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Short report

Sensitivity and specificity of loss of heterozygosity analysis for the classification of rare germline variants in *BRCA1/2*: results of the observational AGO-TR1 study (NCT02222883)

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ABSTRACT

Variant-specific loss of heterozygosity (LOH) analyses may be useful to classify *BRCA1/2* germline variants of unknown significance (VUS). The sensitivity and specificity of this approach, however, remains unknown. We performed comparative next-generation sequencing analyses of the *BRCA1/2* genes using blood-derived and tumour-derived DNA of 488 patients with ovarian cancer enrolled in the observational AGO-TR1 trial (NCT02222883). Overall, 94 pathogenic, 90 benign and 24 VUS were identified in the germline. A significantly increased variant fraction (VF) of a germline variant in the tumour indicates loss of the wild-type allele; a decreased VF indicates loss of the variant allele. We demonstrate that significantly increased VFs predict pathogenicity with high sensitivity (0.84, 95% CI 0.77 to 0.91), poor specificity (0.63, 95% CI 0.53 to 0.73) and poor positive predictive value (PPV; 0.71, 95% CI 0.62 to 0.79). Significantly decreased VFs predict benignity with low sensitivity (0.26, 95% CI 0.17 to 0.35), high specificity (1.0, 95% CI 0.96 to 1.00) and PPV (1.0, 95% CI 0.85 to 1.00). Variant classification based on significantly increased VFs results in an unacceptable proportion of false-positive results. A significantly decreased VF in the tumour may be exploited as a reliable predictor for benignity, with no false-negative result observed. When applying the latter approach, VUS identified in four patients can now be considered benign.

Trial registration number NCT02222883.

INTRODUCTION

In cancer genetics, individual risk stratification and the choice of targeted therapies are increasingly dependent on the germline mutation status in disease-associated genes such as *BRCA1* (MIM: 113705) and *BRCA2* (MIM: 600185). Thus, the unambiguous classification of germline variants identified in a routine diagnostic setting is vitally important for the clinical management of the individuals seeking advice. Criteria for *BRCA1/2* germline variant classification were continuously standardised, in particular through the work of the Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA), the International Agency for Research on Cancer (IARC) and the American College of Medical Genetics and Genomics (ACMG).[1–4] While common *BRCA1/2* germline variants with a minor allele frequency (MAF) ≥1% in the general population are considered benign by default, the classification of rare *BRCA1/2* germline variants with a MAF <1% remains challenging, especially for those that cannot be predicted protein-truncating based on their mutation type. For intronic and missense variants, the multifactorial likelihood analysis demonstrated utility for quantitative assessment of variant pathogenicity, a model based on variant location, in silico prediction of variant effect, cosegregation, family cancer history, co-occurrence with a pathogenic variant in the same gene, tumour pathology and case–control information.[3] The multifactorial likelihood analysis, however, requires input data that may not be available for all rare *BRCA1/2* germline variants.

In tumours with a hereditary disease cause, it is generally suggested that the heterozygous germline inactivation of a predisposition gene may be accompanied by a somatic inactivation of the wild-type allele by another deleterious variant, loss of the wild-type allele or promoter methylation.[5] In 473 patients with ovarian cancer (OC (MIM: 167 000)) enrolled in the observational AGO-TR1 trial, we demonstrated that pathogenic germline variants in the *BRCA1/2* genes very rarely associate with deleterious somatic variants or promoter methylation.[6] In OC, more than 80% of the pathogenic *BRCA1/2* germline variants showed significantly increased proportion of reads that support the variant allele (variant allele fractions (VFs)) in the tumour-derived versus blood-derived DNA, indicating loss of the wild-type alleles.[6–8] Based on these findings, it was suggested that loss of heterozygosity (LOH) analyses might be useful to classify rare germline

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variants in the *BRCA1/2* genes.[9 10] For rare germline variants in (candidate) cancer predisposition genes showing significantly increased VFs in the tumour, a potential role in cancer susceptibility was frequently suggested, as for example in the analyses of 429 patients with OC included in The Cancer Genome Atlas (TCGA) project.[7] However, sensitivity along with specificity of LOH analyses for germline variant classification was not assessed so far. Thus, our study aims to quantify the sensitivity and the specificity of LOH analyses and their potential benefit for the classification of rare *BRCA1/2* germline variants in a well-characterised study sample of 488 patients with OC enrolled in the observational AGO-TR1 trial (NCT02222883).

METHODS

Study sample

A total of 523 consecutive patients with invasive epithelial OC were enrolled. All patients were older than 18 years at study inclusion and provided written informed consent prior to enrolment. Venous blood samples were available from all 523 patients and formalin-fixed paraffin-embedded (FFPE) tumour samples were available from 496 patients. Genomic DNA was isolated from blood samples and from FFPE tumour samples as described previously.[6] Briefly, 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, Bonn, Germany); that is, tumour areas containing >80% tumour nuclei were chosen for DNA isolation (see online supplemental materials and methods for details).

Next-generation sequencing (NGS)

Targeted NGS of blood and tumour samples of 496 patients was performed using a customised gene panel covering the coding regions and exon-flanking sequences (±15 nt) of the *BRCA1* (NM_007294.3) and *BRCA2* (NM_000059.3) genes.[6] The hybridisation capture-based NGS method (Agilent SureSelect XT protocol optimised for 200 ng of genomic DNA) was suitable for the analysis of DNA derived from either blood or FFPE tumour samples. Sequencing was performed on a HiSeq4000 device (Illumina, San Diego, California, USA). NGS analyses with a mean read coverage of at least 100× were considered successful. NGS data derived from both blood and corresponding FFPE tumour samples of 488 individuals achieved this threshold. The clinical characteristics of the 488 individuals were described in the online supplemental table 1. For the 488 individuals included, the mean read coverage was 455× (range 171×–882×) for NGS of blood-derived DNA and 570× (range 110×–1802×) for tumour-derived DNA. Bioinformatic analyses, including variant calling, were carried out using the VARBANK V.2.10–2.24 pipeline of the Cologne Center for Genomics and the DDM1 platform (Sophia Genetics, Saint-Sulpice, Switzerland).

Germline variant classification

We employed criteria based on the ENIGMA and ACMG Guidelines for variant classification.[4] Rare variants were defined as variants with a MAF <1% in large outbred control reference groups. Common variants with a MAF above this threshold were generally considered benign and excluded from this investigation. All rare variants in splice regions and non-synonymous single-nucleotide/indel variants were included in this investigation. CNVs were not considered. To determine MAFs, we used Exome Aggregation Consortium (ExAC)[11] data of individuals of European, non-Finnish ancestry, excluding samples from TCGA. All rare *BRCA1/2* germline variants were classified using a five-tier variant classification system as proposed by the IARC Unclassified Genetic Variants Working Group,[12] namely, pathogenic=class 5, likely pathogenic=class 4, variant of uncertain significance (VUS)=class 3, likely benign=class 2 and benign=-class 1. For reasons of clarity, class 4/5 are referred to as pathogenic variants and class 1/2 variants as benign variants in the following.

LOH analysis

VFs were derived from VARBANK VCF files by division of the number of reads showing the variant allele and the observed read depth. Fold changes, that is, the ratio of tumour and blood VFs, were computed for each rare germline variant. Fisher's exact test was applied to assess the significance level of deviating proportions of reads showing a variant allele between blood and tumour sample, with p values <0.05 after correction for multiple testing using the Benjamini-Hochberg approach[13] considered significant. A significantly increased VF of a variant in the tumour suggests loss of the wild-type allele. A significantly decreased VF of a variant in the tumour suggests loss of the variant allele. Statistical analyses were performed using SPSS Statistics V.25 and the epiR-Package under R V.3.6.2.

