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Abstract

Introduction: International guidelines recommend genetic testing for women with familial breast cancer at an expected prevalence of pathogenic germline variants (PVs) of at least 10%. In a study sample of the German Consortium for Hereditary Breast and Ovarian Cancer (GC-HBOC), we have previously shown that women with TNBC diagnosed before the age of 50 years but without a family history of breast or ovarian cancer (sTNBC) meet this criterion. The present study in-

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vestigates the PV prevalence in *BRCA1, BRCA2,* and nine additional cancer predisposition genes in an extended sTNBC study sample including a cohort of women with a later age at sTNBC diagnosis. ***Patients and Methods:*** In 1,600 women with sTNBC (median age at diagnosis: 41 years, range 19–78 years), we investigated the association between age at diagnosis and PV occurrence in cancer predisposition genes using logistic regression. ***Results:*** 260 sTNBC patients (16.2%) were found to have a PV in cancer predisposition genes (*BRCA1: n* = 170 [10.6%]; *BRCA2: n* = 46 [2.9%], other: *n* = 44 [2.8%]). The PV prevalence in women diagnosed between 50 and 59 years (*n* = 194) was 11.3% (22/194). Logistic regression showed a significant increase in PV prevalence with decreasing age at diagnosis (OR 1.41 per 10 years younger age at diagnosis; 95% confidence interval: 1.21–1.65; *p* < 0.001). The PV prevalence predicted by the model was above 10% for diagnoses before the age of 56.8 years. ***Conclusion:*** Based on the data presented, we recommend genetic testing by gene panel analysis for sTNBC patients diagnosed before the age of 60 years. Due to the still uncertain estimate for women with sTNBC diagnosed above the age of 60 years, further studies are needed.

Introduction

Triple-negative breast cancer (oestrogen/progesterone receptor expression <1%, Her2/neu expression negative) accounts for approximately 10–15% of breast cancer subtypes [1–3]. Approximately 5% of all breast cancer patients carry a pathogenic germline variant (PV) in *BRCA1* or *BRCA2*, with PV prevalence associated with age at first diagnosis and familial history of breast and ovarian cancer. In a review of unselected TNBC cases, PVs in *BRCA1* and *BRCA2* were reported in 9–32% [4]. In a study of 2,733 women with a breast cancer diagnosis before the age of 40 years, PVs in *BRCA1* and *BRCA2* were detected in 24% [5]. TNBC associates with a hereditary context and is detectable in about 66–70% of *BRCA1* and about 16–23% of *BRCA2* PV carriers [6–8]. TNBC patients are described to have an increased prevalence of PVs compared to the other breast cancer subtypes.

The National Comprehensive Cancer Network (NCCN) recommends genetic testing for *BRCA1/2* in TNBC patients with an initial diagnosis age of less than 60 years, regardless of family history [9]. The National Institute for Health and Care Excellence (NICE) recommends genetic testing for a combined *BRCA1/2* mutation carrier probability of at least 10% [10] and for TNBC patients without a family history at an initial age of diagnosis of less than 40 years. According to the health care guidelines in Germany [11], an expected PV detection rate of 10% or higher is currently the basis for the reimbursement of costs of genetic counselling and testing within the framework of special contracts with the statutory and private health insurance. The German Consortium for Hereditary Breast and Ovarian Cancer (GC-HBOC) has comprehensively evaluated clinical criteria for genetic testing in 21,401 families with breast and/or ovarian cancer [12]. Here, the overall frequency for PV in *BRCA1/2* was 24%. These criteria have been included as a prerequisite for the reimbursement of a germline analysis in the uniform assessment standard (EBM) of the National Association of Statutory Health Insurance Physicians (Kassenärztliche Bundesvereinigung). A further analysis of 802 patients with TNBC diagnosed between 19 and 76 years of age with no other family history of breast or ovarian cancer showed a PV prevalence of 15.8%, with a prevalence above 10% until a diagnosis age of 50 years [13]. This led to the introduction of TNBC up to the age of 50 years as an additional inclusion criterion for genetic counselling and testing at GC-HBOC centres (www.konsortium-familiaerer-brustkrebs.de). The first work on PV prevalence in TNBC patients without a familial cancer history [13] only considered the high-risk genes *BRCA1* and *BRCA2*. In the course of the increasing genetic investigation of other cancer predisposition genes in recent years, including gene panel analyses, the PV prevalence in these genes is gaining in importance [14]. In the present study, the age-dependent PV prevalence in the *BRCA1/2* genes and in the further non-*BRCA1/2* breast cancer predisposition genes of the TruRisk® gene panel [14] is to be evaluated in a current collective of unselected TNBC patients without a family history of breast and ovarian cancer.

