Short Report

**BRCA1/2 testing: uptake, phenocopies, and strategies to improve detection rates in initially negative families**


In families with clustering of breast and ovarian cancer, molecular testing of the major susceptibility genes **BRCA1/2** helps to identify patients with disease mutations and healthy persons at high risk who can participate in targeted intervention programs. We investigated 5559 families from the German Consortium for Hereditary Breast and Ovarian Cancer included between 1997 and 2008 and treated under clinical routine conditions. In each family an index patient/person had been screened for deleterious mutations in **BRCA1/2**. Healthy relatives agreed to predictive testing in 888 of 1520 **BRCA1/2** mutation-positive families (58%). Of 2646 eligible unaffected first-degree relatives 1143 decided to be tested (43%). In 325 families with **BRCA1/2**-positive index patients one related BC/OC patient was tested and 39 (12.0%; 95% confidence interval: 8.7–16.0%) discrepant cases found. A second related individual was screened in 163 of 3388 (4.9%) families with **BRCA1/2**-negative index patient and in eight families a **BRCA1/2** mutation was found. In **BRCA1/2** mutation-positive families, BC/OC patients lacking the familial mutation have to be expected at a rather high rate. In families with **BRCA1/2**-negative index patient we recommend a second screening if another patient with a high probability of carrying a **BRCA1/2** mutation is available.

**Conflict of interest**

All authors declare no conflict of interest.

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In families with an accumulation of breast and ovarian cancer cases, molecular testing for the major susceptibility genes BRCA1 and BRCA2 is common practice, as such mutations are considered to be responsible for approximately 20–40% of these cases. Within the German Consortium for Hereditary Breast and Ovarian Cancer (GCHBOC), a complete mutational screening of the BRCA1/2 genes and deletion/duplication analysis of BRCA1 is agreed upon by contract. If a pathogenic mutation is detected in the index patient, diagnostic testing is offered to all affected family members in order to identify further mutation carriers. In addition, predictive genetic testing can be offered to all unaffected family members at risk in order to determine whether they have inherited a deleterious mutation. Affected and unaffected carriers can participate in prevention programs which comprise intensified surveillance and preventive surgery (1, 2).

However, it cannot be excluded that true BRCA1/2 cases and BC/OC cases negative for the familial BRCA1/2 mutation which may occur due to alterations in other breast cancer risk genes coexist within one family. The latter cases have been termed phenocopies (3) or true negatives (4) in the literature. As a prerequisite to detect families with true negatives at least two related BC/OC cases must have had molecular genetic screening or testing. Therefore, we investigated the family structures and patterns of genetic testing in general in the GCHBOC. Our major aims were to find out how many unaffected relatives consented to receive predictive testing and how often related BC/OC patients with and without BRCA1/2 mutation (briefly: discrepant results) were found among relatives.

**Study population and methods**

**Families**

The GCHBOC comprises 12 university centers offering genetic counseling, BRCA1/2 testing and clinical surveillance, and one center responsible for documentation and statistics. Using uniform inclusion criteria and standard operating procedures, families with clustering or early onset of breast or ovarian cancer (see Fig. S1) were registered and tested for the presence of deleterious germ line mutations in BRCA1 and BRCA2. Comprehensive data on familial cancer history including a detailed pedigree, pathology reports, and results of molecular testing were documented in the central database BRCA2006 using standardized electronic case report forms. All patients had previously given their written informed consent for BRCA1/2 analysis and agreed to be enrolled in the registry which had been approved by the institutional review boards of each participating center.

Families were included if one of the following criteria was fulfilled among first- or second-degree relatives: three women with BC; two women with BC, one of them with age at onset ≤50 years; two women, one with BC and one with OC; one woman with BC and age at onset ≤35 years; one woman with bilateral BC and age at onset ≤50 years; one woman with OC ≤40 years, one woman with BC and OC; one male with BC and one female with BC or OC.

