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Penetrances of BRCA1 1675delA and 1135insA with Respect to Breast Cancer and Ovarian Cancer

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Summary

For genetic counseling and predictive testing in families with inherited breast-ovarian cancer, penetrances and expressions of the underlying mutations should be known. We have previously reported two BRCA1 founder mutations in the Norwegian population. Index cases for the present study were found two different ways: through a series of consecutive ovarian cancers (n = 16) and through our family cancer clinic (n = 14). Altogether, 20 of the patients had BRCA1 1675delA, and 10 had 1135insA. Their relatives were described with respect to absence/presence of breast and/or ovarian cancer. Of 133 living female relatives, 83 (62%) were tested for the presence of a mutation. No difference, in penetrance and expression, between the two mutations were found, whereas differences according to method of ascertainment were seen. The overall findings were that disease started to occur at age 30 years and that by age 50 years 48% of the mutation-carrying women had experienced breast and/or ovarian cancer. More ovarian cancers than breast cancers were recorded. Both penetrance and expression (breast cancer vs. ovarian cancer) were different from those in reports of the Ashkenazi founder mutations. Whether the reported differences reflect true differences and/or methodological problems is discussed. An observed excess of mutation carriers could not be accounted for by methodological problems; possible explanations were a "true" low penetrance or preferential segregation.

Introduction

The majority of inherited breast-ovarian cancer and inherited ovarian cancer (MIM 113705) is caused by mutations in the BRCA1 gene (Easton et al. 1995; Narod et al. 1995; Liede et al. 1998). The reported lifetime risks for breast and/or ovarian cancer in female BRCA1-mutation carriers vary from 36% to 95% (Easton et al. 1995; Fodor et al. 1998). It is not clear whether this is due to ascertainment problems in the series analyzed, true differences between penetrance of different mutations, or modifying factors (environmental or genetic) in separate populations (Narod et al. 1995; Katsouyanni et al. 1997). In addition, it is debated whether the ratio between breast and ovarian cancer varies according to the position of the mutation within the gene (Easton et al. 1995; Gayther et al. 1995).

We have approached the problem by describing BRCA1 founder mutations in the Norwegian population. Two such are presently known: 1675delA (Dørum et al. 1997) and 1135insA (Andersen et al. 1996). Both result in frameshift and stop in exon 11. 1675delA is most frequent and has been prospectively demonstrated as a shared BRCA1 haplotype among affected families. Today's living kindreds with this haplotype represent branches of one extensive family (for details, see Dørum et al. 1997). Preliminary data suggest that the families with 1135insA also share one BRCA1 haplotype. There has been a large number of meioses since the founder person living 20–30 generations ago. All index cases in this report are included in the Breast Cancer Information Core database. Consequently, the families today may not share genetic variation outside the BRCA1 haplotype that they have in common. They live geographically separated. This gives an opportunity to assess the penetrance of the mutation-bearing haplotype. It does not, however, solve the problems related to ascertainment. We here describe what we have observed in kindreds with the two Norwegian founder mutations.

Patients and Methods

Identification of Families

Family cancer-clinic series.—Families were either self-referred or referred by a physician and were included if they had (*a*) one member with ovarian cancer who had one first-degree relative (or second-degree relative, through a male) with ovarian cancer or breast cancer at

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Table 1

General Data on Group Studied

	No. of Mutations Demonstrated (No. of Index Cases)/ No. in Whom Mutations Are Absent/No. Not Examined				
	Consecutive Ovarian Cancer–Patient Series		Family–Cancer Clinic Series		
	1675delA	1135insA	1675delA	1135insA	Total
No. of families	11	5	9	5	30
No. of cases of breast cancer:					
Alive	7(2)/0/6	0/0/0	3(3)/2/2	2(5)/1/2	12(10)/3[a]/10
Dead	0/0/13	0/0/5	0/0/15	0/0/6	0/0/39
No. of cases of ovarian cancer:					
Alive	2(11)/0/2	2(5)/0/0	1(6)/0/2	0(1)/1/0	5(23)/1[a]/4
Dead	0/0/25	0/0/9	0/0/18	0/0/8	0/0/60
No. of unaffected sisters:					
Alive	9/9/16	11/7/11	8/9/8	2/5/1	30/30/36
Dead	0/0/8	0/0/5	0/0/11	0/0/4	0/0/28
No. of women:[b]					
Alive	16(11)/9/24	12(5)/7/11	11(9)/11/12	4(5)/7/3	43(30)34/50
Dead	0/0/45	0/0/18	0/0/44	0/0/16	0/0/123
Overall[b]	16(11)/9/69	12(5)/7/29	11(9)/11/56	4(5)/7/19	43(30)/34/173

[a] Mutation-negative phenocopies.