Patients and Methods
Study Samples
The study involved 1,600 women with unilateral TNBC who were diagnosed between 19 and 78 years. The women were consecutively registered at 18 specialised GC-HBOC university centres between July 1999 and August 2021 and were documented in the GC-HBOC central registry. The women stated that they had no relatives with breast or ovarian cancer in their family. All sTNBC patients were counselled and tested in accordance with the German Gene Diagnostics Act, using the same standard operating procedures. The GC-HBOC registry was approved by the respective ethics committees (07-048, March 22, 2007) and registered in the German Clinical Trials Registry (DRKS-ID: DRKS00017837). Written informed consent to participate in the GC-HBOC registry was obtained from all patients evaluated.

Methods

Hormone Receptor and HER2/Neu Status Analysis

Oestrogen, progesterone, and HER2/neu receptor status were assessed according to the national recommendations (https://www.ago-online.de/en/), which closely follow international standards. Triple-negative receptor status is defined as immunohistochemical staining of less than 1% of nuclei for the oestrogen and progesterone receptors and an immunohistochemical result with a DAKO score of less than 3+ and no HER2/neu gene amplification.

Analysis of PVs

Genetic testing of *BRCA1/2* was performed using either next-generation sequencing methods or denaturing high-performance liquid chromatography and high-resolution melting followed by direct Sanger-based sequencing of conspicuous fragments [15, 16]. With the introduction of panel diagnostics in 2015, new genes known to increase the risk of familial breast or ovarian cancer were successively introduced including *ATM, BARD1, BRIP1, CDH1, CHEK2, PALB2, RAD51C, RAD51D,* and *TP53*. These nine genes are considered as "other core genes" in the following [14].

If no pathogenic sequence alterations (PVs) were found in these analyses, the samples were analysed for copy number variations (CNVs) in the BRCA1/2 genes by multiplex ligation-dependent probe amplification (MLPA) using SALSA® MLPA® probe mixes P002 for BRCA1 and P045 for BRCA2 (MRC Holland, Amsterdam, The Netherlands) according to the manufacturer's protocol. Furthermore, the commercial software Sophia DDM™ Genomics (Sophia Genetics, Saint-Sulpice, Switzerland), GeneMapper (Applied Biosystems), CNVHunter (ngs-bits – short-read sequencing tools: GSvar; https://github.com/imgag/ngs-bits), JSI Medical Systems (Ettenheim, Germany), or the CNV module of the CLC Genomics Workbench was used for CNV prediction. Conspicuities were verified by the ExomeDepth programme followed by MLPA using specific SALSA® MLPA® kits for P041 (*ATM*), P042 (*ATM),* and P190 (CHEK2) (MRC Holland). Moreover, detection of deletions and duplications in the genes BRCA1, *BRCA2, CHEK2, RAD51C, RAD51D, PALB2, TP53, CDH1, ATM, BRIP1,* and *BARD1* was performed by using digitalMLPA probe mix D001 Hereditary Cancer Panel 1 (MRC Holland, Amsterdam). All other PVs were confirmed by Sanger sequencing.

Variants were classified according to the guidelines of the International Agency for Research on Cancer (IARC) by the GC-HBOC expert group. The classification as pathogenic or likely pathogenic (class 4 or 5) is based on literature data, multifactorial probability models, and functional analyses of the ENIGMA consortium (htts://enigmaconsortum.org/), which also include genetic data from the GC-HBOC database [17–20].

