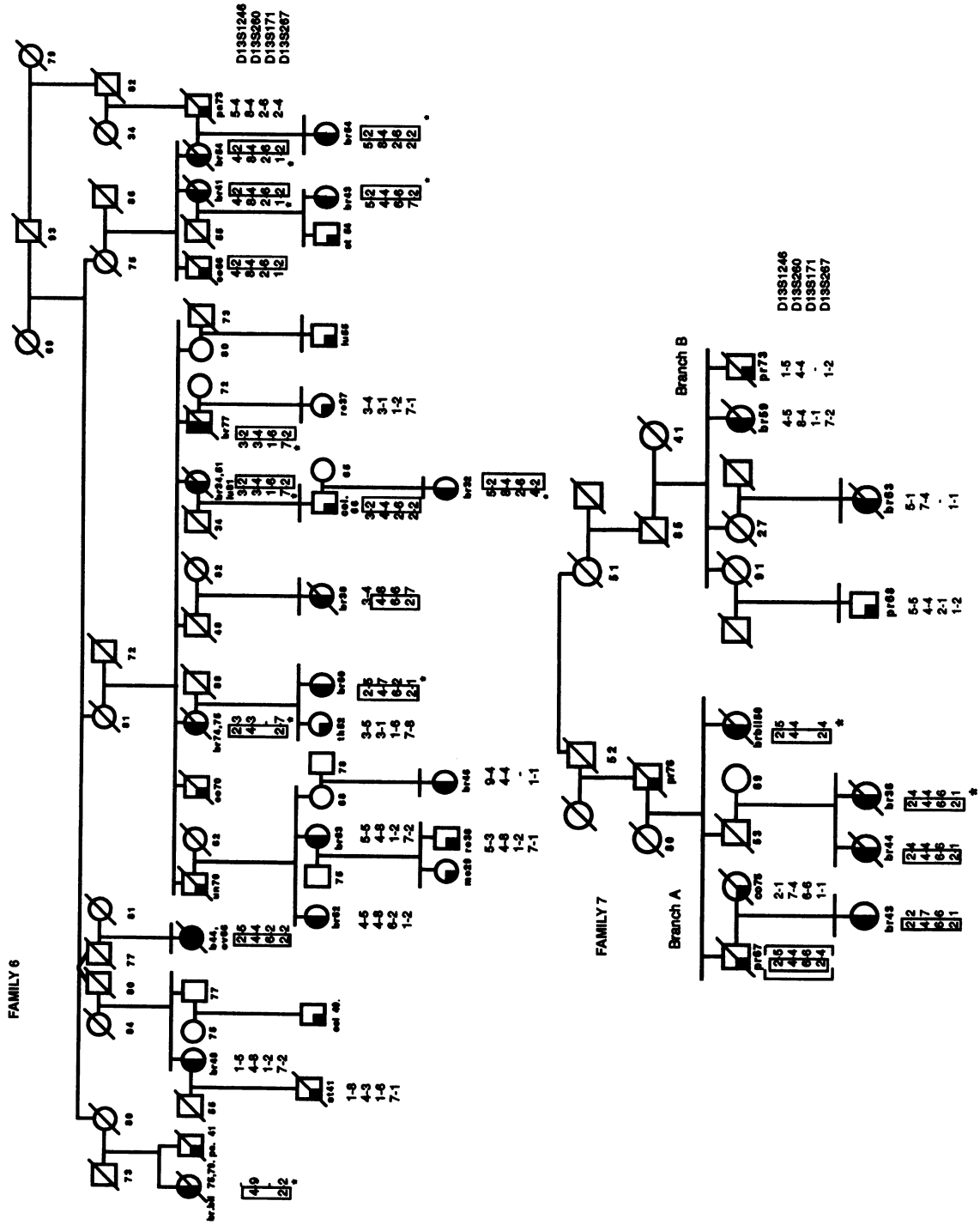


Figure 1 Pedigrees of breast cancer families and chromosome 13q haplotypes. Information about the families is restricted to cancer cases and their parents. Boxes outlining either haplotypes or alleles within recombined haplotypes denote chromosomes compatible with transmission of the trait. Reconstructed genotypes are shown within parentheses. Circles and boxes denote females and males, respectively, and symbols with a slash denote deceased individuals. The number below a symbol denotes the age (in years) at diagnosis, death, or last observation. Shading of symbols denotes the following: left half, breast cancer; right half, ovarian cancer; and bottom left quarter, other cancer. Types of cancer: ad = adrenocortical adenocarcinoma; bl = urinary bladder cancer; br = breast cancer; brbil = bilateral breast cancer; cx = cervical invasive cancer; cxs = cervical cancer; gen = female genital cancer (unknown); le = leukemia; lu = lung cancer: me = mesothelioma: ov = ovarian cancer; ovb = bilateral ovarian cancer; pa = pancreatic cancer; pr = prostate cancer; re=renal cancer; st = stomach cancer; te = testis teratocarcinoma; th = thyroid cancer; and un = carcinoma of unknown origin. # = mutated allele, (patient diagnosed with breast cancer at the age of 46 years in family 5). Breast and ovarian cancer cases marked by an asterisk (*) below the haplotype had lost the wild-type alleles in the tumors.

patients, and 51 (69%) of them were shown to be BRCA2 carriers. Eleven of those were males, and 7 of them had been diagnosed with cancer of the prostate, 2 of the colon, and 1 each of the testis and the breast. The 40 women with cancer who carry the linked BRCA2 haplotype have been diagnosed with 54 primary cancers: 43 cancers of the breast, 7 of the ovaries, 2 of the cervix, and 1 each of the ureter and kidney. The difference in the cancer spectrum between the families is interesting, in light of the fact that four of the families, and possibly all five, carry the same BRCA2 haplotype. Ovarian cancer occurs in three of the five families, two families with one ovarian case each, and four cases in the third. Prostate cancer is found in four of the five families. The family without a male with prostate cancer is the largest family and includes >20 males who are >65 years of age (family 6, fig. 1).

