ORIGINAL ARTICLE

Deleterious somatic variants in 473 consecutive individuals with ovarian cancer: results of the observational AGO-TR1 study (NCT02222883)

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ABSTRACT

Background For individuals with ovarian cancer (OC), therapy options mainly depend on *BRCA1/2* germline status. What is the prevalence of deleterious somatic variants, that is, does genetic tumour testing identify subgroups of individuals who also might benefit from targeted therapy?

Methods Paired analysis of tumour-derived versus blood-derived DNA to determine the prevalence of deleterious somatic variants in OC predisposition genes (*ATM*, *BRCA1/2, BRIP1*, *MSH2/6*, *PALB2*, *RAD51C/D* and *TP53*) and the *PIK3CA* and *PTEN* genes in individuals with OC (AGO-TR1 study, NCT02222883). Results were complemented by *BRCA1*, *PALB2* and *RAD51C* promoter methylation analyses and stratified by histological subtype; 473 individuals were included.

Results The combined analyses revealed that deleterious germline variants in established OC predisposition genes (all: 125/473, 26.4%; *BRCA1/2*: 97/473, 20.5%), deleterious somatic variants in established OC predisposition genes excluding *TP53* (all: 39/473, 8.2%; *BRCA1/2*: 30/473, 6.3%) and promoter methylation (all: 67/473, 14.2%; *BRCA1*: 57/473, 12.1%; *RAD51C*: 10/473, 2.1%; *PALB2*: 0/473) were mutually exclusive, with a few exceptions. The same holds true for deleterious somatic *PIK3CA* and/or *PTEN* variants (33/473, 7.0%) found to be enriched in endometrioid and clear cell OC (16/35, 45.7%); 84.3 % of the deleterious single-nucleotide/indel germline variants in established OC predisposition genes showed significantly higher variant fractions (VFs) in the tumour-derived versus blood-derived DNA, indicating a loss of the wild-type alleles.

Conclusion Tumour sequencing of the *BRCA1, BRCA2, PIK3CA* and *PTEN* genes along with *BRCA1* and *RAD51C* promoter methylation analyses identified large subgroups of germline mutation-negative individuals who may be addressed in interventional studies using PARP or PI3K/AKT/mTOR inhibitors.

Trial registration number NCT02222883

INTRODUCTION

According to the Global Burden of Disease Cancer Collaboration,[1] worldwide, approximately 254 000 women are diagnosed with ovarian cancer (OC), and 165 000 women die from the disease every year. Despite improvements in therapy, the prognosis for OC is still poor, with an average 5-year survival rate of 47.4% (National Cancer Institute, Cancer Stat Facts: Ovarian Cancer, https://web.archive.org/web/20190201115803/https://seer.cancer.gov/statfacts/html/ovary.html). The genetic characterisation of the tumour is important for tailored therapies. The interest in genetic tumour testing for this entity has strongly increased, particularly since the approval of PARP inhibitors for the treatment of recurrent OC in carriers of deleterious somatic or germline *BRCA1/2* variants by the European Medicines Agency in Europe and the Food and Drug Administration in the USA some years ago.[2 3]

The *BRCA1/2* genes are critical in the process of homologous recombination (HR) repair of double-strand DNA breaks. Several studies using gene panel analyses for the detection of pathogenic germline variants in individuals with OC have been published in recent years, revealing the germline status of *BRCA1/2* as a valuable prognostic and/or predictive factor currently widely used for clinical decision making.[4–7] Additional OC predisposition genes (*ATM*, *BRIP1*, *MSH2*, *MSH6*, *PALB2*, *RAD51C*, *RAD51D* and *TP53*) were validated by, for example, Norquist *et al* and Lilyquist *et al* in cohorts sufficiently large to make reliable statements.[8 9] Disease associations for these genes have been clinically validated using the ClinGen clinical validity framework.[10] While associations for *ATM* and *PALB2* are not firmly established and penetrances for genes like *RAD51C/D* and *BRIP1* still have to be investigated in different populations, we defined these eight genes (in addition to *BRCA1/2*) as 'established OC predisposition genes' for this investigation. Data on the prevalence of deleterious

somatic alterations in additional OC predisposition genes are still sparse, and the clinical relevance of these findings is a matter of debate.[4 9 11–15]