Statistical Analysis

The association between age at sTNBC diagnosis and the presence of a PV in *BRCA1, BRCA2,* or the other core genes was analysed using logistic regression. In addition, prevalences of PVs were presented as relative frequencies with 95% confidence intervals grouped by the age at diagnosis from 19 to 29 years and in decades from 30 to 79 years. The confidence intervals were calculated using Wilson's score method [21].

Due to the successive expansion of panel testing to further genes during the study period July 1999–August 2021, complete datasets are not available for all molecular genetic examinations. It was therefore determined how the prevalence of PVs in the additional core genes would increase if all patients had received a complete panel testing of all 11 core genes. Missing values for non-tested genes were replaced as follows. For patients with a PV in one

Table 1. Patient characteristics

	$n = 1,600$ (%)
Status of screening for PVs (PV status)	
Negative	1,340 (83.8)
BRCA1	170 (10.6)
BRCA2	46 (2.9)
Other core genes	44 (2.8)
Age at diagnosis, years[1]	41.2 (19.5–78.0)
Age at diagnosis depending on PV status, years[1]	
Negative	41.7 (19.5–78.0)
BRCA1	35.6 (23.9–66.8)
BRCA2	41.2 (24.8–63.6)
Other core genes	44.0 (24.6–65.9)

[1] Median (range).

of the other genes, it was assumed that no PV would be found in the non-tested genes. For patients without a PV in one of the other genes, the missing values were replaced by the observed proportion of PVs in the respective age group and the respective genes. By summing all values across the core genes, the PV prevalence in the core genes was extrapolated for all patients.

p values <0.05 were considered significant. Statistical analyses were performed using R 4.0.4 for Windows (R Core Team, www.r-project.org, [22]).

Results

The evaluation is based on 1,600 patients with sTNBC from the GC-HBOC central registry. Age at diagnosis and PV status of the patients are listed in Table 1. The median age at sTNBC diagnosis was 41 years (range 19–78 years) and the prevalence for PVs was 16.2% (10.6% for *BRCA1*, 2.9% for *BRCA2*, 2.8% in the other core genes). Patients with PVs in *BRCA1* were younger at diagnosis (median 35 years) than patients with PVs in *BRCA2* (median 41 years) and patients without evidence of PVs (median 41 years), while carriers of PVs in the other core genes had the highest age at sTNBC diagnosis (median 44 years).

Table 2 summarises the prevalences of PVs in *BRCA1, BRCA2,* and the other core genes grouped by age at diagnosis. The PV prevalence decreased with increasing age at diagnosis. While a PV was detected in about one-third of the patients with a very young age of onset (19–29 years) (30.9%), the prevalences in older patients diagnosed between 50 and 59 years and between 60 and 69 years were 11.3% and 13.2%, respectively. Regarding *BRCA1/2*, the proportion of PVs was 27.5% for patients diagnosed up to 29 years of age and 8.2% and 7.5% for patients diagnosed between 50 and 59 years and between 60 and 69 years of age, respectively.

Logistic regression analysis was performed to assess the association between age at diagnosis of sTNBC and

