

# The Contribution of Germline BRCA1 and BRCA2 Mutations to Familial Ovarian Cancer: No Evidence for Other Ovarian Cancer–Susceptibility Genes

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## Summary

To establish the contribution of germline BRCA1 and BRCA2 mutations to familial ovarian cancer, we have analyzed both genes in DNA samples obtained from an affected individual in each of 112 families containing at least two cases of epithelial ovarian cancer. Germline mutations were found in 43% of the families; BRCA1 mutations were approximately four times more common than BRCA2 mutations. The extent of family history of ovarian and breast cancers was strongly predictive of BRCA1-mutation status. Segregation analysis suggests that a combination of chance clustering of sporadic cases and insensitivity of mutation detection may account for the remaining families; however, the contribution of other genes cannot be excluded. We discuss the implications for genetic testing and clinical management of familial ovarian cancer arising from the data presented in these studies.

## Introduction

Familial ovarian cancer occurs as part of two clinically distinct syndromes. The most common of these syndromes is ovarian cancer, which is either apparently site specific or occurs in association with early-onset breast cancer (Easton et al. 1993). Predisposition to ovarian cancer also occurs as part of Lynch type II or hereditary nonpolyposis colorectal cancer (HNPCC) syndrome (Lynch et al. 1982). The results of genetic-linkage studies have suggested that, in >90% of families with multiple

cases of breast and ovarian cancer, the cancers are the result of mutations in either the BRCA1 gene on chromosome 17q12-21 or the BRCA2 gene on chromosome 13q12-13 (Narod et al. 1995; Ford et al. 1998). Linkage data from a small series of families suggest that BRCA1 is also responsible for the majority of “site-specific ovarian cancer” families with three or more cases of epithelial ovarian cancer (Steichen-Gersdorf et al. 1994).

Germline mutations in the BRCA1 and BRCA2 genes have been identified in a large number of families with multiple cases of early-onset breast and/or ovarian cancer (Miki et al. 1994; Shattuck-Eidens et al. 1995; Wooster et al. 1995; Tavtigian et al. 1996; Gayther et al. 1997b). The mutation spectrum is similar in both genes: most germline mutations are predicted to result in protein truncation caused by frameshift, nonsense, or splice-site alterations, and the mutations are spread along the length of the coding region (Breast Cancer Information Core). A small number of founder mutations that are common in specific populations have been described. In particular, these include the 185delAG mutation in BRCA1 and the 6174delT mutation in BRCA2, which are common in Ashkenazi Jews (Struewing et al. 1995; Neuhausen et al. 1996a, 1996b); the 5382insC mutation in BRCA1, which is common in the eastern European population (Gayther et al. 1997a; Ramus et al. 1997); and the 999del5 mutation, which is common in the Icelandic population (Thorlacius et al. 1996). The precise functions of the BRCA1 and BRCA2 proteins are unknown; they have recently been shown to interact with each other, which suggests that they may function in the same pathway (Chen et al. 1998). Both proteins interact in vitro with RAD51, a protein that has been implicated in the repair of double-strand DNA breaks (Scully 1997; Wong et al. 1997). This finding and the results of an analysis of homozygous mutant mice suggest a possible functional role for BRCA1 and BRCA2 in DNA repair and genome integrity (Gayther and Ponder 1998; Zhang et al. 1998).

An immediate consequence of the identification of the BRCA1 and BRCA2 genes is that DNA-based predictive testing has become possible in families when the mu-

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tation is known. Since the search for the mutation is laborious, it is helpful to know the prevalence of BRCA1 and BRCA2 mutations in families with different cancer histories. In this study, we set out to obtain this information in relation to ovarian cancer. One hundred and twelve families were ascertained to have a family history in which at least two first- or second-degree relatives had been given a diagnosis of epithelial ovarian cancer. To search for disease-associated mutations, the entire BRCA1 and BRCA2 coding sequence was analyzed in one affected individual from each family. This enabled us to estimate the proportion of families with ovarian cancer that resulted from these two genes, and it also enabled us to predict whether there are likely to be other highly penetrant susceptibility genes that are responsible for a proportion of cases of familial ovarian cancer.

