*Am. J. Hum. Genet. 62:1381–1388, 1998*

# Haplotype and Phenotype Analysis of Nine Recurrent *BRCA2* Mutations in 111 Families: Results of an International Study

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## Summary

**Several *BRCA2* mutations are found to occur in geographically diverse breast and ovarian cancer families. To investigate both mutation origin and mutation-specific phenotypes due to *BRCA2*, we constructed a haplotype of 10 polymorphic short tandem-repeat (STR) markers flanking the *BRCA2* locus, in a set of 111 breast or breast/ovarian cancer families selected for having one of nine recurrent *BRCA2* mutations. Six of the individual mutations are estimated to have arisen 400–2,000 years ago. In particular, the 6174delT mutation, found in ~1% of individuals of Ashkenazi Jewish ancestry, was estimated to have arisen 29 generations ago (1-LOD support interval 22–38). This is substantially more recent than the estimated age of the *BRCA1* 185delAG mutation (46 generations), derived from our analogous study of *BRCA1* mutations. In general, there was no evidence of multiple origins of identical *BRCA2* mutations. Our study data were consistent with the previous report of a higher incidence of ovarian cancer in families with mutations in a 3.3-kb region of exon 11 (the ovarian cancer cluster region [OCCR]) ($P = .10$); but that higher incidence was not statistically significant. There was significant evidence that age at diagnosis of breast cancer varied by mutation ($P < .001$), although only 8% of the variance in age at diagnosis could be explained by the specific mutation, and there was no evidence of family-specific effects. When the age at diagnosis of the breast cancer cases was examined by OCCR, cases associated with mutations in the OCCR had a significantly older mean age at diagnosis than was seen in those outside this region (48 years vs. 42 years; $P = .0005$).**

Received February 13, 1998; accepted for publication April 10, 1998; electronically published May 8, 1998.

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0002-9297/98/6206-0015$02.00

## Introduction

The isolation of *BRCA1* (Miki et al. 1994) and *BRCA2* (Wooster et al. 1995; Tavtigian et al. 1996), two genes predisposing to early-onset breast cancer and ovarian cancer, has resulted in rapid identification of a large number of families with mutations in these genes (Breast Cancer Information Core) (Couch et al. 1996*b;* Szabo and King 1997). Although both genes exhibit a large number of distinct mutations, several mutations have been found to recur in a number of independently as-

certained families of apparently diverse geographical origin, as well as in families largely confined to a single population.

Genes responsible for inherited cancer, like many other disease genes, have been associated with a wide diversity of expression. This is seen not only in variability in the age at diagnosis of cancer but also in the anatomical site at which the tumor originates. More important, at least from the clinical perspective, is the degree to which specific mutations and accompanying genetic backgrounds influence the expression of *BRCA2* in terms of site and age at diagnosis. For *BRCA2,* Gayther et al. (1997) have provided evidence that mutations in an ~3.3-kb nucleotide region of exon 11 (denoted the "ovarian cancer-cluster region" [OCCR]) are associated with a higher incidence of ovarian cancer relative to breast cancer. In that study, this was highly significant, with an ovarian: breast cancer ratio of 11:45 inside, and 22:282 outside, the OCCR. In the present studies, four of the mutations examined were within the OCCR, whereas the other five were outside this region. This allowed us to examine, with the present data set, the OCCR hypothesis.

In a previous paper (Neuhausen et al. 1996*b*), we analyzed six recurrent *BRCA1* mutations for haplotype conservation, over a 3-Mb segment containing the *BRCA1* gene, using nine STR markers. We also investigated the relationship between the position of the mutation and the phenotype (in terms of both age at diagnosis of breast cancer and proportion of ovarian cancer) of the families carrying each mutation. In the present article, we have undertaken a similar study of recurrent *BRCA2* mutations, addressing both mutation origin and the relationship between mutation and phenotype. To do this, we constructed a haplotype of 10 polymorphic STR markers flanking the *BRCA2* locus in a set of 111 families (selected to contain one of nine *BRCA2* mutations that had been identified a minimum of three times) and analyzed the phenotype associated with each mutation. For five mutations for which sufficient haplotype data existed, we estimated the age of the mutation, using a modified version of our mathematical model developed for our *BRCA1* analysis.

