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Familial Site-specific Ovarian Cancer Is Linked to *BRCA1* on 17q12-21

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Summary

In a study of nine families with "site-specific" ovarian cancer (criterion: three or more cases of epithelial ovarian cancer and no cases of breast cancer diagnosed at age <50 years) we have obtained evidence of linkage to the breast-ovarian cancer susceptibility gene, *BRCA1* on 17q12-21. If the risk of cancer in these families is assumed to be restricted to the ovary, the best estimate of the proportion of families linked to *BRCA1* is .78 (95% confidence interval .32–1.0). If predisposition to both breast and ovarian cancer is assumed, the proportion linked is 1.0 (95% confidence interval .46–1.0). The linkage of familial site-specific ovarian cancer to *BRCA1* indicates the possibility of predictive testing in such families; however, this is only appropriate in families where the evidence for linkage to *BRCA1* is conclusive.

Introduction

With the identification of a locus, on chromosome arm 17q, that predisposes to breast and ovarian cancer (Hall et al. 1990; Narod et al. 1991), it is possible to offer genetic diagnosis to individuals at risk in families. Risk estimates for unaffected individuals are calculated from the prior probability that the disease in that family is linked to *BRCA1*, a probability that is derived from epidemiological data, and from the linkage data derived from affected individuals in the family itself. However, in many families for which genetic diagnosis may be requested, the linkage evidence that can be obtained within the family is weak, either because the families are small or because many of the affecteds are deceased and there are no pathology samples available for analysis. In these families, risk estimates will rely more heavily on the prior probability of linkage. It is therefore important to know with what probability different types of family are linked to *BRCA1*.

Already, we know that ⩾79% of families with multiple cases of breast and ovarian cancer, as well as a much smaller percentage of families with breast cancer only, are linked to *BRCA1* (Easton et al. 1993). The occurrence of ovarian cancer in families with multiple cases of breast cancer greatly increases the prior probability that such a family is linked to *BRCA1*. Hence, since breast-ovarian cancer families have a high prior probability of being linked to *BRCA1*, they are good candidates for genetic diagnosis at the *BRCA1* locus, and several studies are in progress. Families containing ovarian cancer only may, however, represent a distinct group (Lynch et al. 1986). Although there is some expectation that such "site-specific ovarian cancer" families will be linked to *BRCA1*, there are so far no reports to this effect. To address this question, we have analyzed nine families that were ascertained for three or more cases of epithelial ovarian cancer and no cases of breast cancer diagnosed at age <50 years. We report that most of these families appear to be linked to *BRCA1*.

Families and Methods

Families

Nine families met the following minimum criteria: three or more cases of epithelial ovarian cancer at any age and no cases of breast cancer diagnosed at age <50 years. Ovarian cancer was confirmed by pathology report, death certificate, or hospital records. Three of the families (8101,

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8102, and 8103) were ascertained in the United States, two (8104 and 256) in France, and the remainder in the United Kingdom.

Extraction of DNA

Genomic DNA was prepared from peripheral blood lymphocytes or from lymphocyte cell lines immortalized by Epstein-Barr viral transformation, by using an Applied Biosystems 340A nucleic acid extractor. DNA was extracted from archival pathology blocks by incubating individual 5-μm sections in 0.5 ml of 10 mM Tris pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 100 μg of BSA/ml, 0.45% Tween-20, 0.45% Nonidet NP40, and 100 μg of proteinase K/ml. The sections were incubated for ≥8 h at 55°C, then were transferred to a boiling water bath for 10 min, and then were cooled on ice. Adjacent sections were mounted on glass slides and were stained with hematoxylin and eosin for histopathological examination.

Typing of DNA Polymorphisms

Polymorphisms were typed by PCR using 100 ng of lymphocyte DNA or 1–5 μl of DNA extracted from archival pathology samples. One of the PCR primers was either end-labeled using [^{32}P]γ-ATP and T4 polynucleotide kinase or labeled with a fluorochrome, and the DNAs were amplified through 35 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C, with a final extension at 72°C for 7 min. The products were resolved by electrophoresis through denaturing 6% polyacrylamide gels, followed by autoradiography. PCR products that were labeled with a fluorochrome were analyzed on an automatic sequencer (Applied Biosystems 373A) using Genescan software. A total of 177 individuals, of whom 21 were affected, were typed at four loci on 17q12-21. Five of the families were typed with the genetic markers D17S250, D17S579, D17S588, and *NME1;* three of the families (8102, 8103, and 8104) were typed at *THRA1* but not at *NME1;* and family 256 was typed at D17S250, *THRA1,* D17S800, and D17S579. For details of the primers, see the report by Easton et al. (1993).

