Abstract

Purpose: It is not clear that the published estimates of the breast and ovarian cancer penetrances of mutations in \textit{BRCA1} and \textit{BRCA2} can be used in genetic counseling in countries such as Spain, where the incidence of breast cancer in the general population is considerably lower, the prevalence of \textit{BRCA2} mutations seems to be higher, and a distinct spectrum of recurrent mutations exists for both genes. We aimed to estimate these penetrances for women attending genetic counseling units in Spain.

Experimental Design: We collected phenotype and genotype data on 155 \textit{BRCA1} and 164 \textit{BRCA2} mutation carrier families from 12 centers across the country. Average age-specific cumulative risks of breast cancer and ovarian cancer were estimated using a modified segregation analysis method.

Results: The estimated average cumulative risk of breast cancer to age 70 years was estimated to be 52\% [95\% confidence interval (95\% CI), 26\%-69\%] for \textit{BRCA1} mutation carriers and 47\% (95\% CI, 29\%-60\%) for \textit{BRCA2} mutation carriers. The corresponding estimates for ovarian cancer were 22\% (95\% CI, 0\%-40\%) and 18\% (95\% CI, 0\%-35\%), respectively. There was some evidence (two-sided $P = 0.09$) that 330A\textgreater{}G (R71G) in \textit{BRCA1} may have lower breast cancer penetrance.

Conclusions: These results are consistent with those from a recent meta-analysis of practically all previous penetrance studies, suggesting that women with \textit{BRCA1} and \textit{BRCA2} mutations attending genetic counseling services in Spain have similar risks of breast and ovarian cancer to those published for other Caucasian populations. Carriers should be fully informed of their mutation- and age-specific risks to make appropriate decisions regarding prophylactic interventions such as oophorectomy.
Deleterious mutations in BRCA1 and BRCA2 are found in a high proportion (around 20%) of families with multiple cases of breast cancer, especially if they also contain ovarian cancer cases, and only very rarely carried by cases with no family history (1–5). Testing for these mutations in high-risk individuals has become routine in family cancer clinics and genetic counseling centers across the developed world. Once a mutation has been identified in a family, genetic counseling and testing for that particular mutation is then usually offered to other family members. Providing accurate information about the breast and ovarian cancer risks of female mutation carriers is an integral part of this process.

Estimates of the average cumulative risk of breast and ovarian cancer to age 70 years in carriers (the breast and ovarian cancer penetrances of the mutations carried) vary by study, between 40% and 85% for breast cancer and 10% and 65% for ovarian cancer (6–16). It seems that penetrance may depend on the population studied, with higher risks faced by carriers from multiple-case families (17–19). It has also been observed that penetrance may vary by gene and by mutation within the same gene (7, 20, 21), as well as according to the generation of carriers being considered (7, 11, 22). These considerations have led some investigators to refer to “the penetrances” of mutations in BRCA1 and BRCA2, arguing that when penetrance is estimated, it should be referred to as the average penetrance of a defined set of mutations, in a defined population (23).

Indeed, recently published studies have estimated the average penetrance in particular populations (8, 12). However, no estimates are available for the Spanish population and so genetic counseling services depend on estimates from other predominantly Caucasian populations. It is not clear that this is appropriate because, compared with these populations, the cumulative risk of breast cancer in the general Spanish population is considerably lower (estimated to be 4.8% to age 70 years; ref. 24), the prevalence of BRCA2 mutations among multiple-case families seems to be higher (25–27) and a distinct spectrum of recurrent mutations exists in both BRCA1 and BRCA2 (1).

The aim of this study was therefore to estimate the penetrance of mutations in BRCA1 and BRCA2 that are carried by women attending genetic counseling centers in Spain, and in particular (a) to estimate the average cumulative risks of breast and ovarian cancer to age 70 years for female carriers of mutations in BRCA1 and BRCA2; (b) to test whether breast and ovarian cancer risks are different for carriers of recurrent mutations; and (c) to test whether these risks differ by position of the mutation in the gene. To address these aims, we collected and analyzed genotype and phenotype data on 319 mutation-positive families recruited at 12 centers across Spain.

Materials and Methods

Study subjects and genetic testing. Families testing positive for deleterious mutations in BRCA1 at BRCA2 were recruited at 12 genetic counseling centers across Spain. The number of eligible mutation-positive families and the period during which they were recruited at each center are detailed in Table 1.