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Fig. 1. Association between age at sTNBC diagnosis and the prevalence of a pathogenic germline variant (PV) in *BRCA1/2* and the other core genes. The bars show the PV prevalence for *BRCA1* (red), *BRCA2* (blue), and the other core genes (green), and the error bars show the 95% confidence intervals for the PV prevalence in *BRCA1/2* and the other core genes (combined) in the individual age groups. The middle curve shows the PV prevalence predicted by the logistic regression model (with the 95% confidence interval as dashed curves). The dashed horizontal line (red) indicates the prevalence level of 10%, above which molecular genetic testing is recommended.
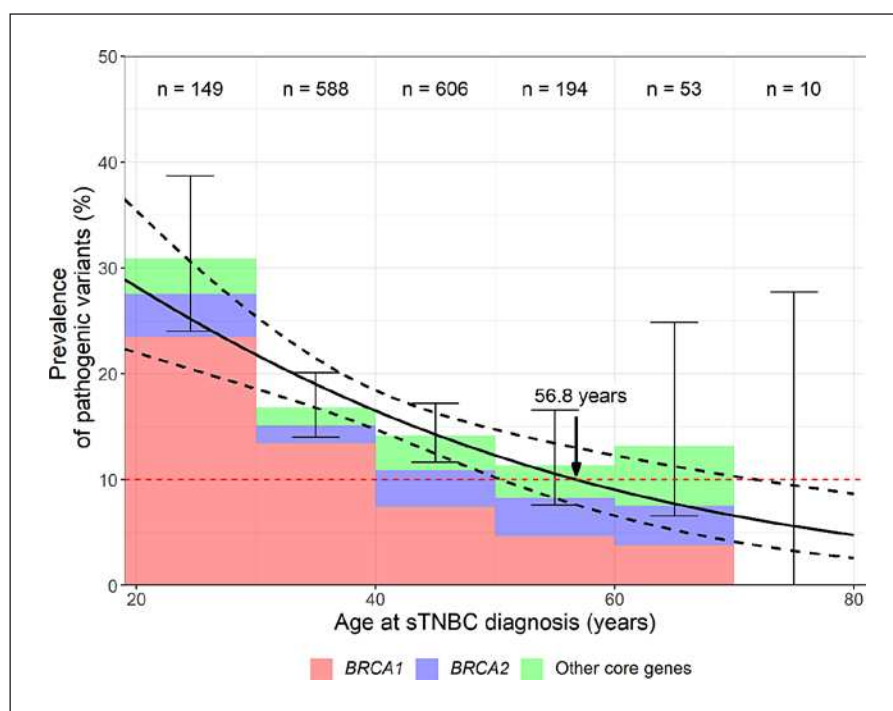
Table 2. Prevalence of PVs in *BRCA1*, *BRCA2*, and the other core genes

Age at sTNBC diagnosis (years)	n	BRCA1	BRCA2	Other core genes	BRCA1/2	All core genes
19–29	149	35 (23.5) [17.4–30.9]	6 (4.0) [1.9–8.5]	5 (3.4) [1.4–7.6]	41 (27.5) [21.0–35.2]	46 (30.9) [24.0–38.7]
30–39	588	79 (13.4) [10.9–16.4]	10 (1.7) [0.9–3.1]	10 (1.7) [0.9–3.1]	89 (15.1) [12.5–18.3]	99 (16.8) [14.0–20.1]
40–49	606	45 (7.4) [5.6–9.8]	21 (3.5) [2.3–5.2]	20 (3.3) [2.1–5.0]	66 (10.9) [8.7–13.6]	86 (14.2) [11.6–17.2]
50–59	194	9 (4.6) [2.5–8.6]	7 (3.6) [1.8–7.3]	6 (3.1) [1.4–6.6]	16 (8.2) [5.1–13.0]	22 (11.3) [7.6–16.6]
60–69	53	2 (3.8) [1.0–12.8]	2 (3.8) [1.0–12.8]	3 (5.7) [1.9–15.4]	4 (7.5) [3.0–17.9]	7 (13.2) [6.5–24.8]
70–79	10	0 (0.0) [0.0–27.8]	0 (0.0) [0.0–27.8]	0 (0.0) [0.0–27.8]	0 (0.0) [0.0–27.8]	0 (0.0) [0.0–27.8]
Total	1,600	170 (10.6) [9.2–12.2]	46 (2.9) [2.2–3.8]	44 (2.8) [2.1–3.7]	216 (13.5) [11.9–15.3]	260 (16.2) [14.5–18.1]

Statistics: *n*, *n* (%) [95% confidence interval %].

the prevalence of PVs in the core genes (Fig. 1). This analysis revealed a significant negative association between age at diagnosis and the presence of PVs (odds ratio 1.41 per 10 years younger age at diagnosis, 95% confidence interval: 1.21–1.65, *p* < 0.001). Up to an age at diagnosis of 56.8 years, the predicted PV prevalence was above 10%.

