Variation in *BRCA1* Cancer Risks by Mutation Position[1]

Deborah Thompson, Douglas Easton,[2] and the Breast Cancer Linkage Consortium[3]

Cancer Research Campaign Genetic Epidemiology Unit, Strangeways Research Laboratories, University of Cambridge, Cambridge CB1 8RN, United Kingdom

Abstract

Previous studies have reported variation in *BRCA1* breast and ovarian cancer risks with mutation position, suggesting that mutations toward the 3′ end of the gene are associated with lower ovarian cancer risks. We evaluated the evidence for genotype-phenotype correlations in 356 families with protein-truncating *BRCA1* mutations. In contrast to previous reports, the ovarian:breast cancer ratio associated with mutations in a central region of the gene (nucleotides 2401–4190) was significantly higher than for other mutations [odds ratio, 1.70 ($P = 0.017$) compared with nucleotides 1–2400; odds ratio, 1.89 ($P = 0.02$) compared with nucleotides 4191–end]. The risks of breast and ovarian cancer conferred by mutations in different regions of the gene were estimated separately by conditional maximum likelihood. According to the best fitting model, the breast cancer risk associated with mutations in the central region was found to be significantly lower than for other mutations (relative risk, 0.71; 95% confidence interval, 0.58–0.86; $P = 0.0002$), whereas the ovarian cancer risk associated with mutations 3′ to nucleotide 4191 was significantly reduced relative to the rest of the gene (relative risk, 0.81; 95% confidence interval, 0.66–1.00; $P = 0.044$). The cancer risks associated with missense mutations in the RING domain in exon 5 appear to be similar to those associated with protein-truncating mutations toward the 3′ end of *BRCA1*, based on nine additional families.

Introduction

BRCA1 (MIM 113705)[4] is a breast and ovarian cancer susceptibility gene located on chromosome 17q (1, 2). *BRCA1* is not completely penetrant, and since its isolation in 1994, there have been numerous attempts to estimate the risks of cancer conferred by different germ-line mutations. Published cumulative risk estimates by age 70 vary between 45 and 87% for breast cancer and between 36 and 66% for ovarian cancer (3–5). More than 400 distinct cancer-associated *BRCA1* mutations have been reported (according to the BIC[5] database[6]), prompting discussion as to whether different mutations confer different cancer risks. The first formal evidence of a genotype-phenotype correlation came from a study of 33 families, which identified a significantly lower ovarian:breast cancer ratio in families with mutations 3′ to exon 12 (6). A further study of 134 patients with truncating *BRCA1* mutations found the that frequency of ovarian cancer relative to breast cancer associated with mutations 3′ to the granin motif (nucleotides 3760–3787) was significantly lower than for other mutations ($P = 0.004$; Ref. 7). However, an analysis of six recurrent *BRCA1* mutations found only a marginally significant difference in the proportion of cases affected by ovarian cancer relative to breast cancer between specific mutations ($P = 0.07$; Ref. 8). In an attempt to confirm or refute these genotype-phenotype correlations in *BRCA1*, we studied a much larger group of 356 families with PT *BRCA1* mutations. We used these data to estimate separately the risks of breast and ovarian cancer associated with mutations in different regions of *BRCA1*. Using nine additional families, we also estimated the cancer risks conferred by MS mutations in the *BRCA1* RING domain.

Materials and Methods

Subjects. The study was based on information from 369 families collected by the Breast Cancer Linkage Consortium from 20 centers in eight Western European and North American countries. For entry into the study, each family was required to contain at least one affected individual known to carry a germline *BRCA1* mutation believed to be disease causing (*i.e.,* frameshift and nonsense mutations, large deletions and insertions, splice site mutations, and MS mutations categorized as disease causing by BIC). The main analysis was restricted to the 356 families with PT mutations. (Although categorization by BIC as a MS mutation, rather than as a polymorphism or unknown variant, does not guarantee that a mutation is associated with disease, it is an indication that there is compelling evidence of disease association). The nine families with MS mutations in the RING domain were considered separately (five

Received 6/15/01; revised 12/7/01; accepted 1/17/02.
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] The coordination and analysis of this study were supported by the Cancer Research Campaign and by a Concerted Action from the European Union. Data collection was supported by the Cancer Research Campaign, the Imperial Cancer Research Fund, The Swedish Cancer Society, Foundation de France, Biomed2 BMH4-CT96-1133, SAF 96/0192, the Nordic Cancer Union, The Cancer Society and Cancer Registry of Finland, the Canadian Breast Cancer Research Initiative, NIH Grant RO1 CA77415 (to S. L. Neuhausen), American Cancer Society Grant 35 RPG-99-181-01-CCE (to S. L. Neuhausen), and the Utah Cancer Registry (supported by NCI Grant NO1-CN-67000, with additional support from the Utah State Department of Health and the University of Utah).
[2] To whom requests for reprints should be addressed, at CRC Genetic Epidemiology Unit, Strangeways Research Laboratory, Worts Causeway, Cambridge, CB1 8RN, United Kingdom. Phone: (44) 1223 740160/740157; Fax: (44) 1223 740159; E-mail: douglas@srl.cam.ac.uk.
[3] Consortium members who contributed families are listed fully at the end of the report.

[4] Online Mendelian Inheritance in Man (OMIM). http://www.ncbi.nlm.nih.gov [for inherited breast cancer type 1 (MM 113705)].
[5] The abbreviations used are: BIC, Breast Cancer Information Core; PT, protein truncating; MS, missense; CRC, Cancer Research Campaign; OR, odds ratio; RR, relative risk; CI, confidence interval; OCCR, ovarian cancer cluster region.
[6] http://www.nchgr.nih.gov/Intramural_research/Lab-transfer/Bic.

Downloaded from http://aacrjournals.org/cebp/article-pdf/11/4/329/1740713/ce0402000329.pdf by guest on 24 August 2022

families with Cys61Gly, two families with Cys64Gly, one family with Cys47Tyr, and one with Cys64Tyr). The three families with MS mutations elsewhere in *BRCA1* (Met1Ile, Arg1751Gln, and Met1775Arg were each seen once) were excluded from all analyses because there were insufficient data to allow a separate analysis of this group. A single family in which both 185delAG and 5382insC were found was also excluded from all analyses.