## Subjects and Methods

### *Family Ascertainment*

Families with one of the nine mutations were from 24 centers located in 13 countries in Europe and North America. The families had been previously ascertained for a variety of reasons, including research studies, directed screening of case series of ovarian or male breast cancer, or attendence at a cancer genetics clinic. Appropriate informed consent was obtained from all participants. When possible, pedigree information was obtained, although, for several centers, no such history was available and, for other centers, only a limited family history could be obtained. All cases of breast and ovarian cancer reported in the pedigree were included in the study, with the exception of cases who were known to not carry the *BRCA2* mutation segregating in the family. No independent verification of diagnosis was obtained, and, for a small proportion of cases, age at diagnosis was not available.

Samples for the 982del4 mutation were from the United States and France; those for 2041insA, from Germany, Canada, and the United States; those for 3034del4, from Belgium, Canada, Spain, France, Switzerland, Italy, and the United States; those for 4486delG, from Sweden; those for 5573insA, from the Netherlands; those for 6174delT, from Canada, France, Israel, Hungary, Sweden, the United Kingdom, and the United States; those for 6503delTT, from Belgium, the Netherlands, Sweden, and the United Kingdom; those for 9254del5, from France and Spain; and, those for 9326insA, from Hungary, Sweden, and the United Kingdom.

### *Genotyping of 13q Markers*

Genotyping was performed at four centers. The families collected by the University of Washington in Seattle, the National Institute of Oncology in Budapest, and the Fundacion Jimenez Diaz in Madrid were genotyped in their respective laboratories; all other families were genotyped in the Genetic Epidemiology Laboratory at the University of Utah. At all centers, the same five DNA samples were used as controls, and a similar protocol was followed. All 10 markers genotyped were STR loci assayed by PCR, with standard procedures. All the results in the tables are from analyses of all 10 markers. For all mutations except 6174delT, allele frequencies used in the likelihood calculations were as reported in Genome Database, from typings of ~80 independent CEPH chromosomes. For analysis of family samples of Ashkenazi Jewish ancestry carrying the 6174delT mutation, we estimated marker-allele frequencies from the haplotype data of the non–mutation-bearing chromosomes. In all cases, allele sizes were matched according to the genotype of CEPH reference individual 1347-02, who was used as a control on each gel. The genetic map assumed for the haplotype analyses was derived from physical-mapping data (Couch et al. 1996*a;* S. L. Neuhausen, unpublished data), under the assumption that 1.5 cM = 1 Mb. Note that this rate is higher than the usual 1:1 ratio assumed as a genomewide average; this was done to ensure that the total distance of the map was in agreement with that of the published genetic map (Dib et al. 1996). None of the markers were located intragenic to *BRCA2*. The assumed map order and dis-

**Table 1**

**Summary of STRs Used in Haplotype Analysis**

| Marker | Position (cM) | No. of Alleles | Heterozygosity[a] (%) | Size (Frequency) of Common Allele (bp) | Genotype of 1347-02 (bp) |
|---|---|---|---|---|---|
| D13S290 | 2.70 | 6 | 46 | 176 (.71), 190 (.11), 188 (.11) | 190/176 |
| D13S1444 | 1.35 | 9 | 80 | 167 (.41), 169 (.24), 177 (.11) | 177/167 |
| D13S1700 | 1.20 | 18 | 89 | 308 (.12), 312 (.09), 258 (.09) | 320/254 |
| D13S260 | 1.00 | 9 | 78 | 163 (.41), 161 (.13), 171 (.09) | 163/161 |
| D13S1699 | .72 | 6 | 67 | 150 (.54), 146 (.37) | 156/146 |
| D13S1698 | .63 | 10 | 63 | 152 (.35), 154 (.30) | 168/160 |
| BRCA2 | .0 | | | | |
| D13S171 | −.60 | 6 | 72 | 241 (.32), 231 (.32), 227 (.25) | 231/231 |
| D13S1695 | −.96 | 11 | 79 | 245 (.37), 247 (.23) | 249/235 |
| D13S310 | −2.10 | 5 | 70 | 146 (.40), 144 (.24), 140 (.24) | 146/146 |
| D13S267 | −3.12 | 6 | 69 | 148 (.44), 160 (.29), 154 (.17) | 160/148 |

[a] Determined from genotyping of 80–100 chromosomes.

tances and the descriptions of the markers used are given in table 1.