## Material and Methods

### *Patient Material*

One hundred and three families in which at least two first-degree relatives had epithelial ovarian cancer diagnosed at any age and nine families in which two cases of epithelial ovarian cancer were diagnosed in second-degree relatives were identified through the Familial Ovarian Cancer Register of the United Kingdom Coordinating Committee on Cancer Research (UKCCCR). To be included in the register, families must have at least two cases of ovarian cancer confirmed by pathology reports or by a death certificate. Borderline cases of ovarian cancer were not included in the study. Fifty-three families had ovarian cancer only, whereas the remaining 59 families had at least one case of breast cancer, in addition to ovarian cancer, diagnosed in a family member before the age of 60 years. These families included a total of 330 cases of ovarian cancer and 133 cases of breast cancer diagnosed before the age of 60 years. Confirmation of diagnosis was obtained from either pathology reports or a death certificate, for a total of 281 cases of ovarian cancer and 70 cases of breast cancer. Initial DNA analysis was performed with genomic DNA obtained from one affected individual from each family, and, when material was available, mutations were confirmed in other affected individuals from the family.

### *Mutation Analysis*

BRCA1 and BRCA2 were both screened for germline mutations, by use of a combination of the protein-truncation test (PTT) and nonradioactive single-strand conformation analysis/heteroduplex analysis (SSCA/HA). PTT was performed, as described elsewhere (Friedman et al. 1997), for the two largest exons of BRCA2 and for the largest exon of BRCA1. For SSCA/HA, coding exons 2, 3, 5-10, and 12-24 of BRCA1 and coding

exons 2-9 and 12-27 of BRCA2 were amplified from genomic DNA. The 5' and 3' splice boundaries of exon 11 of BRCA1 and exons 10 and 11 of BRCA2 were also amplified from genomic DNA. Nonradioactive SSCA/HA was performed as described elsewhere, and gels were visualized by silver staining (Gayther et al. 1995). Direct sequence analysis was used to characterize the nucleotide alteration associated with PTT and/or SSCA/HA variants, as described elsewhere (Gayther et al. 1995).

We also screened for a recently described 6-kb duplication near exon 13 of BRCA1 (Puget et al. 1999) in families in which no BRCA1 or BRCA2 coding mutation was identified. Duplication-specific primers (forward primer, 5'-GATTATTTCCCCCAGGCTA-3'; reverse primer, 5'-AGATCATTAGCAAGGACCTGTG-3') will only produce a PCR-amplification product if the duplication is present in the DNA sample. Two positive genomic DNA controls for the duplication were included in each experiment. In addition, for each sample, a second, independent PCR reaction was simultaneously performed with the use of forward primer 5'-TCACA-ATTCCGAGACATC-3' and reverse primer 5'-AACGGCTACTGCACAGTTCT-3'. This second reaction amplified a product with a size similar to that of the duplication and was used to control both for the efficacy of each genomic DNA sample and for PCR amplification.

### *Statistical Analysis*

The effects, on mutation prevalence, of the numbers of individuals affected with breast and ovarian cancer were assessed with the use of  $\chi^2$  tests for trend. To evaluate the evidence for existence of a third susceptibility gene, in addition to BRCA1 and BRCA2, we performed segregation analysis using the MENDEL program (Lange et al. 1988). To allow for the ascertainment of families on the basis of multiple affected individuals, analyses were based on the conditional likelihood  $Lik(M, D/D)$ , where  $M$  was the observed mutation status of the index case and  $D$  was the observed disease phenotype in the family. BRCA1 and BRCA2 were initially assumed to confer the age-specific risks for breast and ovarian cancer given in previous analyses (Ford et al. 1995). Carriers of a susceptibility allele, at a hypothetical third gene locus (OVCA) and unlinked to BRCA1 and BRCA2, had an increased relative risk ( $r$ ) of ovarian cancer compared with noncarriers, but they had no increased risk for breast cancer. Dominant, recessive, and codominant models for OVCA were explored. The absolute risks by genotype were determined by the constraint that the overall incidence of the disease must agree with the incidence rates for England and Wales. We then estimated  $r$  and the frequencies  $p_1$ ,  $p_2$ , and  $p_3$  of the susceptibility alleles of BRCA1, BRCA2, and

OVCA, respectively, according to maximum likelihood. The sum  $p_1 + p_2$  was constrained to be no higher than .003, which is the combined frequency, given by the model of Claus et al. (1991), of breast cancer-susceptibility alleles and the highest plausible frequency for BRCA1 and BRCA2 mutations combined. We also allowed for a sensitivity parameter,  $\gamma$ , which was used to determine the probability of detecting a mutation, if one was present (the probability was assumed to be the same for BRCA1 and BRCA2).