It is suggested that the heterozygous germline inactivation of OC predisposition genes may be accompanied by a somatic inactivation of the second allele by another deleterious variant, loss of heterozygosity (LOH) or promoter methylation, resulting in an HR deficiency and limited DNA repair capacity of the tumour cells. However, it has recently been proposed that *BRCA1* promoter hypermethylation rarely occurs in individuals with an underlying deleterious *BRCA1/2* germline variant, although this phenomenon has not been studied extensively, and the evidence from individual studies is limited.[16] Therefore, this investigation was initiated to determine whether additional tumour testing identifies a sufficiently large subgroup of individuals with deleterious somatic variants who may be addressed in upcoming interventional studies. We conducted a paired next-generation sequencing (NGS) analysis of blood-derived and tumour-derived DNA from formalin-fixed paraffin-embedded (FFPE) tumour samples obtained from 473 OC individuals enrolled in the observational AGO-TR1 study.[17 18] This investigation was complemented by promoter methylation analyses of the *BRCA1*, *PALB2* and *RAD51C* genes, which were the genes with the most frequent deleterious germline variants in the AGO-TR1 study sample. The analysis of *BRCA2* promoter methylation was omitted based on previous study results.[19–21] In addition, we investigated the occurrence of deleterious somatic *PIK3CA* and *PTEN* variants and stratified our findings by histological subtypes.

STUDY SAMPLE AND METHODS

Study sample

A total of 523 consecutive individuals with invasive epithelial OC were recruited and counselled in 20 centers of the Arbeitsgemeinschaft Gynaekologische Onkologie (AGO), Germany. All individuals were older than 18 years and provided written informed consent prior to enrolment in the trial. Demographic data, disease characteristics, family history and medical history were documented as described previously.[17] FFPE tumour samples were available from 496 of 523 individuals enrolled in the AGO-TR1 study.

Next-generation sequencing

All DNA samples were centrally analysed (Center for Hereditary Breast and Ovarian Cancer, University Hospital Cologne, Germany) by targeted NGS covering the entire coding regions and exon-flanking sequences (±15 nt) of 27 genes (*ATM*, NM_000051.3; *BARD1*, NM_000465.3; *BRCA1*, NM_007294.3; *BRCA2*, NM_000059.3; *BRIP1*, NM_032043.2; *BUB1B*, NM_001211.5; *CDH1*, NM_004360.4; *CHEK1*, NM_001330427.1; *CHEK2*, NM_007194.3; *FAM175A*, NM_139076.2; *FANCM*, NM_020937.3; *MAP3K1*, NM_005921.1; *MLH1*, NM_000249.3; *MRE11A*, NM_005591.3; *MSH2*, NM_000251.2; *MSH6*, NM_000179.2; *NBN*, NM_002485.4; *PALB2*, NM_024675.3; *PIK3CA*, NM_006218.3; *PMS2*, NM_000535.6; *PTEN*, NM_000314.6; *RAD50*, NM_005732.3; *RAD51C*, NM_058216.2; *RAD51D*, NM_002878.3; *STK11*, NM_000455.4; *TP53*, NM_000546.5; and *XRCC2*, NM_005431.1). The methodologies and the results of the germline analysis of 25 genes (excluding *PIK3CA* and *MAP3K1*) were described previously.[17] The hybridisation capture-based NGS method (Agilent, Santa Clara, California, USA) was suitable for the analysis of DNA derived from either blood or FFPE tumour samples (Agilent SureSelect XT protocol optimised for 200 ng of genomic DNA). For the isolation of DNA from FFPE tumour samples, H&E-stained 3 µm tissue sections were centrally investigated (Institute of Pathology, University Hospital Bonn, Germany); that is, tumour areas containing >80% tumour nuclei were chosen for DNA isolation. DNA isolation from FFPE tumour samples was conducted using standard procedures (see supplementary Materials and Methods). Sequencing was performed on MiSeq or HiSeq4000 devices (Illumina, San Diego, California, USA). Of the 496 tumour samples, 488 samples were successfully analysed by targeted NGS with a mean read coverage of at least 100×. For these samples, the mean read coverage was 570× (range 110× –1802×). Bioinformatic analyses were carried out using the SOPHiA DDM platform (Sophia Genetics, Saint-Sulpice, Switzerland) with a minimum cut-off value of 5% for VFs.

