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The results of multigene panel sequencing in Slovak HBOC families

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Hereditary breast and ovarian cancer (HBOC) is primarily associated with mutations in the *BRCA1/2* genes. However, causal variants in other high, moderate, and low penetrance genes proportionally increase the risk of breast/ovarian cancer. This study aims to provide data about the mutation spectrum of HBOC-associated genes in Slovak HBOC families and estimate the ratio of *BRCA* versus non-*BRCA* causal variants. We used panel sequencing containing 22 high/moderate-risk susceptibility genes and parallel MLPA analysis of *BRCA1/2*, *CHEK2* genes, to analyze 94 individuals with a strong family/personal history of breast and/or ovarian cancer. The analyzed group consisted of 80 patients diagnosed with cancer (85.1%) and 14 healthy individuals (14.9%) with a positive family history of HBOC syndrome. In total, we have identified 22 causal DNA variants (23.4%) showing 15 primary findings in *BRCA1/2* genes (68.2%) and 7 positive secondary findings in *CHEK2*, *PALB2*, *CDH1*, and *MUTYH* genes (31.8%). The most frequent pathogenic alterations were BRCA1 mutations c.181T>G and CNV variant (c.5573-?_c.5701+?)del, known as deletion of exons 21-22. Besides known mutations, the *BRCA1* variant c.2794del (p.Val932Leufs*68) and variant c.2480dup (p.Tyr827*) in the *CDH1* gene represent the novel, previously unpublished variants that might be population-specific. In conclusion, we provide the first report of multigene panel testing in Slovak HBOC families demonstrating that almost one-third of pathogenic mutations are situated in susceptibility genes other than *BRCA1/2*. Although multigene panel testing requires precise data filtration and interpretation, it might bring the relevant data for clinical management of the patients.

Key words: hereditary breast and ovarian cancer, BRCA1, BRCA2, panel sequencing, pathogenic variants, secondary findings

According to 2018 GLOBOCAN statistics, breast cancer is the leading type of malignancy diagnosed in women worldwide, representing the most common cause of cancer death in the female population. Ovarian cancer is ranked at 8th place of global cancer incidence in women [1], associated with one of the highest mortalities of all gynecological cancers in developed countries. In Slovakia, GLOBOCAN data show estimated cumulative risks of breast and ovarian cancer at 6.59% and 1.3%, respectively [1]. According to very recent Eurostat data from EU Member states (data extracted in August 2020), Slovakia and Luxembourg show the highest standardized death rates for breast cancer among women (40.7/100,000 and 40.3/100,000, respectively) [2].

Hereditary breast and ovarian cancer (HBOC) syndrome is an inherited oncological syndrome characterized by the high frequency of breast and ovarian cancer in family history. The comprehensive molecular-genetic screening of HBOC families has led to the identification of a great number of genes associated with increased risk of breast/ovarian cancer. According to the findings, the vast majority of known HBOC susceptibility genes encode tumor suppressors involved in genome stability pathways [3]. The group of HBOC-associated genes with high penetrance consists of *BRCA1, BRCA2, CDH1, PTEN, STK11,* and *TP53* genes. In addition, *PALB2, BRIP1, ATM, CHEK2, BARD1, NBN, NF1, RAD51C, RAD51D*, *EPCAM*, and mismatch repair genes (*MLH1, MSH2, MSH6, PMS2)* have been characterized as moderate and low penetrance HBOC-associated genes [4]. However, HBOC syndrome is most often associated with pathogenic variants (PV) or likely pathogenic DNA variants (LPV) in the tumor-suppressor genes *BRCA1* or *BRCA2*. Thus, both genes remain the most important part of genetic testing as approximately 25% of HBOC cases link to pathogenic variants in high-penetrant *BRCA* genes [5]. In general,

the estimated risk of breast and ovarian cancers in *BRCA1* mutation carriers ranges from 57–65% and 20–50%, respectively [6, 7], *BRCA2* mutations are associated with 35–57% and 5–23% average risks of breast and ovarian cancers, respectively [6]. Importantly, the risk of male breast cancer is significantly increased to 5–10% with the *BRCA2* mutation and 1–5% with a *BRCA1* mutation, compared to a risk of 0.1 % in the general population [8]. Besides breast and ovarian cancer, *BRCA1/2* mutation carriers are prone to develop other malignancies, including melanoma, pancreatic, and prostate cancer [9]. Recently, Exome Aggregation Consortium (ExAC) reported the prevalence of *BRCA1* and *BRCA2* variants in the general population at 0.26% and 0.36%, respectively [10]. However, certain geographical regions and ethnic groups are characterized by a higher frequency due to the mutation founder effect. Examples of such populations are island populations (Sardinia, Iceland), Ashkenazi Jews, or French Canadians [11].

Importantly, pathogenic variants in both *BRCA* genes do not explain all HBOC families. Further search for more susceptibility genes has identified several high-penetrance genes besides *BRCA1* and *BRCA2*, detected in 5% of patients. Moreover, other medium and low penetrance genes were reported in 5–14% of cases [12]. PV/LPV in the high penetrant HBOC-associated genes *TP53, CDH1*, and *PTEN* genes significantly increase the risk of ovarian cancer and are associated with the estimated lifetime risk of breast cancer of 25–79%, 39–52%, and 25–85%, respectively [13–15]. According to the findings, germline PV/LPV in the *STK11* gene are associated with the lifetime risk of distinct tumor types, including breast and ovarian tumors [16]. Very recently, multiple, complementary analyses on 150 breast cancer-associated regions analyzed in more than 200,000 individuals of European origin identified more than 200 high-confidence risk signals and at least 191 target genes supported by strong evidence. These data and subsequent studies might provide an important insight into the biology underlying breast cancer susceptibility [17].

Progress in next-generation sequencing (NGS) techniques, together with the development of sophisticated bioinformatics algorithms led to the introduction of massive parallel sequencing of cancer gene panels into the clinical practice [18]. The implementation of such multigene panel testing increases the chance for causal variant detection. On the other hand, the NGS in routine genetic testing inevitably leads to the detection of multiple DNA variants, and many of these are DNA variants of uncertain classification, which complicates the interpretation of results. Rules for annotation and interpretation of identified DNA variants have been published by several organizations, e.g., ACMG (American College of Medical Genetics and Genomics), AMP (Association for Molecular Pathology), ACGS (Association for Clinical Genetic Science), and NHS (National Health Service). In 2013, ACMG issued recommendations for reporting the random findings of DNA variants [19]. Subsequent recommendations issued by ACMG-AMP have defined 28 criteria that individually focus on different aspects of variants' interpretation [20]. Later, ACMG introduced the concept of secondary findings. Variants in specifically analyzed genes are primarily associated with the phenotype, while the remaining incidental findings are identified in a secondary manner [21]. The European Society for Human Genetics (ESHG) favors the term unsolicited findings for all secondary identified DNA variants [22]. The issue of secondary DNA findings is actively addressed by the ACMG working group, and a minimum list of random findings that should be reported as part of clinical sequencing results has been developed [21].

The undisputed advantage of multigene panel testing is cost-effectivity and versatility – this technique has been widely used for genetic testing of various diseases, including hereditary breast cancer [23, 24]. Such a comprehensive approach for screening of HBOC-associated genes provides important data regarding the mutation spectrum among different populations and ethnicities [25–30].

Here we report for the first time the use of massively parallel sequencing of high/moderate-penetrant HBOC-associated genes on the multigene panel in Slovak HBOC families and provide data that could be used in the clinical management of the patients. Furthermore, we aimed to bring the information about PV/LPV in the *BRCA1/2* genes, as well as, in other susceptibility genes, and estimate the ratio of *BRCA* versus non-*BRCA* causal variants in our cohort of Slovak HBOC families.

Patients and methods

Patients. We studied a cohort of 94 cases, which included 80 HBOC patients (85.1%) and 14 healthy asymptomatic individuals (14.9%) with a positive family history of breast or ovarian cancer fulfilling the indication diagnostic criteria for HBOC analysis. The samples were sent to our laboratory from the regional hospitals all over Slovakia between 2018 and 2020. All patients have signed informed consent prior to the testing. Patients` characteristics describing the primary diagnosis and average age of onset are shown in Table 1.

DNA isolation. DNA was isolated by innuPREP Blood DNA Mini Kit (Analytik Jena) and fluorometrically measured on Qubit™ Fluorometer v2.0 (Invitrogen) using Qubit™ 1× dsDNA HS Assay Kit (Invitrogen).

Amplicon sequencing gene panel approach. The analysis of genes associated with HBOC was performed using a commercial kit HEVA screen (4 bases SA), validated for MiSeq genetic analyzer (Illumina). Kit contains 22 genes associated with HBOC syndrome: *ATM, APC, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, MLH1, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, PTEN, RAD50, RAD51C, RAD51D, STK11, TP53*, and technically was based on amplicon sequencing approach.