The most seriously affected available patient (bilateral BC, BC and OC, early age of onset) was chosen as index patient and screened for BRCA1/2 mutations. If no patient was available, an as yet unaffected family member (‘index person’) could be screened if she had a remaining lifetime risk for breast cancer of 30% or higher and/or a heterozygote risk of ≥20% calculated with CYRILLIC V 2.1.3 (27). If a BRCA1/2 mutation was found in the index patient/person, all family members that contacted the genetic counseling unit were directly informed of the availability of genetic testing and were asked to pass on an informative family letter to eligible relatives.

If the screening of the index patient does not reveal a causative germ line mutation in BRCA1/2, further molecular testing in the family was not generally provided.

We restricted our analyses to families recruited between January 1997 and July 2008 with at least one documented molecular screening result of BRCA1/2 and more than five family members >18 years.
Mutation analysis

Genomic DNA was isolated according to standard procedures (5). Mutational screening was carried out using denaturing high-performance liquid chromatography of polymerase chain reaction products encompassing all coding exons of the BRCA1/2 genes and subsequent sequencing of conspicuous amplicons or by direct sequencing of all BRCA amplicons (6). Sequences of both genes were evaluated based on the NCBI cDNA reference sequences U14680.1 (BRCA1 gene) and U43746.1 (BRCA2 gene). Molecular analysis for deletions or duplications of the BRCA1 gene was carried out by multiplex ligation-dependent probe amplification using a commercial kit (MRC Holland, Amsterdam, The Netherlands) (7).

Statistical analysis

Statistical analyses were performed using IBM PASW 18 (8).

Results

Patients and families

Five thousand five hundred nine families fulfilled the inclusion criteria of the study. The distribution of family sizes is skewed with a median family size of 24 and an interquartile range of 20.

In 5025/5559 (90.5%) families an index patient was available for mutational screening whereas in 534 families (9.5%) an unaffected person at risk was screened for BRCA1/2 mutations (Table 1). The median age of onset in the index patients with breast cancer was 44 years with an interquartile range of 13.7 years, and 50 years with an interquartile range of 14.3 years for ovarian cancer.

Molecular genetic testing

In 28.6% (1435/5025) of the families with a BC/OC index patient and in 16.9% (90/534) of the families with an unaffected index person a mutation in BRCA1/2 was identified. Generally, further testing in the families depended on the result of the index person as depicted in Table 2 (see also Table S1).

In 91.2% of the index-negative families (neither a pathogenic mutation nor a UV) no further persons were screened. In 4.9% (169 families) of these families, a second related patient was screened for mutations in the BRCA1/2 genes. These families were larger (median family size of 28 members compared to 23) and had significantly more BC/OC patients with a lower age at onset. The second screening revealed a BRCA1/2 mutation in 8/169 families (4.7%). Additionally, in 129 families a second patient in a non-genetically related branch of the family was screened for mutations in the BRCA1/2 genes, and four mutations (3.1%) were found.

If the index patient/person showed a mutation either in BRCA1 or BRCA2, another affected or yet unaffected family member was tested in 63% or 71.4% of families, respectively. Families in which the index patient had a mutation in BRCA1 and BRCA2 or variants of unknown clinical significance (UV) will not be further discussed in this paper.

The pattern of predictive testing in families where the index patient carried a BRCA1/2 mutation is summarized in Table 3.

To analyze the uptake of genetic testing we focussed on unaffected living female first-degree relatives of mutation carriers older than 18 years (HF1DR) (Table 3). One thousand two hundred seven families had HF1DR and in 62% of them predictive testing was performed. Among the 2646 HF1DR 43% decided to be tested.

Discrepant test results among patients within one family

In 325 families with a BRCA1/2-positive index patient one ore more additional BC/OC patients underwent molecular testing. We found 53 families in which an affected relative did not carry the familial mutation representing 16.3% [95% confidence interval (CI): 12.5–20.8%]. This rate was slightly higher in BRCA1 families than in BRCA2 families but not significantly
Table 3. Number of BRCA1/2 index-positive families with predictive testing by number of female family members >18 years eligible for predictive testing

