High frequency of BRCA1/2 germline mutations in 42 Belgian families with a small number of symptomatic subjects

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Abstract

***Aim*—The initial risk assessments for BRCA1/2 mutation carriers and estimates of carrier frequencies were based on extended pedigrees with a large number of symptomatic subjects. When counselling based on BRCA gene mutation analysis was initiated, we faced requests for counselling mostly from members of small families with only two or three affected members. We report on the likelihood of finding a BRCA mutation in such small families.**

***Methods*—In the first 100 families that came for oncogenetic counselling since September 1994, a BRCA1/2 gene mutation screen was initiated if there were two or more symptomatic first degree relatives, if one of them had ovarian cancer, or if one breast cancer was diagnosed before the age of 50 years.**

***Results*—BRCA gene mutations were found and confirmed by sequencing in 14 out of 42 families (33%); 10 mutations were in the BRCA1 gene and four in the BRCA2 gene. Our findings indicate an increased probability of detecting a BRCA gene mutation when ovarian cancer occurred in the family. There is no increased probability of detecting a mutation with increasing numbers of breast cancers. Only 22% of the eligible presymptomatic family members opted for testing. The presymptomatic female carriers currently prefer breast surveillance rather than prophylactic surgery.**

***Conclusion*—BRCA1/2 gene mutation testing can be done with reasonable efficiency in the Belgian population when there are two symptomatic family members. The availability of testing does not lead to a high frequency of requests for testing by presymptomatic family members.**

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In more than 90% of the large breast cancer families enrolled in research protocols, the hereditary cause could be traced by linkage analysis to either the BRCA1 gene located on chromosome 17q or the BRCA2 gene on chromosome 13q. In these large kindreds the cumulative risk for breast cancer in women with BRCA mutations is estimated to be as high as 85% by the age of 70 years.[1–4] Mutations in the BRCA genes are also known to confer an important risk for ovarian cancer. For men there is a risk for breast cancer associated with BRCA2 mutations and a threefold increase in risk for prostate cancer associated with BRCA1 mutations. BRCA1 mutations also confer a fourfold increase in risk for colon cancer for men as well as women.

The sequence of BRCA1 was published in October 1994 and the sequence of BRCA2 in December 1995.[5 6] This made genetic counselling based on mutation analysis possible in breast and ovarian cancer families. We started BRCA mutation analysis based genetic counselling in September 1994. At that time there was no published model that covered the clinical implementation of this knowledge. Our counselling procedure was derived from the procedures established for Huntington's disease families and was multidisciplinary from the onset. We did profit from published research data and formal guidelines.[7–12] The procedure was modified to include aspects of cancer prevention counselling and progressively adapted, based on feedback from the actual practice. More recent publications did not induce us to make major adjustments in the counselling procedure.[13–15]

In contrast to the large pedigrees with numerous cancer cases used to identify the predisposing genes, the demand for oncogenetic counselling in clinical practice came mostly from members of small families with only two or three affected members. We report on the likelihood of finding a BRCA mutation in these small families, and on the subsequent need for testing presymptomatic subjects in these families.

Methods

PATIENTS

Formal multidisciplinary oncogenetic counselling started at our centre at the time of the sequencing of the BRCA1 gene in September 1994. Probands were referred by general practitioners as well as specialists. We report on the first 100 families that presented for oncogenetic counselling, which coincides with the first two years of activity. A BRCA1/2 gene mutation screen was initiated in these families when there were two or more symptomatic first degree relatives, if one of them had ovarian cancer, or if one breast cancer was diagnosed before the age of 50. Because a BRCA gene mutation carries a relatively low risk for cancer in males, they were excluded in determining

the degree of relationship. The decision to search for a mutation was taken on the basis of the information provided by the family members who came for counselling and who were interested in testing. This information was assessed by the oncogenetic team, consisting of at least a medical oncologist, a molecular biologist, a geneticist, a psychologist, a philosopher-ethicist, a gynaecologist, a surgical oncologist, and the counsellor who took the family history. A BRCA gene mutation screen was then performed on a blood sample from an affected family member whose informed consent to use a sample to try to identify the mutation included permission to use the information thus gained to test others for carriership. When the mutation in a particular family was identified, we informed the affected family member(s) who provided the diagnostic sample, as well as those relatives who had already come for counselling, that individual testing was now possible. These subjects then transmitted the information to other relatives with the option to contact us for more information. The women who opted for testing and turned out to be carriers of the BRCA gene mutation were informed of the known cancer risk data linked to these mutations, and of the current uncertainty of the efficiency of preventive surgery versus continued screening. In our protocol, surveillance starts at the age of 25 and consists of a yearly clinical examination, mammography, transvaginal ultrasound, and determination of serum CA-125 level, as well as monthly breast self-examination. We informed the women that some retrospective data suggest that prophylactic surgery (mastectomy or oophorectomy) could be successful in significantly lowering cancer risks.[16 17]