Amplification of the coding regions including the intron-exon boundaries of 22 genes was carried according to the manufacturer's instructions. Initially, 20 ng of DNA was used in 3 independent multiplex PCR reactions amplifying the target regions. Then products were pooled for each sample, 1 µl of Reagent A was added and samples were incubated at 50 °C/10 min, 55 °C/10 min, and 60 °C/20 min. After that, a ligation mix was added, and samples continued in incubation at 22 °C/30 min and 72 °C/10 min. Prepared libraries were purified using Agencourt AMpure beads (Beckman Coulter) and eluted into a volume of 40 µl. Purified libraries underwent the insertion of adapters and indexes in the Index PCR profile and then again purification with Agencourt AMpure beads (Beckman Coulter). Normalization of each sample was performed using the Qubit dsDNA HS Assay kit (Invitrogen) on Qubit™ Fluorometer v2.0 (Invitrogen). Libraries were further diluted to a concentration of 4 nM according to the manufacturers´ instructions and samples were pooled to one final library at equimolar ratios. This was further denatured using 0.2 N NaOH, diluted with HT1 buffer to 12 pM concentration, and 20% of PhiX Control v3 was added (Illumina).

Library products were analyzed by massive parallel sequencing using MiSeq Reagent Kit v2 (500-cycle) (Illumina) on the Illumina platform, MiSeq in 2×251 cycle profile.

Sequence analysis. Generated reads of 526 amplicons were aligned to the reference sequence (hg19), variant calling and interpretations of the data were performed using the optimized algorithms included in the Amplicon Suite software (4 bases SA). The minimal coverage lower than 30 reads was reviewed and analyzed by capillary sequencing if suspect. The potential variants with a variant allele frequency (VAF) threshold of 25% were selected and each one was assessed for pathogenicity interpretation.

All described variants were confirmed by Sanger Sequencing from the DNA sample of a tested individual (primer sequences and conditions available upon request) according to manufacturers´ instructions. Briefly, PCR products were purified using FastAP (Thermo Scientific) and Exo I (Thermo Scientific) enzymes. The sequencing reactions were carried out from 1.5 µl purified PCR product using the BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems,). Sequencing reaction products were purified with recommended standard ethanol purification. Electrophoresis of sequencing products was performed on SeqStudio™ Genetic Analyzer (Applied Biosystems).

CNV analysis. Analysis of Large Genomic Rearrangements (LGR) for the high penetrant genes associated with HBOC syndrome was carried out, specifically *BRCA1*, *BRCA2*, and part of *CHEK2*. Multiplex Ligation-dependent Probe Amplification (MLPA) analysis was performed for each sample analyzed by the amplicon sequencing method using the MLPA probe mix and according to manufacturer's instructions: SALSA BRCA1 P002 and SALSA BRCA2 P045 (MRC Holland). SeqStudio™ Genetic Analyzer (Applied Biosystems) was used for electrophoresis and the Coffalyser software (MRC Holland) was used for the analysis.

Variant annotation and interpretation. The variants identified by Amplicon Suite software were further annotated using free databases Varsome [https://www.varsome.com] and ClinVar [http://www.ncbi.nlm.nih.gov/clinvar/]. The clinical interpretation of identified variants was analyzed using the standards and guidelines set by the American College of Medical Genetics and Genomics [20]. Population allele frequencies were examined through access to population database Genome Aggregation Database [https://gnomad.broadinstitute.org]. Clinical information about detected variants and mutated genes were retrieved from OMIM [http://www.omim.org] and Orphanet [https://www.orpha.net/consor/cgi-bin/index.php/], respectively. The prediction of substitutions with uncertain clinical significance on protein structure and function was analyzed using the Varsome tool, where 12 different prediction software programs were accessible.

Table 1. Characteristics of the study cohort regarding the cancer type, the average age at diagnosis, and the presence of causal mutations.

Cancer type	Number of probands	Average age of onset (range)	Mutation positive cases (% in appropriate group)	Average age of onset in positive cases (range)
Healthy	14	40.8*	1 (7.14%)	–
Br ca uni	56	43.7 (29–72)	15 (26.8%)	43.5 (30–72)
Br ca bil	7	45.6 (39–60)	1 (14.3%)	41.5 (39–44)
Br ca +	6	49.5 (36–67)	2 (33.3%)	38 (36–40)
Br Ov ca	2	58.8 (50–65)	0	–
Ov ca uni	4	46 (36–57)	1 (25%)	37
Ov ca bil	2	51.5 (47–56)	0	–
Ov ca +	1	62	1 (100%)	59.5 (57–62)
Other ca	2	44 (25–63)	0	–
Summary	**94**	**50.1 (25–72)**	**21 (22.34%)**	**43.7 (30–72)**

Note: *age at the time of testing. Abbreviations: Br ca uni – unilateral breast cancer; Br ca bil – bilateral breast cancer; Br ca + – breast cancer and other associated cancer; Br Ov ca – breast and ovarian duplex cancer; Other ca – other types of cancer, analogously for the Ov ca groups.

Results

We used a panel of 22 high/moderate-risk genes associated with HBOC combined with MLPA analysis of *BRCA1/2* genes (and selected regions of *CHEK2* gene) to analyze 94 individuals with a strong family/personal history of breast and/or ovarian cancer. Together, both methods have together identified 22 causal DNA variants (23.4%) in 21 patients (one patient carried two causal variants). Two CNV variants in 4 samples were identified by the MLPA method. All described point variants were confirmed by Sanger sequencing and classified as PV/LPV according to the ACMG guidelines [20]. Table 3 summarizes all primary and secondary findings found in the analyzed set of samples.

The analyzed group of 94 HBOC suspected probands consisted of 80 patients diagnosed with cancer (85.1%) and 14 healthy individuals (14.9%) with a positive family history of HBOC syndrome. The mean age of breast cancer diagnosis among 71 tested women with breast cancer was 44.87 years (range 29–72), while the mean age of ovarian cancer patients (n=9) was higher – 51.56 years (range 36–65) (Table 1). Each occurrence of breast, and/or ovarian cancer was included in the average age summary calculation. In the case of bilateral and duplex cancer, the age of each occurrence was calculated as a separate case. Other malignancies were not included into the calculation of average age and range of age.

The class of ovarian and other associated cancer types consists of only one patient who was diagnosed with malignant melanoma at the age of 57. There are 6 patients in the class of breast and other associated cancer types, who were diagnosed with breast cancer combined with pancreatic cancer, malignant melanoma, rectosigmoid cancer, cervix cancer, and thyroid cancer (two cases). The class with other types of cancer (except breast or ovarian) consisted of 2 patients diagnosed with prostate tumor (at the age of 63, and one case of breast cancer in the family) and bilateral adrenal glands cancer (at the age of 25, and 2 cases of breast cancer in the family). Finally, the class of healthy probands comprised of 3 individuals with a positive family history of ovarian cancer, 10 individuals with a positive family history of breast cancer, and 1 individual with a positive family history of breast and ovarian cancer.

The largest group of patients was represented by 56 individuals diagnosed with unilateral breast cancer (Table 1), of

Table 2. Summary of the total number of samples and pathogenic DNA variants identified as primary, or secondary findings.

Diagnosis	Number of samples	Positive cases	Pathogenic variants	Primary findings	Secondary findings
HBOC	80	20	21	14	7
Asymptomatic cases	14	1	1	1	0
Overall	**94**	**21**	**22**	**15**	**7**
Frequency		22.34%	23.4%	15.96%	7.44%

Table 3. The complete list of primary and secondary findings detected by multigene panel sequencing in the studied group of Slovak HBOC patients.