When possible, haplotypes associated with each mutation were inferred from multiple samples of related individuals within each kindred known to have the same mutation; otherwise, multilocus genotypes were compared. When haplotypes could not be determined with certainty, all possible haplotypes (to a maximum of 64) consistent with the observed multilocus genotypes were considered in the likelihood analysis, in a manner analogous to the phase calculations in multipoint linkage analysis.

### *Analysis of Haplotype Data*

The estimation of the age of the mutations was performed by use of the same statistical model that had been used in our previous analysis of *BRCA1* (Neuhausen et al. 1996*b*), with several minor modifications. In brief, the joint likelihood of the *BRCA2* haplotypes (or all possible haplotypes from families with a given mutation, relative to a presumed ancestral haplotype) is written as a function of the recombination fraction between the disease and each marker; the number of generations, *G,* since the mutation arose; and the mutation rate and allele frequencies at each marker locus. The marker D13S1700 was assumed to have a higher mutation rate (.01) than the other markers (.002 for a tetranucleotide repeat and .0006 for a dinucleotide repeat), on the basis of both the large number of alleles and the observation of mutations within families. We also included another parameter, $\mu_D$, the proportion of families with an independent mutation identical to that of the presumed ancestral haplotype. This parameter is analogous to genetic heterogeneity in standard linkage analysis and can be estimated from the data.

The method of maximum likelihood was used to find the value of *G* that, among families with identical mutations, best fitted the pattern of haplotype sharing at the 10 marker loci. Approximate support intervals for the age of each mutation were calculated by finding the value of *G* on either side of the most likely value that had a $\geqslant$10-fold decrease in likelihood. A test for heterogeneity of mutation origin was performed by comparing the likelihood at the maximum-likelihood estimates of *G* and $\mu_D$ with the analogous likelihood, assuming $\mu_D = 0$. Each generation is estimated to be 20 years.

### *Analysis of Phenotype Data*

For each mutation, the number of families with that mutation, the number of female and male breast cancer cases, and the number of ovarian cancer cases were tabulated. To partially counter any effects of ascertainment of those directed-screening cases of breast and ovarian cancers, we also examined the data only in those families in which there were at least three cases of cancer, where a case is defined as a female breast cancer at age <60 years, an ovarian cancer, or a male breast cancer. In this second tabulation, only cases of female breast cancer at age <60 years were counted in the breast cancer results; this was done in order to increase the probability that they were associated with the *BRCA2* mutation segregating in the family.

To test for heterogeneity, in the proportion of affected individuals who had ovarian cancer, as a function of whether the mutation associated with a given family was inside or outside the hypothesized OCCR, a randomization test was performed. Specifically, random permutations of families with the nine mutations were performed, in which the number of families with each mutation was kept equal to that present in the actual data set. After this permutation step, the mutations were grouped according to their location respective to the OCCR. Each such permutation resulted in a different

**Table 2**

**Results of Haplotype Analysis of Nine Mutations**

| Mutation | No. of Families[a] | No. of Countries[b] | Core Haplotype at | | | | | Consistency Index[c] | *G* | 1−LOD Interval |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | D13S260 | D13S1699 | D13S1698 | D13S171 | D13S1695 | | | |
| 982del4 | 5 (3/2) | 2 | 161 | 146 | 154 | 231 | 253 | 5/5 | 18 | (4–43) |
| 2034insA | 5 (3/2) | 3 | 163 | 150 | 166 | 241 | 247 | 3/5 | 36 | (13–64) |
| 3034del4 | 11 (4/7) | 7 | 163 | 146 | 154 | 227 | 245 | 2/11 | 80 | (46–134) |
| 4484delG | 4 (0/4) | 1 | 169 | 156 | 166 | 241 | 247 | 4/4 | Not calculated | |
| 5573insA | 3 (3/0) | 1 | 165 | 146 | 154 | 227 | 245 | 2/3 | Not calculated | |
| 6174delT | 69 (22/47) | 7 | 161 | 146 | 152 | 239 | 251 | 45/69 | 29 | (22–38) |
| 6503delTT | 7 (5/2) | 4 | 163 | 150 | 158 | 227 | 245 | 3/5 | 52 | (24–98) |
| 9254del5 | 3 (2/1) | 2 | 163 | 150 | 154 | 231 | X | 2/3 | Not calculated | |
| 9326insA | 4 (1/3) | 3 | 171 | 152 | 152 | 231 | 245 | 2/4 | Not calculated | |