For the purposes of this study, the family members included were restricted to the tested mutation carriers, women with breast cancer diagnosed below age 60, women with ovarian cancer at any age, men with breast cancer at any age, and the first-degree relatives of individuals in any of these four categories, regardless of their own carrier status. The families included in the analysis contained 7627 individuals (median individuals per family, 17). There were 1174 women with a first breast cancer diagnosed below age 60 and 679 women with ovarian cancer. Thirty-eight percent of these cancer diagnoses (including 34% of breast cancers and 46% of ovarian cancers) were confirmed by pathological review, pathologist's report, cancer registry record, clinical record, or death certificate. The median number of female breast cancers before age 60 and ovarian cancers per family was four; 66 families had less than three cases, and 126 had six or more. Seven families contained one case of male breast cancer. A total of 162 distinct mutations were observed in the families analyzed, 158 of which were PT. The number of distinct nucleotides at which PT mutations were observed was 147.

The 356 families with PT mutations included 31 that had been studied previously (6); the information on these families has since been updated. These families are referred to as the "original CRC set."

Seventy-nine families carried one of the *BRCA1* Ashkenazi Jewish founder mutations, 185delAG or 5382insC. The comparatively high frequency of these mutations, together with the *BRCA2* 6174delT mutation, in the Ashkenazi population (estimated combined population frequency of 2–2.5%; Ref. (9) has led to some Ashkenazi families being offered mutation screening on the basis of a lower-risk family history of cancer than would be required of a non-Ashkenazi family. This could lead to underestimation of the risks associated with these mutations compared with other mutations. To avoid this possible bias, Ashkenazi and non-Ashkenazi families within a given center were considered as separate strata. Eleven of the 20 ascertainment centers recruited one or more families with an Ashkenazi mutation; therefore, for the purposes of this analysis we used 31 distinct strata. Although some families with these mutations may not be of Ashkenazi origin, they were classified as such for the purposes of this analysis.

Statistical Methods. Variations in the incidence of breast and ovarian cancer associated with mutations in different parts of *BRCA1* were initially tested for by splitting the gene into two, using the exon 12–13 breakpoint suggested by Gayther *et al.* (6), and computing an OR for the ratio of ovarian to breast cancer cases in the 5′ *versus* the 3′ region of *BRCA1*. For the purpose of these analyses, only breast cancers diagnosed in women before age 60 years were considered. For women with bilateral breast cancer, only the first cancer was included. The OR was adjusted for ascertainment center/strata, using logistic regression in Splus (version 3.4). The significance was estimated by simulation, permuting mutations among families within center, as described by Gayther *et al.* (6) and Thompson *et al.* (10). This procedure accounts for the nonindependence of risks within each family.

The ORs were similarly computed for every possible breakpoint along the gene to identify the optimal two-way division for maximizing the deviance. In this case, because the optimal breakpoint was not chosen *a priori*, the deviance was also maximized over all breakpoints in every simulation to obtain the significance level. Taking the optimum breakpoint as fixed, we repeated the procedure to test for the presence and location of a second breakpoint bisecting the larger region, *i.e.,* defining three separate risk regions. Each analysis was performed with and without the set of 31 original CRC families.

We estimated the risks of breast cancer and ovarian cancer conferred by mutations in each of these three risk regions, using a conditional maximum likelihood approach. The frequency of each specific mutation in each population is unknown; therefore, the likelihood must be conditioned on the set of mutations observed in the families from each center. The derivation of the conditional likelihood is discussed in more detail in Ref. 10, and the procedure used here is essentially the same, except that mutations were pooled into three, rather than two, groups.

The conditional likelihood took the form:

$$\mathrm{L} = \prod_{\text{center}\,i} \frac{\prod_{j=1}^{N_i} \mathrm{L}(\underline{\mathrm{D}}_j, \underline{\mathrm{C}}_j | \mathrm{M}_j, \underline{\lambda}, \underline{\beta})}{\sum_{\pi} \prod_{j=1}^{N_i} \mathrm{L}(\underline{\mathrm{A}}_j | \mathrm{M}_{\pi(j)}, \underline{\lambda}, \underline{\beta})} \quad \text{(A)}$$

where M_j is the mutation in the *j*th family, and $\mathrm{M}_{\pi j}$ is the mutation assigned to the *j*th family by the πth permutation. $\underline{\mathrm{C}}_j$ is the vector of the carrier statuses, $\underline{\mathrm{D}}_j$ is the vector of phenotypic information, and $\underline{\mathrm{A}}_j$ contains all of the information involved in the ascertainment of family *j*.

The phenotypic information $\underline{\mathrm{D}}_j$ on each individual was based on follow-up from birth until their death, 70th birthday, loss to follow-up, diagnosis with ovarian cancer, or oophorectomy. Thus, information on ovarian cancer subsequent to breast cancer was included in the analysis, but information on breast cancer subsequent to ovarian cancer or oophorectomy was not because oophorectomy could substantially alter breast cancer risk. Because the actual ascertainment event for any given family was poorly defined, we used a conservative approach in which we conditioned on all information that could have influenced ascertainment. Thus, $\underline{\mathrm{A}}_j$ included all phenotypic information up until and including the earliest of death, 70th birthday, loss to follow-up, breast cancer, ovarian cancer, prophylactic bilateral mastectomy, or oophorectomy. $\underline{\mathrm{A}}_j$ also included the carrier status of the first tested carrier (the proband), but not that of other relatives. Restricting the analysis to confirmed carriers would bias the results because affected family members and those with a very strong family history of cancer might be more inclined to pursue mutation testing and because deceased individuals would be excluded. The MENDEL program weights correctly the likelihood contributions of untested individuals according to their probability of being a carrier, which is estimated from their cancer history and the cancer histories and carrier statuses of their relatives.

The parameter vector $\underline{\beta}$ consisted of the estimated age-specific risks of breast cancer and ovarian cancer to women with mutations in the 5′ region of *BRCA1*, taken as the baseline risk category for carriers. Cancer risks relative to this baseline for women with mutations in the central or 3′ regions were parameterized by the log-RR parameters in the vector $\underline{\lambda}$. The four RRs (central breast, 3′ breast, central ovarian, and 3′ ovarian) relative to carriers of 5′ mutations were assumed to be

Downloaded from http://aacrjournals.org/cebp/article-pdf/11/4/329/1740713/ce0402000329.pdf by guest on 24 August 2022

independent of age (although, of course, incidence rates in *BRCA1* carriers relative to general population rates are strongly age dependent). Noncarrier risks, by 5-year age group, were fixed at those given in *Cancer Incidence in Five Continents* (11). The permutations, π, were performed within each center to account for possible differences in mutation frequencies and ascertainment policies between centers. The mutations were recoded as 0, 1, or 2, depending on the region of the gene in which they were situated. Ideally, every possible permutation of family to mutation group within a center would be used, but within six of the larger centers this number was prohibitively large. For each of these centers, 10,000 permutations were randomly sampled, with replacement, from the complete set. Although the number of permutations sampled affects the absolute value of the likelihood, it did not appear to affect the maximum likelihood estimates or the differences in log-likelihood between nested models. The same random sample of permutations was retained for each estimation; hence differences in likelihood between models are genuinely attributable to the differences in the models and not to any differences in the sample of permutations used.