Statistical Methods

Multipoint LOD scores for linkage between *BRCA1* and the flanking markers, D17S579 and D17S250, were calculated using the LINKAGE program (Lathrop et al. 1984). *BRCA1* was assumed to lie 2.7 cM distal to D17S250 on the male genetic map and 5.3 cM distal to D17S250 on the female genetic map. Disease susceptibility was assumed to be conferred by an autosomal dominant allele with a population frequency of .003. All families were analyzed under two models. In model 1 the disease gene was assumed to confer a risk of ovarian cancer only (i.e., the risk of breast cancer was assumed to be the same as in noncarriers); and in model 2 the disease gene was allowed to confer risks of both breast and ovarian cancer. The age-specific risks of ovarian and breast cancer in *BRCA1* carriers were the same as those estimated by Easton et al. (in press). Risks of cancer in noncarriers were estimated from population incidence rates for England and Wales during 1979–82 (Muir et al. 1987).

In four families some data on allele loss at the *BRCA1* locus in tumors was available. These data can be used to provide further linkage information, since there is strong evidence that the wild-type chromosome is invariably lost in tumor DNA in *BRCA1* families (Smith et al. 1992; Kelsell et al. 1993). Thus, each allele loss in an affected carrier provides effectively an additional meiosis from which to assess linkage. To incorporate these allele loss data into the linkage calculations, we computed an adjusted LOD score from

$$\mathrm{LOD}(\theta,\varnothing) = \log 10 \frac{\Pr(\text{marker + disease segregation, + allele loss} \mid \theta,\varnothing)}{\Pr(\text{marker + disease segregation, + allele loss} \mid 1/2,1/2)},$$

where θ is the recombination fraction between the markers and the disease locus and ∅ is the corresponding probability that allele losses at the marker loci were on the same chromosome as that presumed if the allele losses affect the putative wild-type chromosome (Smith et al. 1992). Given the tight linkage of these markers to *BRCA1,* ∅ was assumed to be zero.

Evidence for heterogeneity was evaluated using the admixture model in which a proportion of families were assumed to be linked to *BRCA1* and the remainder of cases were assumed to be due to other loci (Smith 1961). LOD scores assuming heterogeneity were computed using the HOMOG program (Ott 1985).

Results

The most likely marker haplotypes for the nine families are shown in figures 1–3. In all but two of the families (8102 and 8103) the haplotypes were consistent with linkage to *BRCA1.* Furthermore, two of the families, 817 and 8167, contained allele losses, in ovarian tumors, that were consistent with them being linked to *BRCA1;* in each case the allele losses affected the putative wild-type chromosome. Family 8101 contained an informative recombinant in individual 406, placing the disease gene below D17S250, which has been reported elsewhere (Smith et al. 1994).

Two families contained some evidence against linkage to *BRCA1.* In family 8102, the two ovarian cancer cases (276 and 279) for whom samples were available for analysis did not share a common haplotype. In family 8103, individual 304, who was diagnosed with ovarian cancer at age 64 years, was inconsistent with linkage to *BRCA1.* The