Web resources

OMIM: http://www.omim.org/
ClinVar: https://www.ncbi.nlm.nih.gov/clinvar/
ENIGMA: https://enigmaconsortium.org/

RESULTS

Germline analysis revealed 208 rare variants in 181 of the 488 patients (37.1%). One hundred and fifty-seven patients carried one (32.2%), 21 carried two (4.3%) and 3 patients carried three rare germline variants (0.6%) (online supplemental figure 1A). Of the 208 rare variants, 94 were pathogenic (class 4/5), 90 were benign (class 1/2) and 24 were of unknown significance (VUS, class 3). The combined *BRCA1/2* genotypes of the 181 patients with rare variants are illustrated in online supplemental figure 1B). All rare variants were listed in the online supplemental table 2).

All rare germline variants were also detected in the corresponding tumour samples. Of the 94 pathogenic germline variants, 79 (84.0%) showed significantly increased VF in the tumour suggesting loss of the wild-type alleles, with fold changes ranging from 1.15 to 2.05 (figure 1, online supplemental table 2). The VF differences of the remaining 15 class 4/5 variants (16%) were statistically not significant with fold changes ranging from 0.85 to 1.13. Of note, none of the class 4/5 variants showed a significantly decreased VF in the tumour. Of the 90 class 1/2 variants, 33 (36.7%) showed significantly increased VFs in the tumour with fold changes ranging from 1.22 to 2.02, 34 showed non-significant differences (37.8%, fold changes ranging from 0.87 to 1.16) and for the remaining 23 variants, VFs were significantly decreased in tumour samples (25.6%, fold changes ranging from 0.06 to 0.84) (figure 1, online supplemental table 2).

Variant classification based on significantly increased VFs shows a high sensitivity of 0.84 (95% CI 0.77 to 0.91), but a poor specificity of 0.63 (95% CI 0.53 to 0.73) and a poor positive predictive value (PPV) of 0.71 (95% CI 0.62 to 0.79). For this approach, the positive likelihood ratio (LR+) for pathogenicity is 2.29 (95% CI 1.72 to 3.05). Briefly, variant classification based on significantly increased VFs is hampered by the random distribution of VFs observed for benign variants. At least in a routine diagnostic setting, classification of rare *BRCA1/2*

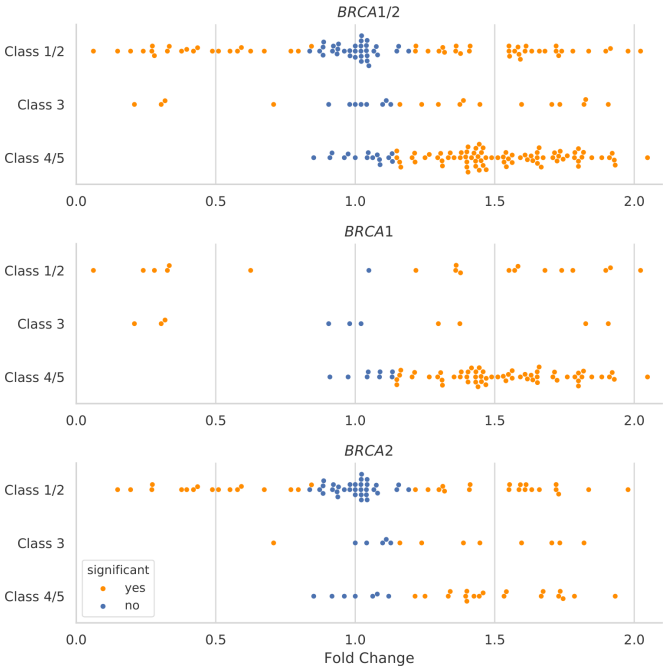

Figure 1 Fold change of VF between blood-derived and tumour-derived DNA observed for 208 class 4/5, class 3 and class 1/2 germline variants in the *BRCA1/2* genes observed in the overall study sample. Significant differences in VFs between blood-derived and tumour-derived DNA were indicated by orange dots. Non-significant differences in VFs between blood-derived and tumour-derived DNA were indicated by blue dots. VFs, variant fraction.

germline variants may not be based on significantly increased VFs due to an unacceptable proportion of false-positive results.

As an alternative approach, a significantly decreased VF of a variant in the tumour, suggesting loss of the variant allele, may be useful to classify a rare *BRCA1/2* germline variant as benign. Significantly decreased VFs were specific for benign variants and were not observed for pathogenic germline variants (figure 1).

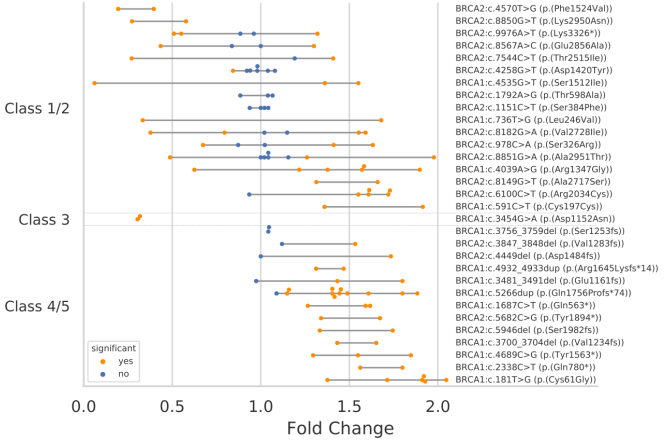

Figure 2 Fold change of VF observed for 31 recurrent class 4/5, class 3 and class 1/2 germline variants in the *BRCA1/2* genes observed in the overall study sample. Significant differences in VFs between blood-derived and tumour-derived DNA were indicated by orange dots. Non-significant differences in VFs between blood-derived and tumour-derived DNA were indicated by blue dots. VFs, variant fractions.

Of the benign variants observed in 90 patients, 17 were recurrent and found at least twice in the sample set. For most of the recurrent benign variants, we found a high variability of fold changes, occasionally ranging from a significant decrease to a significant increase (figure 2). Classification of benign *BRCA1/2* germline variants based on significantly decreased VFs results in a low sensitivity of 0.26 (95% CI 0.17 to 0.35) but a high specificity of 1.0 (95% CI 0.96 to 1.00) and a high PPV of 1.0 (95% CI 0.85 to 1.00). For this approach, the LR+ for benignity was 49.07 (95% CI 3.02 to 795.93) after Haldane-Anscombe correction (online supplemental table 3). A significantly decreased VF of a variant in the tumour may be exploited as a reliable predictor for benignity, with no false-negative result observed. This also holds true when analysis were performed for both genes separately (figure 1, online supplemental table 3). When applying this approach to the 24 VUS identified in our study sample, three distinct VUS found in four patients, that is, *BRCA1* p.(Val525Ile), *BRCA1* p.(Asp1152Asn) and *BRCA2* p.(Lys2498del), may be considered benign (figure 2).

DISCUSSION

It was controversially discussed whether the results of LOH analyses may be useful for the classification of rare *BRCA1/2* germline variants.[7–10 14–18] Information from LOH analyses has not been implemented in the current ENIGMA variant classification system[3 19] based on the previously published data[16] suggesting that LOH analyses are not sufficiently reliable. Using paired analyses of blood-derived and tumour-derived DNA, we demonstrated that rare germline variants in the *BRCA1/2* genes might be classified benign based on significantly decreased VFs in the tumour. This approach reached a specificity of 1.0 (95% CI 0.96 to 1.00), a PPV of 1.0 (95% CI 0.85 to 1.00) and a LR+ of 49.07 (95% CI 3.02 to 795.93). Given the fact that changes in VFs of benign variants occur randomly (figure 2), this approach shows a limited sensitivity of only 0.26 (95% CI 0.17 to 0.36). As of March 2020, more than 6.100 distinct *BRCA1/2* germline VUS were listed in the ClinVar database, indicating the need for additional sources for the classification of *BRCA1/2* germline variants. We suggest that large-scale comparative germline/tumour NGS analyses with sufficient read depths may significantly reduce the number of VUS, especially for VUS for which data regarding cosegregation, family cancer history, co-occurrence with a pathogenic variant in the same gene and case–control information are not available.[3]

Limitations of the study

In the overall study sample of patients with OC enrolled in the observational AGO-TR1 study, pathogenic germline mutations in non-*BRCA1/2* OC predisposition genes such as *RAD51C/D* and *BRIP1* were observed. However, the prevalence of pathogenic germline mutations in these genes was too low to perform meaningful calculations. Larger studies are required to quantify the sensitivity and the specificity of LOH analyses for the classification of rare germline mutations in additional OC predisposition genes. Moreover, this investigation was focused on patients with OC and FFPE samples with a high tumour content. It remains elusive to which extent our approach may be transferred to breast tumour analyses that are usually associated with lower *BRCA1/2* LOH rates[20] and probably lower tumour contents in FFPE samples.