The conditional likelihood was maximized over the risk and log-RR parameters, using the program MENDEL (12). To test the significance of each log-RR parameter, models were run under which every combination of one or more of the parameters were fixed at zero and likelihood ratio tests on the nested models were used to find the optimum model. Parameter estimates were used to obtain cumulative cancer risks for the different groups of mutations, and a log-log transformation was used for the 95% CIs.

MS Mutations. The cancer risks associated with MS mutations in the functional RING zinc-finger domain of *BRCA1* (exon 5) were explored in a separate analysis that included the nine families with mutations meeting this description. The ovarian:breast cancer ratio for these MS mutations was compared in turn with the ratios for mutations in each of the three regions defined in the main analysis and with the combined set of all PT mutations. ORs were adjusted for ascertainment center, as in the main analysis.

Maximum likelihood estimates of the risks of breast cancer and ovarian cancer associated with RING MS mutations were computed in MENDEL (12), using an adaptation of the conditional likelihood technique used in the main analysis (Eq. A). The complete set of 365 families (356 with PT mutations and 9 with MS mutations) was used; mutations were split into four groups (the three risk regions defined for the PT mutations, and a fourth group consisting of the MS mutations), and the baseline was again taken to be the group of 5′ PT mutations, with all other risks estimated relative to this. The likelihood was also maximized for models under which one or more of the MS log-RR parameters was fixed at zero, to test their significance.

Results

Risk Ratio Analysis. Logistic regression was used to test the independence of mutation position and breast/ovarian cancer risk and to define the optimal breakpoint(s). Using the exon 12–13 breakpoint (6), we found an OR for the ovarian:breast cancer ratio in 5′ mutations, compared with 3′ mutations, of 1.40 ($P = 0.037$), confirming the earlier observation. When we allowed the position of the breakpoint to vary, its optimal position was found to be between nucleotides 4185 and 4191, toward the 3′ end of exon 11. As anticipated, mutations 5′ of this breakpoint were associated with a higher ratio of ovarian to breast cancer cases than 3′ mutations (OR, 1.51; $P = 0.102$). Although this breakpoint gave the strongest association, the significance level was lower than for the exon 12–13 breakpoint because that position was taken as fixed, whereas here the significance was from maximizing over all possible breakpoints at every permutation. This breakpoint is slightly 5′ to that previously identified on exon 13, which was between nucleotides 4304 and 4446 (6). However, it lies well within the previously reported 95% CI (nucleotides 2025–5298).

Inspection of the ovarian:breast cancer ratios by family suggested further heterogeneity of risk among families with mutations in the 5′ region. We estimated that the optimal position of the breakpoint subdividing the 5′ region is between nucleotides 2388 and 2401. For convenience, the two breakpoints will be referred to as being at nucleotides 2401 and 4191, although the precision with which they can be identified is limited by the set of mutations observed in the families. The region between nucleotides 2401 and 4190 had a significantly higher ratio of ovarian to breast cancer than the region 5′ to 2401 (OR, 1.70; $P = 0.017$) or than the region 3′ to 4191 (OR, 1.89; $P = 0.02$). Fig. 1 shows that all three regions have distinct patterns of cancer incidence, with mutations in the central region being associated with the highest ratio of ovarian cancer to breast cancer cases (47.6% of cancers were ovarian).

Maximum Likelihood Estimation. We used the conditional maximum likelihood to estimate disease-specific RRs by mutation position. The optimal breakpoints (nucleotides 2401 and 4191) were used to split the mutations into three groups, and the risks of breast cancer and ovarian cancer associated with mutations in the central region and in the 3′ region were estimated relative to the risks associated with 5′ mutations. The likelihood was also maximized for all models under which one or more of these parameters were fixed at the null, and likelihood ratio tests were used to find the best fitting model. The log-likelihoods for a selection of these models are presented in Table 1. The RRs for breast cancer in the central and 3′ regions are referred to as $\Gamma_{b(c)}$ and $\Gamma_{b(3)}$, respectively; the corresponding ovarian RRs are referred to as $\Gamma_{o(c)}$ and $\Gamma_{o(3).}$

Starting with the null model (uniform cancer risks across *BRCA1*), the parameter whose addition gave the most significant increase in likelihood was $\Gamma_{b(c)}$ (model 2). The only parameter that gave a significant improvement in the fit after including $\Gamma_{b(c)}$ was $\Gamma_{o(3)}$ (model 4). Neither $\Gamma_{b(3)}$ nor $\Gamma_{o(c)}$ significantly improved the fit (models 5 and 6, respectively). Hence, model 4 is the most parsimonious model to describe the data. The maximum likelihood estimates of the parameters under model 4 are presented in Table 2, along with their asymptotic 95% CIs. According to this model, the risk of breast cancer is the same in the 5′ and 3′ regions of *BRCA1*, but is significantly lower in the central region (RR, 0.71; 95% CI, 0.58–0.86; $P = 0.0002$). The pattern is different for ovarian cancer, for which the risks in the 5′ and central regions are the same, but with a significantly lower risk in the 3′ region (RR, 0.81; 95% CI, 0.66–1.00; $P = 0.044$). Parameter estimates are also shown for the full model containing all four RR parameters (Table 1).

When the original 31 CRC families were excluded, model 2 provided the best fit. Under this model, the risk of ovarian cancer is the same in all three regions, but the risk of breast cancer is significantly lower for mutations in the central section (RR, 0.73; 95% CI, 0.60–0.89; $P = 0.0007$).