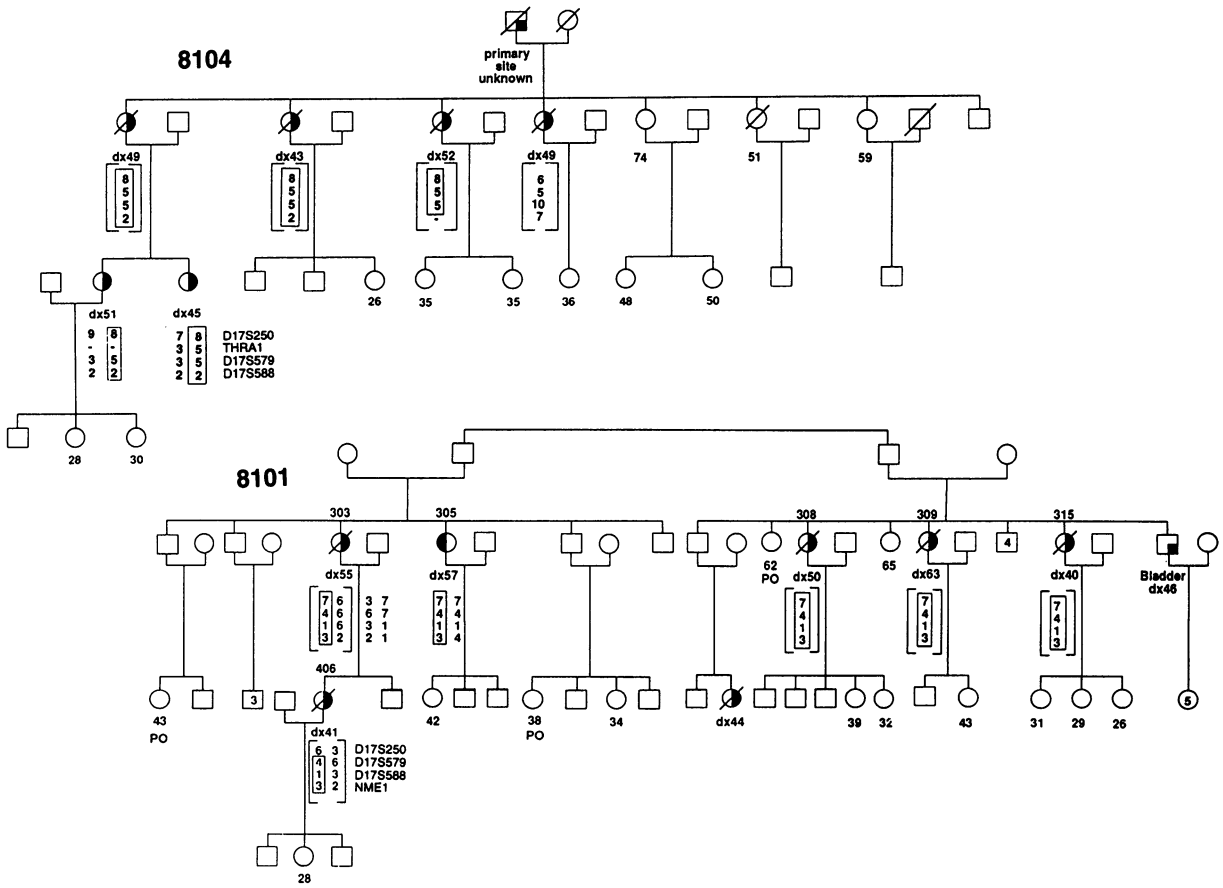

Figure 1 Pedigrees and haplotypes of two ovarian cancer families, 8104 and 8101. Circles represent females and squares represent males. Symbols with a line through them represent deceased individuals. Left-sided-blackened symbols represent breast cancer, right-sided-blackened symbols represent ovarian cancer and quarter-blackened symbols represent any other cancer. Age at diagnosis of cancer is indicated by dx; NA indicates that the age at diagnosis was not available; and the ages of unaffected females or age at death are indicated immediately below symbols. PO = prophylactic oophorectomy. Almost all of the individuals indicated as alive, with the exception of children <18 years of age, were genotyped. To disguise the carrier status of unaffected individuals, only those haplotypes that are necessary to demonstrate the linkage are shown. Haplotypes enclosed within square brackets have been reconstructed. The boxed haplotype indicates the putative linked chromosome.

two individuals who did share a haplotype, 319 and 271, were sisters who were diagnosed with ovarian cancer at age 46 years and 67 years, respectively. However, in one of these cases, individual 319, the "shared" haplotype was lost in the tumor, indicating that this haplotype was unlikely to be linked to cancer predisposition in this family.

The multipoint LOD scores for linkage between the disease and flanking markers are shown in table 1. The total LOD score is 2.42 under homogeneity if the disease gene confers a risk of ovarian cancer only (model 1) and is 2.86 if the gene confers a risk of breast cancer as well (model 2). Under model 1 the best estimate of the proportion of linked families is .78 (95% confidence interval .32–1.0), and under model 2 the best estimate is 1.0 (95% confidence interval .46–1.0).

While the overall LOD scores are very similar under the two models, it is interesting to note that the LOD scores for an individual family may vary substantially. For example, family 445 has a LOD score of 0.89 if *BRCA1* is assumed to confer a risk of ovarian cancer only and has a LOD score of 0.02 if *BRCA1* confers a risk of breast and ovarian cancer. This can be explained by the observation that four individuals, three of whom are 40–50 years of age and one of whom is >50 years of age, share the linked haplotype but are not affected. Unaffected individuals count more heavily against linkage under the second model, where the penetrance of *BRCA1* is 57% by age 50 years and 65% by age 60 years, than under the first model (penetrance 16% and 31%, respectively). In contrast, the evidence against linkage is reduced in families 8102 and 8103 under model 2, since the probability that an ovarian cancer case who does not share the linked haplotype is a phenocopy is greater under model 2 than under model 1.