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Sensitivity and specificity of loss of heterozygosity analysis for the classification of rare germline variants in *BRCA1/2*: Results of the observational AGO-TR1 study (NCT02222883)

Supplementary Materials and Methods

DNA isolation from FFPE tumor samples

Tumour areas containing >80% tumour nuclei were chosen for DNA isolation, macrodissected from unstained 10 µm sections, and incubated in the presence of 1ml limonene (30 min, 60°C, 1,000 rpm). Deparaffinized tissues were rehydrated (consecutive washing with 98% and 70% ethanol) and incubated (8 h, 60°C) in 200 µl of lysis buffer (50 mM Tris-HCl, pH 8.4, 1 mM EDTA, 0.5% [v/v] Tween®20 and 2 mg/ml proteinase K [Carl Roth, Karlsruhe, Germany]). Another 20 µl of Proteinase K (20 mg/ml) was added. Following incubation (16 h, 60°C, 1,000 rpm), 200 µl of tissue lysate was mixed with 250 µl of binding buffer (6 M guanidinium thiocyanate, 0.1 M Tris, pH 7.5), 250 µl of ethanol, transferred onto a NucleoSpin Extract II spin column (Macherey & Nagel, Dueren, Germany), and centrifuged (3 min, 14,000x g). The bound DNA was washed twice with 700 µl of wash buffer (150 mM Tris pH 7.4, 85% ethanol). The wash buffer was removed (1 min, 14,000x g). A total of 50 µl of water was added, incubated for 1 min, and the DNA was eluted (1 min, 14,000x g). The DNA concentration was quantified using a Nanodrop® ND-1000 photometer (NanoDrop Technologies, Wilmington, DE, USA).

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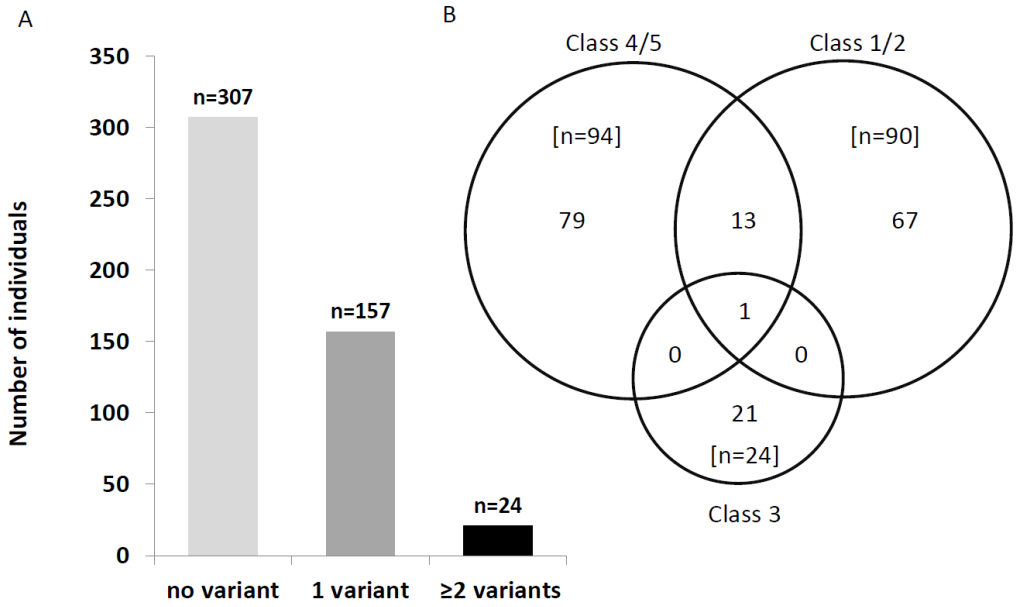
Supplementary Figure 1: A) Rare *BRCA1/2* germline variants identified in the overall study sample of 488 patients with OC. The majority of individuals (307, 62.9%) carried no rare germline variant in the *BRCA1/2* genes; 157 individuals carried one (32.2%), 21 individuals carried two (4.3%), and three individuals carried three rare germline variants (0.6%). **B)** *BRCA1/2* genotypes of the 181 patients with rare *BRCA1/2* variants. 79 patients carried class 4/5 variants only. Of those, one patient carried a combination of one *BRCA1* and one *BRCA2* class 4/5 variant. 13 patients carried one class 4/5 *BRCA1/2* variant in addition to one class 1/2 variant; another patient carried one class 4/5 variant, one class 3, and one class 1/2 variant. 67 patients carried class 1/2 variants only (60 with one variant, five with two, and two with three variants). 21 patients carried class 3 variants only (19 with one variant and two with two variants).



Supplementary Table 1: Characteristics of the 488 individuals with ovarian cancer included in this investigation. Of the 488 patients, 266 were enrolled at primary diagnosis of ovarian cancer and 222 were enrolled with recurrent ovarian cancer.

Age at ovarian cancer diagnosis (mean, range)	58 years, 16-93 years
Histological subtypes (% of the overall study sample)	
- High grade serous	382 (78.3)
- High grade endometrioid	22 (4.5)
- Serous/papillary, grade unknown	20 (4.1)
- Low grade serous	17 (3.5)
- Mucinous	8 (1.6)
- Low grade endometrioid	7 (1.4)
- Clear cell	6 (1.2)
- Other/unspecified	19 (3.9)
- Missing	7 (1.4)
Tumour grade (% of the overall study sample)	
- G1	29 (5.9)
- G2	69 (14.1)
- G3	355 (72.7)
- G4	6 (1.2)
- Not specified	29 (5.9)

Supplementary Table 2: Rare germline variants in the *BRCA1* and *BRCA2* genes observed in 488 OC patients. For each variant, the results of paired NGS analyses of the DNA sample derived from blood and the DNA sample derived from the corresponding tumour are shown, i.e. the number of reads showing the variant allele and the number of reads showing reference allele. ExAC % = Frequency of the germline variant in percent according to the Exome Aggregation Consortium (ExAC) in a dataset of individuals of European, non-Finnish ancestry, excluding samples from The Cancer Genome Atlas (TCGA). This dataset comprises a total of 27,173 samples that were analyzed by whole-exome sequencing. * = Fisher's Exact test was applied to assess the significance level of deviating variant fractions in blood vs tumour samples, with p values <0.05 considered significant after correction for multiple testing using the Benjamini-Hochberg approach.

