Identification of the best index case significantly improves mutation detection rates in families with hereditary breast and ovarian cancer

Short title: Identification of the best index case in HBOC

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Abbreviations: BC: breast cancer; OC: ovarian cancer; HBOC: hereditary breast and ovarian cancer; GC-HBOC: German Consortium for Hereditary Breast and Ovarian Cancer; CI: confidence interval

Definitions: *Mutation detection rate* refers to the detection of expected mutations according to pretest risk calculations; *Mutation prevalence* refers to the proportion of patients with a mutation of the whole cohort.

Keywords: Hereditary, Breast Cancer, Ovarian Cancer, Best Index Case, Patient Selection, Mutation Detection Rate

Abstract

To date, a disease-causing mutation can be found in 15-30% of families with hereditary breast and ovarian cancer (HBOC) and it is believed that more than half of the cases still remain unsolved. Usually it is intended to perform genetic analyses in the family member with the most severe phenotype, which, however, may not always be possible. Moreover, no standard criteria have been established to define the person who is most suitable for genetic testing within a family: 'the best index case'. We therefore established clinical criteria to identify the best index case in HBOC and analyzed the impact on genetic testing. 130 patients who presented at our department from 2016 to 2018 were divided into two groups. In group A (N = 98) genetic analyses were performed in the best index case based on our criteria. In group B (N = 32) at least one other family member was considered a better index case. The detection rate of expected mutations was significantly higher for group A (64.3% vs. 32.0%, p = 0.034) while there was no significant difference of calculated mutation carrier risks between these groups. We conclude that the mutation detection rate in families with HBOC is notably higher after identifying the best index case for genetic testing according to the clinical selection criteria reported here.

Introduction

Approximately 12.8% of all women in Germany are affected by breast cancer (BC) throughout their lives (1). Thus, BC represents the most common type of cancer in women. The lifetime risk for male breast cancer is estimated at about 0.1% (1), the risk for ovarian cancer (OC) at 1.4% (2). Familial clustering suggestive of hereditary breast and ovarian cancer (HBOC) is seen in 30% of cases (1). Mutations in the high-penetrance susceptibility genes *BRCA1* (OMIM 113705) and *BRCA2* (OMIM 600185) are well known causes for HBOC (3). Lately, associations of BC with mutations in other genes such as *CHEK2* (OMIM 604373), *PALB2* (OMIM 610355) and *CDH1* (OMIM 192090), among others, have been described additionally (4, 5). Some of these genes are also involved in other cancer syndromes like hereditary pancreatic cancer in the case of *PALB2* (6, 7) or hereditary diffuse gastric cancer in the case of *CDH1* (8, 9).

In the last years, standard genetic analyses for HBOC within a clinical setting covered up to ten genes in Germany (10). Identification of a disease-causing mutation can be of major importance for the treatment and surveillance of affected persons as well as for healthy relatives (1, 2, 3, 10). In Germany, eighteen centers of the German Consortium for Hereditary Breast and Ovarian Cancer (GC-HBOC) attend affected patients and their families (11). Genetic counseling is performed for all cases and includes risk stratification with the software BRCAPRO. This software implements a statistical model based on Mendelian genetics and Bayes' theorem for calculating an individual's probability of carrying a mutation on the basis of the individual's cancer status and the reported family history (12, 13). According to most studies, a disease-causing mutation can be found in 15-30% of families with HBOC and it is believed that more than half of the cases still remain unsolved (14, 15, 16).

Divergent inclusion criteria and *a priori* mutation carrier risks are certainly reasons for the different mutation prevalences, making it difficult to compare the respective studies. Not only the inclusion criteria for genetic testing but also the selection criteria for the patient who is most suitable for genetic testing within a family (henceforward *the best index case/patient*) varies among the different studies. Moreover, if the best index case of a family is not available, genetic analyses are frequently performed in other family members. There usually exists more than one affected person with BC and/or OC in families suggestive of HBOC. Not all of them must necessarily carry the disease-causing mutation which would be expected in the family. Occasionally, family members can also be affected by a sporadic cancer which developed independently of the family burden. Therefore, correct selection of the best index case for genetic testing is crucial in order to detect a disease-causing mutation in affected families. It is usually intended to perform genetic analyses in the family member with the 'most severe phenotype' (14). However, no standard criteria have been defined for the selection of the best index case in families with HBOC so far, leaving the decision at the discretion of the respective genetic counselor. In some families identification of the index patient with the most severe phenotype can be straightforward. However, in many other families this is not the case. As an example, the question whether a woman with a single BC at age 38 (youngest age of onset), a woman with two independent BCs at ages 44 and 46 (older age of onset but multiple cancers) or a woman with OC at age 49 (oldest age of onset but less frequent type of cancer) should be chosen for genetic analyses within a family needs to be addressed by ranking the severity of these cases. Furthermore, it is of interest if a more standardized approach for the selection of the best index cases would improve the results of genetic analyses in terms of mutation detection rates.