[b] The total number of women with breast and/or ovarian cancer is less than the sum of such cases because 11 (including 3 index cases) had experienced both types of cancer.

age <60 years of age *and/or* (*b*) one member with both breast cancer at age <60 years of age *and* ovarian cancer (for details, see Møller et al. 1998). DNA from affected individuals in 222 separate breast-ovarian cancer kindreds were examined. We found nine families that had BRCA1 1675delA and five families that had 1135insA.

Consecutive ovarian-cancer series.—A series of 727 consecutive ovarian-cancer patients from a defined geographic area (southern Norway) from May 1993–January 1996 were invited to participate. After informed consent was given, we obtained blood samples from 615 patients and demonstrated 1675delA in 11 of the families and 1135insA in 5 of the families. The families from the two initial reports (Andersen et al. 1996; Dørum et al. 1997) are included in the present report.

Index Patients

In the family cancer-clinic series, one index patient per family was identified, who was either the referred patient, if affected, or the closest related affected relative (or the younger/youngest if the family contained two or more affected same-degree relatives). Of the consecutive ovarian-cancer families, 15 had one index case each. One family had three incident cases included in the series. The first case encountered was considered to be the index case, and the family was counted once only. Index patients are identified in all tables if included there, and the effect of inclusion/exclusion from the analyses is discussed.

Pedigree Extension

The pedigrees were extended in all directions. Reported cancers were verified whenever possible. Under the assumption that dominantly inherited breast-ovarian cancer affected the families, the highest penetrance (i.e., the lowest number of unaffected female obligate carriers) was determined in each family. All living affected individuals or obligate carriers were approached to verify carrier status by testing. Subsequently, all sisters and adult daughters of demonstrated carriers, untested affected individuals, and obligate carriers were invited to participate in predictive mutation testing. All procedures were performed in a health-service setting and according to Norwegian legislation. Relatives were approached through the index patients. Written informed consent was obtained for all predictive testing. All results were included in medical files. The present report reflects the information filed as of July 1, 1998.

Statistical Analyses

Actuarial calculations of age-related penetrances were performed by the Kaplan-Meier algorithm in the SPSS computer program. Each person was censored at either last observation or death. Thirteen women who had prophylactic oophorectomy were censored at the date of oophorectomy, in calculations including ovarian cancer as event. None of the women had undergone prophylactic mastectomy. In each calculation, events were considered at the date of demonstrated disease. In historical cases in which age at diagnosis could not be determined,