At each participating center, the first family member to attend was designated the proband. In general, families were selected for mutation testing if they contained (a) at least three cases of breast or ovarian cancer in the same family line; (b) at least two first-degree relatives diagnosed with breast cancer before age 50 years; (c) at least one case of breast cancer and one case of ovarian or bilateral breast cancer in the same family line; (d) at least one woman with both breast and ovarian cancer; and/or (e) at least one case of male breast cancer. The first family member tested was in most cases the proband if they had breast or ovarian cancer. If the proband was unaffected, the youngest living affected relative was tested first, where possible. Mutation testing was carried out using a range of methods (1, 25, 26, 28–31). Mutations in BRCA1 and BRCA2 were defined as deleterious if (a) they were classified as “clinically important” by the Breast Cancer Information Core (BIC)23; (b) they produced a premature stop codon at or before codon 1853 in BRCA1 (32); (c) they were protein truncating mutations occurring before exon 27 in BRCA2 (33); (d) they were single base changes occurring at highly conserved bases of the splice donor or acceptor site and were predicted to adversely affect splicing or shown to have other functional consequences; and/or (e) they produced an amino acid change with strong evidence of pathogenicity (see Supplementary Tables 1A and B). Once a mutation was identified in a family, genetic counseling and testing was offered to other family members. Families were eligible for inclusion in this study only if at least one other member was tested for the family mutation once a mutation had been identified (see Statistical Analysis). All participants gave informed consent.

Data collection. At all centers, a detailed family history was obtained from the proband before any genetic testing was considered. This included, for each family member for which information could be obtained, degree of relatedness, details of any cancers diagnosed and the corresponding ages at diagnosis, vital status, age last known to be alive, and details of any prophylactic interventions (including age at occurrence). This information was confirmed and updated with other family members who subsequently attended the center. Attempts were made to confirm details of all reported cancers, including requesting pathology reports where possible.

Statistical analysis. Family characteristics (the number of individuals tested for mutations and number of cancers diagnosed) were compared, by gene mutation, using the Mann-Whitney test. The distribution of mutations across each gene was described by counting the number of families with mutations in each exon. This was then compared with the corresponding distribution of mutations described in the BIC by, for each exon, applying Fisher’s exact test to the counts for that exon versus those for all other exons combined. P values were adjusted empirically via a permutation test.

The average cumulative risks of breast and ovarian cancer to age 70 years among mutation carriers were estimated simultaneously, but separately for BRCA1 and BRCA2, via a maximum likelihood method using modified segregation analysis implemented in the pedigree analysis software MENDEL. This method has been previously described in detail (7, 34, 35). For each family, we maximized the conditional likelihood of observing all genotypes and disease phenotypes in the family, given the genotype of the first individual to test positive and all disease phenotypes in the family at the time the first mutation was discovered. Therefore, only families in which at least two members were genotyped were informative. Furthermore, if we define prevalent cases to be those diagnosed with cancer before the identification of the family mutation, and incident cases to be those diagnosed afterward, in effect
only prevalent cases were included in the conditioning of the likelihood and so incident cases were much more informative.

The incidence rates for mutation carriers were assumed to follow a Cox proportional hazards model \( \lambda(t) = \lambda_0(t) \exp\{G(t)\} \), where \( \lambda_0(t) \) is the baseline cancer incidence rate for noncarriers and \( \exp\{G(t)\}\) the age-specific hazard ratio (HR), or relative risk, in carriers compared with noncarriers. The function \( \lambda_0(t) \) was estimated using age-specific, population-based breast and ovarian cancer incidence rates for Spain, collated by the IARC for the period 1993 to 1997 (24). Individuals were followed from birth and were censored at the first of the following events: breast cancer diagnosis; ovarian cancer diagnosis; other cancer diagnosis; death; prophylactic intervention (mastectomy or oophorectomy); last contact with the study center or last contact with a relative at which vital status was confirmed; and living to age 70 years. Age at last contact was imputed for <1% unaffected individuals, known to be alive when the proband first attended the study center, as a function of the ages of their relatives. It was assumed that parents were at least 15 years older than their children; children were a maximum of 50 years younger than their mothers and a maximum of 35 years separated any two siblings; and the minimum age complying with these assumptions was chosen. Individuals whose censored age coincided with their age at diagnosis of breast cancer or ovarian cancer were considered breast cancer failures or ovarian cancer failures, respectively. The 2.7% (\( n = 34 \)) affected individuals with missing age at diagnosis were censored at age zero, effectively excluding all but their genotype information from likelihood model.

The basic model consisted of 10 variables, five representing the natural logarithm of the hazard ratio \( \lnHR = G(t) \) for female breast cancer associated with being a mutation carrier at ages 20-29, 30-39, 40-49, 50-59, and 60-69 years, and five representing the corresponding \( \lnHR \)'s for ovarian cancer. Neither the average male breast cancer penetrance nor the penetrance for other cancers in either sex was estimated due to the small number of cases. However, for \( BRCA2 \), we evaluated the influence on the penetrance estimates for women, of modeling the increased risk of breast cancer among male mutation carriers. This was done by including a single additional variable for the \( \lnHR \) for breast cancer associated with being carrier among men, assuming that this was constant over all ages. For this latter analysis, men were followed up until age 90 years, rather than age 70 years, so that male cancers at advanced ages were included, given that these are so rare in the general population.