[a] Data in parentheses are number of families in which haplotypes could be determined/number of families for which only multilocus genotype data were available.

[b] For names of countries, see the Subjects and Methods section.

[c] Number of samples/families consistent with core haplotype for all five markers listed.

$2 \times 2$ table with an associated $\chi^2$ statistic calculated in the standard fashion. The $\chi^2$ statistic associated with the observed aggregation of cases and mutations was compared with those calculated from 2,000 random permutations of families and mutations. The rank of the observed $\chi^2$ statistic among those from 2,000 replicates is the nominal *P* for testing the association between the prevalence of ovarian cancer and a specific mutation. The S-Plus package (StatSci) was used to perform the randomization test. Phenotypic analysis of age at diagnosis, among mutations, was performed by the T-TEST, GLM, and VARCOMP procedures of the SAS statistical analysis package.

## Results

### *Haplotype Analysis and Age of Mutations*

The mutations described in this report span the *BRCA2* gene and are small insertions or deletions that cause protein truncation. In table 2, the mutations are characterized as to the number of families studied, the numbers of genotypes and haplotypes obtained, and the geographic diversity (as based on the number of countries from which samples were contributed). The most common haplotype associated with each of the nine mutations studied, as well as the estimated *G*, support interval, and estimated heterogeneity for those mutations with at least five haplotypes to analyze are also shown in table 2. Although the estimation of the ages of the mutations incorporated data from all 10 markers, we report the consensus haplotype at the six markers closest to *BRCA2,* since, in many cases, the haplotype beyond these markers was difficult to determine. For four of the five mutations examined, the estimated fraction of families in which cancer was due to an independent mutational event was 0; for 6503delTT, the estimated proportion was .11, which is not significantly different from 0. For 6174delT, the 1-LOD upper bound for the proportion attributable to one (or more) independent identical mutations was .06. In all cases, there was no significant evidence of mutational heterogeneity, indicating that, for each mutation studied, all families with the mutation represent derivations from a single ancestral haplotype on which the mutation arose. The estimates of *G* are based on assumptions about mutation rates and recombination rates and therefore may be more appropriately considered as relative indications of time since the mutation originated, rather than as absolute values. We estimate the 982del4, as an example, to have occurred relatively recently—that is, 18 generations ago (1-LOD support interval 4–43), or ~360 years ago (1-LOD support interval 80-860 years).

### *Association between Phenotypic Variation and Mutations*

A summary of the number of cases of breast and ovarian cancers and the ages at diagnosis of the breast cancer cases, stratified by *BRCA2* mutation type, is shown in table 3, for all families with all breast cancer cases and for those "high-risk" families (as described in the Subjects and Methods section) that have breast cancer cases diagnosed at age <60 years. There was significant variation in age at diagnosis among the nine mutations tested when all cases in all families were considered ($P = .0007$, by nested ANOVA), as well as when the analysis was restricted to high-risk families and cases diagnosed at age <60 years ($P = .015$), although only ~8% and ~6%, respectively, of the variance was explained by individual mutation. In both analyses, there was no evidence of significant variation between families, for any mutation group, and the variance due to this effect was estimated to be zero in both cases.