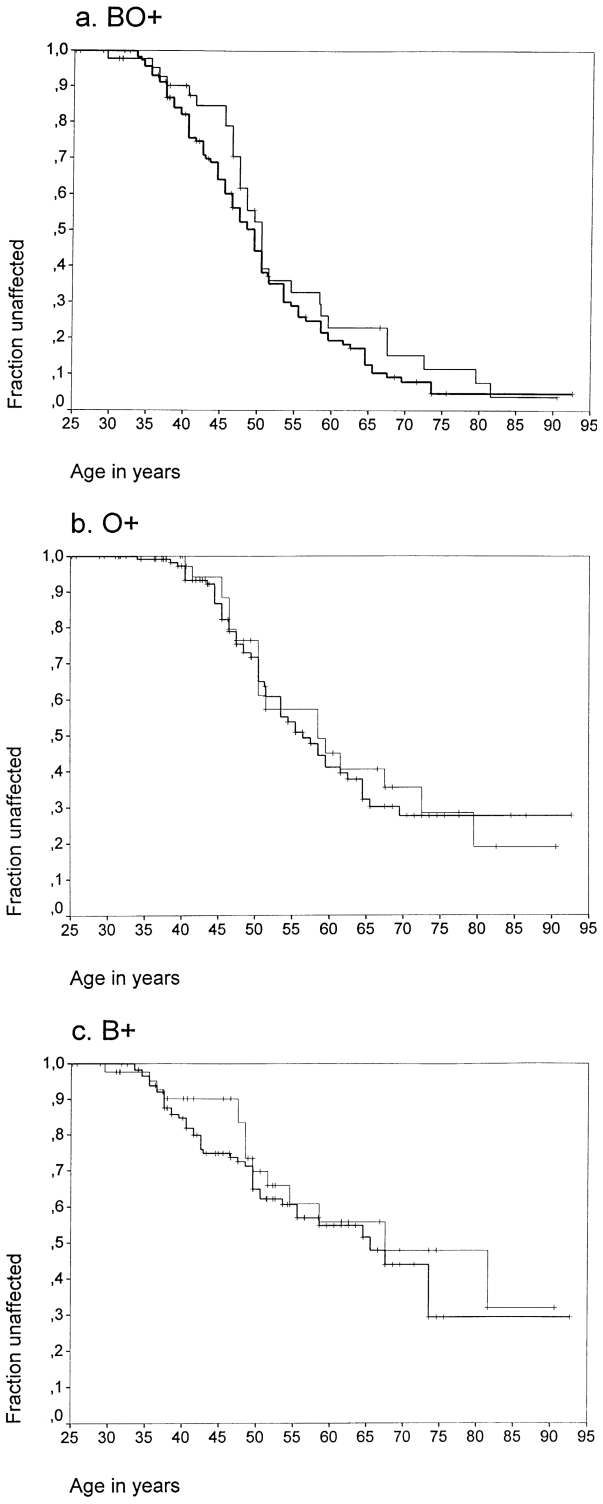

Figure 1 Age-related penetrances of BRCA1 1675delA (*thicker line*) and 1135insA (*thinner line*) in mutation carriers (demonstrated, affected, or obligate). Index cases are not included. *a*, Breast and/or ovarian cancer (whichever came first) scored as event. *b*, Ovarian cancer scored as event (breast cancer considered as unaffected). *c*, Breast cancer scored as event (ovarian cancer considered as unaffected).

age at death was used. Events were considered in three ways: as breast or ovarian cancer (whichever came first), as ovarian cancer (with breast cancer considered as unaffected), or as breast cancer (with ovarian cancer considered as unaffected). Since both ovarian cancer and breast cancer are associated with BRCA1 mutations, censoring for the one when considering the other as event implies the problem of informative censoring (Collett 1994, p. 274); because both expressions are lethal and the patients inevitably are censored at death, this cannot be avoided. Censoring at age at diagnosis as well as at age of death was employed (see the Informative Censoring subsection, below).

For the analyses of penetrances, we included all living or dead female mutation *carriers* (either as demonstrated by either mutation *testing* or *affected* state or as identified as unaffected *obligate* carriers). In addition, we included all of their sisters, as described below.

Contralateral breast cancer was not considered. Occurrences of multiple primary tumors (bilateral breast or breast+ovarian) were not addressed.

As detailed in the Results section (see below), the series were initially examined for differences related to ascertainment criteria and the nature of the mutation. Because such differences were not found, the series were grouped together as one series for further analyses.

Phenocopies were expected, both as in-married carriers of distinct mutations, in the family cancer-clinic series, and as sporadic age-related cancers, in both series. All phenocopies demonstrated were young and in the family cancer-clinic series, as would be expected if they are caused by in-married mutations (see Discussion below). Sporadic age-related cancers were expected to occur at later ages. We stopped the final analysis of penetrance at age 69 years, thereby excluding the older putative sporadic cancers, and we considered all calculated penetrances at >50 years of age to be possibly influenced by the presence of phenocopies and by fluctuations in small numbers.

Mutation Analyses

Peripheral ethylenediaminetetraacetate-treated blood was collected, and DNA was extracted from frozen blood by means of the Nucleon kit (Amersham Life Sciences).

Fragments analysis.—50 ng of sample DNA was used for a multiplex PCR-based fragment analysis. The reaction was performed in 50 μl at pH 8.3, containing 0.1 M Tris, 0.5 M KCl, and 1.5 mM $MgCl_2$, 1 mM of each dNTP, 50 pmol of each of the four primers (BRCA1A, BRCA1B [for analysis of a fragment containing 1135insA], 1675U, and 1675L [for analysis of a fragment containing 1675delA]; MedProbe), and 1 unit of *Taq* polymerase produced in house. The 30 PCR cycles