Detection of CNVs

The germline analyses covered the detection of CNVs. All 523 blood-derived DNA samples were screened for CNVs in the *BRCA1/2* genes by Multiplex Ligation-dependent Probe Amplification (MLPA) using the SALSA MLPA probemixes P002 (*BRCA1*) and P045 (*BRCA2*) (MRC-Holland, Amsterdam, The Netherlands). In addition, NGS data were used to identify CNVs in non-*BRCA1/2* genes using an in silico CNV detection tool incorporated in the SOPHiA DDM platform. Neither CNV calling using the in silico CNV detection tool of the SOPHiA DDM platform nor MLPA-analysis were suitable for FFPE-derived DNA samples. Thus, CNV detection in tumour samples was not included in this investigation.

Variant classification

Variants were considered somatic if they were not identified in a paired germline analysis of the corresponding blood sample. Variant classification was performed as previously described.[18 21 22] All genetic variants were classified using a five-tier variant classification system as proposed by the International Agency for Research on Cancer Unclassified Genetic Variants Working Group, namely, deleterious=class 5, likely deleterious=class 4, variant of uncertain significance=class 3, likely benign=class 2 and benign=class 1. Variants reported to occur in large outbred control reference groups at an allele frequency of >1% were generally considered benign. Class 4/5 variants were subsequently defined as 'deleterious variants'.

Quantitative methylation analysis

Bisulfite conversion of 2 µg of DNA was performed using the innuCONVERT Bisulfite Basic Kit (Analytik Jena, Jena, Germany). Quantitative methylation-specific PCR assays were designed to allow specific amplification of the bisulfite-converted methylated gene promoter sequences of *BRCA1*, *PALB2* and *RAD51C*. The assays were located in regions previously described to be methylated in OC[23–25] (online Supplementary Figure 1). The assays and the oligonucleotide sequences are described in the online supplementary table 1. PCR was performed in 20 µL reaction volumes with PCR buffer[26]; per cent methylation was calculated using the $^{\Delta\Delta}$CT method.[27] Triplicate measurements were carried out for each sample, and median methylation levels were computed with values ≥5% considered positive. Of the 496 tumour samples, methylation

analyses for all three target genes were successfully performed for 473 samples.

Statistical analyses

Statistical analyses were conducted using SPSS Statistics V.25. Fisher's exact test and Student's t-test were used to calculate levels of significance, with p values <0.05 considered significant.

RESULTS

Prevalence of deleterious germline variants in 473 consecutive individuals with OC

We focused on 473 individuals enrolled in the AGO-TR1 study for which NGS data for the tumour samples along with promoter methylation analyses of the *BRCA1*, *PALB2* and *RAD51C* genes were available (CONSORT-like diagram, online Supplementary Figure 2). In this subgroup, comprising 90.4% of the overall AGO-TR1 study sample (473/523), the prevalence of deleterious germline variants was similar to that observed in the overall group, that is, deleterious germline variants in established OC genes (*ATM*, *BRCA1*, *BRCA2*, *BRIP1*, *MSH2*, *MSH6*, *PALB2*, *RAD51C*, *RAD51D* and *TP53*)[8 9] were identified in 125 of 473 individuals (26.4%, online Supplementary Table 2) versus 140 of 523 individuals (26.8%) in the overall sample.[17 18] In the subgroup, deleterious germline variants were most prevalent in *BRCA1* and *BRCA2* (97 of 473 individuals; 20.5%), followed by *RAD51C* (13 of 473 individuals, 2.7%) and *PALB2* (4 of 473 individuals, 0.8%). Regarding all 27 genes analysed, 142 deleterious germline variants (129 single-nucleotide/indel variants and 13 germline CNVs; online Supplementary Table 2) were identified in 133 of 473 individuals (28.1%; nine individuals carried two deleterious germline variants; online Supplementary Table 2).