We restricted consideration to those genetic models that gave familial risks of ovarian cancer that were consistent with those observed in epidemiological studies. Stratton et al. (1998) have previously estimated that, among first-degree relatives of patients with ovarian cancer, the relative risk for ovarian cancer is 2.4, with a 95% confidence interval of 1.9–3.2, on the basis of a meta-analysis of published cohort studies. We incorporated this estimate into our analysis by adding a “penalty” to the log-likelihood, to reflect deviation of risk from 2.4:  $P = [\log(2.4) - \log(\lambda_o)]^2/2v$ , where  $\lambda_o$  is the predicted familial relative risk from the model and where  $v = .0177$  is the variance of the log (relative risk) from the meta-analysis.

## Results

Germline mutations, which are thought to be disease associated, were detected in 48 (43%) of the 112 families. These data are listed in table 1. Twenty-nine different BRCA1 mutations were identified in 40 (36%) of the 112 families. The spectrum of BRCA1 mutations in this data set is illustrated in figure 1a. Mutations are spread quite evenly along the length of the gene. Six mutations were detected on more than one occasion; these six mutations represent 43% of all BRCA1 mutations identified in this study. Haplotype analysis does not indicate a common founder mutation in the British population (Gayther et al. 1995; Neuhausen et al. 1996b). Thirty-five (88%) of the 40 mutations are either frameshift or nonsense mutations that would be predicted to result in premature truncation of the BRCA1 protein. Four mutations occur at the boundaries between exons and introns and are expected to affect splicing. The results of analysis of reverse-transcribed RNA samples obtained from two affected individuals with splice variants (4304G→A in family OV161 and 4476+6T→C in family OV003) have previously shown that these alterations cause aberrant splicing (Gayther et al. 1995). There was no available RNA with which to test the effect of the remaining two splice-site mutations, although both are predicted to abolish highly conserved splice-site consensus sequences. Pro1749Arg, the putative missense mutation in family OV073, has not been described elsewhere and was not detected in 346 normal chro-

mosomes. The functional significance of this missense mutation has been assessed elsewhere (Chapman and Verma 1996). The ability of the C terminus of BRCA1 to cause transcriptional activation in vitro was abolished by a GAL4-BRCA1 fusion protein containing the Pro1749Arg missense alteration, a finding that suggests this change is functionally significant.

Seventy-two families without identifiable BRCA1 mutations were analyzed for mutations throughout the BRCA2 coding sequence. Seven different mutations that were considered to be pathogenic were identified in eight (7%) of these families. The spectrum of these mutations throughout the gene is illustrated in figure 1b. In seven families, the mutation was a frameshift deletion, and, in one family, it was a nonsense mutation. One mutation, 6503delTT, was detected in two different families. Two distinct mutations seen in families OV085 and OV027 occur at nucleotide 5573, which is the beginning of a repeat of seven adenines; this finding possibly suggests this region is a mutation hotspot. Three putative missense mutations were also identified: A75P in exon 3 (family OV120), R2502H in exon 15 (family OV222), and Y3098H in exon 25 (family OV045). These mutations were not detected in 344, 326, and 310 normal chromosomes, respectively. However, because these mutations have an unclear disease association and because there is no available functional assay with which to further investigate the significance of the mutations, they have not been included in the statistical analyses.

Subsequent to our analysis of BRCA1 and BRCA2 for coding mutations, large genomic alterations involving the BRCA1 gene have been reported. These alterations would not have been detected by the PCR-based approaches to mutation screening used in our study. One of these genomic alterations, a duplication and insertion involving 6 kb around exon 13 of BRCA1, is predicted to lead to protein truncation and may be of British origin (Puget et al. 1999). A PCR-based assay has been specifically designed to detect this mutation. We were able to screen for the exon 13 duplication in 55 families that did not have a coding mutation in BRCA1 or BRCA2; the mutation was not detected in any of the families (data not shown).