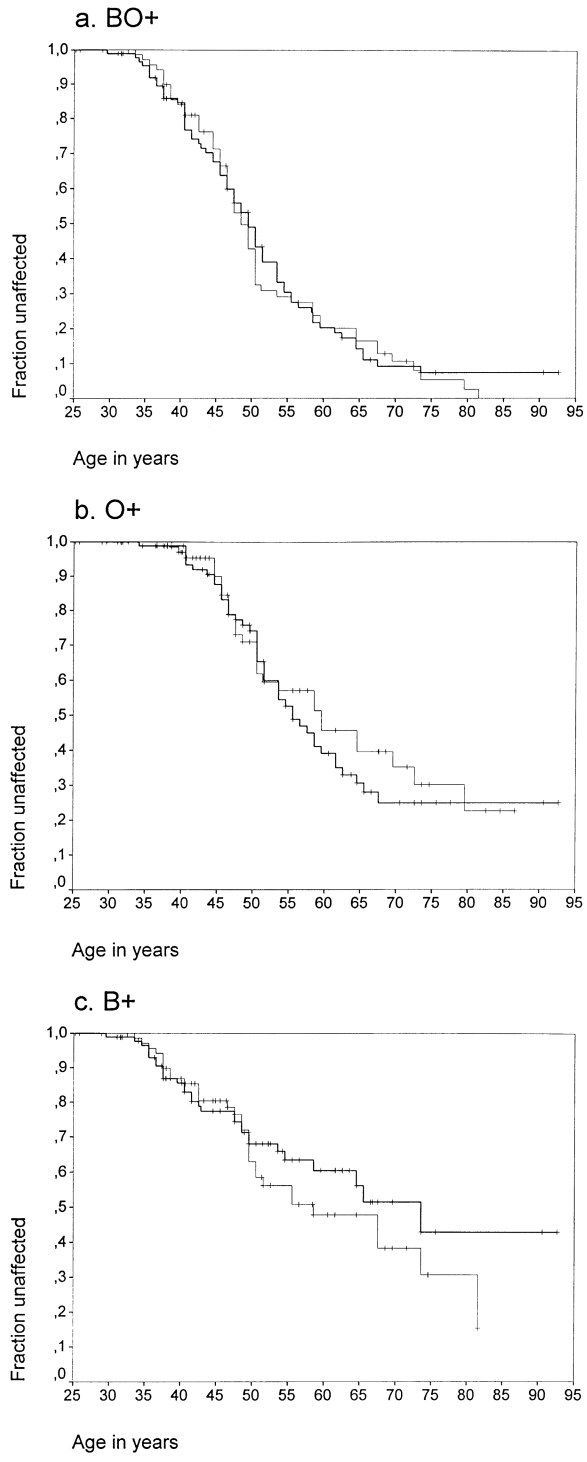

Figure 2 Age-related penetrances of BRCA1 1675delA and 1135insA in mutation carriers (demonstrated, affected, or obligate). Consecutive ovarian-cancer series (*thicker line*) and family cancer-clinic series (*thinner line*). Index cases are not included. *a*, Breast and/or ovarian cancer (whichever came first) scored as event. *b*, Ovarian cancer scored as event (breast cancer considered as unaffected). *c*, Breast cancer scored as event (ovarian cancer considered as unaffected).

were performed in a GeneAmp System 7600 cycler (PE-AB) at 94°C , 60°C, and 72°C, for 30 s at each step. The resulting two fragments were subjected to gel electrophoresis after denaturation by means of the Alf Express™ system (Pharmacia Biotech) for 160 min, and both fragments were scored for single insertions or deletions (i.e., fragments with primer BRCA1A/B or 1675U/L, respectively).

Sequencing.—After identification of probable mutants, the verification procedure consisted of sequencing of a PCR product. The PCR amplification was performed under the same conditions, with some modifications. The two fragments were amplified separately, either by means of 50 pmol of non–Cy5-labeled primers BRCA1A and BRCA1B, for insA1135, or by means of a different primer set, 11624 and 11716, for delA1675. The 25 PCR cycles were performed at 94°C, 55°C, and 72°C, for 30 s at each step. Cycle sequencing was performed with 1 μl of PCR product, by means of a Thermo Sequenase 7-deaza-dGTP kit (Amersham Life Sciences). The 30 PCR cycles were performed at 94°C for 45 s, at 58°C for 45 s, and at 72°C for 30 s. For 1135insA the BRCA1A primers was 5′-Cy5-labeled, and for 1675delA the 1675L primer was 5′-Cy5-labeled. Electrophoresis of the sequencing products was performed by means of the Alf Express, under the same conditions as were used for fragment analysis.

