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Appendices

Prevalence of genetic susceptibility for breast and ovarian cancer in a non-cancer related study population: secondary germline findings from a Swiss single centre cohort

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Appendix 1

Table S1: Individuals with documentation of family history of cancer.

	n	**%**
Total no. of individuals	28/400	7.0
Carrier ID	**Known family history of cancer**	
41653	Uncle pat: lung cancer (smoker, D/O ~65); cousin pat: leukaemia (Dx nk)	
63408	Brother: brain tumour (D/O 43)	
53804	Father: lung cancer (smoker, Dx nk)	
53805	Niece: ovarian cancer (Dx 12/13, parents 1st cousins); mother: liver cancer (Dx ~85)	
57429	Grandmother pat: intestinal cancer (D/O 66)	
60545	Brother: testicular cancer (D/O 31)	
60546	Uncle pat: other (D/O >60); grandmother mat: oesophageal cancer (smoker, D/O >80)	
62040	Father: lung cancer (Dx nk)	
65390	Mother: benign breast cancer (Dx nk)	
65391	Mother: intestinal cancer (Dx nk); father: prostate cancer (Dx ~60-70)	
65760	Grandmother mat: intestinal cancer (Dx 72)	
67255	Mother: non-Hodgkin lymphoma (Dx nk)	
68567/68568	Son (brother of NDD index): astrocytoma (Dx 3)	
71741	Father: other (D/O nk)	
71136	Mother: breast cancer (Dx nk)	
72678	Grandmother mat: recurrent breast cancer (exposition to radioactive material, Dx ~50)	
73328	Father: liver cancer (D/O nk)	
74232	Aunt pat: breast cancer (D/O ~40), grandmother mat: breast cancer (D/O 49)	
74275	Father: benign thyroid cancer; aunt pt: lung cancer? (non-smoker, D/O ~40)	
74623	Grandfather mat: pancreatic cancer (Dx nk)	
74624	Mother: breast cancer (D/O ~20)	
74639	Uncle mat: intestinal cancer (D/O 45)	
74657	Grandfather mat: lung cancer (non-smoker, D/O 42); grandmother mat: colon cancer (D/O ~70)	
74658	Grandmother mat: breast cancer/leukemia (D/O ~60)	
74659	Grandmother pat: pancreatic cancer (D/O ~85)	
76029	Several uncles and aunts: other (D/O nk)	
78686	Father: glioblastoma (D/O 58)	

D/O = (age of) death of diagnosis (years); Dx = (age at) diagnosis (years); mat = maternal; NDD = neurodevelopmental delay; nk = not known; pat = paternal

Appendix 2

Table S2: Synopsis of all detected secondary variants including annotations and criteria for pathogenicity assessment.