With this study we now aim to establish clinical criteria applicable to most families suggestive of HBOC to identify the best index case and analyze the impact on genetic testing. To this goal we divided 130 patients who presented at our clinic from 2016 to 2018 into two groups. In group A genetic analyses were performed in the best index patient according to our criteria, while in group B at least one other family member was considered a better index patient. We then compared the results of genetic analyses for both groups with their average pretest mutation risk probabilities according to BRCAPRO calculation.

Materials and Methods

Patients

The study includes 130 Caucasian patients and their families from Germany who presented at the center for HBOC at the University Hospital Münster for genetic counseling for the first time from 2016 to 2018. Informed consent was obtained from all patients and all cases fulfilled the requirements of the GC-HBOC to offer genetic testing (11, 14). The software Cyrillic (v2.1) was used for pedigree drawing and pretest mutation risk calculation with BRCAPRO based on the reported family history. Families were selected for the study by the criteria shown in supplementary table 1 to ensure reliable pretest risk calculation.

Selection of the best index cases

The 130 families were divided into two groups depending on whether genetic testing was performed in one of the best index cases of a family (Group A, N = 98) or whether there was at least one affected family member that was more suitable but not available for genetic testing (Group B, N = 32). The clinical criteria shown in table

1 were applied in this study in order to determine if there was a better index case within a family.

Genetic analyses

Sequence analyses were performed by next-generation sequencing on an Illumina MiSeq/NextSeq system for the coding exons and adjacent intronic regions of the genes *BRCA1*, *BRCA2*, *RAD51C*, *CHEK2*, *PALB2*, *ATM*, *RAD51D*, *NBN*, *CDH1* and *TP53* according to the specified genes of the GC-HBOC (10). Coverage was at least 100 and Phred-Score was at least 30 for all nucleotides. All detected mutations were confirmed by Sanger sequencing. In four cases of group A and one case of group B sequence analyses were limited to the genes *BRCA1*, *BRCA2*, *RAD51C*, *CHEK2* and *PALB2* after a disease-causing mutation was identified in one of these genes. For the genes *BRCA1* and *BRCA2* multiplex ligation-dependent probe amplification (MLPA; MRC-Holland) was additionally performed in all cases to detect larger alterations such as deletions and duplications of whole exons. Mutations classified as pathogenic (Class 5) or likely pathogenic (Class 4) according to the guidelines of the International Agency for Research on Cancer (17) and the American College of Medical Genetics (18) were considered as disease-causing.

Statistics

The software IBM SPSS statistics (v25) was used for statistical analyses. Comparison of the average pretest mutation carrier probability for the index cases of groups A and B was performed with Mann–Whitney *U* test. The difference of correct results and false negative results (missed mutations) according to pretest risk calculations between groups A and B was analyzed with chi-square test. Values for $p < 0.05$ were considered statistically significant.

Results

Average mutation carrier probability was 33.3% (95% CI: 26.8-39.8) for group A and 39.1% (95% CI: 27.4-50.8) for group B (Table 2). Mutation carrier probability was 34.7% (95% CI: 29.1-40.4) for the whole cohort. The difference of average carrier probabilities between groups A and B was statistically not significant ($p = 0.386$). According to pretest calculations, the expected number of patients with a disease-causing mutation was 32.7 for group A and 12.5 for group B. A disease-causing mutation was found in 21 patients of group A and in 4 patients of group B. The mutation detection rate was significantly higher in group A (64.3 %) compared to group B (32.0%, $p = 0.034$) (Table 2, Supplementary Figure 1). The distribution of the mutations per gene is summarized in supplementary table 2. It should be noted that one patient of group A carried a disease-causing mutation both in *CHEK2* and *CDH1.*

Discussion

To date, no standard criteria have been defined for the selection of the best index case in families with HBOC. With this study we now establish clinical criteria for the identification of the best index case in HBOC and show its importance for the outcome of genetic analyses.