Primer sequences.—The primer sequences were as follows (where the BRCA1 cDNA base numbering is from the start codon [A = +1]): BRCA1A (950), 5′-Cy5-AACATAACAGATGGGCTGGAAGT-3′; BRCA1B (1090), 5′-GATTCTCTGAGCA-TGGCAGTTTC-3′; 1675U (1524), 5′-TACAT-CAGGCCTTCATCCT-3′; 1675L (1587), 5′-Cy5-AG-GAGTCTTTTGAACTGC-3′; 11624 (1505), 5′-TAAAGCGTAAAAGGAGAC-3′; and 11716 (1614), 5′-TTGGTTAGTTCCCTGATT-3′.

Results

Altogether, 74 cases of breast cancer and 93 cases of ovarian cancer were noted in 156 women. Of the 93 cases of ovarian cancer, 75 were verified, by histology reports, as epithelial cancer; the remaining 18 cases of ovarian cancer were clinically described and had the same age at onset as the 75 histologically verified cases (data not shown); and all 93 cases were grouped together as cases of ovarian cancer, for further analyses.

Eleven of the women had both breast cancer and ovarian cancer. Three patients with breast cancer at ages 42, 51, and 52 years and one with ovarian cancer at age 40 years were mutation negative on testing; they were all from the family cancer-clinic series.

Table 1 details the overall findings. Breast cancer and

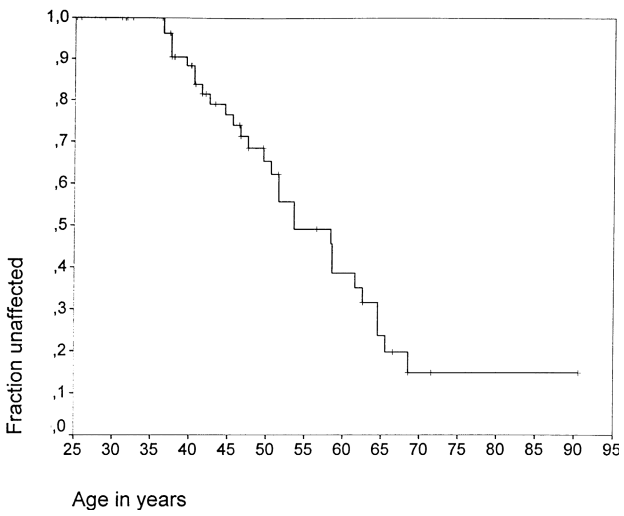

Figure 3 Age-related penetrance in the combined series, including demonstrated mutation carriers and obligate carriers only, with index cases excluded.

ovarian cancer are specified as they correspond with figures 1*b* and *c* and 2*b* and *c*. Also, the four phenocopies are included. Thus, the sum of cancer cases was 152 affected with breast or ovarian cancer (carriers or untested) + 11 with both breast cancer and ovarian cancer + 4 phenocopies = 167. The 152 affected women had 124 unaffected sisters; 96 of these 124 unaffected sisters were alive, 60 (63%) of whom were tested, and 30 (50%) of whom were found to be carriers. Table 2 details carrier status according to age.

Differences between the two mutations were sought in carriers (demonstrated, affected, or obligate). As can be seen in figure 1, no differences were found. The two mutations were therefore grouped together for the rest of the analyses.

Differences according to selection of families were then sought in carriers (demonstrated, affected, or obligate) (fig. 2). As expected, an overrepresentation of early-onset breast cancer was seen in the family cancer-clinic series (log-rank $P = .04$). Removal of the index cases made this difference disappear. In the series grouped together as one series (index cases excluded), the median age at onset was 49.5 years; when the calculation considered demonstrated mutation carriers and obligate carriers only, the median age at onset was 53.5 years (fig. 3).

Table 3 gives the final estimates of penetrances, according to age. In column A, carriers (demonstrated, affected, or obligate) were considered. In the results presented in column B, all untested unaffected sisters (dead or alive) were considered to be carriers. Thus, column A gives the maximum penetrance, and column B the minimum penetrance, that could be derived from the present series. Then the proportions of healthy sisters in each age group who were demonstrated to be carriers/noncarriers were used to calculate the figures in column C, as the interpolated estimate between the results in column A and those in column B.