Detection of deleterious germline variants in the corresponding tumour samples

In the paired NGS analyses of tumour-derived versus blood-derived DNA, all 129 single-nucleotide/indel variants were also detected in the corresponding tumour samples (online Supplementary Table 2). Of the 129 single-nucleotide/indel variants, 115 variants were observed in the established OC predisposition genes (*ATM*, *BRCA1*, *BRCA2*, *BRIP1*, *MSH2*, *MSH6*, *PALB2*, *RAD51C*, *RAD51D* and *TP53*).[8 9] The vast majority of the deleterious single-nucleotide/indel variants affecting the established OC predisposition genes (97/115; 84.3%) showed significantly higher VFs in the tumour-derived versus blood-derived DNA (p<0.05, Fisher's exact Test; online Supplementary Table 2), indicating a loss of the wild-type (WT) alleles in the neoplastic tissue. The remaining 14 deleterious single-nucleotide/indel variants were observed in nine of the additional genes, and significantly increased VFs in the tumour tissue were observed for only five variants (35.7%; *NBN* [2/2], *BUB1B* [1/1], *FAM175A* [1/1], *XRCC2* [1/1], *FANCM* [0/3], *CHEK2* [0/2], *MRE11A* [0/2], *CHEK1* [0/1] and *RAD50* [0/1]).

Prevalence of deleterious somatic variants in 473 consecutive individuals with OC

Deleterious somatic *TP53* variants (386 variants) were identified in 383 of 473 individuals (81.0%, table 1, figure 1). The majority of the deleterious somatic *TP53* variants were missense variants (238/386; 61.7%; figure 1). Of note, deleterious somatic *TP53* variants were most prevalent in individuals with high-grade serous OC (88.5%) and serous/papillary OC (94.7%). Excluding *TP53*, deleterious somatic variants predominantly affected the *BRCA1* (18 variants; 18 of 473 individuals, 3.8%; table 1) and *BRCA2* genes (12 variants; 12 of 473 individuals, 2.5%; table 1). Deleterious somatic variants in all other established OC predisposition genes were rare (14 variants; 12 of 473 individuals, 2.5%; 4 × *ATM*, 1 × *BRIP1*, 1 × *MSH2*, 4 × *MSH6*, 3 × *PALB2*, 1 × *RAD51D;* table 1). Among the additional genes analysed, deleterious somatic variants were most prevalent in the *PIK3CA* gene (26 variants; 24 of 473 individuals, 5.1%), mostly missense variants described to be activating (online Supplementary Table S3, figure 1). Deleterious somatic *PTEN* variants (24 variants) were identified in 16 of 473 individuals (3.4%). Deleterious somatic variants in *PIK3CA* and/or *PTEN* were most prevalent in individuals with high-grade endometrioid, low-grade endometrioid or clear cell OC (16 of 35 individuals, 45.7%; table 1). Notably, we observed the co-occurrence of deleterious somatic *PIK3CA* and *PTEN* variants in 5 of 22 individuals (22.7%) with high-grade endometrioid OC (online Supplementary Table 3).

Table 1 Study sample stratified by histological subtype, mutation and methylation status. Information on histological subtype was not available for six tumours ('missing').