Of course, in some families the best index patient may not be available or declines genetic analyses. In other cases direct genetic testing of a different person can be justified if the cancer treatment depends on an early testing result. Nevertheless, the

mutation detection rate in our study drops to half, if the best index case was not chosen for genetic analyses. Therefore, identification of the best index case based on our clinical criteria must be emphasized whenever possible. If genetic analyses have already been performed in another family member without detecting a disease-causing mutation and the best index case in the family is still alive, additional genetic testing for the best index case should be offered in order to not miss a mutation in the family. Also, if the best index patient is already deceased, efforts should be made to investigate if blood or tissue samples are eventually still available for genetic analyses.

All patients were selected rigorously to participate in our study according to the enrollment criteria found in supplementary table 1, in order to ensure reliable calculations of mutation carrier risks and hence comparability of groups A and B. The downside of this approach is the limitation to a rather small sample size of the cohort, which, however, does not affect statistical significance of the results ($p = 0.034$). We suggest to perform a larger multicenter trial to confirm our findings and validate our selection criteria for clinical practice.

Although statistically not significant, the average mutation carrier risk was slightly higher for group B (39.1% vs. 33.3%, $p = 0.386$). This may seem paradox since the patients of this group are not the best index cases of their respective families. However, both the average mutation carrier risk and the probability that there can be found a better index case raise simultaneously with the number of persons affected by BC/OC within a family. In this context we additionally calculated the average mutation carrier risk for the best index patients of group B who were not available for genetic testing. We found no noteworthy difference between them and the tested

patients of group B (41.9% vs. 39.1%, p = 0.672), which shows the limitation of using the mutation carrier risk alone for finding the best index patient within a family.

In group B 68% of the expected mutations were missed compared to only 35.7% in group A (Supplementary Figure 1), suggesting that a reasonable number of women with BC/OC do not carry the disease-causing mutation which would be expected in the family. A plausible explanation for this could be the possibility of being affected by a cancer which developed independently of the family burden. This option seems to be probable especially when taking into account the high incidence of sporadic BC in the population.

Independently, even in group A the mutation detection rate does not reach 100%. This has been seen in previous studies as well (13, 19) albeit differences in cohort composition, pretest risk calculations and methods of genetic analyses hinder the comparison of the results. Mutations in other or currently undiscovered susceptibility genes and biased reporting of family histories are possible explanations for the fact that still not all cases of HBOC seem to be solved by genetic analyses. Also, BRCAPRO calculation just produces an estimation of mutation carrier risks. At the time the program was designed, *BRCA1/2* were the only susceptibility genes known for HBOC. Independent of this, the creators stated that other susceptibility genes would also be covered by BRCAPRO calculation if disease penetrances were comparable to those of *BRCA1/2* (13), which can be assumed at least for *TP53 (20,21), PALB2* (1, 22) and *CDH1(21)* with regard to the genes which are analyzed by the GC-HBOC. Generally, BRCAPRO can be applied for comparing the overall mutation risk between two groups of the same cohort since any hypothetical impreciseness of risk calculation would affect both. Furthermore, the mutation detection rate in our study is also double as high for group A when considering

mutations in *BRCA1/2* only. Finally, BRCAPRO is still the only software in use by the GC-HBOC since it has the highest diagnostic accuracy (23) and other software has not been approved for the clinical use yet.

In summary, we establish clinical selection criteria which can be easily applied to most families suggestive of HBOC in order to identify the best index case for genetic testing. Mutation detection rates are significantly higher when genetic analyses are performed in the best index case according to our criteria compared to other affected family members. We suggest that the selection criteria reported here can be used as a guideline for genetic counseling of HBOC.

Author Contributions:

Margot Wyrwoll: Selection of patients, data collection.

Lea Fuchs: Preparation of pedigree charts for genetic counseling.