Discussion

We identified 152 affected individuals, and only 3 unaffected mutation carriers, who were ⩾60 years of age—that is, regardless of the way in which we looked at the data by excluding index persons and by assuming different degrees of intermediate/truncate selection with regard to the estimation of the probability that untested unaffected sisters were carriers, the calculated pene-

Table 2

Mutation-Carrier Status in Different Age Groups, for All Sisters in Sibships with Demonstrated, Affected, or Obligate Carriers

Age (years)	No. of Mutations Demonstrated (No. of Index Cases)/No. in Whom Mutations Are Absent/No. Not Examined			Proportion of Carriers Among	
	Affected	Unaffected	Total	Tested Unaffected	Untested Unaffected[a]
⩽29	0(0)/0/ 1	3/ 5/ 7	3(0)/ 5/ 8	.38	2.66
30–39	5(8)/0/ 18	11/ 6/ 6	16(8)/ 6/ 24	.65	3.9
40–49	6(12)/0/ 45	11/ 6/ 6	17(12)/ 6/ 51	.65	3.9
50–59	1(6)/0/ 29	2/ 6/11	3(6)/ 6/ 40	.25	2.75
60–69	1(3)/0/ 11	2/ 5/ 6	3(3)/ 5/ 17	.29	1.74
⩾70	0(1)/0/ 5	1/ 2/28	1(1)/ 2/ 33	.33	9.24
Overall	13(30)/0/109	30/30/64	43(30)/30/173	.50	24.19[b]

[a] Number of untested unaffected multiplied by observed proportion of carriers among tested unaffected in same age group.

[b] The four mutation-negative affected (phenocopies) are not included in this table; therefore, the total is 4 less than that shown in table 1.

Table 3

Cumulative Age-Related Penetrances of Breast and/or Ovarian Cancer (Whichever Came First) in Carriers (Demonstrated, Affected or Obligate) and in Their Unaffected Sisters Not Tested

	Proportion Affected (after Removal of One Index Case per Family)					
	A: Mutation Carriers[a]		B: Category A with the Assumption That All Unaffected Sisters Not Tested Are Carriers[b]		C: Intermediate between A and B[c]	
Age (years)	Mean ± SE Cumulative Proportion of Affected at End of Period	Annual Incidence, 10-Year Period (%)	Mean ± SE Cumulative Proportion of Affected at End of Period	Annual Incidence, 10-Year Period (%)	Mean ± SE Cumulative Proportion of Affected at End of Period	Annual Incidence, 10-Year Period (%)
≤29	.006 ± .006		.005 ± .005		.005 (.005)	
30–39	.15 ± .030	1.4	.13 ± .023	1.3	.14 (.15)	1.4 (1.4)
40–49	.53 ± .044	3.8	.40 ± .037	2.7	.48 (.46)	3.4 (3.2)
50–59	.80 ± .036	2.7	.62 ± .039	2.2	.67 (.67)	1.9 (2.1)
60–69	.90[d] ± .029	1.0	.70[e] ± .039	.8	.76 (.73)	.9 (.8)

[a] Results are from Kaplan-Meier calculations. Demonstrated, affected, and obligate mutation carriers are included.

[b] Results are from Kaplan-Meier calculations and includes all unaffected sisters not tested but assumed to be carriers, regardless of whether they are dead or alive.

[c] Based on probabilities, given in table 2, that untested unaffected sisters are carriers. Data in parentheses are for consecutive ovarian-cancer series only.

[d] Increasing to .97 at age 81 years (which is not included in table because of low numbers for calculations and possible phenocopies at high age).

[e] Increasing to .76 at age 81 years (which is not included in table because of low numbers for calculations and possible phenocopies at high age).

trance was high but was subject to fluctuation in small numbers of individuals >50 years of age. Of the 137 sisters <50 years of age, 75 (55%) were affected. Calculated numbers of phenocopies in this group were fewer than three, potentially reducing the incidence of mutation-associated disease to 53%. Thus, calculated penetrance at age ≤50 years was considered robust with respect to phenocopies; at age >50 years, both phenocopies and fluctuation in small numbers may have influenced the results. We found it inappropriate to calculate penetrance at age >69 (table 3) and did, in this way, exclude from the calculations the five oldest individuals with cancer.

Our calculated penetrances seems to be higher than those in previous reports of other frequent mutations (Levy-Lahad et al. 1997; Struewing et al. 1997; Fodor et al. 1998). Because of the limited numbers of individuals included in them, most reports have wide confidence limits for the calculated penetrances. Conclusions about the differences between the reported series may not be made until more data become available; meanwhile, one may discuss a number of factors potentially influencing the estimated penetrances.