We assumed that the allele frequency for all deleterious mutations combined was 0.001 for each gene, although the sensitivity of results was assessed for frequencies of 0.0001 and 0.005. Possible differences in breast and ovarian cancer risks among subgroups of carriers were assessed by including an additional variable for each subgroup, apart from an arbitrarily chosen reference subgroup, and for each disease. These additional variables represented the disease-specific \( \lnHR \)'s associated with each subgroup in question, relative to the reference subgroup, assuming that these were constant over all ages. Differences were tested for using the likelihood ratio test, considering the basic 10-variable model as reference, with degrees of freedom (df) equal to the number of additional variables included. For each subgrouping, the first model tested included variables for all categories (except the reference), for both breast and ovarian cancer. Where possible differences were identified, further analysis of nested models was carried out. This analysis was applied to subgroups defined by center (A, B, C, D, E, other, for \( BRCA1 \) carrier families; A, B, C, D, F, other, for \( BRCA2 \) carrier families) as well as by recurrent mutation (each of the most recurrent, all others pooled) and by location in the gene (proximal to exon 11, exon 11, distal to exon 11). This analysis was also applied to subgroups of mutations in \( BRCA1 \) defined according to their role in activating the mechanism of nonsense-mediated mRNA decay, with categories determined based on the results of Perrin-Vidoz et al. (ref. 36; activating: producing a codon stop at codons 81-1672; nonactivating: producing a codon stop at or before codon 39 or at or after codon 1829; unknown: all others; see Supplementary Table 1A). For \( BRCA2 \) mutation carriers, it was applied to subgroups defined with reference to the ovarian cancer cluster region (OCCR), first as defined by Gayther et al. (ref. 37; proximal to OCCR: occurring at nucleotides upstream of 3035; OCCR: occurring at nucleotides 3035-6629; distal to OCCR: occurring at nucleotides downstream of 6629), and second as redefined by Thompson et al. (ref. 21; OCCR2: occurring at nucleotides 3059-4075; non-OCCR2: occurring at nucleotides 6503-6629). Variation in the average penetrance over time was evaluated by applying a similar method to subgroups of birth cohort (<1915, 1915-1939, 1940-1964, and \( \geq 1965 \)), but fitting a single linear effect to values -2, -1, 0, and 1 for these subgroups, respectively, thereby considering carriers born in 1940 to 1965 (i.e., the largest subgroup) as reference. Because year of birth was collected only for mutation-tested individuals (19% of the data set), individuals were allocated to these birth year subgroups by first accurately classifying the first-tested family members and then imputing the subgroup of each of their relatives, assuming 25 years difference between generations. This effectively created a variable representing the generation of each individual, with generations aligned between families according to the year of birth of the first tested in each family.

All tests of statistical significance were two-sided and comparisons for which associated \( P \) values were <0.05 were considered statistically significant.

## Results

### Description of families recruited.
A total of 155 families with deleterious mutations in \( BRCA1 \) and 164 with deleterious mutations in \( BRCA2 \) were eligible for inclusion in this study. The number of subjects genotyped per family was similar across
Cancer, and the majority (81%, n = 6%) (P = 0.01) had only one individual with breast or ovarian cancer, and the majority (81%, n = 126) had at least three affected individuals. The corresponding proportions for families carrying mutations in BRCA2 were 4% (n = 6) and 77% (n = 127), respectively. Despite observing equal medians of three cases per family for each gene, there tended to be more breast cancer cases among BRCA2 mutation-carrier families than among BRCA1 mutation-carrier families (P = 0.01). On the contrary, there were more cases of ovarian cancer in families with BRCA1 mutations than in families with BRCA2 mutations (median = 1 and 0, respectively; P < 0.001). The total number of cases of breast and ovarian cancer, by type (incident versus prevalent), are presented in Table 2. Ten of cases of breast and ovarian cancer, by type (incident versus prevalent) and by gene mutated, are presented in Table 2. Ten of cases of breast and ovarian cancer, by type (incident versus prevalent) and by gene mutated, are presented in Table 2. Ten of breast and ovarian cancer subsequent to the identification of the mutation carriers were diagnosed with cancer (9 breast and 1 ovarian). The other two Jewish founder mutations 5382insC and 6174delT, located on exon 20 of BRCA1, and 185delAG is also a Jewish founder mutation, representing 25% of all mutations in BRCA1 described in the BIC. Other recurrent Spanish mutations were 330As>G (also referred to as R71G, exon 5, 14% families) and A1708E (exon 18, 9% families), both in BRCA1, and 3036del4 (exon 11, 13% families) and 9254del5 (exon 23, 14% families), both in BRCA2. The estimated average cumulative risks. The estimated average age-specific cumulative risks of breast cancer and ovarian cancer for female carriers of mutations in BRCA1 and BRCA2 from multiple-case families are presented in Fig. 1A and B, respectively. The estimated age-specific HRs, along with those of Antoniou et al. (7), are provided in Table 3A and B. For BRCA1 mutation carriers, the penetrance to age 70 years.