ID	Chromosome	Position	Gene	Transcript	cHGVS	pHGVS	ExAC %	Variant allele blood	Reference allele blood	Variant fraction blood	Variant allele tumour	Reference allele tumour	Variant fraction tumour	Fold change	P-Value *
Class 4/5 variants															
P32	17	41245210	*BRCA1*	NM_007294.3	c.2338C>T	p.(Gln780*)	0.0000	507	507	0.50	643	71	0.90	1.80	0.00000
P51	17	41223242	*BRCA1*	NM_007294.3	c.4689C>G	p.(Tyr1563*)	0.0000	409	480	0.46	685	121	0.85	1.85	0.00000
P48	17	41226488	*BRCA1*	NM_007294.3	c.4533_4534del	p.(His1511Glnfs*)	0.0000	256	288	0.47	1363	260	0.84	1.79	0.00000
P63	17	41209079	*BRCA1*	NM_007294.3	c.5266dup	p.(Gln1756Profs*74)	0.0166	258	315	0.45	593	139	0.81	1.80	0.00000

P60	17	41215362	*BRCA1*	NM_007294.3	c.5177_5180del	p.(Arg1726Lysfs*3)	0.0000	212	269	0.44	602	150	0.80	1.82	0.00000
P476	13	32905140	*BRCA2*	NM_000059.3	c.771_775del	p.(Asn257fs)	0.0000	243	310	0.44	300	53	0.85	1.93	0.00000
P42	17	41243843	*BRCA1*	NM_007294.3	c.3700_3704del	p.(Val1234fs)	0.0000	446	523	0.46	562	177	0.76	1.65	0.00000
P54	17	41222994	*BRCA1*	NM_007294.3	c.4936del	p.(Val1646Serfs*12)	0.0000	294	306	0.49	633	148	0.81	1.65	0.00000
P31	17	41245210	*BRCA1*	NM_007294.3	c.2338C>T	p.(Gln780*)	0.0000	304	329	0.48	1091	364	0.75	1.56	0.00000
P30	17	41245591	*BRCA1*	NM_007294.3	c.1953_1956del	p.(Lys653fs)	0.0000	520	611	0.46	397	125	0.76	1.65	0.00000
P26	17	41245861	*BRCA1*	NM_007294.3	c.1687C>T	p.(Gln563*)	0.0074	340	353	0.49	594	168	0.78	1.59	0.00000
P84	13	32912940	*BRCA2*	NM_000059.3	c.4449del	p.(Asp1484fs)	0.0000	134	139	0.49	612	108	0.85	1.73	0.00000
P44	17	41243776	*BRCA1*	NM_007294.3	c.3770_3771del	p.(Glu1257fs)	0.0018	281	316	0.47	335	79	0.81	1.72	0.00000
P65	17	41209079	*BRCA1*	NM_007294.3	c.5266dup	p.(Gln1756Profs*74)	0.0166	431	505	0.46	460	161	0.74	1.61	0.00000
P10	17	41276080	*BRCA1*	NM_007294.3	c.34C>T	p.(Gln12*)	0.0000	110	110	0.50	482	66	0.88	1.76	0.00000
P27	17	41245861	*BRCA1*	NM_007294.3	c.1687C>T	p.(Gln563*)	0.0074	282	319	0.47	487	154	0.76	1.62	0.00000
P18	17	41258504	*BRCA1*	NM_007294.3	c.181T>G	p.(Cys61Gly)	0.0056	146	179	0.45	220	36	0.86	1.91	0.00000
P95	13	32936830	*BRCA2*	NM_000059.3	c.7976G>A	p.(Arg2659Lys)	0.0000	175	189	0.48	483	121	0.80	1.67	0.00000
P24	17	41246355	*BRCA1*	NM_007294.3	c.1193C>G	p.(Ser398*)	0.0000	290	314	0.48	494	165	0.75	1.56	0.00000
P19	17	41258473	*BRCA1*	NM_007294.3	c.212G>C	p.(Arg71Thr)	0.0000	190	206	0.48	183	27	0.87	1.81	0.00000
P90	13	32914174	*BRCA2*	NM_000059.3	c.5682C>G	p.(Tyr1894*)	0.0000	219	228	0.49	288	63	0.82	1.67	0.00000
P57	17	41215957	*BRCA1*	NM_007294.3	c.5084_5085del	p.(Phe1695Cysfs*3)	0.0000	109	118	0.48	553	121	0.82	1.71	0.00000
P37	17	41244280	*BRCA1*	NM_007294.3	c.3268C>T	p.(Gln1090*)	0.0000	238	238	0.50	462	138	0.77	1.54	0.00000
P16	17	41258504	*BRCA1*	NM_007294.3	c.181T>G	p.(Cys61Gly)	0.0056	99	162	0.38	315	117	0.73	1.92	0.00000
P68	17	41209079	*BRCA1*	NM_007294.3	c.5266dup	p.(Gln1756Profs*74)	0.0166	106	141	0.43	224	53	0.81	1.88	0.00000
P92	13	32914437	*BRCA2*	NM_000059.3	c.5946del	p.(Ser1982fs)	0.0388	225	217	0.51	138	17	0.89	1.75	0.00000
P21	17	41256182	*BRCA1*	NM_007294.3	c.397del	p.(Arg133fs)	0.0000	297	309	0.49	366	122	0.75	1.53	0.00000
P40	17	41244056	*BRCA1*	NM_007294.3	c.3481_3491del	p.(Glu1161fs)	0.0000	244	453	0.35	221	130	0.63	1.80	0.00000
P53	17	41222997	*BRCA1*	NM_007294.3	c.4932_4933dup	p.(Arg1645Lysfs*14)	0.0000	435	490	0.47	402	181	0.69	1.47	0.00000
P29	17	41245504	*BRCA1*	NM_007294.3	c.2043dup	p.(Asn682fs)	0.0000	110	140	0.44	623	230	0.73	1.66	0.00000
P96	13	32954272	*BRCA2*	NM_000059.3	c.9253dup	p.(Thr3085fs)	0.0000	254	350	0.42	151	50	0.75	1.79	0.00000
P17	17	41258504	*BRCA1*	NM_007294.3	c.181T>G	p.(Cys61Gly)	0.0056	68	95	0.42	177	42	0.81	1.93	0.00000
P82	13	32912337	*BRCA2*	NM_000059.3	c.3847_3848del	p.(Val1283fs)	0.0225	202	246	0.45	407	183	0.69	1.53	0.00000