**Table 3**

**Summary of Phenotypic Data Associated with Mutation**

| | All Families[a] | | | | Families with ⩾3 Cases[b] | | | |
|---|---|---|---|---|---|---|---|---|
| | | No. of Cancer Cases | | | | No. of Cancer Cases | | |
| Mutation | No. | Female Breast (Age [years]) | Ovarian | Male Breast | No. | Female Breast at Age <60 Years (Age [years]) | Ovarian | Male Breast |
| 982del4 | 5 | 25 (41) | 1 | 4 | 4 | 20 (38) | 1 | 4 |
| 2041insA | 5 | 16 (41) | 4 | 5 | 4 | 11 (39) | 3 | 5 |
| 3034del4 | 11 | 37 (42) | 6 | 2 | 9 | 33 (42) | 5 | 2 |
| 4486delG | 5 | 16 (48) | 0 | 3 | 1 | 6 (44) | 0 | 0 |
| 5573insA | 3 | 5 (47) | 7 | 0 | 2 | 2 (40) | 7 | 0 |
| 6174delT[c] | 67 | 119 (49) | 29 | 12 | 22 | 60 (46) | 12 | 8 |
| 6503delTT | 7 | 20 (44) | 12 | 1 | 6 | 18 (44) | 12 | 1 |
| 9254del5 | 3 | 16 (48) | 3 | 3 | 3 | 11 (43) | 3 | 3 |
| 9326insA | 4 | 9 (34) | 0 | 2 | 1 | 3 (35) | 0 | 1 |
| Total | 110 | 263 (45.6) | 62 | 32 | 52 | 164 (42.7) | 43 | 24 |

[a] Includes all families on which at least some phenotypic information was available. Breast cancer tabulation contains all cases of breast cancer, regardless of age, as well as those cases for which age at diagnosis is unknown.

[b] Families with at least three cases of cancer, where a case is defined as a female breast cancer at age <60 years, an ovarian cancer, or a male breast cancer. Only the cases of female breast cancer at age <60 years are included in the results.

[c] In 13 families obtained from a consecutive series of Ashkenazi Jewish ovarian cancer patients tested only for the 6174delT mutation, the ovarian cancer proband was omitted from this table and subsequent analyses; however, the proband was used in determining whether the family had three or more cases.

### *Examination of the OCCR*

The randomization test described in the Subjects and Methods section was used to examine possible differences in the relative proportions of cases of breast and ovarian cancers, for mutations inside and outside the OCCR. These results are shown in table 4. It is clear that there is a higher proportion of ovarian cancer cases associated with families with mutations in the OCCR region, although this difference is not significant for either the complete data set ($P = .12$) or the high-risk subset ($P = .11$). The odds ratio for the entire set of families is 2.1. Interestingly, when we examined the age at diagnosis of the breast cancer cases in terms of OCCR status, we found that most of the age-at-onset variation between mutations could be ascribed to the location relative to the OCCR. This difference, of older age at onset for the OCCR region, was highly significant, both for the nested analysis of variance with between-family variation used as the error term and by ordinary *t*-test. Because the 6174delT mutation group was the largest and had the oldest age at onset, we also performed the analysis of age at onset and OCCR again, without this group. When we removed the cases with a 6174delT mutation, the effect of the mutation location in the OCCR is still present but is not significant ($P = .09$).

## Discussion

In this paper, we have analyzed genotypic and phenotypic data from a series of breast cancer families and from isolated cases with one of nine recurrent mutations in the *BRCA2* gene. These data appear to include both population-specific sequence variants, as well as those found in more geographically diverse populations of northern European Caucasian ancestry. The mutation with the oldest estimated age, 3034del4, was found in the most diverse set of samples (except for the 6174delT mutation in the Ashkenazi population), both in multiple centers in the same country and in seven different countries. For the mutations studied, the multiple instances of specific mutations generally appear to represent founder effects many generations in the past, rather than independent mutational events. This is in contrast to the *BRCA1* mutations—4184del4, Arg1443ter, and 185delAG—which, on the basis of the multiple origins of these mutations, may represent hot spots (Neuhausen et al. 1996*b*).