| Description and distribution of mutations. The mutations identified in the 319 families are detailed in Supplementary Table S1A and S1B, whereas their distribution by exon compared with that of mutations described in the BIC is presented in Supplementary Table S2A and S2B. For both BRCA1 and BRCA2, the proportion of mutations in exon 11, where the majority mutations are found, was very similar to that observed in the BIC (35% versus 36% for BRCA1 and 60% versus 64% for BRCA2). Further comparison of the distribution of mutations by exon with that of those described in the BIC highlighted a significantly higher proportion of mutations in exons 3 (4% versus <1%, adjusted P < 0.0001), 5 (16% versus 4%, adjusted P < 0.0001), 18 (20% versus 2%, adjusted P < 0.0001), 23 (2% versus <1%, adjusted P = 0.02), and 24 (1% versus <1%, adjusted P = 0.02) of BRCA1 and exon 23 of BRCA2 (18% versus 2%, adjusted P < 0.0001), and a significantly lower proportion of mutations in exons 2 (12% versus 26%, adjusted P = 0.0002) and 20 (2% versus 15%, adjusted P < 0.0001) of BRCA1. These differences were largely explained by the presence of distinct recurrent mutations in the Spanish multiple-case families and the inclusion of Jewish founder mutations among those described in the BIC (see Supplementary Table S2A and S2B). One of the most common Spanish mutations was 185delAG, located on exon 2 of BRCA1 and identified in 11% (n = 17) of BRCA1 mutation–positive families. 185delAG is also a Jewish founder mutation, representing 25% of all mutations in BRCA1 described in the BIC. Other recurrent Spanish mutations were 330As>G (also referred to as R71G, exon 5, 14% families) and A1708E (exon 18, 9% families), both in BRCA1, and 3036del4 (exon 11, 13% families) and 9254del5 (exon 23, 14% families), both in BRCA2. The two other Jewish founder mutations 5382insC and 6174delT, located on exon 20 of BRCA1 and exon 11 of BRCA2, respectively, were not observed among the Spanish families, consistent with the findings of Diez et al. (38). When mutations classified in the BIC as having unknown clinical significance were excluded from comparisons, the differences in frequency previously observed for exons 23 and 24 disappeared.

| Table 2. Total number of breast and ovarian cancer cases, by gene mutated |
|----------------|------------------|
|               | BRCA1 (155 families) | BRCA2 (164 families) |
| Breast cancer cases—total | 445 (1) | 566 (35) |
| Prevalent cases | 432 (1) | 553 (35) |
| Excluding the first tested per family* | 318 (1) | 414 (26) |
| Mutation carriers | 63 (0) | 70 (5) |
| Noncarriers | 12 (0) | 9 (0) |
| Untested | 243 (1) | 335 (21) |
| Incident | 13 (0) | 13 (0) |
| Mutation carriers | 9 (0) | 7 (0) |
| Noncarriers | 0 (0) | 2 (0) |
| Untested | 4 (0) | 4 (0) |
| Ovarian cancer cases—total | 141 | 43 |
| Prevalent cases | 140 | 42 |
| Excluding the first tested per family* | 107 | 27 |
| Mutation carriers | 18 | 8 |
| Noncarriers | 1 | 0 |
| Untested | 88 | 19 |
| Incident | 1 | 1 |
| Mutation carriers | 1 | 1 |
| Noncarriers | 0 | 0 |
| Untested | 0 | 0 |

NOTE: The number of male cases is presented in parenthesis.
*Of the 155 first-tested individuals from families with mutations in BRCA1, 114 had developed breast cancer, 33 developed ovarian cancer, and 8 were unaffected when the family mutation was identified. One of the unaffected patients subsequently developed breast cancer. Among the 164 first-tested individuals from families with mutations in BRCA2 (9 of which were men), 139 (all 9 men) had developed breast cancer, 15 ovarian cancer, and 10 were unaffected when the family mutation was identified.
was estimated to be 52% (95% confidence interval (95% CI), 26-69%) for breast cancer and 22% (95% CI, 0-40%) for ovarian cancer. The estimated relative risk of breast cancer was higher before age 40 years (HR, 32 and 51 for ages 20-29 and 30-39 years, respectively) and then decreased with increasing age (HR, 18, 10, and 10 for ages 40-49, 50-59, and 60-69 years, respectively). The pattern of relative risks was similar for ovarian cancer, with higher estimated HRs before age 50 years (HR, 32 and 51 for ages 20-29 and 30-39 years, respectively), and then decreasing with age, although to a lesser extent than for breast cancer. For carriers of mutations in \( \text{BRCA}_2 \), the estimated average cumulative risk of breast cancer to age 70 years was 47% (95% CI, 29-60%), whereas that of ovarian cancer was 18% (95% CI, 0-35%). There appeared to be a slight increase in the estimated relative risk of breast cancer up to intermediate ages (HR, 10, 15, and 25 for ages 20-29, 30-39, and 40-49 years, respectively), and then reduction thereafter (HR, 13 and 3.3 for ages 50-59 and 60-69 years, respectively). In contrast to the pattern of risk of ovarian cancer observed for \( \text{BRCA}_1 \) mutation carriers, the estimated HR for ovarian cancer increased with age. The comparison of Fig. 1A and B reveals that the estimated cumulative risk of both breast and ovarian cancer was higher at younger ages for \( \text{BRCA}_1 \) mutation carriers than \( \text{BRCA}_2 \) mutation carriers.