P79	13	32911322	*BRCA2*	NM_000059.3	c.2830A>T	p.(Lys944*)	0.0000	273	296	0.48	113	23	0.83	1.73	0.00000
P59	17	41215391	*BRCA1*	NM_007294.3	c.5153-2del	p.(?)	0.0000	128	163	0.44	325	127	0.72	1.64	0.00000
P94	13	32936731	*BRCA2*	NM_000059.3	c.7877G>A	p.(Trp2626*)	0.0000	324	324	0.50	464	199	0.70	1.40	0.00000
P73	17	41209079	*BRCA1*	NM_007294.3	c.5266dup	p.(Gln1756Profs*74)	0.0166	400	451	0.47	414	213	0.66	1.40	0.00000
P50	17	41223242	*BRCA1*	NM_007294.3	c.4689C>G	p.(Tyr1563*)	0.0000	251	241	0.51	167	44	0.79	1.55	0.00000
P67	17	41209079	*BRCA1*	NM_007294.3	c.5266dup	p.(Gln1756Profs*74)	0.0166	220	268	0.45	491	264	0.65	1.44	0.00000
P91	13	32914437	*BRCA2*	NM_000059.3	c.5946del	p.(Ser1982fs)	0.0388	464	503	0.48	539	303	0.64	1.33	0.00000
P33	17	41245072	*BRCA1*	NM_007294.3	c.2475del	p.(Asp825fs)	0.0000	148	143	0.51	474	166	0.74	1.45	0.00000
P45	17	41243455	*BRCA1*	NM_007294.3	c.4093T>G	p.(Leu1365Val)	0.0000	250	250	0.50	513	231	0.69	1.38	0.00000
P14	17	41258504	*BRCA1*	NM_007294.3	c.181T>G	p.(Cys61Gly)	0.0056	190	263	0.42	117	45	0.72	1.71	0.00000
P34	17	41245032	*BRCA1*	NM_007294.3	c.2515del	p.(His839fs)	0.0000	597	646	0.48	352	189	0.65	1.35	0.00000
P70	17	41209079	*BRCA1*	NM_007294.3	c.5266dup	p.(Gln1756Profs*74)	0.0166	249	281	0.47	440	226	0.66	1.40	0.00000
P61	17	41215348	*BRCA1*	NM_007294.3	c.5193+1del	p.(?)	0.0000	190	222	0.46	136	45	0.75	1.63	0.00000
P72	17	41209079	*BRCA1*	NM_007294.3	c.5266dup	p.(Gln1756Profs*74)	0.0166	470	510	0.48	248	117	0.68	1.42	0.00000
P41	17	41243843	*BRCA1*	NM_007294.3	c.3700_3704del	p.(Val1234fs)	0.0000	295	375	0.44	301	176	0.63	1.43	0.00000
P13	17	41258504	*BRCA1*	NM_007294.3	c.181T>G	p.(Cys61Gly)	0.0056	97	133	0.42	54	9	0.86	2.05	0.00000
P89	13	32914174	*BRCA2*	NM_000059.3	c.5682C>G	p.(Tyr1894*)	0.0000	239	270	0.47	725	426	0.63	1.34	0.00000
P25	17	41245927	*BRCA1*	NM_007294.3	c.1621C>T	p.(Gln541*)	0.0000	120	130	0.48	356	152	0.70	1.46	0.00000
P86	13	32913829	*BRCA2*	NM_000059.3	c.5338del	p.(Glu1780fs)	0.0000	120	130	0.48	356	153	0.70	1.46	0.00000
P77	13	32907418	*BRCA2*	NM_000059.3	c.1805del	p.(Gly602fs)	0.0000	377	409	0.48	208	102	0.67	1.40	0.00000
P78	13	32910889	*BRCA2*	NM_000059.3	c.2399dup	p.(Asn801fs)	0.0000	189	231	0.45	257	139	0.65	1.44	0.00000
P22	17	41246432	*BRCA1*	NM_007294.3	c.1116G>A	p.(Trp372*)	0.0000	314	327	0.49	140	55	0.72	1.47	0.00000
P87	13	32913987	*BRCA2*	NM_000059.3	c.5496dup	p.(Asn1833fs)	0.0000	208	225	0.48	104	37	0.74	1.54	0.00000
P46	17	41234567	*BRCA1*	NM_007294.3	c.4210del	p.(Leu1404fs)	0.0000	425	442	0.49	319	179	0.64	1.31	0.00000
P97	13	32972315	*BRCA2*	NM_000059.3	c.9666del	p.(Cys3222fs)	0.0018	302	302	0.50	154	66	0.70	1.40	0.00000
P62	17	41209079	*BRCA1*	NM_007294.3	c.5266dup	p.(Gln1756Profs*74)	0.0166	113	138	0.45	163	80	0.67	1.49	0.00000
P56	17	41219625	*BRCA1*	NM_007294.3	c.5074G>C	p.(Asp1692His)	0.0018	213	174	0.55	123	37	0.77	1.40	0.00000
P11	17	41276044	*BRCA1*	NM_007294.3	c.68_69del	p.(Glu23fs)	0.0406	148	148	0.50	137	53	0.72	1.44	0.00000
P474	17	41245861	*BRCA1*	NM_007294.3	c.1687C>T	p.(Gln563*)	0.0074	382	397	0.49	383	235	0.62	1.27	0.00000

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P15	17	41258504	*BRCA1*	NM_007294.3	c.181T>G	p.(Cys61Gly)	0.0000	102	111	0.48	440	226	0.66	1.38	0.00001
P38	17	41244056	*BRCA1*	NM_007294.3	c.3481_3491del	p.(Glu1161fs)	0.0000	85	146	0.37	653	579	0.53	1.43	0.00002
P85	13	32913457	*BRCA2*	NM_000059.3	c.4965C>G	p.(Tyr1655*)	0.0000	275	253	0.52	419	226	0.65	1.25	0.00003
P12	17	41276047	*BRCA1*	NM_007294.3	c.66dup	p.(Glu23fs)	0.0000	87	84	0.51	153	57	0.73	1.43	0.00004
P49	17	41223242	*BRCA1*	NM_007294.3	c.4689C>G	p.(Tyr1563*)	0.0000	264	335	0.44	310	234	0.57	1.30	0.00005
P76	13	32907111	*BRCA2*	NM_000059.3	c.1499del	p.(Gly500fs)	0.0000	175	197	0.47	114	56	0.67	1.43	0.00005
P69	17	41209079	*BRCA1*	NM_007294.3	c.5266dup	p.(Gln1756Profs*74)	0.0166	105	145	0.42	160	102	0.61	1.45	0.00006
P52	17	41222997	*BRCA1*	NM_007294.3	c.4932_4933dup	p.(Arg1645Lysfs*14)	0.0000	120	130	0.48	437	257	0.63	1.31	0.00014
P75	17	41197784	*BRCA1*	NM_007294.3	c.5503C>T	p.(Arg1835*)	0.0000	406	345	0.54	315	170	0.65	1.20	0.00045
P28	17	41245670	*BRCA1*	NM_007294.3	c.1874_1877dup	p.(Val627fs)	0.0000	113	138	0.45	271	188	0.59	1.31	0.00082
P20	17	41256985	*BRCA1*	NM_007294.3	c.213-12A>G	p.(?)	0.0000	112	137	0.45	57	27	0.68	1.51	0.00106
P66	17	41209079	*BRCA1*	NM_007294.3	c.5266dup	p.(Gln1756Profs*74)	0.0166	517	584	0.47	423	360	0.54	1.15	0.00743
P93	13	32920978	*BRCA2*	NM_000059.3	c.6952C>T	p.(Arg2318*)	0.0000	163	225	0.42	386	370	0.51	1.21	0.01077
P74	17	41199683	*BRCA1*	NM_007294.3	c.5444G>A	p.(Trp1815*)	0.0000	236	245	0.49	396	298	0.57	1.16	0.01927
P36	17	41244439	*BRCA1*	NM_007294.3	c.3108dup	p.(Lys1037fs)	0.0000	117	131	0.47	402	304	0.57	1.21	0.02414
P47	17	41234556	*BRCA1*	NM_007294.3	c.4222C>T	p.(Gln1408*)	0.0000	231	261	0.47	461	393	0.54	1.15	0.03756
P64	17	41209079	*BRCA1*	NM_007294.3	c.5266dup	p.(Gln1756Profs*74)	0.0166	222	282	0.44	319	306	0.51	1.16	0.04923
P35	17	41244526	*BRCA1*	NM_007294.3	c.3018_3021del	p.(His1006fs)	0.0000	290	355	0.45	243	233	0.51	1.13	0.11470
P81	13	32912337	*BRCA2*	NM_000059.3	c.3847_3848del	p.(Val1283fs)	0.0225	294	294	0.50	198	155	0.56	1.12	0.19348
P58	17	41215947	*BRCA1*	NM_007294.3	c.5096G>A	p.(Arg1699Gln)	0.0056	258	229	0.53	125	84	0.60	1.13	0.27217
P478	13	32914209	*BRCA2*	NM_000059.3	c.5722_5723del	p.(Leu1908fs)	0.0000	390	422	0.48	245	311	0.44	0.92	0.35063
P477	13	32911371	*BRCA2*	NM_000059.3	c.2880del	p.(Lys960fs)	0.0000	185	209	0.47	58	87	0.40	0.85	0.39229
P71	17	41209079	*BRCA1*	NM_007294.3	c.5266dup	p.(Gln1756Profs*74)	0.0166	277	338	0.45	245	256	0.49	1.09	0.45857
P23	17	41246420	*BRCA1*	NM_007294.3	c.1127del	p.(Asn376fs)	0.0000	548	643	0.46	131	131	0.50	1.09	0.54472
P55	17	41222948	*BRCA1*	NM_007294.3	c.4964_4982del	p.(Ser1655Tyrfs*16)	0.0018	132	268	0.33	169	393	0.30	0.91	0.76483
P475	17	41243788	*BRCA1*	NM_007294.3	c.3756_3759del	p.(Ser1253fs)	0.0000	447	504	0.47	411	427	0.49	1.04	0.83360
P37	13	32914758	*BRCA2*	NM_000059.3	c.6267_6269delinsC	p.(Glu2089Aspfs*2)	0.0000	131	213	0.38	238	342	0.41	1.08	0.85183
P88	13	32914137	*BRCA2*	NM_000059.3	c.5645C>A	p.(Ser1882*)	0.0037	116	126	0.48	458	440	0.51	1.06	0.88660
P80	13	32911755	*BRCA2*	NM_000059.3	c.3264dup	p.(Gln1089fs)	0.0000	241	231	0.51	196	205	0.49	0.96	1.10306