Ascertainment

Under the assumption of single selection, the index patient is excluded, and 50% of the sisters are expected to be mutation carriers. We have identified many families in a small population and should have intermediate selection. In such a situation, fewer than half the sisters are expected to be carriers. Also, because the index person is affected, another mutation-carrying sister would be less likely to be affected. The lower the penetrance, and the smaller the sibships, the more pronounced this effect would be. When the penetrance is examined, it is not valid to use penetrance estimates to select individuals for analyses and then to use the same individuals to establish the original penetrance estimate. For these reasons, not only the index patients but all their sisters should be excluded. It may be that some previous reports have underestimated penetrance when sisters are not excluded. In our family cancer-clinic series, most affected individuals were initially known and were used for ascertainment. It follows that the whole branch of the family should be excluded before analysis. These arguments led to the same conclusions as have been presented above: we tested all available unaffected sisters to determine their carrier status, made no assumptions about the ascertainment model, and used the observed proportions of mutation-carrying sisters, categorized according to age, for our calculations.

Paternal Inheritance

In half the cases, the mutation was expected to be inherited from the father. Because of the sex-limitation character of the trait, the clinical criteria for selection of families are less sensitive for paternal inheritance. If the mother or one of her close relatives in such a situation

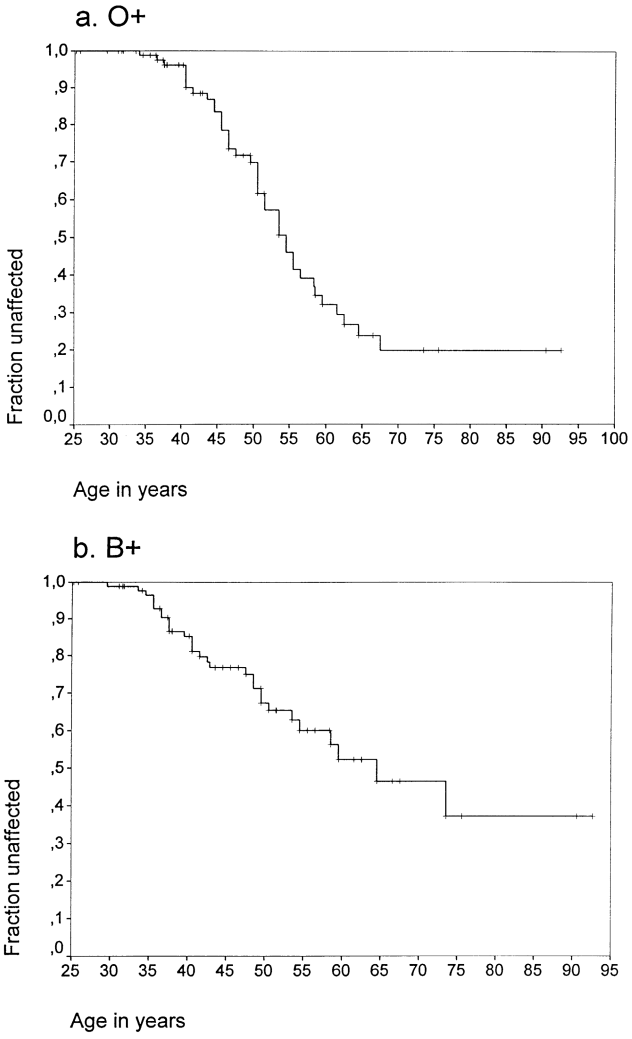

Figure 4 Age-related penetrances of BRCA1 1675delA and 1135insA in the consecutive ovarian-cancer series. Only mutation carriers (demonstrated, affected, or obligate) are included; index cases are excluded. *a,* Ovarian cancer scored as event (patients with breast cancer censored at age at diagnosis). *b,* Breast cancer scored as event (patients with ovarian cancer censored at age at diagnosis).

were to be affected (phenocopies), that part of the family may be interpreted as containing the mutation. This effect may erroneously reduce the calculated penetrance. The father's family has to be extensively examined *also* when the mother and/or her relatives are affected. This problem was expected to occur in the family cancer-clinic cases, and phenocopies were actually found in 4 (29%) of 14 of these families, all on the mother's side.

Extension of Families

If families are extended laterally enough, the problems merge with the concept of ascertainment: eventually, truncate selection for our founder mutations may be reached, and the problems will be solved. In truncate selection, we cannot exclude the index branches—there would be nothing left.