The above analyses were repeated, considering different assumed allele frequencies for all mutations combined in each gene, and HR estimates did not change substantially (data not shown). The estimated penetrances to age 70 years were also

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**Fig. 1.** A, average cumulative risk of breast and ovarian cancer for female carriers of mutations in \( \text{BRCA}_1 \) (bars, 95% CI). B, average cumulative risk of breast and ovarian cancer for female carriers of mutations in \( \text{BRCA}_2 \) (bars, 95% CI).
very robust to the assumed allele frequency, with estimates for breast cancer among BRCA1 mutation carriers of 52% and 54% for frequencies of 0.01% and 0.5%, respectively. The corresponding estimates for ovarian cancer were 22% and 22%.

Among carriers of mutations in BRCA2, the corresponding estimates were 47% and 45% for breast cancer and 41% and 19% for ovarian cancer. Given the relatively low estimated incidence of female breast cancer in the general population in Spain (24), we also investigated the sensitivity of the penetrance estimates to these. Increasing the assumed breast cancer incidence rates by 75% across all ages (corresponding to a cumulative risk of 8.2% to age 70 years, comparable with rates more commonly observed in other Caucasian populations; ref. 39) did not have a substantial effect either, generating estimates of 56% and 20% for breast and ovarian cancer, respectively, among BRCA1 mutation carriers and of 51% and 16% among BRCA2 mutation carriers.

Recurrent mutations. We next investigated whether breast or ovarian cancer risks were different for any of the recurrent Spanish mutations observed in our families. We tested for differences between carriers of 185delAG, 330A>G, A1708E, and other mutations in BRCA1 pooled and found marginal evidence of heterogeneity in HRs ($P = 0.07$; $df = 6$). Further analyses revealed that, for breast cancer, the best fitting model (among those with a single variable per recurrent mutation) by the likelihood ratio test included different effects for 185delAG and 330A>G and in both cases the risks were lower than for all other mutations pooled ($P = 0.006$; $df = 2$). We therefore compared breast cancer HRs estimated from stratified analyses and present results in Table 4. Although HR estimates were substantially lower at all ages for 330A>G compared with other mutations in BRCA1, this was not the case for 185delAG, which had higher estimated relative risks at younger ages and older ages and very few cases diagnosed at intermediate ages. The stratified estimates of the cumulative risks of breast cancer to age 70 years were 27% (95% CI, 0.5-52%) for 330A>G, 58% (95% CI, 0.9-90%) for 185delAG, and 69% (95% CI, 35-85%) for all other mutations in BRCA1 combined. We tested for differences in HRs between carriers of 330A>G and carriers of all other mutations in BRCA1 combined and found weak evidence ($P = 0.09$; $df = 1$).

### Table 3. HRs and 95% CIs for carriers of mutations in BRCA1 and BRCA2 by age and disease

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>Breast cancer</th>
<th>Ovarian cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Our study</td>
<td>Antoniou et al. (7)</td>
</tr>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>A. BRCA1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-29</td>
<td>32 (1.2-841)</td>
<td>17 (4.2-71)</td>
</tr>
<tr>
<td>30-39</td>
<td>51 (21-120)</td>
<td>33 (23-49)</td>
</tr>
<tr>
<td>40-49</td>
<td>18 (7.7-41)</td>
<td>32 (24-43)</td>
</tr>
<tr>
<td>50-59</td>
<td>10 (3.4-28)</td>
<td>18 (11-30)</td>
</tr>
<tr>
<td>60-69</td>
<td>10 (1.5-72)</td>
<td>14 (6.3-31)</td>
</tr>
<tr>
<td>B. BRCA2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-29</td>
<td>10 (1.0-93)</td>
<td>19 (4.5-81)</td>
</tr>
<tr>
<td>30-39</td>
<td>15 (6.0-39)</td>
<td>16 (9.3-29)</td>
</tr>
<tr>
<td>40-49</td>
<td>25 (14-46)</td>
<td>10 (6.1-16)</td>
</tr>
<tr>
<td>50-59</td>
<td>13 (5.1-35)</td>
<td>12 (7.4-19)</td>
</tr>
<tr>
<td>60-69</td>
<td>3.3 (0.8-13)</td>
<td>11 (6.3-20)</td>
</tr>
</tbody>
</table>

NOTE: —, could not be estimated as no cases were observed in this age range.