P43	17	41243788	*BRCA1*	NM_007294.3	c.3756_3759del	p.(Ser1253fs)	0.0000	108	143	0.43	302	369	0.45	1.05	1.29954
P39	17	41244056	*BRCA1*	NM_007294.3	c.3481_3491del	p.(Glu1161fs)	0.0000	196	307	0.39	350	571	0.38	0.97	1.41640
P83	13	32912940	*BRCA2*	NM_000059.3	c.4449del	p.(Asp1484fs)	0.0000	388	438	0.47	118	134	0.47	1.00	1.84878
Class 3 variants															
P241	17	41245975	*BRCA1*	NM_007294.3	c.1573G>A	p.(Val525Ile)	0.0000	420	456	0.48	51	463	0.10	0.21	0.00000
P338	17	41244094	*BRCA1*	NM_007294.3	c.3454G>A	p.(Asp1152Asn)	0.0000	378	444	0.46	74	452	0.14	0.30	0.00000
P322	13	32911181	*BRCA2*	NM_000059.3	c.2689G>A	p.(Glu897Lys)	0.0000	124	124	0.50	475	47	0.91	1.82	0.00000
P404	13	32914392	*BRCA2*	NM_000059.3	c.5900A>G	p.(Lys1967Arg)	0.0000	232	284	0.45	528	149	0.78	1.73	0.00000
P271	17	41209133	*BRCA1*	NM_007294.3	c.5213G>A	p.(Gly1738Glu)	0.0000	218	255	0.46	297	56	0.84	1.83	0.00000
P338	13	32936722	*BRCA2*	NM_000059.3	c.7868A>G	p.(His2623Arg)	0.0000	343	316	0.52	340	70	0.83	1.60	0.00000
P188	17	41215379	*BRCA1*	NM_007294.3	c.5161_5163del	p.(Gln1721del)	0.0000	101	134	0.43	298	66	0.82	1.91	0.00000
P127	13	32907520	*BRCA2*	NM_000059.3	c.1905T>G	p.(Asp635Glu)	0.0000	130	165	0.44	231	77	0.75	1.70	0.00000
P98	17	41244094	*BRCA1*	NM_007294.3	c.3454G>A	p.(Asp1152Asn)	0.0000	471	600	0.44	16	95	0.14	0.32	0.00000
P156	13	32900240	*BRCA2*	NM_000059.3	c.433_435del	p.(Val145del)	0.0018	162	182	0.47	351	165	0.68	1.45	0.00000
P227	13	32953474	*BRCA2*	NM_000059.3	c.8775G>C	p.(Gln2925His)	0.0000	228	238	0.49	324	153	0.68	1.39	0.00000
P245	17	41215387	*BRCA1*	NM_007294.3	c.5156T>G	p.(Val1719Gly)	0.0000	120	130	0.48	281	145	0.66	1.38	0.00002
P146	17	41244466	*BRCA1*	NM_007294.3	c.3082C>T	p.(Arg1028Cys)	0.0037	117	132	0.47	739	473	0.61	1.30	0.00016
P237	13	32930614	*BRCA2*	NM_000059.3	c.7491_7493del	p.(Lys2498del)	0.0000	179	258	0.41	123	302	0.29	0.71	0.00076
P171	13	32918701	*BRCA2*	NM_000059.3	c.6848C>T	p.(Pro2283Leu)	0.0000	191	263	0.42	200	184	0.52	1.24	0.01158
P241	13	32972525	*BRCA2*	NM_000059.3	c.9875C>T	p.(Pro3292Leu)	0.0018	521	521	0.50	250	181	0.58	1.16	0.01551
P173	13	32912357	*BRCA2*	NM_000059.3	c.3865A>G	p.(Lys1289Glu)	0.0000	300	366	0.45	479	479	0.50	1.11	0.13594
P120	13	32911794	*BRCA2*	NM_000059.3	c.3302A>G	p.(His1101Arg)	0.0000	136	112	0.55	135	82	0.62	1.13	0.26637
P487	17	41245699	*BRCA1*	NM_007294.3	c.1846_1848del	p.(Ser616del)	0.0018	104	143	0.42	269	440	0.38	0.90	0.56528
P415	13	32953965	*BRCA2*	NM_000059.3	c.9032T>C	p.(Leu3011Pro)	0.0000	198	190	0.51	88	70	0.56	1.10	0.73957
P52	13	32915105	*BRCA2*	NM_000059.3	c.6613G>A	p.(Val2205Met)	0.0056	118	122	0.49	263	252	0.51	1.04	1.27614
P386	17	41228555	*BRCA1*	NM_007294.3	c.4434G>T	p.(Glu1478Asp)	0.0000	282	306	0.48	323	336	0.49	1.02	1.41127
P453	17	41226518	*BRCA1*	NM_007294.3	c.4505C>A	p.(Pro1502Gln)	0.0000	480	480	0.50	198	206	0.49	0.98	1.45329
P167	13	32906831	*BRCA2*	NM_000059.3	c.1216_1219delinsACCG	p.(Ala406_Gln407delinsThrGlu)	0.0018	322	349	0.48	340	369	0.48	1.00	1.82212

Class 1/2 variants															
P274	17	41245027	*BRCA1*	NM_007294.3	c.2521C>T	p.(Arg841Trp)	0.2099	506	506	0.50	201	1237	0.14	0.28	0.00000
P218	17	41226488	*BRCA1*	NM_007294.3	c.4535G>T	p.(Ser1512Ile)	0.3129	492	555	0.47	12	404	0.03	0.06	0.00000
P342	17	41243509	*BRCA1*	NM_007294.3	c.4039A>G	p.(Arg1347Gly)	0.6424	222	231	0.49	649	49	0.93	1.90	0.00000
P211	13	32913062	*BRCA2*	NM_000059.3	c.4570T>G	p.(Phe1524Val)	0.0018	443	500	0.47	59	586	0.09	0.19	0.00000
P221	17	41246092	*BRCA1*	NM_007294.3	c.1456T>C	p.(Phe486Leu)	0.0294	289	368	0.44	388	48	0.89	2.02	0.00000
P221	17	41245900	*BRCA1*	NM_007294.3	c.1648A>C	p.(Asn550His)	0.0295	358	358	0.50	532	66	0.89	1.78	0.00000
P121	13	32937521	*BRCA2*	NM_000059.3	c.8182G>A	p.(Val2728Ile)	0.3257	588	522	0.53	186	742	0.20	0.38	0.00000
P211	13	32914592	*BRCA2*	NM_000059.3	c.6100C>T	p.(Arg2034Cys)	0.4810	419	419	0.50	482	79	0.86	1.72	0.00000
P243	13	32914592	*BRCA2*	NM_000059.3	c.6100C>T	p.(Arg2034Cys)	0.4810	304	329	0.48	652	134	0.83	1.73	0.00000
P384	13	32937488	*BRCA2*	NM_000059.3	c.8149G>T	p.(Ala2717Ser)	0.1675	337	337	0.50	719	147	0.83	1.66	0.00000
P243	17	41246812	*BRCA1*	NM_007294.3	c.736T>G	p.(Leu246Val)	0.0394	326	313	0.51	124	606	0.17	0.33	0.00000
P283	13	32930673	*BRCA2*	NM_000059.3	c.7544C>T	p.(Thr2515Ile)	0.1012	466	504	0.48	60	401	0.13	0.27	0.00000
P221	17	41251803	*BRCA1*	NM_007294.3	c.536A>G	p.(Tyr179Cys)	0.0294	265	265	0.50	431	64	0.87	1.74	0.00000
P400	17	41226488	*BRCA1*	NM_007294.3	c.4535G>T	p.(Ser1512Ile)	0.3129	424	441	0.49	739	234	0.76	1.55	0.00000
P196	17	41243512	*BRCA1*	NM_007294.3	c.4036G>A	p.(Glu1346Lys)	0.0074	225	234	0.49	105	551	0.16	0.33	0.00000
P412	17	41246812	*BRCA1*	NM_007294.3	c.736T>G	p.(Leu246Val)	0.0394	340	340	0.50	318	61	0.84	1.68	0.00000
P187	13	32906593	*BRCA2*	NM_000059.3	c.978C>A	p.(Ser326Arg)	0.1039	271	282	0.49	502	125	0.80	1.63	0.00000
P215	17	41244246	*BRCA1*	NM_007294.3	c.3302G>A	p.(Ser1101Asn)	0.0184	396	396	0.50	29	211	0.12	0.24	0.00000
P109	13	32893207	*BRCA2*	NM_000059.3	c.68-7T>A	p.(?)	0.2918	130	102	0.56	17	188	0.08	0.15	0.00000
P1	13	32914815	*BRCA2*	NM_000059.3	c.6323G>A	p.(Arg2108His)	0.0818	230	230	0.50	189	709	0.21	0.42	0.00000
P367	13	32953550	*BRCA2*	NM_000059.3	c.8851G>A	p.(Ala2951Thr)	0.4400	113	138	0.45	210	26	0.89	1.98	0.00000
P121	13	32907000	*BRCA2*	NM_000059.3	c.1385A>G	p.(Glu462Gly)	0.0407	366	429	0.46	464	172	0.73	1.59	0.00000
P405	13	32937521	*BRCA2*	NM_000059.3	c.8182G>A	p.(Val2728Ile)	0.3257	236	245	0.49	501	141	0.78	1.59	0.00000
P3	13	32914592	*BRCA2*	NM_000059.3	c.6100C>T	p.(Arg2034Cys)	0.4810	287	336	0.46	420	147	0.74	1.61	0.00000
P354	13	32953604	*BRCA2*	NM_000059.3	c.8905G>A	p.(Val2969Met)	0.0663	125	125	0.50	319	52	0.86	1.72	0.00000
P168	13	32914592	*BRCA2*	NM_000059.3	c.6100C>T	p.(Arg2034Cys)	0.4810	258	268	0.49	327	87	0.79	1.61	0.00000
P206	13	32972626	*BRCA2*	NM_000059.3	c.9976A>T	p.(Lys3326*)	0.8407	432	416	0.51	138	394	0.26	0.51	0.00000
P329	13	32913062	*BRCA2*	NM_000059.3	c.4570T>G	p.(Phe1524Val)	0.0018	239	259	0.48	64	272	0.19	0.40	0.00000