The identification of recombination events in affected individuals from informative families is critical to the precise mapping of susceptibility genes. In this respect, family 5 is a valuable resource, with 11 individuals affected with either breast or ovarian cancer, or both, and a maximum LOD score of 3.19 (table 1). The family has two distantly related branches named A and B (fig. 1). Branch B includes three affected sisters who share a haplotype that has identical alleles with the suspected haplotype segregating in family 4, 6, and 7 (table 2). Branch A includes eight breast and ovarian cancer cases all segregating a common haplotype that share alleles with the haplotype segregating in branch B at markers telomeric to D13S260 and centromeric to D13S263. LOH studies in tumors from members of both branches showed retention of shared alleles at markers telomeric to D13S260 (fig. 2).

Table 2

Chromosome 13q Haplotypes Segregating with Breast Cancer in Five Icelandic Families Compatible with Linkage to the BRCA2 Locus

Marker	Frequency (%)	Family 2	4	5a	5b	6	7a
D13S283	...	3	1	2	2	...	...
D13S217	10	4	■	5	■	3	■
D13S290	11	2	■	1	■	■	■
D13S1246	16	6	■	5	■	■	■
D13S1226	32	1	■	3	■	■	■
D13S260	39	■	■	7	■	■	■
D13S171	45	■	■	■	■	■	■
D13S267	6	7	■	■	■	■	■
D13S1293	24	...	3	■	■	■	3
D13S220	23	...	1	■	■	■	1
D13S219	36	4	2	■	■	■	2
D13S263	...	1	1	8	6	...	...

NOTE.—The frequencies of the shaded alleles in the Icelandic population of markers D13S220, D13S267, D13S171, D13S260, and D13S1246 are given in the table. The frequency of the shaded alleles of markers D13S217, D13S290, D13S1226, and D13S219 are according to the Genome Data Base. The relative order of the markers is according to Gyapay et al. (1994) and Dib et al. (in press).