### *Mutation Prevalence, by Family Type*

Table 2 gives the proportion of families with BRCA1 and BRCA2 mutations, according to the number of cases of breast and ovarian cancer in the family. The numbers of ovarian cancers and breast cancers in the family were strongly predictive of BRCA1-mutation status ( $\chi^2 = 18.2, P = .0001$  and  $\chi^2 = 15.29, P < .0001$ , respectively). BRCA2-mutation status was significantly associated with the number of cases of breast cancer in the family ( $\chi^2 = 3.80, P = .05$ ), but there was no significant asso-

Table 1

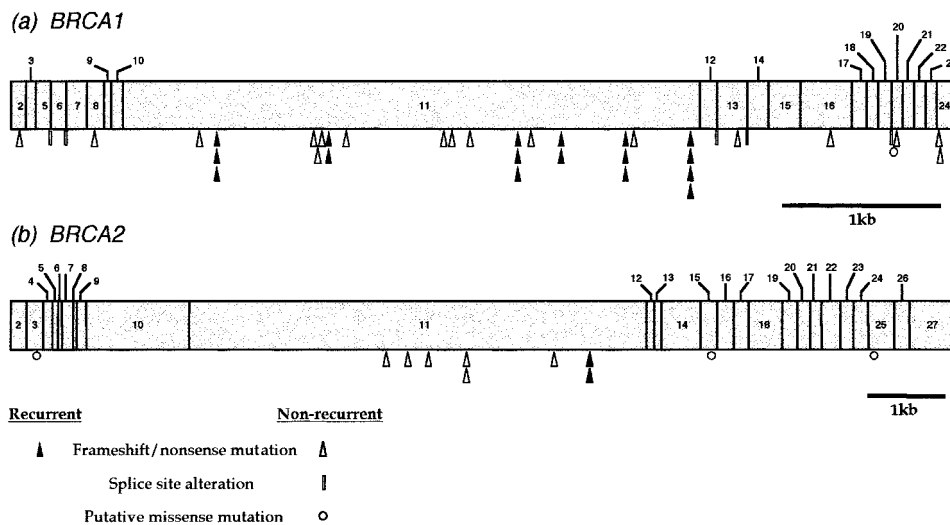
## Germline Mutations in BRCA1 and BRCA2