P239	13	32914277	*BRCA2*	NM_000059.3	c.5785A>G	p.(Ile1929Val)	0.0313	420	438	0.49	245	599	0.29	0.59	0.00000
P362	13	32914839	*BRCA2*	NM_000059.3	c.6347A>G	p.(His2116Arg)	0.0261	105	110	0.49	103	11	0.90	1.84	0.00000
P333	13	32945172	*BRCA2*	NM_000059.3	c.8567A>C	p.(Glu2856Ala)	0.1326	219	258	0.46	66	266	0.20	0.43	0.00000
P367	17	41249263	*BRCA1*	NM_007294.3	c.591C>T	p.(Cys197Cys)	0.1789	83	94	0.47	97	11	0.90	1.91	0.00000
P364	13	32953550	*BRCA2*	NM_000059.3	c.8851G>A	p.(Ala2951Thr)	0.4400	207	298	0.41	114	458	0.20	0.49	0.00000
P68	13	32953549	*BRCA2*	NM_000059.3	c.8850G>T	p.(Lys2950Asn)	0.0718	110	140	0.44	24	172	0.12	0.27	0.00000
P4	13	32914592	*BRCA2*	NM_000059.3	c.6100C>T	p.(Arg2034Cys)	0.4810	121	125	0.49	261	82	0.76	1.55	0.00000
P330	17	41226488	*BRCA1*	NM_007294.3	c.4535G>T	p.(Ser1512Ile)	0.3129	455	514	0.47	366	206	0.64	1.36	0.00000
P393	13	32953549	*BRCA2*	NM_000059.3	c.8850G>T	p.(Lys2950Asn)	0.0718	212	259	0.45	147	420	0.26	0.58	0.00000
P253	13	32906593	*BRCA2*	NM_000059.3	c.978C>A	p.(Ser326Arg)	0.1039	234	225	0.51	304	118	0.72	1.41	0.00000
P128	17	41243509	*BRCA1*	NM_007294.3	c.4039A>G	p.(Arg1347Gly)	0.6424	411	446	0.48	138	323	0.30	0.63	0.00000
P52	17	41243509	*BRCA1*	NM_007294.3	c.4039A>G	p.(Arg1347Gly)	0.6424	103	142	0.42	387	200	0.66	1.57	0.00000
P53	17	41243509	*BRCA1*	NM_007294.3	c.4039A>G	p.(Arg1347Gly)	0.6424	350	311	0.53	266	99	0.73	1.38	0.00000
P22	13	32937521	*BRCA2*	NM_000059.3	c.8182G>A	p.(Val2728Ile)	0.3257	269	303	0.47	120	45	0.73	1.55	0.00000
P20	17	41243509	*BRCA1*	NM_007294.3	c.4039A>G	p.(Arg1347Gly)	0.6424	120	130	0.48	102	32	0.76	1.58	0.00000
P107	13	32937488	*BRCA2*	NM_000059.3	c.8149G>T	p.(Ala2717Ser)	0.1675	335	322	0.51	263	129	0.67	1.31	0.00000
P315	13	32972626	*BRCA2*	NM_000059.3	c.9976A>T	p.(Lys3326*)	0.8407	216	224	0.49	49	131	0.27	0.55	0.00000
P91	13	32906593	*BRCA2*	NM_000059.3	c.978C>A	p.(Ser326Arg)	0.1039	224	263	0.46	172	383	0.31	0.67	0.00000
P154	13	32930673	*BRCA2*	NM_000059.3	c.7544C>T	p.(Thr2515Ile)	0.1012	352	447	0.44	139	85	0.62	1.41	0.00001
P481	13	32972626	*BRCA2*	NM_000059.3	c.9976A>T	p.(Lys3326*)	0.8407	310	310	0.50	126	65	0.66	1.32	0.00037
P472	13	32937521	*BRCA2*	NM_000059.3	c.8182G>A	p.(Val2728Ile)	0.3257	211	219	0.49	433	676	0.39	0.80	0.00122
P54	13	32907129	*BRCA2*	NM_000059.3	c.1514T>C	p.(Ile505Thr)	0.1079	198	190	0.51	354	217	0.62	1.22	0.00240
P352	13	32945172	*BRCA2*	NM_000059.3	c.8567A>C	p.(Glu2856Ala)	0.1326	125	125	0.50	134	72	0.65	1.30	0.00350
P475	13	32912750	*BRCA2*	NM_000059.3	c.4258G>T	p.(Asp1420Tyr)	0.8179	402	387	0.51	353	468	0.43	0.84	0.00382
P445	13	32953550	*BRCA2*	NM_000059.3	c.8851G>A	p.(Ala2951Thr)	0.4400	106	125	0.46	402	291	0.58	1.26	0.00463
P398	17	41243509	*BRCA1*	NM_007294.3	c.4039A>G	p.(Arg1347Gly)	0.6424	215	252	0.46	174	136	0.56	1.22	0.01750
P317	17	41249263	*BRCA1*	NM_007294.3	c.591C>T	p.(Cys197Cys)	0.1789	108	108	0.50	49	23	0.68	1.36	0.02391
P300	13	32929007	*BRCA2*	NM_000059.3	c.7017G>C	p.(Lys2339Asn)	0.0018	120	111	0.52	88	133	0.40	0.77	0.02762
P449	13	32953550	*BRCA2*	NM_000059.3	c.8851G>A	p.(Ala2951Thr)	0.4400	231	283	0.45	141	130	0.52	1.16	0.14920