It has been possible to incorporate allele loss data in

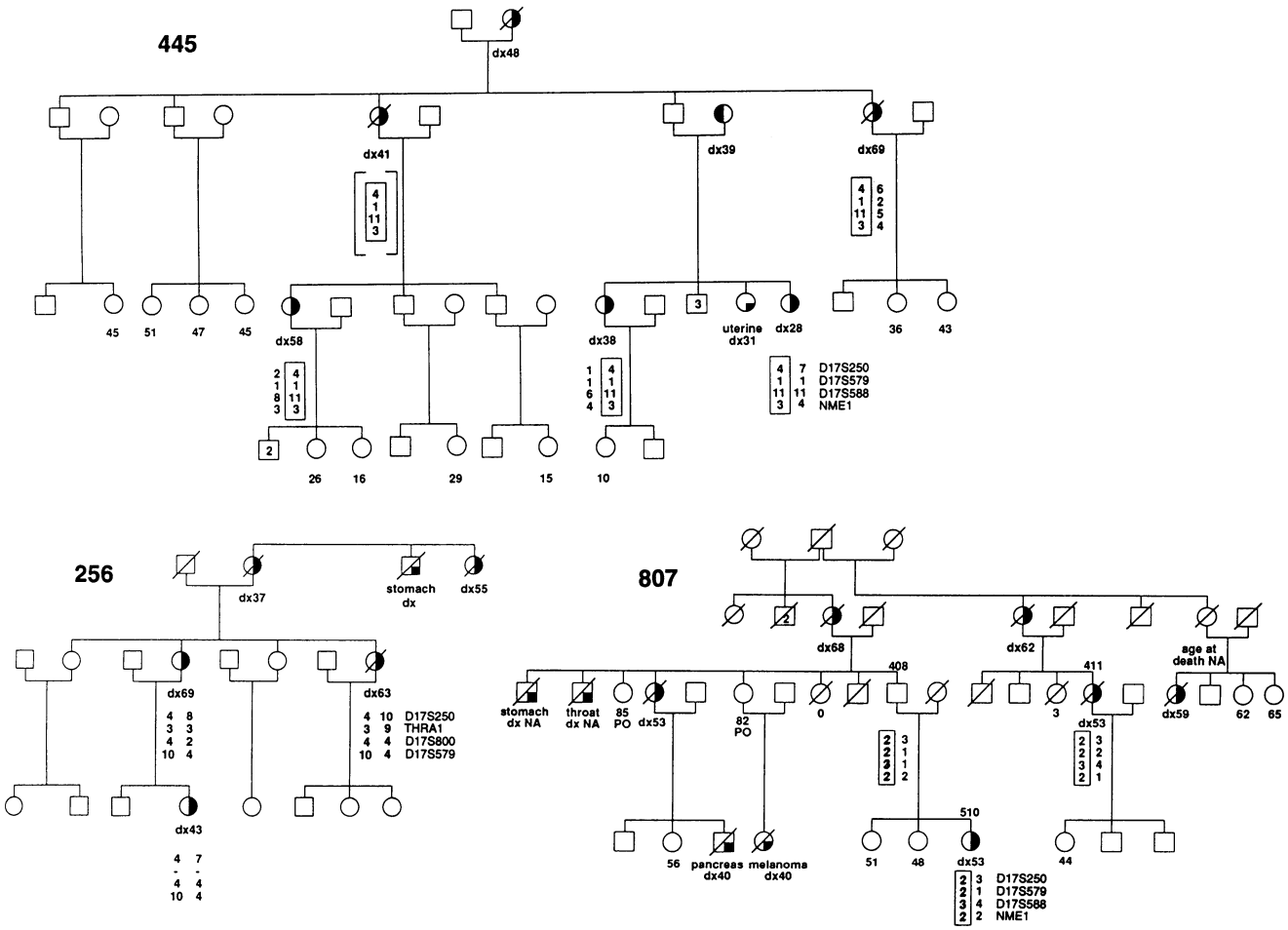

Figure 2 Pedigrees of families 445, 256, and 807. Figure legend is as described for fig. 1.

some of the families into the linkage calculations and thus to increase the power to detect evidence for or against linkage. Family 8167 is an example of a family where the loss of a wild-type chromosome in an ovarian tumor strengthens the evidence for linkage; the LOD score under model 1 is 0.82 when this information is incorporated, 0.53 when it is not. In contrast, family 8103 contains two sisters with ovarian cancer and one cousin who is inconsistent with linkage; if the allele loss information in this family is ignored the LOD score under model 1 is −0.66, but if it is included the LOD score is reduced to −1.36.