FAMILY	NO. OF CONFIRMED (UNCONFIRMED) CASES OF CANCER		DESCRIPTION OF MUTATION					
	Ovarian	Breast	Gene	Exon or Intron	Codon	Nucleotide	Alteration	Type
OV010	3 (1)	1 (3)	BRCA1	Exon 2	23	185	185delAG	Frameshift
OV022	3	1	BRCA1	Intron 5	—	331	331+2T→C	Splice site
OV131	2	1 (2)	BRCA1	Intron 6	—	421	421-2delA	Splice site
OV047	3 (2)	2 (2)	BRCA1	Exon 8	169	624T→G	Gln169ter	Nonsense
OV008	3 (1)	2 (2)	BRCA1	Exon 11	367	1220	1220insC	Frameshift
OV002	6	3 (1)	BRCA1	Exon 11	392	1294	1294del40	Frameshift
OV110	2	1	BRCA1	Exon 11	392	1294	1294del40	Frameshift
OV150	4	2	BRCA1	Exon 11	392	1294	1294del40	Frameshift
OV132	3	0 (1)	BRCA1	Exon 11	607	1938A→T	lys607ter	Nonsense
OV014	3 (3)	0	BRCA1	Exon 11	608	1942	1942del4	Frameshift
OV069	2	0	BRCA1	Exon 11	627	2000	2000del4	Frameshift
OV001	3	1 (1)	BRCA1	Exon 11	652	2073	2073delA	Frameshift
OV020	3	1 (1)	BRCA1	Exon 11	652	2073	2073delA	Frameshift
OV175	2	2 (1)	BRCA1	Exon 11	655	2082	2082insG	Frameshift
OV252	3	2 (2)	BRCA1	Exon 11	690	2187	2190delA	Frameshift
OV181	2	2 (1)	BRCA1	Exon 11	871	2731	2731insT	Frameshift
OV133	2 (1)	1 (1)	BRCA1	Exon 11	885	2774	2774delCT	Frameshift
OV171	2	0	BRCA1	Exon 11	916	2867	2867insA	Frameshift
OV013	2	0	BRCA1	Exon 11	1001	3121	3121delA	Frameshift
OV029	6	1 (1)	BRCA1	Exon 11	1001	3121	3121delA	Frameshift
OV160	2	0	BRCA1	Exon 11	1001	3121	3121delA	Frameshift
OV189	3	0	BRCA1	Exon 11	1056	3286	3286delC	Frameshift
OV012	7 (1)	1	BRCA1	Exon 11	1112	3452	3452del4	Frameshift
OV033	2	0	BRCA1	Exon 11	1112	3452	3452del4	Frameshift
OV007	4 (3)	0 (1)	BRCA1	Exon 11	1252	3875	3875del4	Frameshift
OV206	3 (4)	0	BRCA1	Exon 11	1252	3875	3875del4	Frameshift
OV282	3	1	BRCA1	Exon 11	1252	3875	3875del4	Frameshift
OV025	3 (3)	2	BRCA1	Exon 11	1289	3986	3986delAA	Frameshift
OV019	3	1	BRCA1	Exon 11	1355	4184	4184del4	Frameshift
OV042	2 (2)	1 (5)	BRCA1	Exon 11	1355	4184	4184del4	Frameshift
OV044	3 (7)	0 (7)	BRCA1	Exon 11	1355	4184	4184del4	Frameshift
OV245	3	0 (1)	BRCA1	Exon 11	1355	4184	4184del4	Frameshift
OV161	6	1	BRCA1	Exon 12	1395	4304	4304G→A	Splice site
OV226	2 (1)	0 (1)	BRCA1	Exon 13	1443	4446C→T	Arg1443ter	Nonsense
OV003	2 (1)	2	BRCA1	Intron 13	—	4476	4476+6T→C	Splice site
OV148	2 (1)	2	BRCA1	Exon 16	1648	5061	5061delA	Frameshift
OV073	3	0 (1)	BRCA1	Exon 20	1749	5365C→G	Pro1749Arg	Missense
OV139	2	0 (1)	BRCA1	Exon 20	1755	5382	5382insC	Frameshift
OV074	2	0 (1)	BRCA1	Exon 24	1835	5622C→T	Arg1835ter	Nonsense
OV224	2 (1)	1 (7)	BRCA1	Exon 24	1837	5629	5629delG	Frameshift
OV092	2 (1)	2 (1)	BRCA2	Exon 11	1366	4326	4326insCATC	Frameshift
OV072	3	0	BRCA2	Exon 11	1491	4701	4701delGAAA	Frameshift
OV140	2	1	BRCA2	Exon 11	1602	5032	5032insA	Frameshift
OV027	2	1 (1)	BRCA2	Exon 11	1782	5573	5573delAA	Frameshift
OV085	2	0	BRCA2	Exon 11	1782	5573	5573delA	Frameshift
OV254	2 (1)	0 (2)	BRCA2	Exon 11	1970	6137C→A	Ser1970ter	Nonsense
OV016	3	4	BRCA2	Exon 11	2092	6503	6503delTT	Frameshift
OV162	3	1	BRCA2	Exon 11	2092	6503	6503delTT	Frameshift

ciation with the number of cases of ovarian cancer. BRCA1 mutations were more frequent than BRCA2 mutations in families with large numbers of ovarian cancers; in particular, 14 (35%) of 40 families with BRCA1 mutations had four or more ovarian cancers, whereas none

of the families with BRCA2 mutations fell into this category. However, this difference did not reach formal statistical significance ( $\chi^2 = 2.89$ ,  $P = .08$ ).

As shown in table 2, mutations were found in 66% of families with either three or more reported cases of



**Figure 1** Location and type of germline mutations detected in (a) the BRCA1 gene and (b) the BRCA2 gene, in families containing two or more confirmed cases of epithelial ovarian cancer.

ovarian cancer or four or more reported cases of breast or ovarian cancer; however, they were found in only 20% of families with two cases of ovarian cancer alone. If families are classified only on the basis of confirmed cases, then these frequencies are altered to 71% and 22%, respectively. In the families with a BRCA1 mutation, the average patient age at diagnosis of ovarian cancer (49.9 years) was significantly lower than that in the families without the BRCA1 mutation (53.9 years) ( $P = .003$ ). In the families with the BRCA2 mutation, the average age at diagnosis of ovarian cancer (55.0 years) did not differ significantly from that in either the families with the BRCA1 mutation or the families with no mutation.