The 4486delG mutation has been reported only in Scandanavia (Håkansson et al. 1997). For this study, there were too few haplotypes to determine the age of the mutation. However, all four samples (three from sporadic male breast cancer cases and one large breast cancer family) genotyped with this mutation appeared to share a conserved haplotype over an ~3-cM interval containing the *BRCA2* locus. A similar pattern was observed in the three Dutch families carrying the 5573insA mutation. The 9254del5 mutation has been identified only in two French families of Catalan origin and in a single Spanish family also from this region. The three families share a conserved haplotype over an ~2-cM region spanning the *BRCA2* locus. These three families have different phenotypes, with one family having three cases

**Table 4**

**Examination of OCCR**

| Mutation Location | All Families[a] | | | | Families with ⩾3 Cancer Cases[b] | | | |
|---|---|---|---|---|---|---|---|---|
| | | No. of Cancer Cases | | | | No. of Cancer Cases | | |
| | No. | Female Breast (Age [years]) | Ovarian | Male Breast | No. | Female Breast at Age <60 Years (Age [years]) | Ovarian | Male Breast |
| OCCR$^+$ | 82 | 160 (48.0) | 48 | 17 | 31 | 88 (44.9) | 31 | 9 |
| OCCR$^-$ | 28 | 103 (41.9) | 14 | 16 | 21 | 76 (40.3) | 12 | 15 |

[a] As defined in table 3. For age at diagnosis, $P < .0001$; for breast cancer versus ovarian cancer, $P = .12$.
[b] As defined in table 3. For age at diagnosis, $P < .0005$; for breast cancer versus ovarian cancer, $P = .11$.

of male breast cancer and four cases of female breast cancer, a second family having three cases of ovarian cancer, and a third family having eight site-specific cases of female breast cancer.

By contrast, the 3034del4 mutation has been found in families in seven different western European and North American countries (Belgium, Canada, France, Italy, Spain, Switzerland, and the United States). There was a considerable amount of haplotype diversity among the 11 families examined, accounting for the large value of the estimated age. Although our analysis failed to find significant statistical evidence of multiple independent origins for this mutation (the maximum-likelihood estimate for the proportion due to independent mutation is 0), given the limited number of families available for analysis, statistically we could not rule out the possibility that there were independent mutations for as many as half the families. This mutation is in a region that may be a hot spot for such deletions. Another 4-bp deletion, located only 2 bp downstream, has been reported in five families thus far, and a 2-bp deletion located 4 bp downstream has been reported once (Breast Cancer Information Core).

Of particular interest is the 6174delT mutation found in high frequency in the Ashkenazi Jewish population. Along with the two *BRCA1* mutations (185delAG and 5382insC), it has been estimated that 1 in 50 Ashkenazi Jewish individuals carry one of these three mutations (Struewing et al. 1995, 1997; Oddoux et al. 1996; Roa et al. 1996). These mutations account for ~30% of early-onset breast cancer (Neuhausen et al. 1996*a;* Offit et al. 1996; Tonin et al. 1996) and for as much as 60% of all ovarian cancer in this population (Abeliovich et al. 1997). On the basis of our analysis of haplotypes and genotypes of 69 families with the 6174delT mutation, we estimate that the mutation originated ~29 generations ago (1-LOD support interval 22–38). The corresponding analysis for the age of the *BRCA1* 185delAG, on the basis of our original set of 18 families with this mutation, resulted in an estimate of 46 generations (1-LOD support interval 22–82) and suggested that the cases in ~90% of the families are due to the presumed ancestral Jewish mutation (an estimate reflecting the fact that two families of non-Jewish ancestry were part of the sample). Thus, the 6174delT mutation appears to have originated more recently. Support for the more recent origin of the 6174delT mutation comes from examination of these mutations in 44 non-Ashkenazi Jewish patients. One Iraqi patient had a 185delAG mutation, and none had a 6174delT mutation (Abeliovich et al. 1997). Sher et al. (1996) also reported a 185delAG mutation in an Iraqi Jew, suggesting that this mutation has an origin earlier than that of the 6174delT mutation. More recently, an additional three *BRCA1* 185delAG mutations have been identified, in a sample of 639 Iraqi Jews (Bar-Sade et al. 1997), but, to our knowledge, the 6174delT mutation has never been found outside the Ashkenazi Jewish population.

Our analysis was consistent with the finding by Gayther et al. (1997)—that is, that there is a higher incidence of ovarian cancer relative to breast cancer associated with the OCCR; however, this higher incidence was not statistically significant. One possible reason for the difference between the significance presented here and that reported by Gayther et al. (1997) could be the ill-defined 5′ end of the OCCR. The 3034del4 mutation is on the 5′ border of the OCCR, as defined by Gayther et al. (1997), and its exclusion, rather than inclusion in the OCCR, could have an affect on the analysis.