<table>
<thead>
<tr>
<th>Number of tested female first-degree relatives of BRCA1/2-positive patients</th>
<th>Number of female first-degree relatives of BRCA1/2-positive patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>0</td>
<td>318</td>
</tr>
<tr>
<td>1</td>
<td>241</td>
</tr>
<tr>
<td>2</td>
<td>79</td>
</tr>
<tr>
<td>3</td>
<td>26</td>
</tr>
<tr>
<td>≥4</td>
<td>27</td>
</tr>
<tr>
<td>Sum of families</td>
<td>318</td>
</tr>
<tr>
<td>Families with predictive testing/families with &gt;0 first-degree relatives (%)</td>
<td>241/469 (51.6)</td>
</tr>
<tr>
<td>Individuals with predictive testing, tested/available (%)</td>
<td>241/469 (51.6)</td>
</tr>
</tbody>
</table>

*Exact number of first-degree relatives of BRCA1/2-positive patients included.

Table 4. Characteristics of the 53 families with BRCA1/2-positive index patients and phenocopies

<table>
<thead>
<tr>
<th>Families</th>
<th>BRCA1</th>
<th>BRCA2</th>
<th>BC or OC in both parental lines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>33</td>
<td>20</td>
<td>14</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Degree of relationship between index and the closest non-carrier patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother</td>
</tr>
<tr>
<td>Sister</td>
</tr>
<tr>
<td>Child</td>
</tr>
<tr>
<td>Greater than first-degree relatives</td>
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</tbody>
</table>

Comparison of positive and negative patientsa

<table>
<thead>
<tr>
<th>Breast cancer</th>
<th>Patient without familial mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>45</td>
</tr>
</tbody>
</table>

| Bilateral breast cancer | 19 | 3 |
| Ovarian cancer         | 2  | 5 |
| Breast and ovarian cancer | 3 | 0 |
| Mean age of onset years | 40.79 | 50.1 |
| 95% CI               | 38.3–43.0 | 46.8–53.5 |

aIn families with more than one related patient without mutation the closest relationship was taken.

Discussion

To date national programs for familial breast and ovarian cancer still focus on the molecular diagnosis of germ line mutations in the high risk genes, BRCA1 and BRCA2, in families with clustering or early onset of disease. In our study a deleterious BRCA1/2 mutation was identified in the index patient in 27.5% of families, thus reflecting the successful selection of high risk families. In these families predictive testing is available for all unaffected family members above 18 years. They are informed by the index patient or by another counselee in the family. The uptake rate of 43% (of eligible first-degree relatives of index patients) in our study is in line with an extensive study of non-research families in the literature (10). Here eligible family members were informed in a similar way as in our study. The rate is lower than that reported in publications dealing with fewer families and using proactive approaches (10–13). Our study represents a point of prevalence; some families had 2 years and others up to 10 years for their test decision.

For healthy mutation carriers prospective prevention and surveillance are offered and evaluation studies are different (18.1% vs 15.6%). Characteristics of the 53 families are summarized in Table 4. In 14 families, the index patient had BC/OC cases in both parental lines; here patients without the familial mutation have to be expected. In 32 of the families there was a first-degree relationship between index patient and mutation-negative patient. The age of onset between index patient and negative patients is significantly different, partly because of the selection of the index patient. No patient with BC and OC was found among the mutation-negative patients. Among the non-carrier patients three had bilateral breast cancer (age of onset 77, 52, and 39 years) and five patients had confirmed OC.

We compared the 53 families showing discrepant results and the 272 other BRCA1/2-positive families with at least one additional patient tested. The discrepant families were larger (median family size 35 vs 31) and significantly more persons were molecularly screened or tested (median number 4 vs 3). Both had a similar distribution of age at onset of breast or ovarian cancer, similar distribution of inclusion criteria except for more families with a woman with OC and a male BC patient (4/53 vs 2/273). Among the 74 tested non-index OC cases in these 325 families 69 carry the familial mutation.
underway. For healthy non-carriers guidelines from international expert panels do not recommend any preventive action (14, 15).

Molecular testing is also offered to all BC/OC-affected family members as mutation carriers have an increased risk of secondary BC/OC and contralateral breast cancer (16).

We detected 53 families with one related patient tested negative for the familial mutation. In 14 of them the BC/OC cases were documented in both parental lines of the index patient, thus patients without the familial mutations have to be expected. The remaining 39 families comprise 12.0% (95% CI: 8.7–16.0%). It cannot be excluded that mutation-negative patients – particularly OC patients – carry other mutations in high risk genes.