MUTATION ANALYSIS AND DNA SEQUENCING

Genomic DNA as well as RNA extracted from blood cells of affected patients was used for the mutation analysis.

Exon 11 of BRCA1 was screened by the protein truncation test (PTT) as described by Hogevorst *et al*.[18] Exons 2, 5, and 20 were submitted to a combined single strand conformation polymorphism/heteroduplex analysis (SSCP/HD) according to Futreal *et al*.[19] These exons were chosen because they showed the highest incidence of mutations at the time we initiated our mutation screenings. The BRCA1 gene was also analysed by Southern blotting in order to detect gross genomic alterations that could be missed by polymerase chain reaction (PCR) based techniques.[20] Exons 10 and 11 of BRCA2 were assessed by PTT.

When putative mutations were detected, the corresponding fragments were reamplified from the original genomic DNA, the amplified fragments were purified with the High Pure PCR Product Purification Kit (Boeringer Mannheim), and the mutations confirmed by sequence analysis (Sequenase Version 2.0 DNA Sequencing Kit from USB).

In 11 out of the 42 families, RNA extracted from leucocytes was available for analysis and in these families the smaller exons of BRCA1 and BRCA2 were screened by applying the PTT to this RNA (reverse transcriptase-PTT, RT-PTT). Two reverse transcriptase-polymerase chain reaction (RT-PCR) fragments generated from BRCA1 were analysed as described by Hogevorst *et al*.[18] In the case of BRCA2, the PTT was applied to four RT-PCR fragments covering exons 1 to 10, the junction of exons 10 and 11, exons 11 to 19, and exons 18 to 27, respectively.

BREAST AND OVARIAN CANCERS IN THE FAMILY HISTORY

The data on the families were collected from the pedigrees as these were known to the oncogenetic team at the time of taking the decision to initiate a BRCA mutation screen. In counting breast and ovarian cancers, a case of bilateral breast cancer was counted as one case, but breast and ovarian cancer in a single family member counted as one case of breast cancer and one case of ovarian cancer. The average age at diagnosis of breast cancer and ovarian cancer is the arithmetic mean of the known ages at diagnosis in a family. The number of women in the pedigrees was used as an indicator of the number of women at risk of developing breast and ovarian cancer. Since the age and the age at death of some of the women in the pedigrees was not known, all women were included.

STATISTICAL ANALYSIS

Several hypotheses were tested in our sample: the presence of ovarian cancer cases in the family, the presence of breast and ovarian cancer in the same woman, the presence of bilateral breast cancer, and a low average age at the diagnosis of malignancy in the family possibly increase the likelihood of finding a BRCA gene mutation in breast cancer families. All statistical tests were performed two tailed at the 5% level of significance. The comparison of the frequencies of identified BRCA gene mutations in various subgroups of families (families with and without ovarian cancer, breast and ovarian cancer in the same woman, or bilateral breast cancer) was performed using the Fisher Exact test. The Mann-Whitney U test was used when comparing the average age at the diagnosis of malignancy in the families with and without an identified BRCA gene mutation.

Results

In 42 of the first 100 families that consulted for familial cancer, the BRCA1 and BRCA2 genes were screened for mutations because the criteria (see Methods) for initiating the BRCA screen were met and a blood sample from a breast or ovarian cancer patient was available. As the clinic is open to all familial cancer syndromes, we consider this to be a high proportion, which can probably be explained by the prevalence of familial breast cancer and the attention the media paid to the discovery of the BRCA genes.