### Table 4. Comparison of estimated breast cancer HRs, by age and mutation in BRCA1

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>185delAG (17 families)</th>
<th>330A&gt;G (21 families)</th>
<th>Others* (117 families)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-29</td>
<td>60 (0.1-999)</td>
<td>1.1</td>
<td>26 (0.6-999)</td>
</tr>
<tr>
<td>30-39</td>
<td>15 (0.1-999)</td>
<td>11 (0.7-193)</td>
<td>57 (24-140)</td>
</tr>
<tr>
<td>40-49</td>
<td>2.5 (0.6-10)</td>
<td>13 (2.2-75)</td>
<td>33 (14-75)</td>
</tr>
<tr>
<td>50-59</td>
<td>6.5 (0.6-67)</td>
<td>18 (5.8-58)</td>
<td></td>
</tr>
<tr>
<td>60-69</td>
<td>43 (7.2-258)</td>
<td>1.4 (0.1-21)</td>
<td>16 (1.3-186)</td>
</tr>
</tbody>
</table>

NOTE: —, could not be estimated as no cases were observed in this age range.

* All mutations in BRCA1 except 185delAG and 330A>G.

† Upper confidence limit greater than 999.
No heterogeneity in HRs for ovarian cancer was observed among recurrent mutations in BRCA1 ($P = 0.9; \text{df}, 3$). We also tested for differences in breast and ovarian cancer risk among carriers of 3036del4, 9254del5, and other mutations in BRCA2 pooled, but found no evidence of heterogeneity in their HRs ($P = 0.6; \text{df}, 4$).

**Cancer risks by mutation location.** Overall, there was no evidence of heterogeneity in HRs by mutation location relative to exon 11 for either cancer in either gene (for BRCA1 mutations, $P = 0.4; \text{df}, 4$; for BRCA2 mutations, $P = 0.8; \text{df}, 4$). HRs for breast cancer were less than 1 for mutations at the extreme ends of each gene, relative to those on exon 11. For BRCA1, neither breast cancer nor ovarian cancer risks seemed to differ according to role of the mutation in activating the nonsense-mediated mRNA decay mechanism ($P = 0.4; \text{df}, 4$), and the HRs associated with mechanism-activating mutations were greater than 1. Similarly, for BRCA2, there was no evidence that breast or ovarian cancer risks differed by mutation location relative to the OCCR as defined by Gayther et al. (ref. 37; $P = 0.9; \text{df}, 4$). For both breast and ovarian cancer, relative to mutations in the OCCR, HR estimates were less than 1 for mutations upstream and greater than 1 for mutations downstream. We also found no evidence that risks varied for mutations in the OCCR2 region more recently defined by Thompson et al. (ref. 21; $P = 0.7; \text{df}, 2$). For both breast and ovarian cancer, the HR estimates in our study were generally of similar magnitude to those of Antoniou et al. (7), with similar penetrance estimates and overlapping associated confidence intervals.

**Changing risks over time.** Strong evidence of a cohort effect, whereby the incidence of cancer in carriers has increased over calendar time, was detected for breast and ovarian cancer for BRCA1 mutation carriers ($P = 0.001; \text{df}, 2$). Further analyses suggested that this was almost entirely due to differences in breast cancer risk ($P = 0.0002; \text{df}, 1$), with an estimated HR of 4.7 per generation, on average (95% CI, 1.9-12). This effect was also seen for carriers of mutations in BRCA2 (overall: $P = 0.02; \text{df}, 2$; for breast cancer: $P = 0.006; \text{df}, 1$). The estimated HR per generation, on average, was 3.1 (95% CI, 1.4-6.6). Ovarian cancer HR estimates for later generations were greater than 1 for both BRCA1 and BRCA2 mutation carriers, but these differences were not statistically significant ($P = 0.9; \text{df}, 1$ and $P = 0.9; \text{df}, 1$, respectively).

**Discussion**

This study has combined data from a large number of families carrying deleterious BRCA1 and BRCA2 mutations, recruited at genetic counseling centers throughout Spain. The estimated average cumulative risk of breast cancer to age 70 years was estimated to be 52% (95% CI, 26-69%) for BRCA1 mutation carriers and 47% (95% CI, 29-60%) for BRCA2 mutation carriers. The corresponding estimates for ovarian cancer were 22% (95% CI, 0-40%) and 18% (95% CI, 0-35%) respectively. These results seem to be robust to the assumed allele frequencies and the assumed incidence of breast cancer in the Spanish population, as well as to the modeling of the risk of breast cancer for male BRCA2 mutation carriers. We found some evidence that the mutation 330A>G is associated with reduced breast cancer risk compared with other mutations in BRCA1 and that the average breast cancer penetrance of mutations in BRCA1 and BRCA2 in general, has increased over time.