Histological subtype	Individuals	*gBRCA1* (%)	*gBRCA2* (%)	*gOCgenes* (%)	*sTP53* (%)	*sBRCA1* (%)	*sBRCA2* (%)	*sOCgenes* (%)	*sPIK3CA* (%)	*sPTEN* (%)	*mBRCA1* (%)	*mRAD51C* (%)
High-grade serous	373	66 (17.7)	21 (5.6)	23 (6.2)	330 (88.5)	14 (3.8)	10 (2.7)	9 (2.4)	7 (1.9)	2 (0.5)	50 (13.4)	8 (2.1)
High-grade endometrioid	22	3 (13.6)	0 (/)	2 (9.1)	9 (40.9)	2 (9.1)	1 (4.5)	2 (9.1)	5 (22.7)	6 (27.3)	2 (9.1)	0 (/)
Serous/papillary grade unknown	19	2 (10.5)	0 (/)	1 (5.3)	18 (94.7)	1 (5.3)	0 (/)	0 (/)	1 (5.3)	0 (/)	2 (10.5)	1 (5.3)
Low-grade serous	16	0 (/)	1 (6.3)	1 (6.3)	2 (12.5)	0 (/)	0 (/)	0 (/)	2 (12.5)	1 (6.3)	0 (/)	1 (6.3)
Low-grade endometrioid	7	1 (14.3)	0 (/)	0 (/)	2 (28.6)	1 (14.3)	0 (/)	1 (14.3)	2 (28.6)	4 (57.1)	0 (/)	0 (/)
Clear cell	6	0 (/)	0 (/)	0 (/)	0 (/)	0 (/)	0 (/)	0 (/)	4 (66.7)	1 (16.7)	0 (/)	0 (/)
Mucinous	6	0 (/)	0 (/)	0 (/)	1 (16.7)	0 (/)	0 (/)	0 (/)	0 (/)	1 (16.7)	0 (/)	0 (/)
Other/unspecified	18	2 (11.1)	0 (/)	1 (5.6)	15 (83.3)	0 (/)	0 (/)	0 (/)	3 (16.7)	1 (5.6)	2 (11.1)	0 (/)
Missing	6	1 (16.7)	1 (16.7)	0 (/)	6 (100)	0 (/)	1 (16.7)	0 (/)	0 (/)	0 (/)	1 (16.7)	0 (/)
Total	**473**	**75 (15.9)**	**23 (4.9)**	**28 (5.9)**	**383 (81.0)**	**18 (3.8)**	**12 (2.5)**	**12 (2.5)**	**24 (5.1)**	**16 (3.4)**	**57 (12.1)**	**10 (2.1)**

A total of 18 tumours were summarised as 'other/unspecified' of which 11 tumours showed a mixed histopathology (defined by two or more distinct histological subtypes present based on routine histopathological assessment), 4 tumours were undifferentiated, 2 were adenocarcinomas and 1 was a pleomorphic cellular tumour. In the group of individuals with high-grade serous carcinoma, the mean age at first diagnosis was significantly younger in carriers of deleterious germline *BRCA1/2* variants (52.4 years, range 30–77; p<0.0001, Student's t-test) and in individuals with *BRCA1* promoter methylation (55.5 years, range 32–78; p=0.0205, Student's t-test) compared with all individuals with high grade serous carcinoma (59.2 years, range 21–93).

g, germline; *mBRCA1*, *BRCA1* promoter methylation ≥5%; *mRAD51C*, *RAD51C* promoter methylation ≥5%; s, somatic.