Gene	RefSeq	Detected DNA variants				No. of samples
		cDNA name	Protein name	Pathogenity	rs ID	
Primary findings						
BRCA1	NM_007300.4	c.181T>G	p.Cys61Gly	5	rs28897672	3
BRCA1	NM_007300.4	**c.2794del#**	p.Val932Leufs*68	5	–	1
BRCA1	NM_007300.4	c.4243del	p.Glu1415Lysfs*4	5	rs80357981	2
BRCA1	NM_007300.4	c.5329dup	p.Gln1777Profs*74	5	rs80357906	2
BRCA1	NM_007300.4	(c.5573-?_c.5701+?)del	del exon 21-22	5	–	3
BRCA2	NM_000059.3	c.3076A>T	p.Lys1026*	5	rs80358552	1
BRCA2	NM_000059.3	c.4005dup	p.Phe1336Ilefs*2	5	rs397507701	1
BRCA2	NM_000059.3	c.7595_7596ins	p.Ala2534Leufs*18	5	rs80359666	1
BRCA2	NM_000059.3	c.9403del	p.Leu3135Phefs*28	5	rs80359760	1
Secondary findings						
CDH1	NM_004360.5	**c.2480dup#**	p.Tyr827*	5	–	1
CHEK2	NM_001005735.2	c.599T>C	p. Ile200Thr	3 – 5	rs17879961	1
CHEK2	NM_001005735.2	c.1229del	p.Thr410Metfs*15	4/5	rs555607708	1
CHEK2	NM_001005735.2	(c.1038-?_1224+?)del	del exon 10-11	4/5	–	1
MUTYH	NM_001128425.1	c.1187G>A	p.Gly396Asp	4/5	rs36053993	1
PALB2	NM_024675.4	c.172_175del	p.Gln60Argfs*7	4/5	rs180177143	1
PALB2	NM_024675.4	c.509_510del	p.Arg170Ilefs*14	5	rs515726123	1

Notes: reference sequence of the canonical gene transcript (RefSeq) is stated for each gene; for registered DNA variants, the identification number by dbSNP database (rs ID) is indicated; # novel, not yet published mutation

which 15 were identified as HBOC positive (26.8%). We have found 11 primary findings (8 in *BRCA1*, 3 in *BRCA2* gene) and 5 secondary findings (2 in *CHEK2*, 1 in each of *CDH1, MUTYH*, and *PALB2* genes). The mean ages of onset of the disease in the whole group and positive probands were 43.66 and 43.80 years, respectively (see ranges in Table 1). In one proband, two causal variants were detected simultaneously, namely missense c.181T>G in *BRCA1* gene and deletion of exons 10–11 ((c.1038-?_1224+?)del) in the *CHEK2* gene.

The group of patients diagnosed with bilateral breast cancer consisted of 7 patients, whose average age at the time of diagnosis was 45.6 years. We have identified the *BRCA1* gene variant in one positive sample (14.3%) coming from a 39-year-old patient with the second tumor diagnosed at the age of 44. The group diagnosed with duplex of breast and other associated cancers (excluding ovarian cancer) consisted of 6 patients, whose average age at the time of onset was 49.5 years, and we have identified 2 positive samples (33.3%) with the presence of secondary DNA variants in *PALB2* (breast and pancreatic cancer) and *CHEK2* gene (breast and cervical cancer). Additionally, we have detected one positive sample in the group with unilateral ovarian cancer (n=4), detection rate 25%, and the group with duplex of ovarian and other associated cancers (excluding breast cancer) (n=1), detection rate 100%.

In total, we identified 21 samples (22.34%) with the presence of causal PV/LPV DNA variants (class 4 or 5) in the cohort of 94 samples. Since we have detected the presence of two different variants in one sample, the total number of identified causal variants was 22 (23.4%). Overall, 15 variants were characterized as primary findings (15.96%) in *BRCA1/2* genes, and 7 variants as secondary findings (7.44%) in *CHEK2, PALB2, CDH1*, and *MUTYH* genes (Table 2).

Most of all identified variants (20/22) represented alterations previously associated with HBOC syndrome in publications and databases. In addition, we have identified 2 previously non-described variants, c.2794del in the *BRCA1* gene and c.2480dup in the *CDH1* gene. Out of 15 detected primary findings, 11 were localized in *BRCA1* and 4 in the *BRCA2* gene (Table 3). The most frequent pathogenic *BRCA1* alterations, were c.181T>G and CNV variant (c.5573-?_c.5701+?)del, known as deletion of exons 21–22, which were both identified 3 times. In the *BRCA2* gene, we found 4 previously known pathogenic variants, each in one case. We have also detected 3 mutations in the *CHEK2* gene, and 2 mutations in the *PALB2* gene. Considering the molecular-biological impact of mutation on the protein synthesis, 12 frameshift variants (54.54%), 5 substitution missense variants (22.72%), 1 substitution nonsense variant (4.54%), and 4 CNV deletions (18.2%) have been identified (Table 4).

In total, secondary findings were responsible for almost one-third (31.8%) of mutation carriers, while primary findings were detected in 68.2% of all mutation-positive cases in our cohort. All clinical information of carriers of pathogenic DNA variants and their families is summarized in Table 5. According to the findings, no associations between specific genetic variants and tumor type nor associations with a family history were found in our cohort of HBOC families (Table 5). Further study on a larger cohort of patients may bring some reliable genotype-phenotype correlations.

Discussion

Since the establishment of genetic testing of breast/ovarian cancer susceptibility genes among HBOC families in Slovakia, several studies related to this issue have been reported so far [31–39]. However, most of them were exclusively focused on the molecular-genetic analysis of *BRCA1/2* genes. To find out the ratio of causal variants in genes other than *BRCA1/2* and bring a more accurate picture of the mutation spectrum, we provide the first report of multigene panel testing in Slovak HBOC families. Using a panel of 22 susceptibility genes combined with MLPA analysis we demonstrated that primary findings in *BRCA1/2* represent the majority (68.2%) of identified genetic changes, while secondary findings in other genes represent almost one-third, 31.8%, which represents a ratio of 2.14 : 1. The proportion of identified secondary PV/LPV could be also higher, as was shown in the study of Lerner-Ellis et al. [40], where 55.3% of PV/LPV were localized in other than *BRCA1/2* genes.

Causal DNA variants in the tested Slovak HBOC population were detected at 23.4% frequency (22 PV/LPV in 94 analyzed subjects). In a study of the German population, Kraus et al. reported the frequency of positive findings at 18% (105 out of 581), with variants in the *BRCA1/2* genes representing up to 68.6% [41]. Tedaldi et al. demonstrated a frequency of 29% in the Italian population (74 out of 255), with variants in the *BRCA1/2* genes representing up to 77% [42]. Accordingly, a study in Polish women with a strong family history of breast cancer showed that mutations in *BRCA1/2* genes accounted for 82% of identified variants [43]. Recently, Tsaousis et al. reported causal variants in the Greek, Romanian, and Turkish populations with a frequency of 22.1% (264 of 1,197), with variants in the *BRCA1/2* genes accounting for 43.6% [44]. Although the mentioned studies analyzed cohorts from different European populations, some of them with a significantly higher number of samples, only minimal differences in the detection rate of primary and secondary variants were observed compare to our results.

On the other hand, our results show a higher detection rate of PV/LPV in the Slovak HBOC population (23.4%) than some other recent studies based on multigene panel sequencing. In the French HBOC population, Velásquez et al. [45] and Benusiglio et al. [46] detected 8.3% and 12.1% in the group of 128 and 234 cases, respectively. Foglietta et al. [47] reported a mutation detection rate of 13.8% in the group of 363 cases in the Italian population and Lerner-Ellis et al. [40] detected a mutation detection rate at a level of 9.1% in 3,251 cases in the population of Ontario. Such discrepancies in the identified PV/LPV frequencies between the various

Table 4. Complex molecular-biological characterization of the pathogenic variants identified in Slovak HBOC families.