*Model Fitting*

To evaluate the evidence for further ovarian cancer-susceptibility genes, in addition to BRCA1 and BRCA2, we performed a segregation analysis. We tested several models that allowed for a third gene (OVCA), which was unlinked to BRCA1 or BRCA2 and which had two alleles; in these models, carriers of the susceptibility allele had an increased risk for ovarian cancer, compared with noncarriers, but they had no increased risk for breast cancer. Models were constrained for plausible BRCA-gene frequencies and by the requirement that the predicted overall incidence of breast and ovarian cancer must agree with known incidence. The frequencies of BRCA1 and BRCA2 were estimated in all models. We did not assume any values for these parameters. The only assumption was that their combined frequency should not be  $>.003$ . To assess the goodness of fit of different genetic models to the data, we computed, for

each model, the predicted probability of observing a BRCA1 or BRCA2 mutation in families, in different categories of family history. We then compared these predictions with what we actually observed.

Table 3 summarizes the estimated parameters for some of these models. In all but one of the models (model 4), the relative risk conferred by the OVCA susceptibility allele converged to 1 (i.e., there was no effect). This was true whether OVCA was assumed to be dominant, recessive, or codominant. In the case of model 4, the likelihood differed only slightly from that of the corresponding model showing no effect of OVCA (model 4a). We also fitted models in which the penetrance estimates for BRCA1 and BRCA2 were reduced so that the incidence rates for both breast and ovarian cancer were 50% of the Breast Cancer Linkage Consortium (BCLC) estimates. However, these models also converged to a relative risk of 1 for OVCA (data not shown). Models in which the risk for breast cancer conferred by BRCA1 was given by the BCLC values (models 1 and 2) had a poor fit, with the observed number of BRCA1 and BRCA2 mutations being greater than the predicted number of such mutations. The models with the reduced incidence rates, in which BRCA1 and BRCA2 were at 50% of the BCLC rates, showed a better fit than did the models in which the actual BCLC rates were used; however, they did not show a better fit than did the average heterogeneity rates. Better fits were obtained in the models in which the average of the two penetrance estimates given by Easton et al. (1995) were assumed. In each case, models assuming 90% sensitivity for mutation detection showed better fit than did models assuming 64% sensitivity. Table 2 shows that, when model

**Table 2****Observed and Predicted Number of Mutations, by Family Type**

FAMILY HISTORY OF CANCER AND MUTATION STATUS <sup>a</sup>	NO. (%) OF FAMILIES WITH CANCER			
	Confirmed Cases <sup>b</sup>		All Cases <sup>c</sup>	
	Observed	Predicted <sup>d</sup>	Observed	Predicted <sup>d</sup>
At least two cases of ovarian cancer and at least two cases of breast cancer:				
BRCA1	10 (56)	11.40	18 (56)	20.13
BRCA2	1 (5)	1.18	3 (13)	1.73
None	7 (39)	5.42	11 (31)	10.14
At least three cases of ovarian cancer and no more than one case of breast cancer:				
BRCA1	17 (63)	16.37	12 (52)	11.10
BRCA2	2 (7)	.68	2 (13)	.90
None	8 (30)	9.75	9 (35)	11.00
Two cases of ovarian cancer and one case of breast cancer:				
BRCA1	5 (29)	6.89	5 (26)	6.86
BRCA2	3 (18)	.77	2 (11)	.66
None	9 (53)	9.34	12 (63)	11.49
Two cases of ovarian cancer and no cases of breast cancer:				
BRCA1	8 (16)	8.33	5 (13)	4.91
BRCA2	2 (4)	1.42	1 (3)	.96
None	40 (80)	40.25	32 (84)	32.13
Totals:				
BRCA1	40 (36)	43.00	40 (36)	43.00
BRCA2	8 (7)	4.25	8 (7)	4.25
None	64 (57)	64.76	58 (57)	64.76

<sup>a</sup> Ovarian cancer diagnosed at any age, breast cancer diagnosed at age  $\leq 60$  years.

<sup>b</sup>  $\chi^2 = 4.61$  ( $P = .47$ ) for comparison of observed and predicted numbers.

<sup>c</sup>  $\chi^2 = 4.32$  ( $P = .50$ ) for comparison of observed and predicted numbers.

<sup>d</sup> For the best-fitting model (model 4a) in table 3.

4a was used, the predicted mutation frequencies were close to those observed.