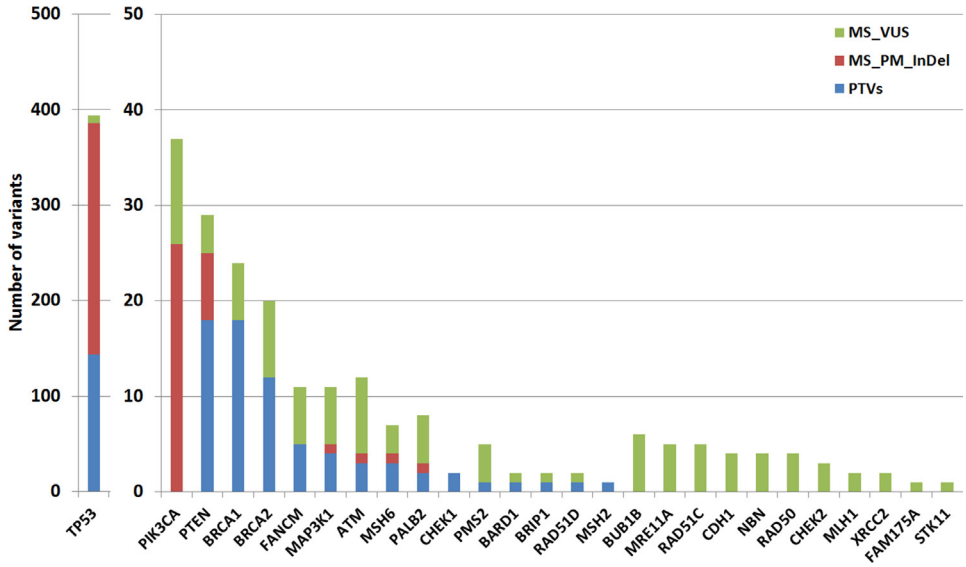

Figure 1 Number of somatic variants (including deleterious variants and VUS) per gene identified in 27 genes in 473 individuals with OC. The number of deleterious variants corresponds to the number of individuals with deleterious variants per gene, with the exception of *TP53* (383 individuals), *PIK3CA* (24 individuals), *PTEN* (16 individuals) and *FANCM* (4 individuals). No deleterious somatic variants were observed in the genes *BUB1B*, *CDH1*, *CHEK2*, *FAM175A*, *MLH1*, *MRE11A*, *NBN*, *RAD50*, *RAD51C*, *STK11* and *XRCC2*. MS_VUS, missense variants and other variants of unknown significance; MS_PM_InDel, deleterious missense variants and indel variants; OC, ovarian cancer; PTVs, protein truncating variants.

Deleterious somatic variants in all additionally tested genes were rare. Deleterious somatic *MAP3K1* variants (five variants) were identified in 5 of 473 individuals (1.1%; figure 1). All other genes affect less than 1% of all individuals for each gene (figure 1; 1 × *BARD1*, 2 × *CHEK1*, 4 × *FANCM*, 1 × *PMS2*).

Promoter methylation analyses of the *BRCA1*, *PALB2* and *RAD51C* genes

Promoter methylation analyses were carried out for the *BRCA1*, *PALB2* and *RAD51C* genes in the 473 tumour samples. *PALB2* gene promoter methylation was not detected in any of our tumour samples (online Supplementary Table S3). *PALB2* promoter methylation was previously described in OC with a clear cell subtype[23] (representing only 1.3% of our study sample; table 1) but not in high-grade serous carcinoma.[28] Methylation of the *BRCA1* gene promoter was identified in 57 of 473 individuals (12.1%; table 1). Methylation of the *RAD51C* gene promoter was identified in 10 of 473 individuals (2.1%; table 1).

Combined analysis of the study results

In summary, 125 of 473 individuals (26.4%) carried deleterious germline variants in the established OC predisposition genes, with LOH representing the second hit necessary for tumour development in a large proportion (84.3%) of these samples (figure 2). Deleterious somatic variants in established OC predisposition genes (excluding *TP53*) were identified in