In a first step, the 42 families were screened for BRCA1/2 mutations at the genomic level with a PTT analysis on the large exons of BRCA1 and BRCA2, and by SSCP/HD on exons 2, 5, and 20 of BRCA1, as described in

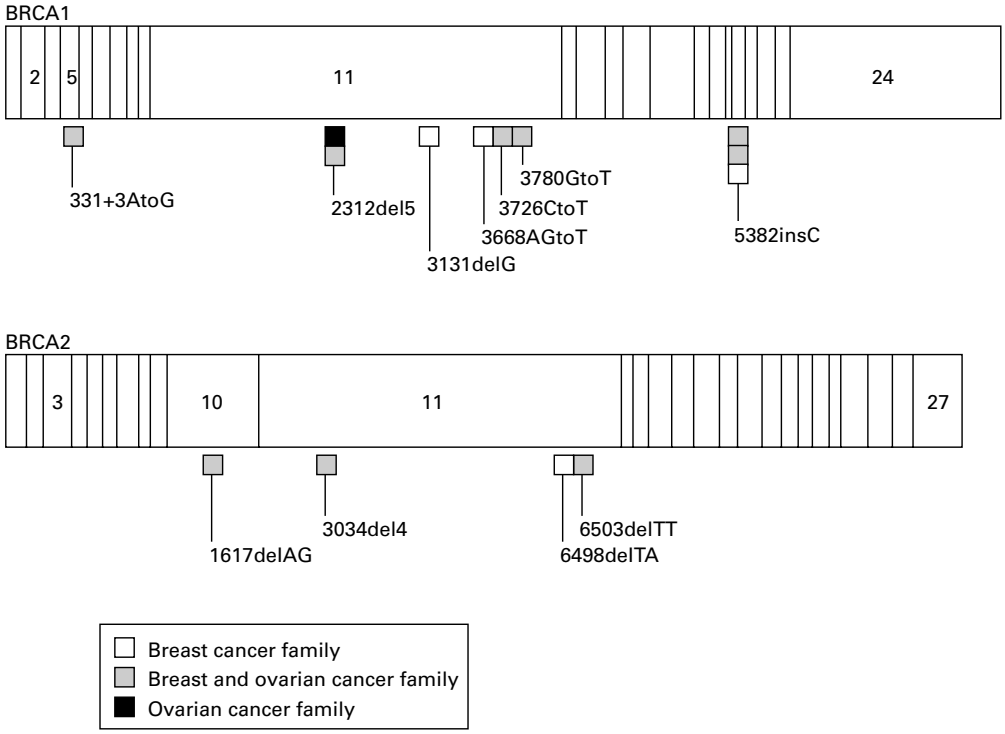

Figure 1 Diagram of distribution of BRCA1/2 gene mutations.

the Methods section. Mutations were found and confirmed by sequencing in 14 families (fig 1, table 1). Seven of these mutations were included in the series reported by Peelen *et al*.[21]

We also analysed 11 families by RT-PTT to identify possible truncating mutations in the remaining smaller exons of BRCA1 and BRCA2. This method was applied for the screening of two RT-PCR fragments generated from BRCA1, while four fragments were analysed in the case of BRCA2 (see Methods). In nine out of the 66 PCR fragments investigated, the PTT generated truncated proteins not observed in control experiments. Sequence analysis of the corresponding complementary DNA fragment indicated that, in each case, the observed protein truncation resulted from abnormal splicing events since whole exons were missing. However, genomic sequence analysis of the intron/exon boundaries did not result in the detection of splice mutations.

The presence of ovarian cancer in the pedigree was a strong and significant (p=0.005) predictor for the probability of finding a BRCA1/2 gene mutation (table 2). The likelihood of finding a mutation increased with a lower average age at diagnosis of breast cancer in a family (p=0.015, table 3). There was a trend for a correlation between finding a mutation and a lower average age at diagnosis of ovarian cancer (p=0.093), the occurrence of breast and ovarian cancer in a single family member (p=0.49), or bilateral breast cancer (p=0.19). The lack of statistical significance could be related to our sample size.