Gene	cDNA name	Protein name	rs ID	X codone/all aa	Exon/all exons	Domain name/ position	GnomAD NFE freq	Preferred population	Publications/ ClinVar
BRCA1	c.181T>G	p.Cys61Gly	rs28897672	No (p.61 of 1885)	4/24	Zinc Finger (p.24-65)	0.0000617	CE EU	Yes/Yes
BRCA1	**c.2794del#**	p.Val932Leufs*68	n.a.	p.1000 of 1885	10/24	before Interaction PALB2 (p.1397-1424)	n. a.	n.a.	No/No
BRCA1	c.4243del	p.Glu1415Lysfs*4	rs80357981	p.1419 of 1885	12/24	Interaction PALB2 (p.1397-1424)	0,00000879	SK, FR	Yes/Yes
BRCA1	c.5329dup	p.Gln1777Profs*74	rs80357906	p.1851 of 1885	20/24	BRCT1 (p.1642-1736) BRCT2 (p.1756-1855)	0.000176	CE EU	Yes/Yes
BRCA1	(c.5573-?_c.5701+?) del	del exon 21-22	n.a.	n.a.	20-21/23	BRCT2 (p.1756-1855)	n.a.	CZ, SK	Yes/n.a.
BRCA2	c.3076A>T	p.Lys1026*	rs80358552	p.1026 of 3419	11/27	POLH, FANCD2, SEM1-binding, NES motif	n.a.	CZ, SK	Yes/Yes
BRCA2	c.4005dup	p.Phe1336Ilefs*2	rs397507701	p.1338 of 3419	11/27	POLH-binding (p.1338-1781)	n.a.	BRZ, CE EU, HU	Yes/Yes
BRCA2	c.7595_7596ins	p.Ala2534Leufs*18	rs80359666	p.2552 of 3419	15/27	Interaction FANCD2 (p.2350-2545) and SEM1 (p.2481-2832)	n.a.	n.a.	Yes/No
BRCA2	c.9403del	p.Leu3135Phefs*28	rs80359760	p.3163 of 3419	25/27	after SEM1 interac-tion (p.2481-2832)	n.a.	PL, GE, SK	Yes/Yes
CDH1	**c.2480dup#**	p.Tyr827*	n.a.	p.827 of 883	16/16	p.811-882 binding α, β, γ-catenins	n.a.	n.a.	No/No
CHEK2	c.599T>C	p. Ile200Thr	rs17879961	No (p.200 of 587)	5/16	FHA (p.113-175)	0.00393	EU	Yes/Yes
CHEK2	c.1229del	p.Thr410Metfs*15	rs555607708	p.425 of 587	12/16	Kinase (p.220-486)	0.00254	EU	Yes/Yes
CHEK2	(c.1038-?_1224+?)del	del exon 10-11	n.a.	n.a.	10-11/16	Kinase (p.220-486)	–	CE EU	Yes/n.a.
MUTYH	c.1187G>A	p.Gly396Asp	rs36053993	No (p.396 of 550)	13/16	Nudix hydrolase (p.364-495)	0.00492	EU	Yes/Yes
PALB2	c.172_175del	p.Gln60Argfs*7	rs180177143	p.67 of 1187	3/13	DNA binding (p.1-579)	0.0000791	PL, U	Yes/Yes
PALB2	c.509_510del	p.Arg170Ilefs*14	rs515726123	p.184 of 1187	4/13		0.0000703	PL, CZ	Yes/Yes

Note: # novel not yet published mutation. Abbreviations: X – STOP codon; aa – amino acids; NFE – Non-Finish European; n.a. – not available; CE EU – Central Europe, EU – Europe; SK – Slovakia; FR – France; CZ – Czechia; BRZ – Brazil; PL – Poland; U – Ukraine; HU – Hungary; GE – Germany.

Table 5. Clinical data of mutation positive carriers detected in the studied group of Slovak HBOC patients.

Gene	DNA variant	No.	Age of onset	Tumor type	Family history
BRCA1	c.181T>G	1	36	Br Ca, TN	negative
		2	44	Br Ca	Br Ca in father's sister (39 y.), St Ca in father (75 y.)
		3[SC]	44	Br Ca	Col Ca in mother´s sister (40 y.), Ut Ca in mother´s mother (44 y.), Ov Ca in father´s sister (43 y.), Lu Ca in father´s brother and father
BRCA1	c.2794del	4	38	Br Ca	Br Ca in mother (36 y.), St Ca in mother´s mother (79 y.), Tes Tu in maternal cousin (35 y.), Ut Ca in father´s mother (79 y.) and sister (50 y.)
BRCA1	c.4243del	5	37	Ov Ca	Br Ca in mother (38 y.), Ov Ca in mother´s sister (39 y.), duplex of Br Ca and Ov Ca in three mother´s father sisters (66, 50, 50 y.), Br Ca in father´s mother (57 y.), Ca Col in father´s sister, Pan Ca in paternal cousin (48 y.)
		6	38	Br Ca	Ov Ca in sister (39 y.) and in daughter (37 y.)
BRCA1	c.5329dup	7	30	Br Ca, TN	Br Ca in mother´s sister (40 y.), Ov Ca in mother´s mother (64 y.)
		8	39	Br Ca	Ov Ca in sister (48 y.)
BRCA1	(c.5573-?_c.5701+?) del	9	50	Br Ca	Br Ca in maternal cousin (42 y.) and Ov Ca in two mother´s sisters (42, 46 y.)
		10	39, 44	Bil Br Ca, TN	Ov Ca in mother´s sister and mother (each in 40 y.), Ut Ca in mother (45 y.)
		11	n.a.	Healthy	Br Ca in mother (70 y.) and mother´s mother (62 y.)
BRCA2	c.3076A>T	12	57, 62	Mal Mel, Ov Ca	Br Ca in mother and mother´s aunt, Col Ca in father, age of diagnosis not known
BRCA2	c.4005dup	13	41	Br Ca	Br Ca in father´s sister (44 y.), Col Ca in father´s brother (60 y.) and mother´s father (73 y.)
BRCA2	c.7595_7596ins	14	43	Br Ca	Ut Ca in mother (63 y.)
BRCA2	c.9403del	15	39	Br Ca	negative
CDH1	c.2480dup	16	39	Br Ca	negative
*CHEK***2**	c.599T>C	17	72	Br Ca	Br Ca in sister (65 y.) and mother (78 y.), Ov Ca in sister´s daughter (27 y.), End Ca in maternal cousin (58 y.)
CHEK2	c.1229del	18	36	Br Ca, Cer Ca	Col Ca in mother (60 y.), Ut Ca in mother´s sister (42 y.), Br Ca in maternal cousin (40 y.), Thyr Ca and leukemia in son (27 r.)
CHEK2	(c.1038-?_1224+?)del	19[SC]			see variant in carrier 3
MUTYH	c.1187G>A	20	33	Br Ca	Br Ca in father´s mother (58 y.), Pro Ca in maternal cousin (33 y.)
PALB2	c.172_175del	21	38, 40	Br Ca, Pan Ca, Ichtyosis	Br Ca in mother and mother´s mother, age of diagnosis not known
PALB2	c.509_510del	22	67	Br Ca	Bil Br Ca in father´s sister (50 y.), Leukemia in father, Col Ca in father´s brother

Note: [SC]same carrier/family. Abbreviations: Bil – bilateral; Br ca – breast cancer; Cer Ca – cervix cancer; Col Ca – colorectal cancer; End Ca – endometrium cancer; Lu Ca – lung cancer; Mal Mel – malignant melanoma; Ov ca – ovarian cancer; Pan Ca – pancreatic cancer; Pro Ca – prostate cancer; St Ca – stomach cancer; Thyr Ca – thyroid cancer; Tes Tu – testis tumor; TN – triple-negative; Ut Ca – uterus cancer; y. – years

studies may be explained by the number and composition of analyzed samples and differences in the indication criteria between several countries.

In this study, we have identified 5 different PV in *BRCA1* (c.181T>G, c.2794del, c.4243del, c.5329dup, (c.5573-?_c.5701+?)del) and 4 different PV in *BRCA2* (c.9403del, c.7595_7596ins, c.4005dup, c.3076A>T). According to the previous studies, six most frequent variants in the *BRCA1* gene (c.68_69del, c.181T>G, c.3700_3704del, c.4243del, c.5329dup, c.843_846del) and four most frequent variants in the *BRCA2* gene (c.3076>T, c.5645C>A, c.9098dup, c.9403del) account for the majority of *BRCA1/2* mutation spectrum in Slovak HBOC families [38, 39]. Ciernikova et al. have described c.5329dup (*previously know as c.5266dupC*) as the most frequent pathogenic alteration accounting for 1/4 of all *BRCA* gene alterations in a cohort of 120 Slovak HBOC families [33]. Konecny et al. later found the high frequencies of *BRCA1* mutations c.181T>G and c.5329dup (17% and 38%, respectively) in genetic screening of Slovak HBOC families using Sanger sequencing [38]. This was confirmed by Zidekova et al. who showed similar frequencies for c.181T>G and c.5329dup (17% and 32%, respectively) [39].

BRCA1 mutation c.5329dup was originally considered as a founder mutation in the Ashkenazi Jewish population. However, several European countries reported a significantly increased frequency of this variant as well. Interestingly, an STR genotyping of 245 carrier families from 14 different European populations (Russian, Latvian, Ukrainian, Czech, Slovak, Polish, Danish, Dutch, French, German, Italian, Greek, Brazilian, and Ashkenazi Jewish) estimated that the mutation arose some 1,800 years ago in the region of Northern Europe, and spread to the various European populations, including the Ashkenazi Jewish population during the following centuries [48]. Several *BRCA1/2* variants identified in our study have been also reported in other European populations. The results showed that *BRCA1* variants c.181T>G and c.5329dupC belonged to the most

frequent mutations not only in Slovak but also in Central European HBOC families [49]. Moreover, *BRCA2* variant c.9403delC occurs also in Polish and Czech HBOC populations [50, 51], and c.4005dupA in *BRCA2* has been also reported in other Central European populations [52].