## Discussion

We identified germline mutations of BRCA1 or BRCA2 in slightly less than half (43%) of 112 families with at least two confirmed cases of epithelial ovarian cancer in close relatives. The ratio of BRCA1 mutations to BRCA2 mutations was 5:1. Our data confirm previous data suggesting that BRCA1 mutations are more commonly associated with inherited susceptibility to ovarian cancer than are BRCA2 mutations. However, they also demonstrate that BRCA2 mutations are responsible for a significant proportion of families in which only cases of ovarian cancer have been observed.

No BRCA1 or BRCA2 mutation was found in ~30% of families with an extensive family history of cancer (families containing either three or more cases of ovarian cancer or two or more cases of both breast and ovarian cancer). Some mutations undoubtedly would have been missed by the methods of mutation detection used in the present study; PTT would have missed missense mutations, and, although SSCA/HA would have been likely to detect all small insertions and deletions, it may have missed some single-base-pair substitutions. Because only the coding sequence of both genes was analyzed, mu-

tations occurring in the regulatory regions, which affect transcription, would not have been detected. Evidence of regulatory mutation was sought by looking for a reduction to homozygosity, in cDNA, for polymorphisms heterozygous in genomic DNA, in the 23 families for which RNA samples were available; however, none was found. Because the methods used in this study were PCR based, large genomic deletions and rearrangements in BRCA1, similar to those that recently have been reported elsewhere (Petrij Bosch et al. 1997; Puget et al. 1997; Swensen et al. 1997), may have been missed. In the present study, for those families in which no BRCA1 or BRCA2 mutation was reported, there was insufficient available material with which to perform Southern-blot analysis to look for similar deletions and rearrangements. However, using a mutation-specific PCR assay, we were able to screen for a recently described duplication in BRCA1 (Puget et al. 1999); this mutation was not detected in any of the families without BRCA1 and BRCA2 mutations. Finally, in the majority of families, a blood sample was available from only one affected individual; therefore, it is possible that, in some families, mutation analysis will have been performed on an individual who was a phenocopy.

That a substantial proportion of families had no observed mutation raises the possibility that other genes (or nongenetic familial factors) may be important de-

**Table 3****Parameters of Genetic Models Fitted to the Ovarian-Cancer Data Set**

MODEL	FREQUENCY OF			RELATIVE RISK OF OVCA	MUTATION- DETECTION SENSITIVITY	ASSUMED RATE	PREDICTED FAMILIAL RISK <sup>a</sup>	LOG LIKELIHOOD
	BRCA1	BRCA2	OVCA					
1	.0011	.0019	0	1	.64	Homogeneity <sup>b</sup>	3.15	-114.1
2	.0010	.0020	0	1	.90	Homogeneity <sup>b</sup>	2.96	-111.2
3	.0017	.0013	0	1	.64	Average heterogeneity <sup>c</sup>	2.00	-95.4
4	.0014	.0016	.41	16,940	.90	Average heterogeneity <sup>c</sup>	2.05	-93.9
4a	.0014	.0016	0	1	.90	Average heterogeneity <sup>c</sup>	1.89	-94.2

<sup>a</sup> Overall familial risk to first-degree relatives that is predicted by the model.

<sup>b</sup> BRCA1 rates are from the model of Easton et al. (1995), under the assumption of homogeneity; BRCA2 risks are from Ford et al. (1998).

<sup>c</sup> BRCA1 rates are the average of incidence rates from the heterogeneity model of Easton et al. (1995); BRCA2 risks are from Ford et al. (1998).

terminants of familial risk. In the families with either three or more ovarian cancers or four or more early-onset breast or ovarian cancers, those cancers are unlikely to be caused by chance. The failure to detect mutations in ~30% of these families is, in fact, consistent with the findings of the BCLC studies (Ford et al. 1998). In those studies, combined linkage and mutation analysis indicated that >95% of all families with multiple cases of breast and ovarian cancer are accounted for by BRCA1 and BRCA2 mutations, if a mutation-detection sensitivity of 64% is assumed. We can therefore conclude, with some confidence, that, if there are additional genes, they are of minor importance, compared with BRCA1 and BRCA2, in families with breast and ovarian cancer. However, because there are wide confidence limits on the aforementioned estimates, the possibility of there being one or more additional highly penetrant genes cannot be completely excluded. To search for evidence for such genes, specifically in relation to ovarian cancer, we have so far analyzed, both by linkage and by loss of heterozygosity at the BRCA1 and BRCA2 loci in tumors, 11 families in which there were at least three cases of ovarian cancer and no more than one case of breast cancer diagnosed at age <60 years and in which no BRCA1 or BRCA2 mutation was detected. The best estimate based on these data (S. Ramus, unpublished data) is that the mutation is in either BRCA1 or BRCA2 in ~50% of such apparently mutation-negative families. Although no single family showed strong evidence against linkage to BRCA1 and BRCA2, it remains possible that some of the disease in these families is the result of a mutation in other genes. One possibility would be the mismatch-repair genes, such as MLH1 and MSH2 (Leach et al. 1993; Papadopoulos et al. 1994). Our preliminary results (P. Harrington, unpublished data) indicate that the replication error-positive (RER+) phenotype expected in such cases is less frequent in tumors from familial cases without mutation than it is in sporadic ovarian cancers. This finding argues against