In 46 patients, a PV was found in one of the other core genes. Of these, 2 patients had a PV in *BRCA1* and in one of the other core genes (*BRCA1* and *RAD51C* or *BRCA1* and *BRIP1*). Table 3 provides an overview of all PVs detected. In addition to *BRCA1* and *BRCA2*, PVs were most frequently observed in *PALB2*, whereas no PV was found in *CDH1*.

The proportion of patients in whom all other core genes in addition to *BRCA1* and *BRCA2* were fully analysed was 45% (patients with PVs are considered to be ful-

ly analysed). The data in Tables 1 and 2 therefore represent a lower bound estimate of the actual PV prevalence in the other core genes as well as the overall PV prevalence. An extrapolation assuming complete panel diagnosis in all patients resulted in a prediction for overall PV prevalence of 12.8% and 18.9% in the patients diagnosed from 50 to 59 years and from 60 to 69 years, respectively (Fig. 2).

Discussion

We investigated the prevalence of PVs in relation to the age of diagnosis in patients with unilateral TNBC and without a self-reported family history of breast and ovarian cancer. To minimise potential underreporting, all pa-

Fig. 2. Association between age at sTNBC diagnosis and the predicted prevalence of a pathogenic germline variant (PV) in *BRCA1/2* and the other core genes, assuming a complete screening of all core genes and the age dependence of the PV prevalence. The bars show the PV prevalence for *BRCA1* (observed: red), *BRCA2* (observed: blue), and the other core genes (observed: green, additional predicted prevalence: light green), and the error bars show the 95% confidence interval for the predicted PV prevalence in *BRCA1/2* and the other core genes in the individual age groups. The dashed horizontal line indicates the prevalence of 10% above which molecular genetic testing is recommended.
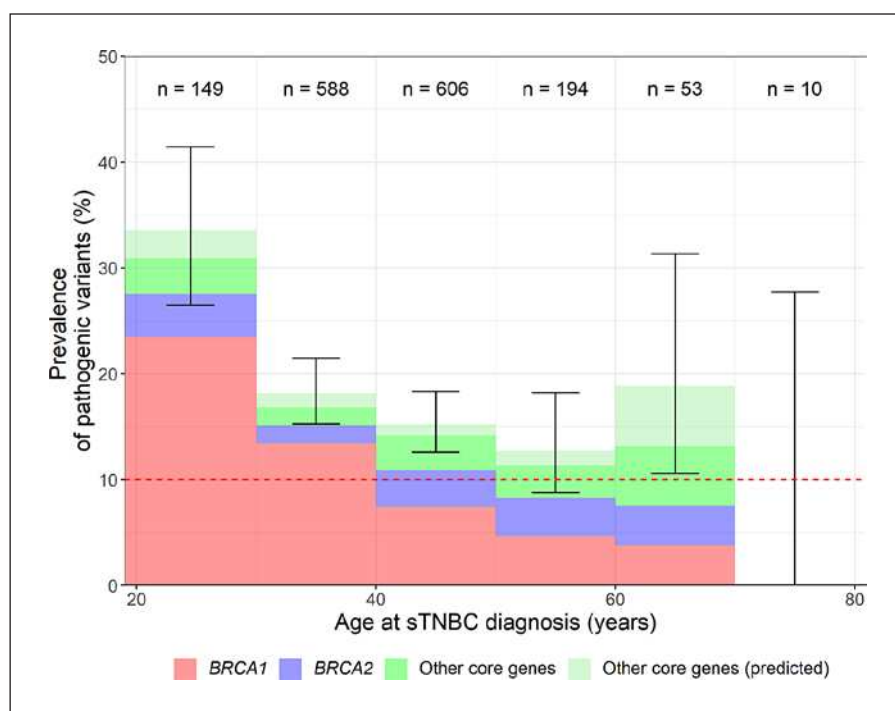
Table 3. Number of pathogenic germline variants in *BRCA1*, *BRCA2*, and the other core genes

	N = 1,602 (%)
Gene	
BRCA1	170 (10.6)
BRCA2	46 (2.9)
PALB2	16 (1.0)
RAD51C	7 (0.4)
ATM	5 (0.3)
BARD1	5 (0.3)
BRIP1	4 (0.2)
CHEK2	3 (0.2)
RAD51D	3 (0.2)
TP53	3 (0.2)
CDH1	0 (0.0)