Ashkenazi Founder Mutations. Seventy-nine of the 356 families with PT mutations carried one of the two common Ashkenazi Jewish founder mutations, 185delAG (46 families) and

Downloaded from http://aacrjournals.org/cebp/article-pdf/11/4/329/1740713/ce0402000329.pdf by guest on 24 August 2022

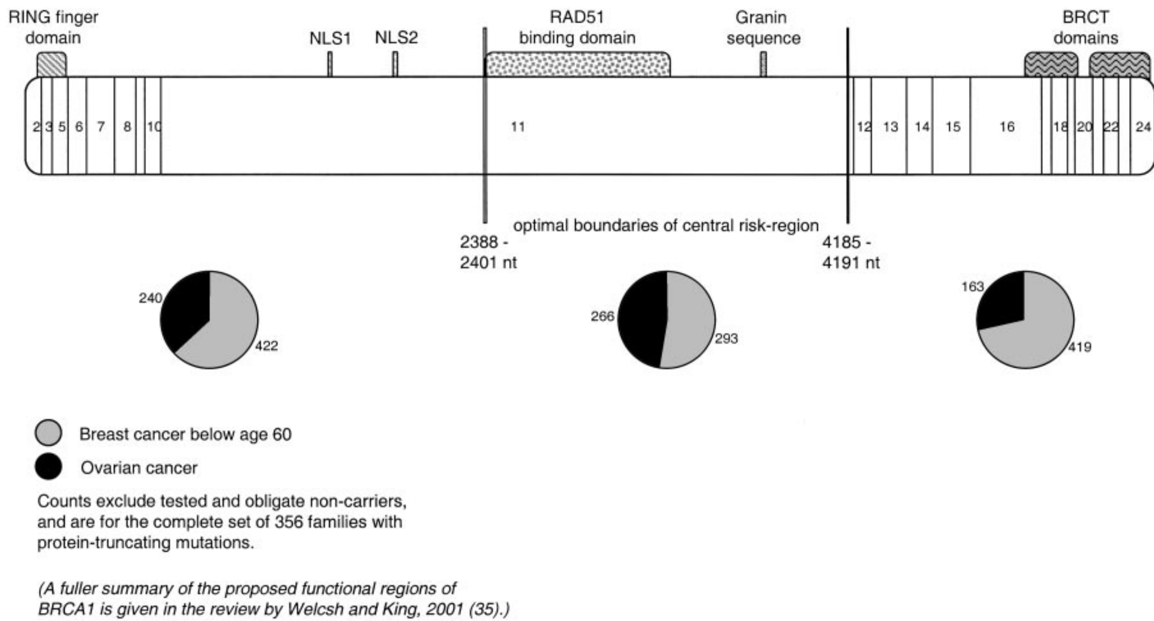

Fig. 1. Counts of breast and ovarian cancer by mutation location within *BRCA1*, for all 356 PT mutations.

Table 1 Log-likelihood ratio tests for nested models describing variation of *BRCA1* cancer risks with mutation position

Likelihood maximized using all 356 families carrying PT *BRCA1* mutations. RR (Γ) in *italics* are those fixed at one under the given model; other entries are the maximum likelihood estimates under the given model. Model 4 (**bold text**) provides the most parsimonious fit to the data.

Model	Breast RR (Γ_b)		Ovarian RR (Γ_v)		Log-likelihood	Likelihood ratio statistic	P
	Centre [$\Gamma_{b(c)}$]	3 [$\Gamma_{b(3)}$]	Center [$\Gamma_{o(c)}$]	3 [$\Gamma_{o(3)}$]			
1 (null)	*1*	*1*	*1*	*1*	−1324.4271		
2	0.69	*1*	*1*	*1*	−1315.5288	17.797	0.00002 *vs.* model 1
3	*1*	*1*	*1*	0.76	−1320.6170	7.620	0.006 *vs.* model 1
4	**0.71**	***1***	***1***	**0.81**	**−1313.5064**	4.045	0.044 *vs.* model 2
						14.221	0.0002 *vs.* model 3
5	0.77	1.11	*1*	0.84	−1312.8588	1.295	0.26 *vs.* model 4
6	0.72	*1*	1.06	0.83	−1313.3870	0.239	0.63 *vs.* model 4
Full	0.76	1.12	1.10	0.88	−1312.5896		

5382insC (33 families). Defining separate centers for Ashkenazi families and stratifying all analyses by center should have minimized the impact of any differences in cancer risks between Ashkenazis and others, but to ensure that the results were not unduly influenced by these families, maximum likelihood estimates were produced for a subset of the data restricted to non-Ashkenazi families. For the purposes of the analysis, all families carrying either of these mutations were conservatively assumed to be Ashkenazi Jewish, although we do not have the ethnicity or haplotype data to confirm this. The optimal breakpoints for this subset were identical to those found for the complete set of families, and the estimated ORs were very similar [OR for nucleotides 2401–4190 *versus* nucleotides 0–2400, 1.70 ($P = 0.03$); OR for nucleotides 2401–4190 *versus* 4191-end, 1.89 ($P = 0.04$)].

The most parsimonious model in the maximum likelihood analysis was model 2, *i.e.,* there was a significantly lower breast cancer risk associated with central mutations, but no significant variation in ovarian cancer risk (breast RR, 0.68; 95% CI, 0.56–0.83; $P < 0.0001$).

A maximum likelihood analysis was also performed using only the 79 Ashkenazi families. Risks for 5382insC carriers were estimated relative to those for 185delAG carriers. Neither the breast nor the ovarian cancer risk differed significantly between the two mutations (breast RR, 1.13; 95% CI, 0.78–1.64; $P = 0.52$; ovarian RR, 0.65; 95% CI, 0.36–1.18; $P = 0.13$). The RR for breast cancer was very close to the corresponding non-Ashkenazi 3′:5′ RR (non-Ashkenazi breast RR, 1.09; 95% CI, 0.88–1.36; $P = 0.40$), whereas the ovarian cancer RR was somewhat stronger than the corresponding non-Ashkenazi 3′:5′ RR (non-Ashkenazi ovarian RR, 0.95; 95% CI, 0.71–1.27; $P = 0.70$).

MS Mutations. The estimated OR for comparison of the ovarian:breast cancer ratio associated with MS mutations in the RING domain compared with all PT mutations, adjusted for center, was 0.41 ($P = 0.023$). When we looked separately at PT mutations in each of the three risk regions, the most significant OR was for the comparison of the MS mutations with PT mutations in the central region (OR, 0.31; $P = 0.003$). The pattern of risk associated with MS mutations was not significantly different from that associated with PT mutations in either

Downloaded from http://aacrjournals.org/cebp/article-pdf/11/4/329/1740713/ce0402000329.pdf by guest on 24 August 2022

Table 2 Maximum likelihood estimates[a] of age-specific incidence of breast cancer and of ovarian cancer and of RRs, estimated under model 4

Cancer site	Baseline mutation group[b]	Age group (yr)	MLE[c]	95% CI	P[d]
Breast	5′ and 3′ mutations	40–49	0.018	0.011–0.025	
		50–59	0.027	0.010–0.043	
		60–69	0.018	0.002–0.034	
RR for center mutations, relative to 5′ and 3′			0.71	0.58–0.88	0.0002
Ovarian	5′ and center mutations	40–49	0.019	0.014–0.024	
		50–59	0.022	0.015–0.030	
		60–69	0.024	0.014–0.035	
RR for 3′ mutations, relative to 5′ and center			0.81	0.66–0.99	0.044

[a] Likelihood maximized using all 356 families carrying PT *BRCA1* mutations, under model 4 (see Table 1).
[b] Age-specific incidence rates are for the baseline group of mutations specified. Risks for other mutations are estimated relative to this baseline, assuming an age-independent ratio. Breast cancer risks associated with 5′ (below nt 2401) and 3′ (at or above nt 4191) mutations are fixed to be identical. For ovarian cancer, the risks associated with 5′ (below nt 2401) and center (at or above nt 2401 and below nt 4191) mutations are fixed to be identical.
[c] MLE, maximum likelihood estimate.
[d] Significance levels are from likelihood ratio tests comparing model 4 with the model in which the parameter of interest is fixed, as in Table 1.

the 5′ or 3′ regions (5′ OR, 0.52; $P = 0.091$; 3′ OR, 0.42; $P = 0.064$).