there being a major contribution of mutations in mismatch-repair genes in these families with ovarian cancer, a conclusion that must be confirmed by direct mutation analysis of germline DNA.

A high proportion of families with only two cases of ovarian cancer and either one or no cases of breast cancer had no detectable BRCA1 or BRCA2 mutation. Even if one-third of the BRCA1 and BRCA2 mutations that were present had been missed, this would have accounted for only approximately one-third of such families. The relative contributions of chance, nongenetic familial factors, and genes to the remaining approximately two-thirds of families are unclear. If, for the mother or daughter of an affected individual, the relative risk of ovarian cancer is 2.4 (Stratton et al. 1998), then the expected proportion of affected relative pairs caused by chance would be 40%. However, this estimate of relative risk encompasses all affected pairs, including those that are part of a more extensive family history of cases; therefore, the proportion of chance occurrences will be higher for families with only two affected members.

To further explore the evidence for there being other ovarian cancer-susceptibility genes in these smaller families as well as in the multiple-case families, we performed a segregation analysis of the entire family set. We tested several models that allowed for the possibility of a third ovarian cancer-specific gene in addition to BRCA1 and BRCA2. These analyses gave no support for the existence of a further ovarian cancer-specific locus. We also looked for suggestions, both from the pathology and from familial patterns of associated cancer, that the families without mutations might include a distinct group. A review of the pathology has shown no difference in the histological subtype of ovarian cancers in familial cases with and without a BRCA1 or BRCA2 mutation, nor has it shown such a difference between familial nonmutation cases and a consecutive hospital-based series (Pharoah et al. 1999). The relatives in our

39 families, in which there are only two cases of ovarian cancer and no detectable BRCA1 or BRCA2 mutation, do not appear to show, at sites other than the ovary and breast, patterns of cancer that are distinct from those seen in the families with known mutations, although the nonuniform ascertainment and small numbers preclude a formal comparison.

What are the implications of our data for genetic testing? They indicate that testing for predisposing mutations is likely to be justified in any family in which two or more close relatives have epithelial ovarian cancer and that testing should be directed first at the BRCA1 gene and, if this is negative, at the BRCA2 gene, possibly starting with (but not being restricted to) the “ovarian cancer–cluster region” (Gayther et al. 1997b). Different germline BRCA1 and BRCA2 mutations are probably associated with different risks for breast and ovarian cancer (Easton 1997). We have previously reported that mutations found in the 3' end of the BRCA1 gene and in a central region of the BRCA2 gene appear to be responsible for a lower risk of ovarian cancer, relative to the risk for breast cancer, compared with mutations elsewhere in the gene (Gayther et al. 1995, 1997b). Both this variation in risk and the fact that these risks may also vary according to family history and other risk factors suggest that some caution should be used when mutation data are used to provide precise risk estimates for clinical management.

In families in which there are only two cases of ovarian cancer and in which no mutation can be found, the risk to unaffected women remains unclear. If many of these families are, in fact, a chance association of cases, the risks may be quite low, and measures such as prophylactic oophorectomy may be inappropriate. Unlike the familial risks of breast cancer, familial risks of ovarian cancer do not seem to be strongly age dependent; therefore, the ages at which the affected individuals were diagnosed may not provide a reliable guide to familial risk. Prospective data from the follow-up of unaffected women at risk in such families will be needed to answer this question.

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## Electronic-Database Information

The URL for data in this article is as follows:

Breast Cancer Information Core Database, [http://www.nhgri.nih.gov/Intramural\\_research/Lab\\_transfer/Bic/](http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/)

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