Besides known *BRCA* mutations, a novel *BRCA1* variant c.2794del (p.Val932Leufs*68) has been identified in an early-diagnosed breast cancer patient with a family history of breast, gastric, and testicular cancer. According to our knowledge, this variant has not been previously described in ClinVar, GnomAD database nor in any available published articles. An interpretation of novel sequence variants and their molecular impact can be predicted by a variety of distinct software packages with various evaluation algorithms based on the combination of several criteria (localization in the functional domain of the protein, the molecular impact of amino acid changes, evolutionary conservation, etc.) [20]. We have performed an in silico analysis in the VarSome tool, which predicts this novel alteration as a null variant leading to the introduction of premature STOP codon at p.1000. In addition, the variant is rare, with unknown frequency in the gnomAD database, and located in the mutation hot spot region of *BRCA1* showing site pathogenicity of 71.1% with a score of 13.516, which exceeds the threshold of 2.472.

Only one positive sample (7.14%) was identified in a group of healthy individuals with a family history of breast/ovarian cancer. Specifically, *BRCA1* large deletion (c.5573-?_c.5701+?)del was found in a 43-year-old healthy proband with 2 breast cancer cases in the family. This may suggest that a broader panel of HBOC-associated genes would be appropriate for clinical testing of asymptomatic probands with positive family history. The same type of variant was identified in the Czech HBOC population by Vasickova et al. [53], however unlike our data, the family history of the carrier was very strong (2 cases of bilateral breast cancer at the age of 36, 43, and 25, 45 years).

Importantly, the multigene panel enabled us to identify other PV/LPV associated with increased risk of breast and ovarian cancer in Slovak HBOC families. In total, 7 positive secondary findings in HBOC-associated genes other than *BRCA1/2* were found, accounting for almost one-third of all identified variants in HBOC-suspected patients (31.8%, n=7 out of 22). Specifically, three (13.63%) *CHEK2* gene variants (c.599T>C, c.1229del, (c.1038-?_1224+?)del), two (9.1%) *PALB2* gene variants (c.172_175del, c.509_510del), one *CDH1* gene variant c.2480dup, and one *MUTYH* c.1187G>A (4.54% each) were identified (Table 3).

Comparison of the spectrum of affected HBOC genes with other studies shows that *BRCA1*, *BRCA2*, *CHEK2*, *PALB2*, and interestingly also *CDH1* and *MUTYH* genes are reported in other HBOC populations as well. On the other hand, causal variants in other HBOC associated genes as *ATM*, *RAD51C*, *BARD1*, *BLM*, *TP53* [40, 45, 54] are missing in our study (but not in the Slovak HBOC population at all, because we have recently detected them in a separate group of HBOC families analyzed by different target-enrichment NGS approach, data not published yet).

Variant c.2480dup (p.Tyr827*) in the *CDH1* gene represents a novel, previously unpublished variant. This variant has not been reported in the ClinVar database, nor in the gnomAD exomes and genomes databases. According to the Varsome tool, this novel *CDH1* mutation was interpreted as a pathogenic variant of class 5 due to its null nonsense effect and generating the premature STOP codon at the p.827 position.

Despite the fact, that *MUTYH* is a recessive gene, we have detected monoallelic c.1187G>A variant in *MUTYH* in a patient with an early onset of breast cancer [30] with a family history of breast and prostate cancer. Our observation is in accordance with other studies supporting the possible contribution of heterozygous PV c.1187G>A in *MUTYH* to cancer risk [45, 55].

Variant c.1229del (*previously known as c.1100delC*) in the *CHEK2* gene, and variants c.172_175del and c.509_510del in the *PALB2* gene were identified as the most common secondary findings in the Polish population [43]. Weischer et al. reported that the presence of a deleterious mutation c.1229del in the heterozygous state led to a threefold increase in the risk of breast cancer in the general female population [56]. The c.1229del variant, together with the c.599T>C substitution, are frequently detected variants of the *CHEK2* gene in patients with HBOC syndrome among several populations of Central Europe [57, 58]. However, the pathogenic effect of the c.599T>C variant (*previously known as c.470T>C, p.Ile157Thr*) has been studied for a long time and its interpretation is rather controversial [59]. Currently, the variant is classified in the ClinVar database as a variant with conflicting interpretations of pathogenicity (class 3 to 5) and in Varsome as a variant of class 5, with relatively high population frequency in the European Non-Finnish population, up to 0.0393 (gnomAD Exomes). This variant has been previously described in association with various syndromes (HBOC, Li Fraumeni, colorectal carcinomas, etc.) [60, 61]. Nevertheless, it is characterized by a reduced penetrance compared to other truncating *CHEK2* mutations. In a large meta-analysis of 18 case-control studies, Han et al. demonstrated that this variant increased the risk for breast cancer less than two-fold (OR=1.58, 95% CI=1.42–1.75) [62]. Moreover, Cybulski et al. reported a smaller odds ratio and decreased risk of breast cancer in *BRCA1* mutation carriers simultaneously carrying *CHEK2* variant c.599T>C [57]. Despite the relatively high prevalence of this variant in the Polish population (in the north of Slovakia) (4.8–5.2%) [57, 63], more recent studies did not confirm its pathogenic role in the Polish population of breast cancer patients [63] nor in patients with ovarian cancer [64]. According to the data from the Czech Republic, the frequency of this variant in the patient cohort was 3.08% (47 out of 1,523) compared to the frequency of 3.1% in controls (104 out of 3,360) [65]. However, Lerner Ellis et al. [40] reported 17 cases of

the mentioned *CHEK2* variant in the large study of 3,251 HBOC patients from the Ontario region, which represents only 0.5%. In our study, we have detected *CHEK2* mutation c.599T>C only in one breast cancer patient (1.06%) with a strong positive family history that included 2 cases of breast cancer, 1 case of ovarian cancer, and 1 case of endometrial cancer. However, the age of breast cancer diagnosis in a positive patient was 72. Accordingly, the age of breast cancer diagnosis in proband´s sister and mother were high (65 and 78 years, respectively). On the contrary, proband´s niece was diagnosed with ovarian cancer at the age of 27. Thus, our findings may reflect the conflicting results described in the literature. The last variant we have identified in the *CHEK2* gene was the CNV variant (c.1038 -?_1224 +?)del, known as deletion of exons 10 and 11. It was detected in a sample of a 44-year-old patient with breast cancer. This extensive deletion has been identified in the Slovak, Czech, and Polish populations [66], and is associated with the occurrence of distinct malignancies, breast cancer included [67]. The origin of this alteration is not fully clarified, but it is suggested to be of Central European or Slavic origin [66].

Finally, we have identified two frameshift variants c.172_175del and c.509_510del in the *PALB2* gene. Both alterations have been documented in the neighboring Polish population, though with the higher occurrence (7 and 12 families, respectively) [43]. Antoniou et al. performed a detailed analysis of a set of patients carrying different variants of the *PALB2* gene. According to the findings, the risk of breast cancer in women carrying *PALB2* gene variants was 6–9 times higher compared to the general population, representing a higher risk than in *BRCA2* mutation carriers [68]. In our study, the patients with *PALB2* positivity were diagnosed with breast cancer at the age of 67 and 38 years, and patients with *BRCA2* positivity were diagnosed with breast cancer at the age of 39, 41, and 43 years. In addition, a broader spectrum of cancers including colorectal cancer and leukemia has been demonstrated in a family history of *CHEK2* and *PALB2* mutation carriers (Table 5).

If we look at the age of cancer onset in the group of positive mutation carriers in Table 5, it is obvious that the family with *CHEK2* controversial variant c.599T>C (p.Ile200Thr) significantly differs from other families. The age of onset is 72 years in the proband and 65 and 78 years in first-degree family relatives. If we remove this family from the calculation, the average age of positive carriers at the age of onset decreases to 40.6 years that represents a 3.1-year reduction and 9.5-year difference from the overall average age of all analyzed samples (50.1 years) (Table 1). These data are certainly influenced by a low number of samples. However, they indicate that:

1./ This is additional evidence suggesting that the controversial *CHEK2* variant c.599T>C (p.Ile200Thr) represents with high probability a low penetrance variant associated with lower susceptibility of cancer risk [62] mainly because it is known that CHEK2 protein affected by this missense variant has a lower activity [65].

2./ The presence of pathogenic mutations in HBOC-associated genes relates to the notable decrease of the age of cancer diagnosis and this fact results in a significant increase of mutation frequency with decreasing age at diagnosis [69].

Overall, the age at cancer diagnosis in our study is relatively low when comparing with other multigene-based studies. For example, Foglietta et al. [47] reported the mean age in the group of breast cancer cases to be 46 years and in the *BRCA1/2* positive cases even higher at 48 years, while the mean age in our group of breast cancer patients was 43.7 and in positive cases at 43.5. The similar average age of breast cancer cases at 43.2 years was reported also in their study by Felicio et al. [70]. However, Lerner Ellis et al. [40] and Benusiglio et al. [546] reported a vastly different overall average age of patients in their study at vastly different levels in their respective studies (56 and 52 years, respectively).