The estimated risk of breast cancer associated with MS mutations relative to 5′ PT mutations was 1.19 (95% CI, 0.78–1.82; $P = 0.43$), and the corresponding ovarian cancer RR was 0.73 (95% CI, 0.35–1.49; $P = 0.36$), neither of which was significant.

Discussion

A tendency for families with mutations toward the 3′ end of *BRCA1* to have a lower than average proportion of ovarian cancer cases has been reported on several occasions (*e.g.,* Refs. 6, 13, 14). The study by Gayther *et al.* (6) found that a linear trend model for the ovarian:breast cancer ratio was less satisfactory than a model dividing the gene into two discrete regions, with the optimal breakpoint between codons 1435 and 1441 and with a lower proportion of ovarian cancers in families with mutations 3′ to this boundary. Holt *et al.* (7) tested the hypothesis that the proportion of ovarian cancer is different for mutations on either side of the granin motif (codons 1214–1223) and found that the proportion of ovarian cancer was significantly lower for mutations 3′ to this motif. Our optimal breakpoint for the change in risks lay between these two estimates (nucleotide 4191, codon 1358), and well within the 95% CI given by Gayther *et al.* (6). Moreover, we found that a division of *BRCA1* into three regions (of roughly equal size), with different risks associated with mutations in each region, improved the fit. The ovarian:breast cancer ratio was similar for the 3′ and 5′ regions, but significantly higher in the center (Fig. 1).

The likelihood analysis allowed us to estimate separately the risks of breast and ovarian cancer associated with mutations in each of the three regions of *BRCA1*. The major effect was an estimated 29% reduction in breast cancer risk associated with mutations in the central region of *BRCA1* compared with mutations in the outer two regions. The risk of ovarian cancer was significantly lower (19%) for mutations 3′ to nucleotide 4191 than for other mutations, although this effect ceased to be significant when either the 31 original CRC families or the 79 families with an Ashkenazi founder mutation were removed from the analysis. However, the breast cancer effect barely changed when the CRC families were removed and increased in magnitude and significance when the Ashkenazi families were excluded. Thus, the reduction in breast cancer risk associated with mutations in the central region appears robust, whereas the observed variation in ovarian cancer risk may be artifactual. All four RR parameters were correlated with one another, and the nature of the estimation procedure means that it is impossible to completely disentangle the breast and ovarian cancer effects.

When the 31 CRC families were excluded, we were unable to find a single breakpoint splitting *BRCA1* into two regions with significantly different ovarian:breast cancer ratios. However, when we fixed the 4191 breakpoint, the optimal division of the 5′ region was at nucleotide 2401, as for the complete set of families (OR, 1.74; $P = 0.01$). Simultaneous estimation of two breakpoints (*i.e.,* maximizing the deviance over every three-way division) gave the optimal positions as nucleotides 2401 and 3896 (comparing the central region with the two outside regions; OR, 1.90; $P = 0.02$). If, as implied by the results of the MENDEL analysis for this group of families, the only variation in cancer risk is a lower breast cancer risk associated with mutations in the central region, then it is not so surprising that a division of the gene into just two contiguous regions does not produce a significant variation in risk.

The distribution of the mutations between the three regions did not differ significantly between the CRC and non-CRC families, and the proportion of breast cancer patients who were tested carriers was similar in the two sets, as was the average number of women per family with a breast cancer followed by an ovarian cancer. However, nearly half of the CRC families were identified specifically on the basis of at least two relatives with ovarian cancer, and a significantly higher proportion of the cancers in the CRC families were ovarian rather than breast (47.8 *versus* 35.7%; $P = 0.0006$), which might explain the stronger evidence for an ovarian effect in the CRC families.

We have noted that only 38% of the cancers were formally confirmed, the remainder having been reported by patients or relatives. Because any misreporting of cancer diagnoses would be independent of mutation and any variation in confirmation rates among centers was accounted for in the stratification, this is unlikely to affect the conclusions. A reanalysis restricted to confirmed cancer cases gave very similar results, although the significance was reduced given the smaller number of cases (*e.g.,* OR for the original exon 12–13 boundary, 1.47; $P = 0.098$).

Ashkenazi Jewish Founder Mutations. The elevated frequency of *BRCA1* and *BRCA2* mutations in the Ashkenazi population and the ease of testing for the three founder mutations compared with screening two entire genes make it likely that Ashkenazi families would be more readily offered mutation screening than would other families with comparable cancer

Downloaded from http://aacrjournals.org/cebp/article-pdf/11/4/329/1740713/ce0402000329.pdf by guest on 24 August 2022

histories. Accordingly, the median numbers of breast and ovarian cancers per family were lower among the Ashkenazi families than the non-Ashkenazi families (median number of breast cancers, 2 *versus* 3; median number of ovarian cancers, 1 *versus* 2, respectively). This explains in part the differences between the results of the full analysis (significantly lower ovarian cancer risk associated with 3′ mutations) and those of the analysis excluding the Ashkenazi families (no variation in ovarian cancer risk observed).

A recent study of 208 Ashkenazi Jewish ovarian cancer patients reported a lower risk of ovarian cancer in carriers of the 5382insC mutation than in 185delAG carriers, but found no difference in breast cancer risk between the two mutations (15). Most other studies have failed to find any significant difference in risk between the two mutations, possibly because the numbers of carriers of each mutation are small, particularly for the less common 5382insC (16, 17).