In a study conducted in an Ashkenazi Jewish population index patients from 700 families were tested for the three Ashkenazi founder mutations in the BRCA1/2 genes resulting in 136 BRCA1/2-positive families (17). They report on five families in which a related patient did not carry the familial mutation representing 3.7% (95% CI: 1.2–8.4% of BRCA1/2-positive families) similar to 2.8% (95% CI: 2.0–3.8%) of all BRCA1/2-positive families in our study.

More recently, in a study based on 277 families Smith et al. (3) identified 32/118 living BC/OC patients 23.7% (95% CI: 16.4–32.4%) that did not carry the familial mutation. However they had less BRCA1/2 families in their sample and tested more affected higher degree relatives.

In the majority of our index patients (n = 4434, 72.5%) no BRCA mutation could be identified. In rare cases, this could be caused by reduced sensitivity of the molecular screening method which is slightly over 95% (18). In these cases the false-negative result for the familial mutation would be replicated in the second patient. Most probably other high and intermediate risk mutations (19–21) or clustering of common low risk variants (22, 23), or just a chance caused the accumulation of breast and ovarian cancer in the large families of our consortium. In some families other patients may carry a BRCA1/2 mutation but BRCA1/2 screening in other family members is not provided in the study guidelines. Generally, additional costs for a second screening are not covered by health insurance companies in Germany. Nevertheless in 163 of the 3338 families with a BRCA-negative index person, a second related patient was screened and a BRCA mutation detected in eight of them.

Testing more than one affected family member for BRCA1/2 mutations may be a useful strategy to further improve the detection rates of BRCA1/2 mutation in high risk families.

In the clinical context, the simple rule ‘a second patient should be screened if the family fulfils the inclusion criteria for the study without taking the disease information of the first tested patient into account’ could be helpful. By using this rule, however, we disregard information and the probability to find a BRCA1/2 mutation is low. Therefore, the decision whether to screen further family members or not, should be based on the statistical probability of the second index patient carrying a BRCA1/2 mutation if a threshold for mutation screening is important. We recommend the use of the BOADICEA model and program which among the published approaches (24, 25, 28) is most appropriate for our purpose since it uses a genetic model with two major genes BRCA1 and BRCA2, an additional genetic component and can be applied to affected and non-affected persons in arbitrary pedigrees. Under the assumptions of this model the probability that in a family with a BRCA1/2-positive index patient at least one additional patient does not have the familial mutation depends on the number of related patients tested, their relationship to the index patient, their age at disease onset and the mutated gene (BRCA1 or BRCA2).

There are some limitations of our study. Data collection are based on selected high risk families, additionally slightly more comprehensive documentation has to be expected in parental lines with BC/OC cases although the guidelines of the consortium comprise the documentation of first- and second-degree relatives in both parental lines of the index patient. Therefore our data set does not allow for unbiased estimates of standardized incidence ratios. In particular, it cannot be addressed whether non-carriers in carrier families have a higher BC/OC risk than the general population (26). In families with BRCA1/2-negative index patients, testing of other patients was not systematically performed.

Conclusions

In more than two-thirds of BRCA1/2 of positive families within the GCHBOC, further relatives received molecular genetic testing. The uptake of genetic testing among healthy first-degree relatives was 43% and should be improved to give more relatives the option of intensified screening, surveillance, and preventive surgery. On the basis of our data, BC/OC patients without the familial mutation have to be expected at a rather high rate which mirrors the high prevalence of breast and ovarian cancer due to other causes. It provides relieving information for genetic counseling of women in selected high risk families. Even if the index patient does not have a BRCA1/2 mutation, the chance is not negligible that other relatives do. We therefore recommend offering molecular screening of BRCA1/2 to an affected relative depending on the probability of carrying a mutation in these genes.

Supporting Information

The following Supporting information is available for this article:
Fig S1. Distribution of family sizes.
Table S1. Comparison of families with and without predictive testing among all families with BRCA1/2 pos index patients
Additional Supporting information may be found in the online version of this article.

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References