The implementation of multigene panel testing for hereditary breast and ovarian cancer offers a quick and cost-effective approach. A decision-analytic model reported by Manchanda et al. for UK and US populations showed that clinical criteria/family-based *BRCA1/BRCA2/RAD51C/RAD51D/BRIP1/PALB2* testing showed to be more cost-effective than *BRCA1/BRCA2* testing alone [71]. Considering our data multigene panel testing of high/moderate-penetrant HBOC-associated genes in Slovak families represents an effective approach, which allows identifying almost 50% more PV/LPV compared to the *BRCA1/2* approach alone. Therefore, multigene panel testing for HBOC risk changes the clinical management for substantially more patients and unaffected relatives harboring PV in cancer-predisposing genes.

The results of our study have also a direct impact on clinical use, while a preventive screening program is recommended for unaffected mutation carriers. Importantly, patients with identified PV in HBOC-associated genes can be targeted for personalized treatment modalities. According to the findings from clinical studies, *BRCA1/2*-associated breast cancers have been shown to respond better to platinum-based chemotherapy [72]. In addition, several studies have demonstrated that *BRCA1/2* and *CHEK2* mutations could determine the tumor PARP inhibitor sensitivity [73]. In this context, PARP inhibitors have been increasingly used to treat advanced breast and ovarian cancer in patients with mutations in *BRCA* genes. Moreover, very recent findings indicated that not only *BRCA1/2* mutation carriers but also metastatic breast cancer patients with germline *PALB2* mutations might benefit from PARP inhibitor Olaparib treatment [74]. Mateo et al. reported that Olaparib was effective in patients with advanced castrate-resistant prostate cancer harboring BRCA mutations [75]. The possibility to use genetic information for treatment selection is in line with the modern concept of personalized medicine [76]. Further research might identify other predictive markers for drug response in HBOC tumors with homologous recombination deficiencies leading to the development of novel targets for therapies.

In conclusion, the main advantage of using the multigene panel approach is that it would lead to the detection of a higher portion of clinically relevant variants, that would otherwise not be found by *BRCA1/2* testing of HBOC families. Another benefit represents higher informative value in the cases when pathogenic predisposition in more than one gene can explain an inherited disease. HBOC syndrome presents an optimal example, in which testing based on personal and family history through a multigene panel test may be more efficient and/or cost-effective.

Modern approaches accelerate fast, and the future dilemma would not be if the panel sequencing approach is suitable for genetic testing but rather what size of panel sequencing is the most effective for testing. There is still an open debate considering which genes should be included in clinical settings, as some of the NGS panels may contain genes that are still under research.

Our study on the Slovak HBOC cohort shows that almost one-third of pathogenic mutations are localized in other genes than the primary-associated ones – *BRCA1/2*. The limit of our study is a relatively small number of the tested subjects. However, we provide the first report of NGS panel sequencing in Slovak HBOC families and relevant data for clinical management of the patients. Identification of causal primary and secondary findings has an impact not only on cancer-preventive management of mutation carriers but also provides important information about eligibility for targeted therapeutic modalities such as PARP-inhibitors.

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References

[1] BRAY F, FERLAY J, SOERJOMATARAM I, SIEGEL RL, TORRE LA et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394–424. Erratum in: CA Cancer J Clin 2020; 70: 313. https://doi.org/10.3322/caac.21609

[2] EUROSTAT Cancer statistics – specific cancers. https://ec.europa.eu/eurostat/statistics-explained/pdfscache/39738.pdf [As accessed on December, 2020]

[3] NIELSEN FC, VAN OVEREEM HT, SØRENSEN CS. Hereditary breast and ovarian cancer: new genes in confined pathways. Nat Rev Cancer 2016; 16: 599–612. https://doi.org/10.1038/nrc.2016.72

[4] ANGELI D, SALVI S, TEDALDI G. Genetic Predisposition to Breast and Ovarian Cancers: How Many and Which Genes to Test? Int J Mol Sci 2020; 21: 1128. https://doi.org/10.3390/ijms21031128

[5] JARA L, MORALES S, DE MAYO T, GONZALEZ-HORMAZABAL P, CARRASCO V et al. Mutations in BRCA1, BRCA2 and other breast and ovarian cancer susceptibility genes in Central and South American populations. Biol Res 2017; 50: 35. https://doi.org/10.1186/s40659-017-0139-2

[6] KUCHENBAECKER KB, HOPPER JL, BARNES DR, PHILLIPS KA, MOOIJ TM et al. Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. JAMA 2017; 317: 2402–2416. https://doi.org/10.1001/jama.2017.7112

[7] REBBECK TR, FRIEBEL TM, FRIEDMAN E, HAMANN U, HUO D et al. Mutational spectrum in a worldwide study of 29,700 families with BRCA1 or BRCA2 mutations. Hum Mutat 2018; 39: 593–620. doi:10.1002/humu.23406

[8] TAI YC, DOMCHEK S, PARMIGIANI G, CHEN S. Breast cancer risk among male BRCA1 and BRCA2 mutation carriers. J Natl Cancer Inst 2007; 99: 1811–1814. https://doi.org/10.1093/jnci/djm203

[9] HOANG LN, GILKS BC. Hereditary Breast and Ovarian Cancer Syndrome: Moving Beyond BRCA1 and BRCA2. Adv Anat Pathol 2018; 25: 85–95. https://doi.org/10.1097/PAP.0000000000000177

[10] MAXWELL KN, DOMCHEK SM, NATHANSON KL, ROBSON ME. Population Frequency of Germline BRCA1/2 Mutations. J Clin Oncol 2016; 34: 4183–4185. https://doi.org/10.1200/JCO.2016.67.0554

[11] JANAVICIUS R. Founder BRCA1/2 mutations in the Europe: implications for hereditary breast-ovarian cancer prevention and control. EPMA J 2010; 1: 397-412. https://doi.org/10.1007/s13167-010-0037-y

[12] MELCHOR L, BENITEZ J. The complex genetic landscape of familial breast cancer. Hum Genet 2013; 132: 845–863. https://doi.org/10.1007/s00439-013-1299-y

[13] MASCIARI S, DILLON DA, RATH M, ROBSON M, WEITZEL JN et al. Breast cancer phenotype in women with TP53 germline mutations: A Li-Fraumeni syndrome consortium effort. Breast Cancer Res Treat 2012; 133: 1125–1130. https://doi.org/10.1007/s10549-012-1993-9

[14] MESTER J, ENG C. Cowden syndrome: Recognizing and managing a not-so-rare hereditary cancer syndrome. J Surg Oncol 2015; 111: 125–130. https://doi.org/10.1002/jso.23735

[15] VAN DER POST RS, VOGELAAR IP, CARNEIRO F, GUILFORD P, HUNTSMAN D et al. Hereditary diffuse gastric cancer: Updated clinical guidelines with an emphasis on germline CDH1 mutation carriers. J Med Genet 2015; 52: 361–374. https://doi.org/10.1136/jmedgenet-2015-103094

[16] LIM W, OLSCHWANG S, KELLER JJ, WESTERMAN AM, MENKO FH et al. Relative frequency and morphology of cancers in STK11 mutation carriers. Gastroenterology 2004; 126: 1788–1794. https://doi.org/10.1053/j.gastro.2004.03.014

[17] FACHAL L, ASCHARD H, BEESLEY J, BARNES DR, ALLEN J et al. Fine-mapping of 150 breast cancer risk regions identifies 191 likely target genes. Nat Genet 2020; 52: 56–73. https://doi.org/10.1038/s41588-019-0537-1

[18] SHAH PD, NATHANSON KL. Application of Panel-Based Tests for Inherited Risk of Cancer. Annu Rev Genomics Hum Genet 2017; 18: 201–227. https://doi.org/10.1146/annurev-genom-091416-035305

[19] GREEN RC, BERG JS, GRODY WW, KALIA SS, KORF BR et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. Genet Med 2013; 15: 565–574. https://doi.org/10.1038/gim.2013.73

[20] RICHARDS S, AZIZ N, BALE S, BICK D, DAS S et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015; 17: 405–424. https://doi.org/10.1038/gim.2015.30

[21] KALIA SS, ADELMAN K, BALE SJ, CHUNG WK, ENG C et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genet Med 2017; 19: 249–255. https://doi.org/10.1038/gim.2016.190

[22] VAN EL CG, CORNEL MC, BORRY P, HASTINGS RJ, FELLMANN F et al. Whole-genome sequencing in health care: recommendations of the European Society of Human Genetics. Eur J Hum Genet 2013; 21: 580–584.