Daniel E.J. Waschk: Conception and design, genetic counseling and BRCAPRO calculation, selection of patients, data collection, statistical analyses, drafting of manuscript.

All authors: Data analysis and interpretation, critical revision and final approval of manuscript.

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Conflict of Interests statement: The authors declare no competing interests.

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Table 1: Clinical selection criteria for identifying the best index case for genetic analyses in families with HBOC that were applied in this study.

BC: Breast cancer; OC: Ovarian cancer; HBOC: Hereditary breast and ovarian cancer

Selection criteria
• When comparing the same type of a single cancer (BC and BC or OC and OC), the patient who was more than five years younger at the time of first diagnosis was considered the better index case. • A woman with two independent BCs was chosen as better index case over a woman with a single BC except for the following conditions: Age at first diagnosis was more than 5 years younger in the woman with a single BC. Age at first diagnosis was within 5 years but the woman with a single BC had not yet reached the age of onset of the second BC of the woman with multiple BC. • In two women with two independent BCs each, one of the women was chosen as better index case if one of the following conditions were fulfilled: Age at first diagnosis of the first BC was more than 5 years younger. Age at first diagnosis was within 5 years but mean age of onset for both BCs was 5 years younger in one of the women. • When comparing a woman with either a single BC or two independent BCs and a woman with OC, the patient with BC was only considered the better index case if either the age at first diagnosis was more than 10 years younger or if both BCs occurred more than 5 years earlier. • In two women diagnosed with BC within 5 years of age, the better index case was the person who additionally had OC independent of its age of onset as long as the other woman had already reached the age of onset of the OC. • A male person with BC was considered one of best index cases independent of his age at diagnosis.

Table 2: Results of pretest risk calculations and genetic analyses.

Group A: Genetic testing was performed in one of the best index cases of a family according to our criteria.

Group B: At least one affected family member was more suitable for genetic testing.

	Group A	Group B	Total
Number of Patients	98	32	130
Average calculated mutation carrier probability	33.3% (95% CI: 26.8-39.8)	39.1% (95% CI: 27.4-50.8)	34.7% (95% CI: 29.1-40.4)
Expected number of patients with a mutation	32.7	12.5	45.2
Number of patients with a detected mutation	21	4	25
Mutation detection rate	64.3%	32.0%	55.3%

Supplementary Table 1: Inclusion and exclusion criteria for study enrollment of patients to ensure reliable pretest risk calculation with BRCAPRO.

BC: Breast cancer; OC: Ovarian cancer; HBOC: Hereditary breast and ovarian cancer