Discussion

The genetic analysis reported here favors linkage of at least a proportion of families with site-specific ovarian cancer to *BRCA1* on 17q12-21. Seven of the nine families studied were consistent with linkage of ovarian cancer to *BRCA1*. Allele losses in the tumors from two of the families were also consistent with linkage to *BRCA1*, because the losses affected the putative wild-type chromosome. In neither of the two families (8102 and 8103) that were apparently unlinked to *BRCA1* was there any evidence for microsatellite instability, which otherwise might have indicated linkage to the locus, *MSH2*, that has recently been reported to be involved in DNA mismatch repair (Leach et al. 1993). A number of families containing the syndrome Lynch type II (hereditary nonpolyposis colorectal cancer)—in which individuals are predisposed principally to colon cancer but also to ovarian cancer, among other cancer sites—have been reported to be linked to *MSH2* on chromosome arm 2p (Peltomaki et al. 1993). Families that are linked to *MSH2* often show microsatellite instability in the tumor DNA, resulting in insertion or deletion of simple repeated sequences (Ionov et al. 1993; Thibodeau et al. 1993). However, there is no evidence to suggest that families 8102 and 8103 are linked to *MSH2*.

The linkage of at least a proportion of families with familial site-specific ovarian cancer to *BRCA1* broadens the description of which types of family are due to the inheri-