[23] EASTON DF, PHAROAH PD, ANTONIOU AC, TISCHKOWITZ M, TAVTIGIAN SV et al. Gene-panel sequencing and the prediction of breast-cancer risk. N Engl J Med 2015; 372: 2243–2257. https://doi.org/10.1056/NEJMsr1501341

[24] BISHOP MR, HUSKEY ALW, HETZEL J, MERNER ND. A research-based gene panel to investigate breast, ovarian and prostate cancer genetic risk. PLoS One 2019; 14: e0220929. https://doi.org/10.1371/journal.pone.0220929

[25] BUYS SS, SANDBACH JF, GAMMON A, PATEL G, KIDD J et al. A study of over 35,000 women with breast cancer tested with a 25-gene panel of hereditary cancer genes. Cancer 2017; 123: 1721–1730. https://doi.org/10.1002/cncr.30498

[26] COUCH FJ, SHIMELIS H, HU C, HART SN, POLLEY EC et al. Associations between cancer predisposition testing panel genes and breast cancer. JAMA Oncol 2017; 3: 1190–1196. https://doi.org/10.1001/jamaoncol.2017.0424

[27] MOMOZAWA Y, IWASAKI Y, PARSONS MT, KAMATANI Y, TAKAHASHI A et al. Germline pathogenic variants of 11 breast cancer genes in 7,051 Japanese patients and 11,241 controls. Nat Commun 2018; 9: 4083. https://doi.org/10.1038/s41467-018-06581-8

[28] THEOBALD KA, SUSSWEIN LR, MARSHALL ML, ROBERTS ME, MESTER JL et al. Utility of expedited hereditary cancer testing in the surgical management of patients with a new breast cancer diagnosis. Ann Surg Oncol 2018; 25: 3556–3622. https://doi.org/10.1245/s10434-018-6581-8

[29] BISHOP MR, OMELER-FENAUD SM, HUSKEY ALW, MERNER ND. Gene panel screening for insight towards breast cancer susceptibility in different ethnicities. PLoS One 2020; 15: e0238295. https://doi.org/10.1371/journal.pone.0238295

[30] SHIN HC, LEE HB, YOO TK, LEE ES, KIM RN et al. Detection of Germline Mutations in Breast Cancer Patients with Clinical Features of Hereditary Cancer Syndrome Using a Multi-Gene Panel Test. Cancer Res Treat 2020; 52: 697–713. https://doi.org/10.4143/crt.2019.559

[31] CIERNIKOVA S, TOMKA M, SEDLAKOVA O, REINEROVA M, STEVURKOVA V et al. The novel exon 11 mutation of BRCA1 gene in a high-risk family. Neoplasma 2003; 50: 403–407.

[32] CIERNIKOVA S, TOMKA M, KOVAC M, STEVURKOVA V, ZAJAC V. Age and Geographical Distribution in Families with BRCA1/BRCA2 Mutations in the Slovak Republic. Hered Cancer Clin Pract 2006; 4: 7–11. https://doi.org/10.1186/1897-4287-4-1-7

[33] CIERNIKOVA S, TOMKA M, KOVAC M, STEVURKOVA V, ZAJAC V. Ashkenazi founder BRCA1/BRCA2 mutations in Slovak hereditary breast and/or ovarian cancer families. Neoplasma 2006; 53: 97–102.

[34] KOVAC M, TOMKA M, CIERNIKOVA S, STEVURKOVA V, VALACHOVA A et al. Multiple gynecologic tumors as rare associated phenotypes of FAP/Gardner syndrome in a family with the novel germline mutation in the APC gene. Clin Genet 2006; 69: 183–186. https://doi.org/10.1111/j.1399-0004.2005.00565.x

[35] KONECNY M, VIZVARYOVA M, WEISMANOVA E, ILENCIKOVA D, MLKVA I et al. The spectrum and incidence of BRCA1 pathogenic mutations in Slovak breast/ovarian cancer families. Neoplasma 2007; 54: 137–142.

[36] KONECNY M, ZAVODNA K, VRANOVA V, VIZVARYOVA M, WEISMANOVA E et al. Identification of rare complete BRCA1 gene deletion using a combination of SNP haplotype analysis, MLPA and array-CGH techniques. Breast Cancer Res Treat 2008; 109: 581–583. https://doi.org/10.1007/s10549-007-9670-0

[37] KONECNY M, VIZVARYOVA M, ZAVODNA K, BEHULOVA R, GERYKOVA BUJALKOVA M et al. Identification of a novel mutations BRCA1*c.80+3del4 and BRCA2*c.6589delA in Slovak HBOC families. Breast Cancer Res Treat 2010; 119: 233–237. https://doi.org/10.1007/s10549-008-0244-6

[38] KONECNY M, MILLY M, ZAVODNA K, WEISMANOVA E, GREGOROVA J et al. Comprehensive genetic characterization of hereditary breast/ovarian cancer families from Slovakia. Breast Cancer Res Treat 2011; 126: 119–130. https://doi.org/10.1007/s10549-010-1325-x

[39] ZIDEKOVA D, WACZULIKOVA I, DOLESOVA L, VAVROVA L, HAMIDOVA O et al. Rapid screening test of most frequent BRCA1/BRCA2 pathogenic variants in the NGS era. Neoplasma 2018; 65: 309-315. https://doi.org/10.4149/neo_2018_170507N328

[40] LERNER-ELLIS J, MIGHTON C, LAZARO C, WATKINS N, DI GIOACCHINO V et al. Multigene panel testing for hereditary breast and ovarian cancer in the province of Ontario. J Cancer Res Clin Oncol 2021; 147: 871–879. Erratum in: Cancer Res Clin Oncol 2020; https://doi.org/10.1007/s00432-020-03399-0

[41] KRAUS C, HOYER J, VASILEIOU G, WUNDERLE M, LUX MP et al. Gene panel sequencing in familial breast/ovarian cancer patients identifies multiple novel mutations also in genes others than BRCA1/2. Int J Cancer 2017; 140: 95–102. https://doi.org/10.1002/ijc.30428

[42] TEDALDI G, TEBALDI M, ZAMPIGA V, DANESI R, ARCANGELI V et al. Multiple-gene panel analysis in a case series of 255 women with hereditary breast and ovarian cancer. Oncotarget 2017; 8: 47064-47075. https://doi.org/10.18632/oncotarget.16791

[43] CYBULSKI C, KLUŹNIAK W, HUZARSKI T, WOKOŁORCZYK D, KASHYAP A et al. The spectrum of mutations predisposing to familial breast cancer in Poland. Int J Cancer 2019; 145: 3311–3320. https://doi.org/10.1002/ijc.32492

[44] TSAOUSIS GN, PAPADOPOULOU E, APESSOS A, AGIANNITOPOULOS K, PEPE G et al. Analysis of hereditary cancer syndromes by using a panel of genes: novel and multiple pathogenic mutations. BMC Cancer 2019; 19: 535. https://doi.org/10.1186/s12885-019-5756-4

[45] VELÁZQUEZ C, LASTRA E, AVILA COBOS F, ABELLA L, DE LA CRUZ V et al. A comprehensive custom panel evaluation for routine hereditary cancer testing: improving the yield of germline mutation detection. J Transl Med 2020; 18, 232. https://doi.org/10.1186/s12967-020-02391-z

[46] BENUSIGLIO PR, KORENBAUM C, VIBERT R, EZENFIS J, GEOFFRON S et al. Utility of a mainstreamed genetic testing pathway in breast and ovarian cancer patients during the COVID-19 pandemic. Eur J Med Genet 2020; 63: 104098. https://doi.org/10.1016/j.ejmg.2020.104098

[47] FOGLIETTA J, LUDOVINI V, BIANCONI F, PISTOLA L, REDA MS et al. Prevalence and Spectrum of BRCA Germline Variants in Central Italian High Risk or Familial Breast/Ovarian Cancer Patients: A Monocentric Study. Genes (Basel) 2020; 11: 925. https://doi.org/10.3390/genes11080925

[48] HAMEL N, FENG BJ, FORETOVA L, STOPPA-LYONNET D, NAROD SA et al. On the origin and diffusion of BRCA1 c.5266dupC (5382insC) in European populations. Eur J Hum Genet 2011; 19: 300–306. https://doi.org/10.1038/ejhg.2010.203

[49] MACHACKOVA E, FORETOVA L, LUKESOVA M, VASICKOVA P, NAVRATILOVA M et al. Spectrum and characterisation of BRCA1 and BRCA2 deleterious mutations in highrisk Czech patients with breast and/ or ovarian cancer. BMC Cancer 2008; 8: 140. https://doi.org/10.1186/1471-2407-8-140