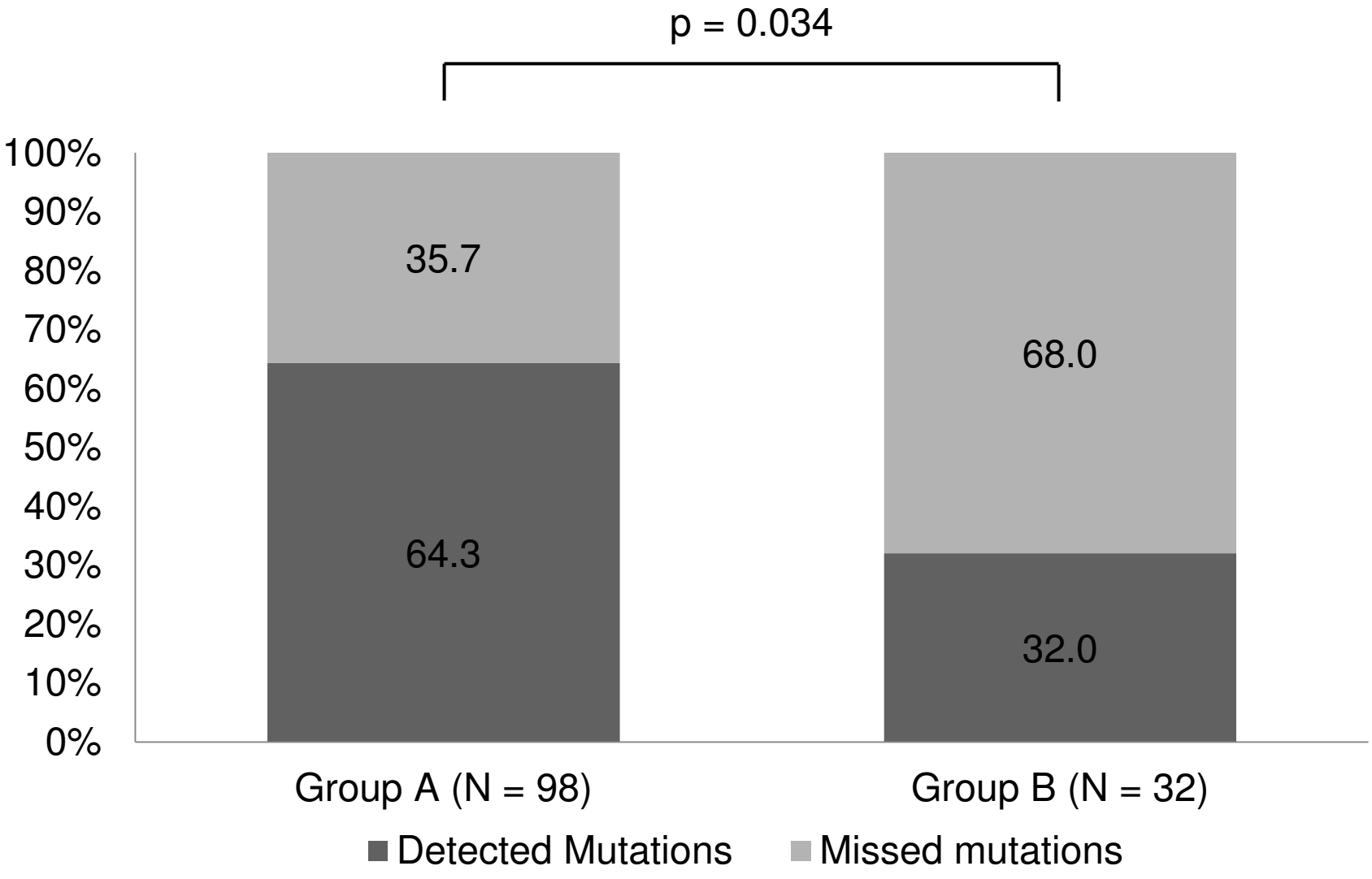
Inclusion criteria
• Family history was complete for at least three generations including ages of onset of BC/OC cases and current ages of unaffected family members. • Genetic analyses were performed in a family member (index case) affected by BC and/or OC as stated in the methods section. • Pathology reports on the subtype of cancer were available at least for the index case. • Genetic counseling and pretest risk calculation were performed for all patients by the author D.E.J. Waschk.
Exclusion criteria
• Incomplete knowledge about ages of onset and types of cancer in affected persons as well as number and current ages of unaffected relatives within three generations of the family. • Pathology reports were not available for the index case. • Pathology reports were conflicting when trying to distinguish a relapse from a *de novo* cancer in cases with metachronous breast cancer. • Consanguinity was reported for the ancestors of the index case. • A disease-causing mutation for HBOC was already known in the family at first presentation. • Other conditions leading to an increased risk of breast cancer were present in the index case such as radiation of the thorax in the treatment of Hodgkin's Lymphoma or genetic conditions like Neurofibromatosis type 1.

Supplementary Table 2: Number of detected mutations for each gene and for the categories '*BRCA1/2*' and 'Other genes'.

†One patient from group A carried a disease-causing mutation both in *CHEK2* and *CDH1.*

Category	Number of Mutations		
	Group A	**Group B**	**Total**
BRCA1/2	14	3	17
Other genes	8	1	9
Gene	**Group A**	**Group B**	**Total**
BRCA1	9	3	12
BRCA2	5	0	5
CHEK2†	5	1	6
RAD51C	0	0	0
PALB2	2	0	2
RAD51D	0	0	0
ATM	0	0	0
CDH1†	1	0	1
NBN	0	0	0
TP53	0	0	0

Supplementary Figure 1: Mutation detection rates for group A and B.



Group A: Genetic testing was performed in one of the best index cases of a family according to our criteria.
Group B: At least one affected family member was more suitable for genetic testing.