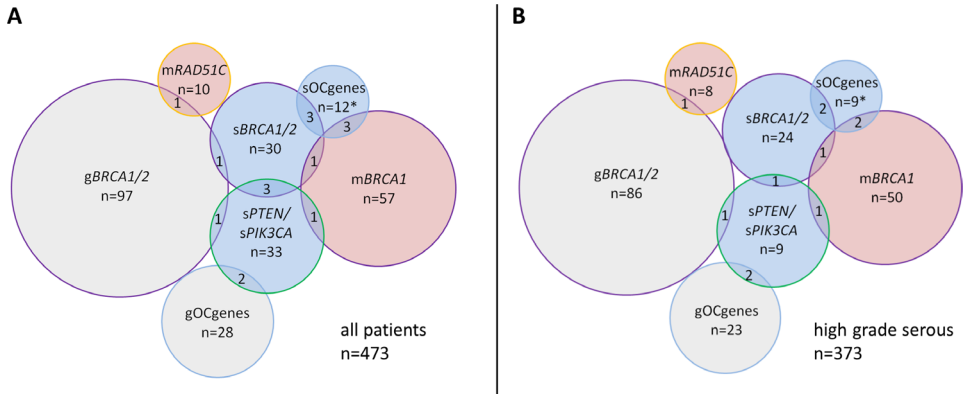

Figure 2 Schematic representation of individuals with deleterious germline and somatic alterations identified in tumour samples obtained from 473 OC individuals for all histological tumour subtypes (A) and high grade serous histological subtype (B). Germline or somatic deleterious variants in *BRCA1/2* as well as in the other OC genes and methylation of *BRCA1* or *RAD51C* could be detected in 224 (47.4%) of the 473 investigated individuals (an overlap between the groups, that is, the presence of two changes could be detected for only 10 individuals. In addition, somatic changes in *PIK3CA* or *PTEN* could be detected in 33 individuals, of which seven individuals showed somatic changes in both genes and another seven individuals showed an overlap with one of the other groups. *One individual with a deleterious germline *MSH6* variant carried additional deleterious somatic variants in *BRIP1* and *MSH6* (individual #119, online Supplementary Table S3). OCgenes: eight non-*BRCA1/2* genes (*ATM*, *BRIP1*, *MSH2*, *MSH6*, *PALB2*, *RAD51C*, *RAD51D* and *TP53*) significantly associated with OC. Deleterious somatic *TP53* variants were excluded from the 'sOCgenes' subgroup. g, germline; m, methylation; OC, ovarian cancer; s, somatic.

39 of 473 individuals (8.2%, figure 2). Promoter methylation of the *BRCA1* and *RAD51C* genes was observed in 57 of 473 individuals (12.1%) and 10 of 473 individuals (2.1%), respectively (figure 2). With a few exceptions, these somatic single-nucleotide/indel variants and methylation of *BRCA1* and *RAD51C* and deleterious germline variants were mutually exclusive. The same holds true for the subgroup of 33 individuals (7.0%) carrying deleterious somatic *PIK3CA* and/or *PTEN* gene variants (figure 2).

DISCUSSION

It is generally assumed that the heterozygous germline inactivation of cancer predisposition genes may be accompanied by a somatic inactivation of the second allele by deleterious variant, LOH or promoter methylation in the neoplastic tissue. In this large investigation of 473 individuals with OC, we demonstrated that the majority of the deleterious germline single-nucleotide/indel variants affecting established OC predisposition genes (97/115; 84.3%) showed significantly higher VFs in tumour-derived versus blood-derived DNA samples (online Supplementary Table 2), indicating the loss of the WT alleles by either large deletions (ie, LOH with copy number losses) or copy number neutral LOH.[29] Regarding individuals with deleterious *BRCA1/2* germline variants, only 1 of 97 individuals (1.0%, figure 2) carried a deleterious germline variant plus a different deleterious somatic variant in the *BRCA2* gene (individual #92; online Supplementary Table 3). Regarding other established OC predisposition genes, only 1 of 28 individuals (3.6%, figure 2) carried a deleterious germline variant plus a different deleterious somatic variant in the *MSH6* gene (individual #119; online Supplementary Table S3). The *BRCA1/RAD51C* promoter methylation was not found in individuals carrying a deleterious germline or somatic variant in the same gene. Thus, deleterious somatic single-nucleotide/indel variants or promoter methylation rarely represent the second hit in the development of hereditary OC.