Discussion

In this paper, we report linkage analyses in seven large breast cancer families by using markers around the BRCA2 gene on chromosome 13q. Five of the families show strong evidence of 13q linkage, while results from one family are inconclusive. Haplotype analysis strongly suggests that at least four of the families have inherited the defect from a common ancestor. The quarter of a million inhabitants of Iceland are believed to be descended from a few thousand people who immigrated to Iceland from Norway and Ireland ~1,100 years ago (Bjarnason et al. 1973). It is likely that the high frequency of BRCA2-linked Icelandic breast cancer families is due to a founder effect. Furthermore, in a study of 17 sister pairs diagnosed with breast cancer at ≤50 years of age, none were indicative of 17q linkage (Barkardottir et al. 1995). A more recent analysis of these and 27 more sister pairs with breast cancer <60 years of age identified 16 sister pairs with evidence of BRCA2 linkage, while only one sister pair showed evidence of BRCA1 linkage (A. Arason, A. Jonasdottir, R. B. Barkardottir, J. Th. Bergthorsson, and V. Egilsson, unpublished data). This suggests that even in small family clusters mutations in BRCA2 may be more significant than those in BRCA1 for Icelandic families.

Inspection of the BRCA2 linked families presented in this paper demonstrates that there is an elevated risk of other cancer types in BRCA2 carriers, particularly ovarian and prostate cancers. A link between breast and ovarian cancer is well known (Schildkraut et al. 1989; Narod et al. 1991), and epidemiological studies have also suggested a link between breast and prostate cancer (Thissen 1974; Anderson et al. 1992; Tulinius et al. 1992; Sellers et al. 1994; Ford et al. 1994). LOH studies in tumors of different types occurring in BRCA2 carriers from these families showed a high incidence of LOH compatible with loss of the normal BRCA2 allele (Gudmundsson et al. 1995). Because the retained BRCA2 allele in these tumors shared a haplotype with that of breast cancer patients in the families, it is likely that it is involved in the development of these different cancer types. The difference in the cancer spectrum among the families, and the clustering in some of the BRCA2 linked families of cancer in individuals who do not carry the suspected BRCA2 haplotype, suggest that other factors may also be involved. It could be specific environmental factors, modifying genes, or different predisposing gene(s) segregating within the families.

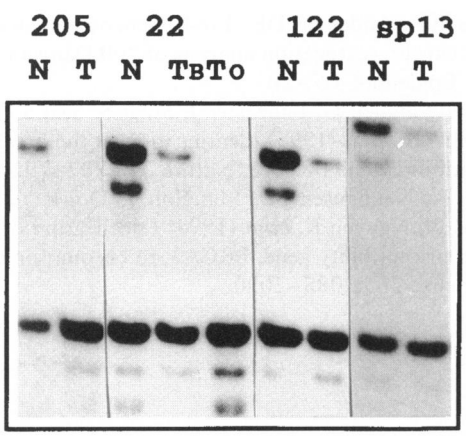


Figure 2 Loss of heterozygosity for marker D13S267 in tumors from BRCA2 carriers of branch A and B in family 5. Samples marked 205, 22, and 122 are from members of branch B, and sample sp13 from a member of branch A. N and T represent, respectively, DNA isolated from normal tissue and breast tumor. To represents DNA from ovarian cancer.

A recombination event in family 5 (see fig. 1) located the BRCA2 gene telomeric to D13S260. Combined with previously published data where the BRCA2 gene was shown to be located centromeric to D13S267 (Wooster et al. 1994), the BRCA2 region was narrowed down to ~3 cM. Further refinement of the BRCA2 region was achieved by using additional markers to map the smallest region in common between the Icelandic families of the BRCA2-linked haplotype. By this method, the region was minimized to 600 kb around marker D13S171, which was an important step in the cloning of the *BRCA2* gene recently reported by Wooster et al. (1995).

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