When we excluded either the Ashkenazi families or the original CRC families, the apparent absence of variation in ovarian cancer risk with mutation position concurred with the findings of a recent study of cancer risks in first-degree relatives of a population series of ovarian cancer patients (18). In contrast to our results, they reported a significant upward trend in breast cancer risk with increasingly 3′ mutation position. However, of the 39 *BRCA1* ovarian cancer patients in their study, only 21 had at least one female first-degree relative with breast and/or ovarian cancer; hence, the numbers of cases associated with mutations in any region of *BRCA1* were necessarily small.

Comparison with *BRCA2*. The pattern of breast cancer risk variation is reminiscent of that observed for *BRCA2*, where a lower risk of breast cancer is associated with mutations in the central third of the gene named the OCCR (19). The *BRCA2* reduction in breast cancer risk is slightly larger than for *BRCA1* (RR, 0.63; 95% CI, 0.46–0.84; $P = 0.001$; Ref. 10). This reduction in OCCR breast cancer risk is accompanied by a less significant increase in OCCR ovarian cancer risk, which was not seen in *BRCA1*.

In BRCA2, the OCCR coincides with the eight BRC repeat motifs, at least six of which have been shown to bind RAD51, a gene involved in the homologous repair of double-strand DNA breaks (20–22). BRCA1 also binds RAD51 via a domain in exon 11 (23), suggesting that BRCA1, BRCA2, and *RAD51* may operate in a common pathway. In the light of the BRCA2 result, it is intriguing that the 5′ boundary of the BRCA1 central region defined in this study coincides with the start of the BRCA1 RAD51-binding domain and that the 3′ boundary is coincident with the end of exon 11 (Fig. 1). Although the biological mechanism underlying the genotype-phenotype correlation is not known, this observation adds weight to the hypothesis that BRCA1/2 proteins truncated midway through the RAD51-binding domain have bound incompletely, and behave differently from truncated proteins that have either bound correctly or not at all. However, it is possible that the positions of the risk-region boundaries relative to the RAD51-binding domain are merely coincidental and that some other mechanism involving, *e.g.,* BRCA1 splice variants, may be at work.

MS Mutations. We also attempted to evaluate the risks associated with MS mutations in *BRCA1*. Although large numbers of single-amino acid substitutes in BRCA1 have been reported (summarized on the BIC database), most are probably not strongly disease associated. We therefore restricted out attention to those variants classified as mutations by BIC.

Twelve of the families in our set had MS mutations in *BRCA1*, 9 of which were located in the RING zinc-finger domain near to the NH_2 terminus, a cysteine-rich motif thought to be involved in DNA binding or protein-protein interactions (codons 20–68 in exons 2–5; Fig. 1; Refs. 2, 24). The RING domain is known to bind to BARD1 and BAP1 (25, 26) and displays perfect homology between human and murine *BRCA1* (27, 28), providing strong evidence of the functional significance of mutations in this region. The nine families all had mutations that change a cysteine into a glycine or a tyrosine and, hence, would be expected to affect DNA binding. This study provides direct evidence that RING finger mutations are pathological. Although the small number of families studied meant that cancer risks did not differ significantly from the 5′ PT baseline, the results suggest that MS mutations in the RING domain confer risks of breast cancer similar to those associated with PT mutations in the 3′ region of *BRCA1* and risks of ovarian cancer that are similar to or lower than those for 3′ PT mutations.

Of the other MS mutations seen in the set of families, two were located in the BRCT region located toward the COOH terminus of BRCA1. The region contains two tandem copies of a BRCT motif (codons 1649–1736 and 1756–1855; Fig. 1). The BRCT region is highly conserved between species (27–29) and shows strong similarity to a human protein known to bind p53; hence, the BCRT region is believed to be functional (30). Members of the two families with MS mutations in this region had a total of 3 ovarian cancers and 19 female breast cancers diagnosed below age 60. Unfortunately, we did not have enough data to estimate the risks associated with mutations in this region.

Male Breast Cancer. A recent study found a 7% risk of breast cancer in male *BRCA2* mutation carriers by age 80 years; this is ~80 times higher than in the general population, but it still only accounts for ~10% of male breast cancers (10). Although Struewing *et al.* (31) reported that 4 of 110 Israeli Jewish male breast cancer patients carried germ-line 185delAG *BRCA1* mutations, the general consensus seems to be that *BRCA1* mutations account for only a very small proportion of male breast cancers in other populations (32–34). Our data support this: in 365 families, we observed just seven cases of male breast cancer (mean age, 55 years). Three cases carried germ-line mutations, and the other four had not been tested. All were from different families, and as the mutations in these families are spread across exons 2, 8, 11, 17, and 18, there is no suggestion of any *BRCA1* genotype-phenotype correlation for male breast cancer.

Conclusions. Although we were able to demonstrate differences in cancer risks between three regions of *BRCA1*, the true variation may be more complicated than this. Given the many mutations, it may be impossible to provide estimates for each individual mutation, although functional analysis may allow a more rational classification of mutations. This study has provided hypotheses about differences in risk that can be tested, *e.g.,* in population-based studies. If these differences can be observed in other studies, they will improve the accuracy of the predicted risks available to patients undergoing genetic testing.

Acknowledgments

We thank Debbie Howe for help in preparing this manuscript.

Appendix

Following are the contributing centers and the names of the principal investigators. The number of families contributed by each center is given in brackets:

Downloaded from http://aacrjournals.org/cebp/article-pdf/11/4/329/1740713/ce0402000329.pdf by guest on 24 August 2022

CRC Genetic Epidemiology Unit, Cambridge, United Kingdom (coordinating center): D. Easton, D. Thompson, L. McGuffog

University of Pennsylvania, Pittsburgh, PA: B. Weber, L. Campeau [97 families]

Institut Curie, Paris, France: D. Stoppa-Lyonnet, S. Gad [77 families]

CRC Human Cancer Genetics Research Group, Cambridge, United Kingdom: B. Ponder, S. Gayther, A. Taylor [44 families]

University of Lund, Sweden: A. Borg, N. Loman, O. Johannsson, H. Olsson [32 families]

Institute of Cancer Research, Sutton, United Kingdom: M. Stratton, D. Ford, J. Peto, R. Eeles [25 families]

University Central Hospital, Departments of Oncology and Obstetrics and Gynaecology, Helsinki, Finland: H. Eerola, H. Nevanlinna [20 families]

Creighton University, Omaha, NE, and IARC, Lyon, France: H. T. Lynch, S. Narod, D. Goldgar, G. Lenoir, O. Sinilnikova [16 families]

Duke University Medical Center Comprehensive Cancer Center, Durham, NC: A. Futreal [15 families]

University of Leiden and Foundation for the Detection of Hereditary Tumors, Leiden, the Netherlands: P. Devilee, H. Vasen, C. J. Cornelisse [8 families]