Deleterious somatic variants in OC predisposition genes (excluding *TP53*) were predominantly found in the *BRCA1* (18 variants; 18 of 473 individuals, 3.8%) and *BRCA2* genes (12 variants; 12 of 473 individuals, 2.5%). The prevalence of deleterious somatic *BRCA1/2* variants is comparable with those reported in previous studies.[4 11 13 30] Deleterious somatic variants in other established OC predisposition genes (excluding *TP53*) were generally rare, affecting less than 1% of all individuals for each gene (figure 1). Consequently, our analyses demonstrate that the vast majority of deleterious variants found in the tumour were of germline origin. Regarding *BRCA1/2*, 128 deleterious variants were found in the tumour, of which 98 (76.6%) were proven germline variants. For all genes significantly associated with OC (excluding *TP53*), 168 deleterious variants were found in the tumour, of which 126 (75.0%) were proven germline variants. Thus, tumour testing without prior germline testing may require genetic counselling due to its potential implications for the stratification of the risk of the individual for other malignancies (ie, breast cancer) and, most importantly, the stratification of the cancer risks of family members who may benefit from predictive testing and prophylactic measures. Moreover, genetic tumour testing alone may miss deleterious germline CNVs in OC predisposition genes.[31]

Due to the high prevalence of deleterious germline variants in OC predisposition genes, germline testing is generally recommended in individuals with OC. We suggest that germline mutation analysis of established or proposed OC predisposition genes should be complemented with genetic tumour testing in patients without deleterious germline variants in these genes. Genetic tumour testing of OC predisposition genes, the *PIK3CA* and *PTEN* genes and *BRCA1* and *RAD51C* promoter methylation analyses identified a large subgroup of germline mutation-negative individuals (125/348, 35.9%), which may be addressed in future interventional studies using poly ADP ribose polymerase (PARP) or phosphoinositide 3-kinase (PI3K)/ protein kinase B (AKT)/ mechanistic target of rapamycin (mTOR) inhibitors.[32] Matulonis *et al*[33] already described the activity of olaparib in combination with a PI3K-inhibitor. However, they focused only on high-grade serous OC. Our data suggest that treatment options with PI3K inhibitors could also be further investigated in other histological subtypes. In our series, the highest rates of deleterious somatic *PIK3CA* and/or *PTEN* variants were shown in the subgroup of individuals with endometrioid and clear cell tumours (table 1).

In summary, germline, somatic or epigenetic alterations that likely impair OC predisposition gene activity were identified in 224 of 473 individuals (47.4%, figure 2), with an even higher prevalence of (epi)genetic alterations in the subgroup of individuals with high-grade serous OC (figure 2B). Recently, the phase 3 SOLO1 trial (NCT01844986) revealed that maintenance therapy with the PARP inhibitor olaparib provided a substantial benefit with regard to progression-free survival among women with newly diagnosed advanced OC and a deleterious *BRCA1/2* variant, with a 70% lower risk of disease progression or death with olaparib compared with placebo.[34] The question emerges, however, whether the presentation of *BRCA1/RAD51C* promoter methylation or a deleterious somatic *BRCA1/2* variant represents a robust predictive marker for therapy response, especially regarding PARP inhibitor therapy. The response to PARP inhibition was found to depend on HR deficiency and is not restricted to deleterious *BRCA1/2* germline variants,[16] and in vitro analyses suggested that *BRCA1* hypermethylation and *BRCA1* deleterious variants conferred the same degree of sensitivity to PARP inhibition.[5] Further prospective studies will help clarify the clinical significance of these somatic (epi)mutations as biomarkers to direct targeted therapy.

Limitations of this study

Due to the mean read coverage of 570×, we used a VF cut-off value of at least 5%. Therefore, we cannot exclude the existence of low-abundance variants in the tumour samples investigated in this study.

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