There were fewer mutations found in the families that just met the lowest criteria for selection of two symptomatic first degree relatives, but the chance of finding a BRCA gene mutation did not correlate with the number of breast cancer cases in the family; there were on average 2.1 breast cancers in the families with an identified BRCA mutation versus 2.9 breast

Table 1 BRCA1 and BRCA2 mutations and cancer phenotype

Gene	*Mutation*	*No of women in pedigree*	*No of breast ca*	*Average age at diagnosis of breast ca (y)*	*No of ovarian ca*	*Average age at diagnosis of ovarian ca (y)*	*Breast and ovarian ca*	*Bilateral breast ca*
BRCA1	5382 ins C	11	3	43.3	2	55.0	1	
BRCA1	3780 (G→T)	11	1	45.0	3	43.0	1	
BRCA1	5382 ins C	19	2	42.5				
BRCA1	2312 del 5	14			4	46.3		
BRCA1	IVS5 + 3 (A→G)	9	2	35.5	1	40.0		
BRCA1	2312 del 5	24	3	45.3				
BRCA1	3668 (AG→T)	7	5	44.0				2
BRCA1	3726 (C→T)	13	1	29.0	1	45.0		
BRCA1	3131 del G	3	2	53.0				1
BRCA1	5382 ins C	9	1	45.0	1	58.0		1
BRCA2	3034 del 4	13	2	51.0	1	56.0	1	
BRCA2	6498 del TA	8	2	42.5				
BRCA2	1617 del AG	9	3	43.7	1			
BRCA2	6503 del TT	19	2	49.5	1	49.0		1
Total		169	29	Median: 44	15	Median: 47.6	3	5

Table 2 Distribution of the identified BRCA gene mutations according to family characteristics

	No of families	*No of mutations BRCA1 or 2*	*% of families mutation found*
All families	42	14	33
Ovarian cancer			
Present in family	14	9	64
Not present in family	28	5	18
Breast and ovarian cancer*			
In a single person	3	3	100
In separate people	8	5	63
Bilateral breast cancer†			
Present in family	7	4	57
Not present in family	32	9	28

* Only families with breast and ovarian cancer cases are taken into account.
† Only families with breast cancer cases are taken into account.

Table 3 Distribution of the identified BRCA gene mutations according to the average age at diagnosis of breast cancer and of ovarian cancer

	No of families	*No of mutations BRCA1 or 2*	*% of families mutation found*
Average age at diagnosis of breast cancer among family members (y)*			
<35	2	1	50
35–39	2	1	50
40–44	8	5	63
45–49	13	4	31
50–54	10	2	20
55–59	1	0	0
>59	3	0	0
Total	39	13	33
Average age at diagnosis of ovarian cancer among family members (y)†			
<45	2	2	100
45–49	3	3	100
50–54	2	0	0
55–59	5	3	60
>59	1	0	0
Total	13	8	62

* The median of the average ages at diagnosis of breast cancer was 44 years in the families with a BRCA mutation versus 47.5 years in the families without an identified mutation (p=0.015).
† The median of the average ages at diagnosis of ovarian cancer was 47.6 years in the first group of families and 56 years in the families where no mutation was found (p=0.093).

cancers in the families where no mutation was found. Even when corrected for family size by calculating the number of breast cancer cases per 100 women, no correlation was found: 17 breast cancers per 100 women in the families with an identified mutation versus 23 breast cancers in the families where no mutation was found. Four of the families that only just met the lowest criteria had ovarian cancer versus 10 of the families with a more extensive history of cancer in the family.

All mutations were found in breast/ovarian cancer families with few other cancers except for one BRCA1 mutation identified in a female rectal cancer patient belonging to a family with two (dead) breast cancer patients. Testicular cancer, diagnosed at the age of 25, also occurred in this family.

What is the interest in presymptomatic testing once a BRCA1 or 2 mutation is identified in the family? Family members who had shown an interest in testing initially, before identification of the mutation, consequently opted for testing. In contrast, of the other relatives who were eligible for testing, only a minority requested it. Overall, women tended to be somewhat more interested in the test than men: 14 women out of 52 eligible presymptomatic female relatives opted for testing (27%), whereas only eight men out of 46 eligible did so (17%). All presymptomatic women who tested positive took a conservative approach to their breast cancer risk, while two of the seven women with a previous history of breast cancer requested a prophylactic contralateral mastectomy after being notified of a positive BRCA test result.