McGill University, Montreal, Canada: S. Narod (currently at University of Toronto) [6 families]

National Cancer Institute, Bethesda, MD: J. Struewing [6 families]

Deutsches Krebsforschungszentrum, Heidelberg and University of Würzburg, Germany: J. Chang-Claude, B. H. F. Weber, U. Hamann [5 families]

University of Utah, Salt Lake City, UT: S. L. Neuhausen, L. Cannon-Albright [5 families]

Imperial Cancer Research Fund, Leeds, United Kingdom: D. T. Bishop, G. Crockford [4 families]

Center Jean Perrin, Clermont-Ferrand, France: Y. Bignon [2 families]

Max-Delbrück-Centrum für Molekulare Medizin, Tumorgenetik, Berlin, Germany: S. Scherneck, S. Seitz [2 families]

Karolinska Hospital, Dept of Molecular Medicine, Stockholm, Sweden: A. Lindblom [2 families]

University of Aberdeen, United Kingdom: N. Haites, A. Schofield [1 family]

Institut Paoli Calmettes, Marseille, France: H. Sobol [1 family]

Center for Cancer Epidemiology, Manchester, United Kingdom: G. Evans [1 family]

References

1. Hall, J. M., Lee, M. K., Newman, B., Morrow, J. E., Anderson, L. A., Huey, B., and King, M-C. Linkage of early-onset familial breast cancer to chromosome 17q21. Science (Wash. DC), *250:* 1684–1689, 1990.

2. 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., Rosteck, P., Lai, M., Barett, J. C., Lewis, C., Neuhausen, S., Cannon-Albright, L., Goldgar, D., Wiseman, R., Kamb, A., and Skolnick, M. R. A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA1*. Science (Wash. DC), *266:* 66–71, 1994.

3. The Anglian Breast Cancer Study Group. Prevalence and penetrance of *BRCA1* and *BRCA2* mutations in a large population based series of breast cancer cases. Br. J. Cancer, *83:* 1301–1308, 2000.

4. Antoniou, A. C., Gayther, S. A., Stratton, J. F., Ponder, B. A. J., and Easton, D. F. Risk models for familial ovarian and breast cancer. Genet. Epidemiol., *18:* 173–190, 2000.

5. Ford, D., Easton, D. F., Bishop, D. T., Narod, S. A., Goldgar, D., and the Breast Cancer Linkage Consortium. Risks of cancer in *BRCA1*-mutation carriers. Lancet, *343:* 692–695, 1994.

6. Gayther, S. A., Warren, W., Mazoyer, S., Russel, P. A., Harrington, P. A., Chiano, M., Seal, S., Hamoudi, R., van Rensburg, E. J., Dunning, A. M., Love, R., Evans, G., Easton, D., Clayton, D., Stratton, M., and Ponder, B. A. J. Germline mutations of the *BRCA1* gene in breast and ovarian cancer families provide evidence for a genotype-phenotype correlation. Nat. Genet., *11:* 428–433, 1995.

7. Holt, J. T., Thompson, M. E., Szabo, C., Robinson-Benion, C., Arteaga, C. L., King, M-C., and Jensen, R. A. Growth retardation and tumour inhibition by *BRCA1*. Nat. Genet., *12:* 298–302, 1996.

8. Neuhausen, S. L., Mazoyer, S., Friedman, L., Stratton, M., Offit, K., Caligo, A., Tomlinson, G., Cannon-Albright, L., Bishop, T., Kelsell, D., Solomon, E., Weber, B., Couch, F., Struewing, J., Tonin, P., Durocher, F., Narod, S., Skolnick, M. H., Lenoir, G., Serova, O., Ponder, B., Stoppa-Lyonnet, D., Easton, D., King, M-C., and Goldgar, D. E. Haplotype and phenotype analysis of six recurrent *BRCA1* mutations in 61 families: results of an international study. Am. J. Hum. Genet., *58:* 271–280, 1996.

9. Fodor, F. H., Weston, A., Bleiweiss, I. J., McCurdy, L. D., Walsh, M. M., Tartter, P. I., Brower, S. T., and Eng, C. M. Frequency and carrier risk associated with common *BRCA1* and *BRCA2* mutations in Ashkenazi Jewish breast cancer patients. Am. J. Hum. Genet., *63:* 45–51, 1998.

10. Thompson, D., Easton, D., and the Breast Cancer Linkage Consortium. Variation in cancer risks, by mutation position, in *BRCA2* mutation carriers. Am. J. Hum. Genet., *68:* 410–419, 2001.

11. Parkin, D. M., Whelan, S. L., Ferlay, J., Raymond, L., and Young, J. (eds.). Cancer Incidence in Five Continents. VII. IARC Scientific Publ. No. 143. Lyon, France: IARC, 1997.

12. Lange, K., Weeks, D., and Boehnke, M. Programs for pedigree analysis: MENDEL, FISHER and dGENE. Genet. Epidemiol., *5:* 471–472, 1988.

13. Friedman, L. S., Ostermeyer, E. A., Szabo, C. I., Dowd, P., Lynch, E. D., Rowell, S. E., and King, M-C. Confirmation of *BRCA1* by analysis of germline mutations linked to breast and ovarian cancer in ten families. Nat. Genet., *8:* 399–404, 1994.

14. Shattuck-Eidens, D., McClure, M., Simard, J., Labrie, F., Narod, S., Couch, F., Hoskins, K., Weber, B., Castilla, L., Erdos, M., Brody, L., Friedman, L., Ostermeyer, E., Szabo, C., King, M.-C., Jhanwar, S., Offit, K., Norton, L., Gileski, T., Lubin, M., Osborne, M., Black, D., Boyd, M., Steel, M., Ingles, S., Haile, R., Lindblom, A., Olsson, H., Borg, A., Bishop, D. T., Solomon, E., Radice, P., Spatti, G., Gayther, S., Ponder, B., Warren, W., Stratton, M., Liu, Q., Fujimura, F., Lewis, C., Skolnick, M. H., and Goldgar D. E. A collaborative survey of 80 mutations in the *BRCA1* breast and ovarian cancer susceptibility gene. Implications for presymptomatic testing and screening. JAMA, *273:* 535–541, 1995.

15. Moslehi, R., Chu, W., Karlan, B., Fishman, D., Risch, H., Fields, A., Smotkin D., Ben-David, Y., Rosenblatt, J., Russo, D., Schwartz, P., Tung, N., Warner, E., Rosen, B., Friedman, J., Brunet, J-S., and Narod, S. A. *BRCA1* and *BRCA2* mutation analysis of 208 Ashkenazi Jewish women with ovarian cancer. Am. J. Hum. Genet., *66:* 1259–1272, 2000.