P477	13	32945172	*BRCA2*	NM_000059.3	c.8567A>C	p.(Glu2856Ala)	0.1326	189	196	0.49	78	113	0.41	0.84	0.15434
P69	13	32930673	*BRCA2*	NM_000059.3	c.7544C>T	p.(Thr2515Ile)	0.1012	118	133	0.47	78	61	0.56	1.19	0.26949
P213	13	32972626	*BRCA2*	NM_000059.3	c.9976A>T	p.(Lys3326*)	0.8407	382	352	0.52	98	116	0.46	0.88	0.28469
P465	13	32972695	*BRCA2*	NM_000059.3	c.10045A>G	p.(Thr3349Ala)	0.0018	340	301	0.53	413	311	0.57	1.08	0.33264
P264	13	32906593	*BRCA2*	NM_000059.3	c.978C>A	p.(Ser326Arg)	0.1039	126	142	0.47	154	222	0.41	0.87	0.34299
P62	13	32937521	*BRCA2*	NM_000059.3	c.8182G>A	p.(Val2728Ile)	0.3257	115	129	0.47	135	115	0.54	1.15	0.34833
P434	13	32913562	*BRCA2*	NM_000059.3	c.5070A>C	p.(Lys1690Asn)	0.0225	394	410	0.49	266	325	0.45	0.92	0.36327
P246	13	32912750	*BRCA2*	NM_000059.3	c.4258G>T	p.(Asp1420Tyr)	0.8179	232	222	0.51	324	366	0.47	0.92	0.41658
P413	13	32912750	*BRCA2*	NM_000059.3	c.4258G>T	p.(Asp1420Tyr)	0.8179	457	457	0.50	241	206	0.54	1.08	0.41589
P300	13	32929309	*BRCA2*	NM_000059.3	c.7319A>G	p.(His2440Arg)	0.0018	124	110	0.53	137	155	0.47	0.89	0.42197
P392	13	32906766	*BRCA2*	NM_000059.3	c.1151C>T	p.(Ser384Phe)	0.1049	285	309	0.48	417	510	0.45	0.94	0.58790
P11	13	32907407	*BRCA2*	NM_000059.3	c.1792A>G	p.(Thr598Ala)	0.3706	177	163	0.52	66	77	0.46	0.88	0.59444
P44	13	32912750	*BRCA2*	NM_000059.3	c.4258G>T	p.(Asp1420Tyr)	0.8179	269	269	0.50	335	377	0.47	0.94	0.65887
P429	13	32914592	*BRCA2*	NM_000059.3	c.6100C>T	p.(Arg2034Cys)	0.4810	205	240	0.46	253	336	0.43	0.93	0.73863
P181	13	32953550	*BRCA2*	NM_000059.3	c.8851G>A	p.(Ala2951Thr)	0.4400	257	290	0.47	698	726	0.49	1.04	0.88200
P157	13	32907407	*BRCA2*	NM_000059.3	c.1792A>G	p.(Thr598Ala)	0.3706	105	128	0.45	315	341	0.48	1.07	0.92371
P236	13	32972626	*BRCA2*	NM_000059.3	c.9976A>T	p.(Lys3326*)	0.8407	398	383	0.51	292	304	0.49	0.96	0.98840
P108	13	32906766	*BRCA2*	NM_000059.3	c.1151C>T	p.(Ser384Phe)	0.1049	375	423	0.47	299	311	0.49	1.04	0.99168
P327	13	32953550	*BRCA2*	NM_000059.3	c.8851G>A	p.(Ala2951Thr)	0.4400	169	191	0.47	300	313	0.49	1.04	1.11608
P310	17	41219694	*BRCA1*	NM_007294.3	c.5005G>T	p.(Ala1669Ser)	0.0055	206	297	0.41	161	214	0.43	1.05	1.16985
P135	13	32907407	*BRCA2*	NM_000059.3	c.1792A>G	p.(Thr598Ala)	0.3706	112	112	0.50	475	438	0.52	1.04	1.20733
P324	13	32912750	*BRCA2*	NM_000059.3	c.4258G>T	p.(Asp1420Tyr)	0.8179	123	123	0.50	157	145	0.52	1.04	1.31730
P151	13	32912750	*BRCA2*	NM_000059.3	c.4258G>T	p.(Asp1420Tyr)	0.8179	318	305	0.51	566	566	0.50	0.98	1.35494
P488	13	32906766	*BRCA2*	NM_000059.3	c.1151C>T	p.(Ser384Phe)	0.1049	447	504	0.47	259	280	0.48	1.02	1.37876
P194	13	32912007	*BRCA2*	NM_000059.3	c.3515C>T	p.(Ser1172Leu)	0.0975	270	304	0.47	551	597	0.48	1.02	1.40007
P55	13	32912750	*BRCA2*	NM_000059.3	c.4258G>T	p.(Asp1420Tyr)	0.8179	271	241	0.53	415	384	0.52	0.98	1.40524
P196	13	32937521	*BRCA2*	NM_000059.3	c.8182G>A	p.(Val2728Ile)	0.3257	278	289	0.49	395	395	0.50	1.02	1.41169
P252	13	32953550	*BRCA2*	NM_000059.3	c.8851G>A	p.(Ala2951Thr)	0.4400	259	269	0.49	201	201	0.50	1.02	1.49170
P112	13	32906593	*BRCA2*	NM_000059.3	c.978C>A	p.(Ser326Arg)	0.1039	225	287	0.44	84	103	0.45	1.02	1.62008

P300	13	32972380	*BRCA2*	NM_000059.3	c.9730G>A	p.(Val3244Ile)	0.0018	115	134	0.46	168	189	0.47	1.02	1.62179
P430	13	32953550	*BRCA2*	NM_000059.3	c.8851G>A	p.(Ala2951Thr)	0.4400	86	109	0.44	141	180	0.44	1.00	1.85784
P295	13	32906766	*BRCA2*	NM_000059.3	c.1151C>T	p.(Ser384Phe)	0.1049	203	293	0.41	98	140	0.41	1.00	1.83981
P272	13	32945172	*BRCA2*	NM_000059.3	c.8567A>C	p.(Glu2856Ala)	0.1326	170	185	0.48	154	167	0.48	1.00	1.83092

Supplementary Table 3: Performance of loss of heterozygosity (LOH)-analyses for the classification of rare germline variants in the *BRCA1* and *BRCA2* genes. Test for pathogenicity is based on the assumption that a significantly increased variant fraction in the tumour predicts pathogenicity.Test for benignity is based on the assumption that a significantly decreased variant fraction in the tumour predicts benignity. * = calculation was performed using the Haldane-Anscombe correction[1]. PPV = positive predictive value; LR+ = positive likelihood ratio.

	***BRCA1/2* (n=184)**	***BRCA1* (n=88)**	***BRCA2* (n=96)**
Test for pathogenicity			
Sensitivity (95%CI)	0.84 (0.75-0.91)	0.88 (0.78-0.95)	0.73 (0.52-0.88)
Specificity (95%CI)	0.63 (0.53-0.73)	0.35 (0.15-0.59)	0.71 (0.59-0.82)
PPV (95%CI)	0.71 (0.61-0.79)	0.82 (0.71-0.90)	0.49 (0.32-0.65)
LR+ (95%CI)	2.29 (1.72-3.05)	1.36 (0.97-1.89)	2.56 (1.65-3.96)
Test for benignity			
Sensitivity (95%CI)	0.26 (0.17-0.36)	0.30 (0.12-0.54)	0.24 (0.15-0.36)
Specificity (95%CI)	1.00 (0.96-1.00)	1.00 (0.95-1.00)	1.00 (0.87-1.00)
PPV (95%CI)	1.00 (0.85-1.00)	1.00 (0.54-1.00)	1.00 (0.80-1.00)
LR+ (95%CI)*	49.07 (3.02-795.93)	42.71 (2.51-727.20)	13.31 (0.83-213.68)

Reference:

1. Haldane JB. The estimation and significance of the logarithm of a ratio of frequencies. *Ann Hum Genet* 1956;**20**(4):309-11 doi: 10.1111/j.1469-1809.1955.tb01285.x