Among the mutations, there were significant differences associated with age at diagnosis of breast cancer. Much of the variation was associated with mutation location relative to the OCCR. However, when we removed the cases with a 6174delT mutation, the effect of the mutation location in the OCCR, although still present, was not significant. The later age at onset of breast cancer in the cases with the 6174delT mutation could be due to ease of screening families for this common mutation. However, the age effect is still present in those families with three or more cancer cases who would likely be screened in any testing program, suggesting that mutations within the OCCR and/or, more specifically, the 6174delT mutation do confer a later age at onset of breast cancer. On the basis of previous studies

of two common mutations, there is a suggestion that mutations in the OCCR are less penetrant for breast cancer at a younger age. In the Icelandic studies of the 999del5 mutation, which is outside the OCCR, 28% of Icelandic breast cancer cases of age <40 years carry this mutation, which has a population prevalence of 0.50%. In contrast, for the 6174delT mutation, which is within the OCCR, 8% of Ashkenazi Jewish breast cancer cases of age <40 years carry this mutation, which has a population prevalence of 1.2%. Therefore, with a prevalence twofold higher for the 6174delT mutation, there is a large difference, in comparison with the Icelandic mutation, for age at onset of breast cancer, suggesting lower penetrance at age <40 years.

As a first step in mutation detection, comparison of an observed haplotype in a family examination of haplotypes can be useful to identify common mutations. In addition to this set of haplotypes for recurrent mutations, we are also constructing a haplotype database of any mutations, so that others can compare their haplotypes (for further information, please contact S.L.N.). A haplotype database of Dutch mutations is available from a Leiden University Medical Center Department of Human Genetics Website. Since multiple families with identical mutations on identical genetic backgrounds can be ascertained, this will allow us to better elucidate additional genetic and environmental factors that contribute to the observed variation in phenotype. Similarly, studies of families with identical mutations but with different origins will allow us to examine better the possible effect of genetic modifier loci. A copy of the revised version of the haplotype-analysis program is available, on request, from D.E.G.

## Acknowledgments

The authors wish to thank the many families who participated in the research studies described in this article. This work was supported by U.S. Department of Defense grants DAMD-17-94-J-4260 and DAMD 17-96-I-6266 (both to S.L.N.), by the Canadian Breast Cancer Foundation and U.S. Department of Defense grant DAMD-17-J-4299 (both to S.A.N.), by National Institutes of Health grant HG-00571 (to D.G.), by Hungarian research grants OMFBIEU 96-09-004 and OTKA T19307 (both to E.O.), by Spanish Ministry of Education grant CICYT 96/0192 (to A.O. and J.B.), by National Institutes of Health grant R01 CA27632 (to E.S. and M.-C.K.), by Swedish Cancer Society grants (to A.B.), by an Italian Association for Cancer Research grant (to M.A.C.), and by Cancer Research Campaign program grants. B.A.J.P. is a Gibb Fellow of the CRC. This work was also supported by the Utah Cancer Registry, which is funded by National Cancer Institute contract N01-CN-6700, with additional support from the Utah Department of Health and the University of Utah. We also want to thank clinicians and researchers at all the institutions, including Håkan Olsson, Oskar Johannsson, Niklas Loman, and Ulf Kristoffersson from the Oncogenetic Group at University Hospital in Sweden; and Mary Daly, Josephine Costalas, and JoEllen Dangel from Fox Chase Cancer Center. We also thank Thao Tran for performing the genotyping at the University of Utah. A.O. is a fellow of the Funación Conchita Rábago.

## Electronic-Database Information

URLs for data in this article are as follows:

Breast Cancer Information Core, http://www.nchgr.hih.gov/intramural_research/Lab_transfer/Bic

Leiden University Medical Center Department of Human Genetics ("Haplotypes carrying *BRCA1* mutations found repeatedly in the Dutch population"), http://ruly70.medfac.leidenuniv.nl/~devilee/hapover.htm

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