Many studies have estimated the penetrance of mutations in BRCA1 and BRCA2. Antoniou et al. combined data from 22 international studies of carrier families and estimated the average cumulative risk of breast cancer to age 70 years to be 65% (95% CI, 44-78%) and 45% (95% CI, 31-56%) for BRCA1 and BRCA2 mutation carriers, respectively, and the corresponding cumulative risks of ovarian cancer to be 39% (95% CI, 18-54%) and 11% (95% CI, 2-19%; ref. 7). These estimates have been used in genetic counseling centers across the globe. Other studies before that of Antoniou et al. estimated much higher penetrances of 70% to 80% for breast cancer for mutations in both genes and around 60% and 30% for ovarian cancer in BRCA1 and BRCA2 mutation carriers, respectively (9, 14-16). All these earlier studies were based on families selected because they had multiple cases of breast and/or ovarian cancer. In contrast, all 22 studies combined by Antoniou et al. (7) recruited families of carrier cases unselected for family history. The observed differences in estimates have led some researchers to conclude that carriers from multiple-case families have a higher risk of breast and ovarian cancer than those with no family history (7).

It is therefore intriguing that our main results for breast cancer, based on predominantly multiple-case carrier families, are consistent with those of Antoniou et al. (7), with similar penetrance estimates and overlapping associated confidence intervals. This finding may be explained by the hypothesis that the increased cancer risks associated with mutations in these two genes is reflected in the average HRs and that the penetrance is a function of these and the underlying incidence of breast cancer in the population being considered. That is, the relatively low breast cancer incidence in Spain may also apply to mutation carriers and this may be explained by country-specific rates of exposure to nongenetic causes of the disease that apply to noncarriers and carriers alike. The HR estimates in our study were generally of similar magnitude to those of Antoniou et al. (ref. 7; see Table 3A and B). This was not expected, given that, if genetic modifiers of risk exist among mutation carriers, one would anticipate observing higher risks for carriers from multiple-case families than for carriers unselected for family history.

Our observation that the relative risk of ovarian cancer associated with being a mutation carrier was higher before age 50 years for BRCA1 and higher after age 50 years for BRCA2 is consistent with the results of Risch et al. (13) who found that the majority of hereditary ovarian cancers diagnosed before age 50 years were due to BRCA1, whereas the majority of those diagnosed at later ages were due to BRCA2. This finding, along with the differences in the pattern of relative risks of breast cancer by age, consistently observed in our study and that of Antoniou et al. (ref. 7; see Table 3A and B; Fig. 1A and B), suggests that carriers of mutations in BRCA2 may be able to delay decisions regarding prophylactic oophorectomy more than carriers of mutations in BRCA1, because the risk of cancer, and of ovarian cancer in particular, does not seem to increase sharply in this former group of women until after the age of 40 years, when many have already had children.

Chen and Parmigiani (40) have even more recently carried out a meta-analysis of all BRCA1 and BRCA2 penetrance
studies that both published a minimum set of data, and analytically took the selection of carrier families into account. They included the great majority of the previously cited studies, both of multiple-case families and of families of cases unselected for family history, and found no evidence of heterogeneity in penetrance estimates. The estimated average cumulative risk of breast cancer to age 70 years was 57% (95% CI, 47-66%) for BRCA1 mutation carriers and 49% (95% CI, 40-57%) for BRCA2 mutation carriers, whereas those for ovarian cancer were 40% (95% CI, 35-46%) and 18% (95% CI, 13-23%), respectively (40). Our main results are highly concordant with these estimates, the only exception being our lower estimate for the average ovarian cancer penetrance of mutations in BRCA1, although the confidence intervals overlap. It may be that the apparent variation between studies with distinct recruitment criteria is really due to chance. On the other hand, earlier studies tended to be smaller and so the power to detect between-study differences must have been limited.

We found evidence that the mutations 185delAG and 330A>G have lower associated breast cancer risks than the other mutations in BRCA1 carried by high-risk Spanish women. However, comparison of stratified HR estimates suggested that this was not consistent across all ages for 185delAG. It is not clear why the risk of breast cancer relative to other mutations in BRCA1 would be lower at intermediate ages but not at older or younger ages. This Jewish founder mutation has been included in a number of penetrance studies, none of which found evidence of reduced breast cancer risk relative to other mutations in BRCA1 (11, 19, 41). Furthermore, the mutation-specific estimate of penetrance to age 70 years was not substantially lower for 185delAG. In contrast, the evidence that the mutation 330A>G in BRCA1 has lower breast cancer penetrance to age 70 years was very consistent. This variant has been shown to be deleterious, causing aberrant splicing of the transcript that leads to a premature stop codon (42), and represents an estimated 50% of all mutations detected in women from Galicia, in the northwest of Spain (43). Although evidence was marginal (P = 0.09) that breast cancer risks varied between carriers of 330A>G and carriers of all other mutations in BRCA1 (including 185delAG) combined, the estimated cumulative risks of breast cancer to age 70 years were strikingly different, being 27% (95% CI, 0-52%) and 66% (95% CI, 34-83%), respectively.