[50] FORETOVA L, MACHACKOVA E, NAVRATILOVA M, PAVLU H, HRUBA M et al. BRCA1 and BRCA2 mutations in women with familial or early-onset breast/ovarian cancer in the Czech Republic. Hum Mutat 2004; 23: 397–398. https://doi.org/10.1002/humu.9226

[51] WOJCIK P, JASIOWKA M, STRYCHARZ E, SOBOL M, HODOROWICZ-ZANIEWSKA D et al. Recurrent mutations of BRCA1, BRCA2 and PALB2 in the population of breast and ovarian cancer patients in Southern Poland. Hered Cancer Clin Pract 2016; 14: 5. https://doi.org/10.1186/s13053-016-0046-5

[52] TEA MK, KROISS R, MUHR D, FUERHAUSER-RAPPAPORT C, OEFNER P et al. Central European BRCA2 mutation carriers: birth cohort status correlates with onset of breast cancer. Maturitas 2014; 77: 68–72. https://doi.org/10.1016/j.maturitas.2013.09.012

[53] VASICKOVA P, MACHACKOVA E, LUKESOVA M, DAMBORSKY J, HORKY O et al. High Occurrence of BRCA1 Intragenic Rearrangements in Hereditary Breast and Ovarian Cancer Syndrome in the Czech Republic. BMC Med Genet 2007; 8: 32. https://doi.org/10.1186/1471-2350-8-32

[54] CAVAILLE M, UHRHAMMER N, PRIVAT M, PONELLE-CHACHUAT F, GAY-BELLILE M et al. Feedback of extended panel sequencing in 1530 patients referred for suspicion of hereditary predisposition to adult cancers. Clin Genet 2021; 99: 166–175. https://doi.org/10.1111/cge.13864

[55] WIN AK, REECE JC, DOWTY JG, BUCHANAN DD, CLENDENNING M, ROSTY C et al. Risk of extracolonic cancers for people with biallelic and monoallelic mutations in MUTYH. Int J Cancer 2016; 139: 1557–1563. https://doi.org/10.1002/ijc.30197

[56] WEISCHER M, BOJESEN SE, TYBJAERG-HANSEN A, AXELSSON CK, NORDESTGAARD BG. Increased risk of breast cancer associated with CHEK2*1100delC. J Clin Oncol 2007; 25: 57–63. https://doi.org/10.1200/JCO.2005.05.5160

[57] CYBULSKI C, GÓRSKI B, HUZARSKI T, BYRSKI T, GRONWALD J et al. Effect of CHEK2 missense variant I157T on the risk of breast cancer in carriers of other CHEK2 or BRCA1 mutations. J Med Genet 2009; 46: 132–135. https://doi.org/10.1136/jmg.2008.061697

[58] KLEIBLOVA P, STOLAROVA L, KRIZOVA K, LHOTA F, HOJNY J et al. Identification of deleterious germline CHEK2 mutations and their association with breast and ovarian cancer. Int J Cancer 2019; 145: 1782–1797. https://doi.org/10.1002/ijc.32385

[59] BALMANA J, DIGIOVANNI L, GADDAM P, WALSH MF, JOSEPH V et al. Conflicting Interpretation of Genetic Variants and Cancer Risk by Commercial Laboratories as Assessed by the Prospective Registry of Multiplex Testing. J Clin Oncol 2016; 34: 4071–4078. https://doi.org/10.1200/JCO.2016.68.4316

[60] CYBULSKI C, GÓRSKI B, HUZARSKI T, MASOJĆ B, MIERZEJEWSKI M et al. CHEK2 is a multiorgan cancer susceptibility gene. Am J Hum Genet 2004; 75: 1131–1135. https://doi.org/10.1086/426403

[61] DESRICHARD A, BIDET Y, UHRHAMMER N, BIGNON YJ. CHEK2 contribution to hereditary breast cancer in non-BRCA families. Breast Cancer Res 2011; 13: R119. https://doi.org/10.1186/bcr3062

[62] HAN FF, GUO CL, LIU LH. The effect of CHEK2 variant I157T on cancer susceptibility: evidence from a meta-analysis. DNA Cell Biol 2013; 32: 329–335. https://doi.org/10.1089/dna.2013.1970

[63] BAK A, JANISZEWSKA H, JUNKIERT-CZARNECKA A, HEISE M, PILARSKA-DELTOW M et al. A risk of breast cancer in women – carriers of constitutional CHEK2 gene mutations, originating from the North – Central Poland. Hered Cancer Clin Pract 2014; ; 12: 10. https://doi.org/10.1186/1897-4287-12-10

[64] KOCZKOWSKA M, KRAWCZYNSKA N, STUKAN M, KUZNIACKA A, BROZEK I et al. Spectrum and Prevalence of Pathogenic Variants in Ovarian Cancer Susceptibility Genes in a Group of 333 Patients. Cancers (Basel) 2018; 10: 442. https://doi.org/10.3390/cancers10110442

[65] KLEIBLOVÁ P, STOLAŘOVÁ L, LHOTA F, HOJNÝ J, ZEMÁNKOVÁ P et al. Germline CHEK2 Gene Mutations in Hereditary Breast Cancer Predisposition – Mutation Types and their Biological and Clinical Relevance Klin Onkol. Summer 2019; 32: 36–50. https://doi.org/10.14735/amko2019S36

[66] WALSH T, CASADEI S, COATS KH, SWISHER E, STRAY SM et al. Spectrum of mutations in BRCA1, BRCA2, CHEK2, and TP53 in families at high risk of breast cancer. JAMA 2006; 295: 1379–1388. https://doi.org/10.1001/jama.295.12.1379

[67] CYBULSKI C, WOKOŁORCZYK D, HUZARSKI T, BYRSKI T, GRONWALD J et al. A large germline deletion in the Chek2 kinase gene is associated with an increased risk of prostate cancer. J Med Genet 2006; 43: 863–866. https://doi.org/10.1136/jmg.2006.044974

[68] ANTONIOU AC, CASADEI S, HEIKKINEN T, BARROWDALE D, PYLKÄS K et al. Breast-cancer risk in families with mutations in PALB2. N Engl J Med 2014; 371: 497–506. https://doi.org/10.1056/NEJMoa1400382

[69] ENGEL C, RHIEM K, HAHNEN E, Loibl S, Weber KE et al. Prevalence of pathogenic BRCA1/2 germline mutations among 802 women with unilateral triple-negative breast cancer without family cancer history. BMC Cancer 2018; 18, 265. https://doi.org/10.1186/s12885-018-4029-y

[70] FELICIO PS, GRASEL RS, CAMPACCI N, DE PAULA AE, GALVAO HCR et al. Whole-exome sequencing of non-BRCA1/BRCA2 mutation carrier cases at high-risk for hereditary breast/ovarian cancer. Hum Mutat 2021; 42: 290–299. https://doi.org/10.1002/humu.24158

[71] MANCHANDA R, PATEL S, GORDEEV VS, ANTONIOU AC, SMITH S et al. Cost-effectiveness of Population-Based BRCA1, BRCA2, RAD51C, RAD51D, BRIP1, PALB2 Mutation Testing in Unselected General Population Women. J Natl Cancer Inst 2018; 110: 714–725. https://doi.org/10.1093/jnci/djx265

[72] TUTT A, TOVEY H, CHEANG M, KERNAGHAN S, KILBURN L et al. Carboplatin in BRCA1/2-mutated and triple-negative breast cancer BRCAness subgroups: the TNT Trial. Nat Med 2018; 24: 628–637. https://doi.org/10.1038/s41591-018-0009-7

[73] OHMOTO A, YACHIDA S. Current status of poly(ADP-ribose) polymerase inhibitors and future directions. Onco Targets Ther 2017; 10: 5195–5208. https://doi.org/10.2147/OTT.S139336

[74] TUNG NM, ROBSON ME, VENTZ S, SANTA-MARIA CA, NANDA R et al. TBCRC 048: Phase II Study of Olaparib for Metastatic Breast Cancer and Mutations in Homologous Recombination-Related Genes. J Clin Oncol 2020; 38: 4274–4282. https://doi.org/10.1200/JCO.20.02151

[75] MATEO J, CARREIRA S, SANDHU S, MIRANDA S, MOSSOP H et al. DNA-repair defects and Olaparib in metastatic prostate cancer. N Engl J Med 2015; 373: 1697–1708. https://doi.org/10.1056/NEJMoa1506859

[76] SCHUH A, DREAU H, KNIGHT SJL, RIDOUT K, MIZANI T et al. Clinically actionable mutation profiles in patients with cancer identified by whole-genome sequencing. Cold Spring Harb Mol Case Stud 2018; 4: a002279. https://doi.org/10.1101/mcs.a002279