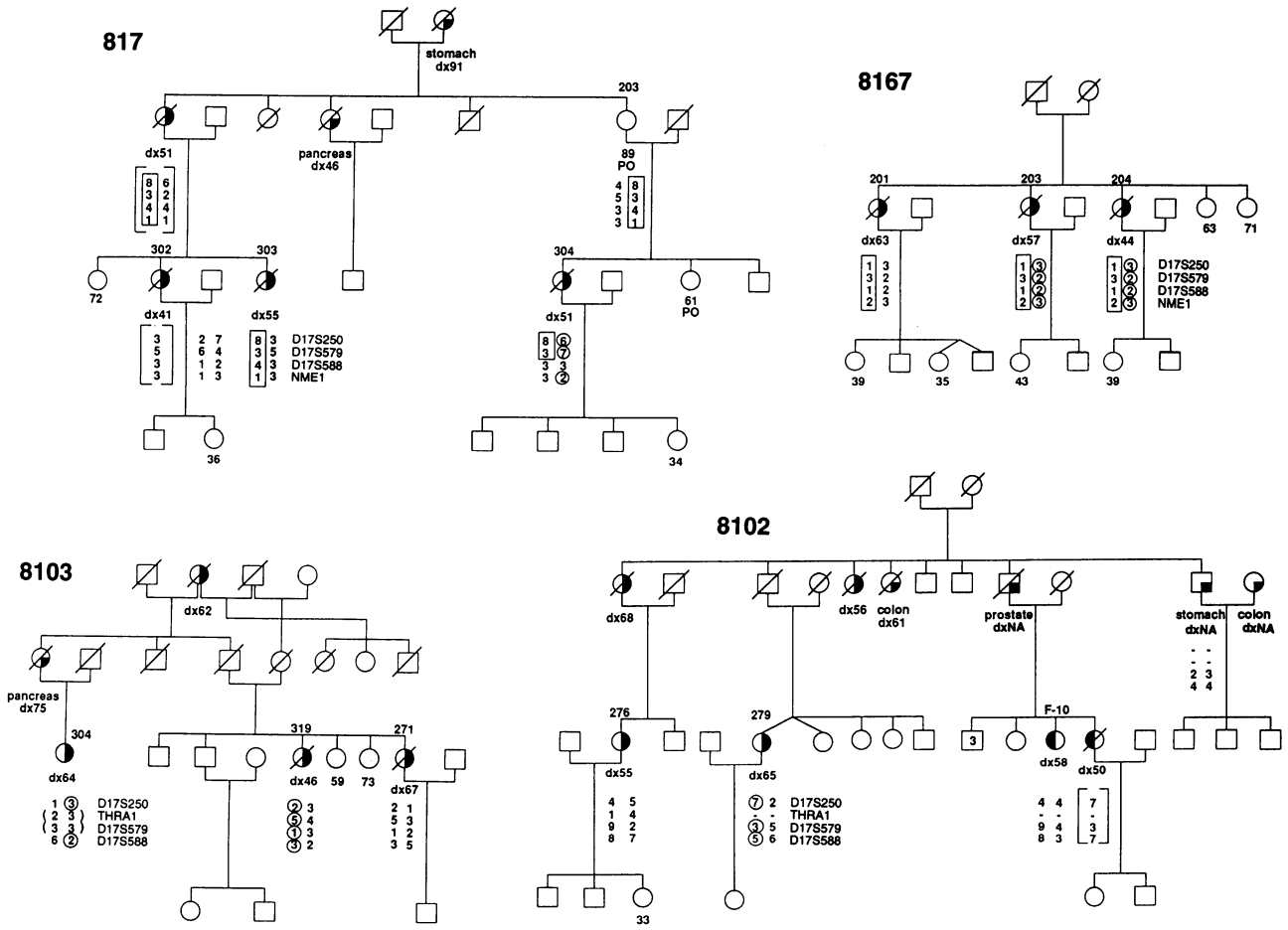

Figure 3 Pedigrees of families 817, 8167, 8103, and 8102. Figure legend is as described for fig. 1; circled alleles were affected by LOH in the tumor.

Table 1

LOD Scores for Linkage to *BRCA1*, under Homogeneity, Obtained from an Analysis of D17S579 and D17S250

Family	Model 1	Model 2
807	.39	.13
817	.78	.52
8167	.82	.65
8104	1.04	1.29
8101	.32	.21
8102	−.82	−.26
8103	−1.36	−.20
445	.89	.02
256	.36	.50
Total	2.42	2.86

NOTE.—Under model 1 *BRCA1* is assumed to confer a risk of ovarian cancer only, and under model 2 *BRCA1* is assumed to confer a risk of breast and ovarian cancer.

tance of mutant *BRCA1* alleles. Approximately 45% of families with breast cancer only, as well as the majority of families with breast and ovarian cancer, are linked to *BRCA1* (Easton et al. 1993). It now appears that a high proportion of families with multiple cases of ovarian cancer only are also linked to *BRCA1*. One possible explanation is that these families represent breast-ovarian cancer families in which, by chance, none of the *BRCA1* carriers have developed breast cancer. However, a more probable explanation for these findings is that there is allelic variation at the *BRCA1* locus. Another study (D. F. Easton, D. Ford, D. T. Bishop, and the Breast Cancer Linkage Consortium, unpublished data) analyzed 33 families that appeared to be linked to *BRCA1*, and it found significant evidence of heterogeneity of risks of breast and ovarian cancer between families. Under that study's best model, which allowed for two *BRCA1* alleles, one allele (which represented 71% of all mutations) conferred, by age 70 years, a breast cancer risk of 91% and an ovarian cancer risk of 32%, while the second allele conferred risks of 70%

and 84%, respectively. It is possible that some of the families studied here are due to *BRCA1* alleles that confer a high risk of ovarian cancer with a reduced risk of breast cancer. Once the *BRCA1* gene has been cloned, it may be possible to resolve the question of allelic heterogeneity, by correlating the spectrum of mutations with disease phenotype in the families.

Other cancer sites that occurred in the nine families studied here include the uterus, stomach, throat, pancreas, bladder, colon, and prostate. In order to address the question of cancer risk at other sites in *BRCA1* carriers, Ford et al. (1994) analyzed 33 families each having a prior probability of linkage to *BRCA1* $\geqslant$90%, and they found a 3.3-fold increased risk of prostate cancer and a 4.1-fold increased risk of colon cancer. No other sites were associated with an elevated cancer risk in *BRCA1* carriers. In none of the 17q-linked families reported here was a *BRCA1* carrier diagnosed with either prostate or colon cancer. However, in family 8102, which contained some evidence against linkage to *BRCA1*, one case each of colon and prostate cancer was diagnosed in individuals who were in the direct line of descent of the cancer-predisposing trait. In the future it may be possible to incorporate such information into linkage calculations and risk estimates, but at the moment the value of such information remains unclear, especially since prostate and colon cancer are common in the general population.

In the meantime, genetic diagnosis in a proportion of families with apparently site-specific ovarian cancer may now be possible. However, until the *BRCA1* gene is cloned and disease carriers are accurately identified by detecting mutations in the *BRCA1* gene in germ-line DNA, predictive testing is only appropriate in families where the evidence for linkage to *BRCA1* is conclusive.

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