Discussion

Previous reports and statistics on BRCA gene testing have mostly been generated based on large pedigrees with a large number of affected subjects. The stringent criteria for BRCA testing derived from these large families are not readily applicable to the commonly encountered situation of a small family in which dominant inheritance of cancer predisposition is apparent. To meet a demand for oncogenetic counselling from members of small families with only two or more affected members, less stringent rules have to be applied. The proposed minimum selection criteria for searching for a BRCA1 or BRCA2 gene mutation in small families with a limited number of cases were two or more first degree relatives with breast cancer if one of these was diagnosed before the age of 50 or if one of them had ovarian cancer at any age. The purpose of this analysis was first to verify whether the application of these selection criteria made testing still worthwhile, and what factors influence the likelihood of finding a mutation. A secondary purpose was to examine the consequences of finding a mutation in these families for the presymptomatic relatives. Forty-two families of the first 100 families that consulted for familial cancer met the criteria put forward for initiating a BRCA1/2 mutation screen and had material available for analysis. We searched for mutations in the large exons of the BRCA1 and BRCA2 genes, and in exons 2, 5, and 20 of BRCA1 that contain frequently recurring mutations described world wide. The analyses can be performed on genomic DNA and are easily incorporated into a routine diagnostic test. In 14 of the 42 families we were able to find a BRCA1 or BRCA2 mutation. The initial mutation screen covered only about 60% of the coding regions of the BRCA1 and BRCA2 genes. However, expanding the PTT analysis to all the remaining smaller exons, for which RNA material is needed, did not lead to the identification of additional truncating mutations. Recently, several authors described large deletions in the BRCA1 gene of several patients belonging to breast cancer families.[22 23] For instance, in the Dutch population two mutations of this type were found to be present in more than 30% of the BRCA1 families.[20] Southern blot analysis of BRCA1 in our series did not show any genomic alteration.

The success rate of 14 mutations identified in 42 families (33%) compares favourably with other data obtained in similar families.[24–26] Various factors could explain this relatively high mutation rate. Mutations in the BRCA1 and BRCA2 genes have been found world wide, but the type of mutations identified can differ considerably from country to country. Only a few specific mutations are dispersed world wide (for example, 5382 ins C). Some mutations recur frequently but only in a particular country (for example, 2312 del 5 only in Belgium and The Netherlands) and

many mutations have been found in only one or a few families.[21] The rather high proportion of ovarian cancers in the families reported here probably increased the yield of mutation detection. However, the BRCA2 gene mutations we could isolate in our breast and ovarian cancer families were not preferentially located in the ovarian cancer cluster region.[27] A mutation was detected in all five families in which the average age at diagnosis of ovarian cancer among family members was less than 50 years. When analysing our site specific breast cancer families, the success rate for detecting a mutation decreases to 18%. This indicates that the mutation responsible for breast cancer "specific" predisposition in our small families cannot be picked up efficiently with the screening methods used. These mutations could be regulatory mutations or missense mutations of BRCA1 or BRCA2, or they may even be localised in a third BRCA gene, which would be responsible for the cancer predisposition in most of the small breast cancer specific families. Sequencing of the two genes to identify point mutations would be especially difficult in these families, as linkage analysis showing the pathogenicity of these point mutations would be difficult if not impossible.

Once a BRCA1 or 2 mutation was found, testing was offered to the family members. Surprisingly, only a minority of the presymptomatic men and women in the families with a BRCA gene mutation subsequently came forward to request testing. Since most of them were not interviewed, the reasons for this abstention are not known. One might speculate that some do not want testing, while others may not have been fully informed by their family members. This should be elucidated in the future as the relative responsibilities of health care workers versus family members for diffusing this knowledge within these families are better defined. The current lack of data on the cost/benefit of preventive surgery versus close observation justifies a neutral counselling position with regard to this matter and we are satisfied that presymptomatic female carriers opt for careful follow up for the moment. It is, however, noteworthy that some of the women with previous cancer and treatment experience chose a more invasive preventive strategy. We expect that the prospective follow up of mutants will help redefine the preventive options in the future.

So far, our experience has shown BRCA gene mutation analysis based genetic counselling to be feasible in small families when there are two or more symptomatic first degree relatives, if one of them had ovarian cancer, or if one breast cancer was diagnosed before the age of 50. It seems appropriate to continue screening small families in view of the fact that a BRCA gene mutation was identified in 24% of the families that only just met the minimal criteria for selection. Technical advances may allow the initial mutation analysis to be expanded. Commercial full mutation analysis may be appropriate in a limited number of families.

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