Two patients were found to have a PV in *BRCA1* and one other core gene (*RAD51C* and *BRIP1*, respectively).

tients were interviewed following a structured anamnestic protocol that explicitly asks for cancer cases of the patient herself and family members on the maternal and the paternal side. Previous work revealed a prevalence of PVs in *BRCA1* and *BRCA2* of more than 10% in 802 sTNBC patients up to the age of about 50 years [13]. According to national consensus, a PV detection probability of 10% is the threshold for offering genetic testing for PVs in breast and ovarian cancer predisposition genes. The study results at that time led to the adaptation and expansion of the criteria for genetic counselling and genetic testing at the centres of the GC-HBOC. The collective examined in

the current study comprised 1,600 sTNBC patients. A PV prevalence of 11.3% was found in women with sTNBC with an age of diagnosis between 50 and 59 years. In a study by Couch et al. [23], of 1,828 sTNBC patients without familial breast and ovarian cancer, a PV prevalence of 10.4% was shown for this age group. In addition to *BRCA1/2*, 15 other cancer predisposition genes were analysed in the study by Couch et al. [23] (*ATM, BARD1, BRIP1, CDH1, CHEK2, MRE11A, NBN, PALB2, PTEN, RAD50, RAD51C, RAD51D, STK11, TP53, and XRCC2*). PVs were detected in 3.7% of unselected TNBC patients, with *PALB2* variants predominating (21/1,828, 1.1%). TNBC is associated more often with *PALB2* [24] than with *ATM* or *CHEK2* variants [25]. In our analysis, we found PVs in the non-*BRCA1/2* core genes in 2.8% of TNBC patients; most were detected in *PALB2* (16/1,600 = 1%). These findings only represent a lower estimate of PV prevalence in genes due to the later introduction of panel diagnostics (Fig. 1).

A limitation of our study is the small number of sTNBC patients diagnosed after the age of 60 years (n = 63). For women diagnosed between the age of 60 and 69 years, the PV prevalence is greater than 10% (13.2%), but the confidence interval is wide (6.5–24.8) due to the low sample size. A study by Shimelis and colleagues [26] reports a PV prevalence of 5% in individuals over the age of 60 years with TNBC and no family history of breast or ovarian cancer, however, without giving confidence intervals for this estimate. Therefore, further studies are needed for more accurate estimates of PV prevalence in this age group.

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Conclusion

Based on these results and a PV prevalence threshold of 10% for the offer of molecular genetic counselling and testing, women with TNBC up to 60 years of age and no family history of breast and ovarian cancer should be screened for PVs in the known risk genes for breast and ovarian cancer. Given the still uncertain estimate for women with sTNBC diagnosed above the age of 60 years, further studies are needed, e.g., within the knowledge-generating care concept of GC-HBOC.

Acknowledgments

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Statement of Ethics

The study protocol was reviewed and approved by the Ethics Committee of the University of Cologne, approval number 07-048, March 22, 2007, and registered in the German Clinical Trials Registry (DRKS-ID: DRKS00017837). Written informed consent to participate in the GC-HBOC registry was obtained from all patients evaluated.

Conflict of Interest Statement

Christopher Schröder reports an institutional grant from Illumina and research grants from BMS Stiftung Immunonkologie outside the submitted work. Julia Gallwas is a member of the aca-demic advisory board of the Bundesaerztekammer and was paid for lectures for Merck Sharp & Dohme and Roche Diagnostics between 2017 and 2019. All other authors have no conflicts to declare.

Author Contributions

K.R., S.Z., C.E., and R.K.S. were responsible for the conception and implementation of the consensus conference and for the drafting of this paper. All authors made significant contributions to data collection and interpretation as part of the consensus process. All authors reviewed and revised the manuscript critically and consented to the final version for publication.

Data Availability Statement

The participants of this study did not give written consent for their data to be shared publicly, so due to the sensitive nature of the research, supporting data is not available.

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