We observed that breast cancer incidence among carriers of mutations in both genes seems to have increased over time, which is consistent with the findings of a number of other studies (7, 11, 22). Although increased surveillance (44), more accurate reporting over time, and possibly even ascertainment bias are likely to explain at least part of this effect, this constitutes strong evidence of the influence of nongenetic modifiers of the breast cancer penetrance of mutations in BRCA1 and BRCA2. If real, this trend over the last century should also be considered in genetic counseling, because it implies that young mutation carriers are now at higher risk of breast cancer than indicated by the average cumulative incidence estimated in women from all birth cohorts. It also suggests that the incorporation of environmental modifiers, once identified, will be important in refining risk prediction models.

On the other hand, we found no evidence that breast or ovarian cancer risk varied by mutation position for either gene, nor that, for BRCA1, risk differed for mutations shown to activate the nonsense-mediated mRNA decay mechanism. HR estimates were in the opposite direction, both to those previously reported (45) and to those expected under the hypothesis that mutations that do not activate the nonsense-mediated mRNA decay mechanism (and therefore generating high levels of truncated proteins) may cause higher risk than those that do (36). We similarly found no evidence that mutations in the most recently defined OCCR (OCCR2) of BRCA2 (21) are associated with increased ovarian cancer risk or decreased breast cancer risk. Although the power to detect differences for ovarian cancer was limited due to lower disease incidence, this finding is largely concordant with that of Al-Saffar and Foulkes (46), who argued that the significance of the OCCR remains uncertain.

One of the potential limitations of this study was missing information on year of birth, age at last contact, and age at diagnosis: The latter was for only a small number of cases; almost all were from the earliest generations. Missing age at last contact was imputed in a highly conservative way and cases with unknown age at diagnosis were censored at age zero. These imputations are unlikely to have caused bias, but will have slightly reduced the precision of estimates. The high proportion (>80%) of missing data on year of birth meant that we could only model changes in penetrance over time in terms of categories reflecting generations within families, and it is possible that there was some misclassification between families. However, the imputation method used to determine categories of birth year was such that any misclassification would be random and therefore unlikely to bias results. An additional limitation was that changes in cancer incidence in noncarriers over time were not modeled because population-based incidence data was not available more than 25 years before the IARC data used. This may also have exaggerated the estimates of the increase in breast cancer risk among carriers from successive generations. Finally, the estimates of the age-specific male breast cancer rates were based on limited and highly variable (between cancer registries) data from the IARC study (24), which is perhaps reflected in the very high HR estimate.

In summary, we have estimated the average cumulative risks of breast cancer and ovarian cancer to age 70 years, for carriers of deleterious mutations in BRCA1 and BRCA2 from families that attend genetic counseling centers in Spain. In general, the results are consistent with those from a recent meta-analysis of practically all previous penetrance studies (40), suggesting that Spanish women found to carry BRCA1 and BRCA2 mutations have similar risks of breast and ovarian cancer to those published for other Caucasian populations. It seems that the recurrent Galician mutation 330A>G in BRCA1 may have lower breast cancer penetrance to age 70 years. Our findings confirm that carriers should be fully informed of their mutation- and age-specific risks of breast and ovarian cancer to make appropriate decisions regarding prophylactic interventions such as oophorectomy. More generally, it seems that the identification of environmental modifiers of risk among carriers will be important in explaining risk heterogeneity.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.
Acknowledgments

We thank the patients and families without whose generous participation this study would not have been possible; Alicia Barroso who conducted all genetic testing at the Centro Nacional de Investigaciones Oncolólicas; Luz Álvarez who assisted with data collection; Guillermo Pita for information technology support; Marina Pollán and Fernando Artalejo from the Universidad Autónoma de Madrid; Drs. Montserrat Baiget and Agustí Barnadas from the Hospital de la Santa Creu i Sant Pau Dra Ana Fernández-Marmiesse from the Fundación Pública Gallega de Medicina Xénonómica; and Evan Esteban who carried out the genotyping for the Grupo de Cáncer Hereditario de la Comunidad Valenciana.

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Penetrance of Spanish BRCA1/2 Mutations
The Average Cumulative Risks of Breast and Ovarian Cancer for Carriers of Mutations in \textit{BRCA1} and \textit{BRCA2} Attending Genetic Counseling Units in Spain

Roger L. Milne, Ana Osorio, Teresa Ramón y Cajal, et al.


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