16. Struewing, J., Hartge, P., Wacholder, S., Baker, S. M., Berlin, M., McAdams, M., Timmerman M. M., Brody, L. C., and Tucker, M. A. The risk of cancer associated with specific mutations of *BRCA1* and *BRCA2* among Ashkenazi Jews. N. Engl. J. Med., *336:* 1401–1408, 1997.

17. Satagopan, J. M., Offit, K., Foulkes, W., Robson, M. E., Wacholder, S., Eng, C. M., Karp, S. E., and Begg, C. B. The lifetime risks of breast cancer in Ashkenazi Jewish carriers of *BRCA1* and *BRCA2* mutations. Cancer Epidemiol. Biomark. Prev., *10:* 467–473, 2001.

18. Risch, H. A., McLaughlin, J. R., Cole, D. E., Rosen, B., Bradley, L., Kwan, E., Jack, E., Vesprini, D. J., Kuperstein, G., Abrahamson, J. L., Fan, I., Wong, B., and Narod, S. A. Prevalence and penetrance of germline *BRCA1* and *BRCA2* mutations in a population series of 649 women with ovarian cancer. Am. J. Hum. Genet., *68:* 700–710, 2001.

19. Gayther, S. A., Mangion, J., Russell, P. A., Seal, S., Basford, R., Ponder, B. A. J., Stratton, M., and Easton, D. Variation in risks of breast an ovarian cancer associated with different germline mutations of the *BRCA2* gene. Nat. Genet., *15:* 103–105, 1997.

20. Bork, P., Blomberg, N., and Niges, M. Internal repeats in the *BRCA2* protein sequence. Nat. Genet., *13:* 22–23, 1996.

21. Wong, A. K. C., Pero, R., Ormonde, P. A., Tavtigian, S. V., and Bartel, P. L. RAD51 interacts with the evolutionarily conserved BRC motifs in the human breast cancer susceptibility gene *BRCA2*. J. Biol. Chem., *272:* 31941–31944, 1997.

22. Gayther, S. A., and Ponder, B. A. J. Clues to the function of the tumour susceptibility gene *BRCA2*. Dis. Markers, *14:* 1–8, 1998.

23. Scully, R., Chen, J., Plug, A., Xiao, Y., Weaver, D., Feunteun, J., Ashley, T., and Livingston, D. M. Association of *BRCA1* with RAD51 in mitotic and meiotic cells. Cell, *88:* 265–275, 1997.

24. Bienstock, R. J., Darden, T., Wiseman, R., Pedersen, L., and Barrett, J. C. Molecular modeling of the amino-terminal zinc ring domain of *BRCA1*. Cancer Res., *56:* 2539–2545, 1996.

25. Wu, L. C., Wang, Z. W., Tsan, J. T., Spillman, M. A., Phung, A., Xu, X. L., Yang, M-C. W., Hwang, L-Y., Bowcock, A. M., and Baer, R. Identification of a RING protein that can interact *in vivo* with the *BRCA1* gene product. Nat. Genet., *14:* 430–440, 1996.

26. Jensen, D. E., Proctor, M., Marquis, S. T., Gardner, H. P., Ha, S. I., Chodosh, L. A., Ishov, A. M., Tommerup, N., Vissing, H., Sekido, Y., Minna, J., Borodovsky, A., Schultz, D. C., Wilkinson, K. D., Maul, G. G., Barlev, N., Berger, S. L., Prendergast, G. C., and Rauscher, F. J. 3rd. BAP1: a novel ubiquitin hydrolase which binds to the *BRCA1* RING finger and enhances *BRCA1*-mediated cell growth suppression. Oncogene, *5*: 1097–1112, 1998.

27. Abel, K. J., Xu, J., Yin, G. Y., Lyons, R. H., Meisler, M. H., and Weber, B. L. Mouse *BRCA1*: localization, sequence analysis and identification of evolutionarily conserved domains. Hum. Mol. Genet., *4:* 2265–2273, 1995.

Downloaded from http://aacrjournals.org/cebp/article-pdf/11/4/329/1740713/ce0402000329.pdf by guest on 24 August 2022

28. Sharan, S. K., Wims, M., and Bradley, A. Murine *BRCA1*: sequence and significance for human missense mutations. Hum. Mol. Genet., *4:* 2275–2278, 1995.

29. Szabo, C. I., Wagner, L. A., Francisco, L. V., Roach, J. C., Argonza, R., King, M-C., and Ostrander, E. A. Human, canine and murine *BRCA1* genes: sequence comparison among species. Hum. Mol. Genet., *5:* 1289–1298, 1996.

30. Koonin, E. V., Altschul, S. F., and Bork, P. *BRCA1* protein products . . . Functional motifs . . . Nat. Genet., *13:* 266–268, 1996.

31. Struewing, J. P., Coriaty, Z. M., Ron, E., Livoff, A., Konichezky, M., Cohen, P., Resnick, M. B., Lifzchiz-Mercerl, B., Lew, S., and Iscovich, J. Founder *BRCA1/2* mutations among male patients with breast cancer in Israel. Am. J. Hum. Genet., *65:* 1800–1802, 1999.

32. Stratton, M. R., Ford, D., Neuhasen, S., Seal, S., Wooster, R., Friedman, L. S., King, M-C., Egilsson, V., Devilee, P., McManus, R., Daly, P. A., Smyth, E., Ponder, B. A. J., Peto, J., Cannon-Albright, L., Easton, D. F., and Goldgar, D. E. Familial male breast cancer is not linked to the *BRCA1* locus on chromosome 17q. Nat. Genet., *7:* 103–107, 1994.

33. Friedman, L. S., Gayther, S. A., Kurosaki, T., Gordon, D., Noble, B., Casey, G., Ponder, B. A. J., and Anton-Culver, H. Mutation analysis of *BRCA1* and *BRCA2* in a male breast cancer population. Am. J. Hum. Genet., *60:* 313–319, 1997.

34. Csokay, B., Udvarhelyi, N., Sulyok, Z., Besznyak, I., Ramus, S., Ponder, B., and Olah, E. High frequency of germ-line *BRCA2* mutations among Hungarian male breast cancer patients without family history. Cancer Res., *59:* 995–998, 1999.

35. Welcsh, P. L., and King, M-C. *BRCA1* and *BRCA2* and the genetics of breast and ovarian cancer. Hum. Mol. Genet., *10:* 705–713, 2001.

Downloaded from http://aacrjournals.org/cebp/article-pdf/11/4/329/1740713/ce0402000329.pdf by guest on 24 August 2022