Informative Censoring

If a mutation carrier dies of ovarian cancer, she cannot contract breast cancer afterward. Both the penetrance of breast cancer or ovarian cancer, considered separately, and the prevalence of both breast cancer and ovarian cancer occurring in the same person are influenced by the probability that that person survives the first cancer. The problem is known as informative censoring, and "there is no satisfactory way to compare survival times of two or more groups of patients in the presence of informative censoring" (Collett 1994, p. 274). This problem has been extensively discussed in relation to inherited retinoblastoma, which also may serve as an example of modified second-cancer risk in relation to treatment for the first cancer. With the improvement in treatment results, it is to be expected that penetrances for both breast cancer and ovarian cancer, considered separately, are increasing, as is the prevalence of affected individuals with both types of cancer. Retrospective reports from different countries reflect different populations, different mutations, and different environments, as well as international differences between health care available today and that available generations ago. Scoring as affected/unaffected and including both breast cancer and ovarian cancer as affected may be the most robust parameter for penetrance, as well as for comparisons between series. When column A of table 3 and figure 4*b* are compared, it is seen that the penetrance in mutation carriers 69 years of age was reduced, from 90% to 54%, when only cases of breast cancer were counted and when there was censoring with regard to age at onset of ovarian cancer (penetrance for the consecutive ovarian-cancer series alone, according to the data in column A of table 3, was 82% [data not shown]); these may be considered two extreme results obtained in the present study, indicating that it is possible that methodological problems may explain some of the differences between previous reports. Since figure 4 is based on consecutive ovarian-cancer series only, we recalculated table 3 for that series separately. The results are given in column C of table 3, for comparison. They seem to be identical to the results for the combined series, indicating that there is no problem of fluctuating results due to small numbers and that there are no major discrepancies, between the series, due to ascertainment problems. The results indicate that, in our series, informative censoring was a problem, whereas the ascertainment differences had less impact.

Differences between the Series

Except for the early-onset cases of breast cancer that were used to ascertain the family cancer-clinic series, we found no difference between the two series. This may be an indication that the problems discussed above have no major impact on the estimated penetrances; however, the problems are complex, and such an interpretation must be regarded with extreme caution. As mentioned, some of the problems discussed are less important when penetrance is high.

Modifiers of Penetrance

The families lived geographically separate, and they were so distant genetically that they may not have shared many factors other than the mutation-carrying BRCA1 haplotype. Theoretically, frequent genetic and/or environmental modifying factors could be present in our population and not elsewhere. Such putative endemic/ethnic factors have, however, not been described.

Breast Cancer versus Ovarian Cancer

The families contained more verified cases of ovarian cancer than the total number of reported breast cancers; after removal of the 16 index cases in the consecutive ovarian-cancer series, this was still the case. This result is in contrast to those in previous reports, but it is compatible with theoretical analyses of the initial families reported by the Breast Cancer Linkage Consortium (Easton 1995). This favors a "true" high expression of ovarian cancer as affected phenotype, and it seems to argue against the putative hypothesis that ascertainment of families through ovarian cancer as an infrequent expression of the trait results in selection of families with additional factors causing generally increased penetrance. If additional factors modified the expression from breast cancer to ovarian cancer, then the proportion of ovarian cancers would decrease with increasing genetic distance to the proband. The series was considered insufficient for analysis of this problem, which will be addressed in further studies.

Random Segregation

After removal of one index person in each of the 30 families that we studied, the sibships contained 246 women. Of these, 122 were affected, 30 were unaffected and mutation carriers, and 24.19 were unaffected untested and were calculated to be carriers (table 2); the sum is 176.19, which is more than the 123 (50%) expected ($\chi^2 = 23.0$, $P < .00001$). The observation of the same number of carriers as noncarriers among the tested unaffected sisters may indicate preferential segregation of the mutation-carrying haplotypes. Neither selection nor genetic drift is expected to produce such a finding; however, because most the affected individuals had died and were unavailable for mutation testing (see above), the question of putative preferential segregation remains unanswered. The findings may also be explained as an indication of a "true" low penetrance. For these reasons, we differentiated the untested unaffected sisters according to possible carrier status, without assuming random segregation of the mutations (table 3).

One practical reason for the present report is that we are performing predictive mutation testing in the healthy sisters. We had to know the predictive value of demonstrating the carrier status in healthy women in these families. We will use the results for genetic counseling in families ascertained as described.

Acknowledgments

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Breast Cancer Information Core, http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/index.html (for BRCA1 1675delA and 1135insA)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for BRCA1/inherited breast-ovarian cancer [MIM 113705])

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