Variant Specification										Population Databases					Computational Evidence								Gene/Disease specific Databases			Final Assessment of Variant Pathogenicity	
Carrier ID (quality parameters of the individual variant: Cov./Score/SR)	**Gene**	**Mutation HGVS ¥**	**het/ hom**	**Function**	**Loc.**	**Genomic Pos. (hg19)**	**SNP db_xref**	**Predicted AA Change**	**InterPro domain ID (v.67.0)**	**1000G** [%]	**ESP6500** [%] EurAm	**ExAC v0.3** frequ¶/allele count/# hom	**gnomAD v2.0.2** frequ¶/allele count/# hom	**FLOSSIES** frequ¶ / allele count (Eur)/# hom	**SIFT v6.2.0 ‡**	**MutTaster v2013†**	**PolyPhen-2 v2.2.2† HumVar**	**CADD Phred**	**GERP+ +_RS**	**Align-GVGD v2007!§**	**UMD predictor**	**Prediction CONSENSUS⌠**		**HGMD PRO v2017.3: variant class/acc.**	**Locus specific databases (LSDB, assessed by the LOVD)$**	**NCBI-ClinVar£**	**Rationale for Classification (ACMG evidence category if meeting)**	**final CLASSIFICATION**
65137 (143/17.1/42.66)	*BRCA1*	c.21C>T	het	syn	E2	chr17:41276093	rs149402012	p.R7=	—	—	0.00	52.2/63/1	56.72/157/1; AFR: 0.60%	334.0/2/0	na	na	na	na	na	na	poly	na	na	not listed	**benign/LCS**; inconsistent[a]: ?/?(4), -/.(1); 1-neutral[e]	**B**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
72266 (342/20.2/33.92)	*BRCA1*	c.135-12delT ‡	het	nonc	I3	chr17:41258562	—	nonc	—	—	—	—	—	—	na	na	na	na	na	na	na	na	na	not listed	not listed	not listed	novel; no evident splicing effect *in silico*[2] (BP7)	VUS
67202 (474/21.2/42.41)	*BRCA1*	c.181T>G	het	miss	E5	chr17:41258504	rs28897672	p.C61G	IPR018957	—	—	6.722/8/0	3.255/8/0	—	del	path	B	25.0	5.17	C65	path	6+	dam	DM/CM940172	**pathogenic**; inconsistent[a]: +/+ (>>30), +/.(>>30), +?/.(3), +?/.(3), ?/.(3), -/.(2), ?/.(8); 5-causal[e]; class 5[f]; class 5[g]	**path**	classified as "pathogenic" by expert panel; recurrent variant with established pathogenicity	PATHOGENIC
75128 (571/22.0/41.86)	*BRCA1*	c.532G>A ‡	het	miss	E8	chr17:41251807	—	p.V178I	—	—	—	—	—	—	tol	poly	B	21.2	4.19	C0	poly	2+	undet	not listed	not listed	not listed	novel	VUS
39649 (494/21.4/54.66) **58893** (498/21.4/47.79) **66650** (214/18.6/60.28)	*BRCA1*	c.536A>G	het	miss	E8	chr17:41251803	rs56187033	p.Y179C	—	0.02	0.03	27.18/33/0	25.97/72/0	50.6/5/0	del	path	PD	25.9	5.16	C35	path	7+	dam	DM?/CM03786	**benign/LCS**; inconsistent[a]: -/-(8),?/.(6); -/.(14), +?/.(1); 1-neutral[e]; class 1[f]; class 1[g]	**B**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
68840 (545/21.8/49.72)	*BRCA1*	c.693G>A	het	syn	E11	chr17:41246855	rs62625298	p.T231=	—	—	—	4.004/4/0	8.01/22/0	10.1/0/0	na	na	na	na	na	na	poly	na	na	DM?/CS125434	**likely benign**; inconclusive[a]; probably neutral[b]: -?/-(1); 1-neutral[e]	**LB**	MAF within pathogenic range (PM2); functional studies: BRCA1Δ11 increased[2] [1-3], clinical relevance unclear (PS3?)	VUS
74275 (353/20.3/47.88)	*BRCA1*	c.736T>G	het	miss	E11	chr17:41246812	rs28897675	p.L246V	—	0.02	0.03	21.81/26/0	29.71/82/0	60.7/5/0	tol	poly	PrD	8.0	2.26	C0	poly	2+	undet	DM?/CM045534, DM/CS042532	**benign/LCS**; inconsistent[a]: -/-(4), ?/?(1), ?/.(3), +/.(3); 1-neutral[e]; class 1[f]; class 1[g]	**B**	classified as "benign/LCS" by expert panel; detected *in trans* with deleterious *BRCA1* variants [4-5, UMD database] (BP2); various functional studies show no deleterious effect [6-7], inconsistency of splicing studies [8-9] (BS3)	(LIKELY) BENIGN
75894 (443/21.0/45.60)[@] **75895** (415/20.8/58.31)[@]	*BRCA1*	c.823G>A	het	miss	E11	chr17:41246725	rs8176153	p.G275S	—	0.04	—	66.91/81/1;	59.55/146/2; SAS: 0.48%	—	tol	poly	PrD	18.0	3.98	C0	prob path	3+	undet	DM/CM076033	**not yet reviewed**; inconclusive[a]; 1-neutral[e]	confl.: B(1), LB(6), VUS(1)	high to very high MAF in SAS, gnomAD: 2 homozygous individuals; no statistical increased prevalence in affected individuals in a case-control study from a mult-ethnic South Asian cohort [10]	(LIKELY) BENIGN
72979 (527/21.6/51.99) **74409** (613/22.2/44.86)	*BRCA1*	c.981A>G	het	syn	E11	chr17:41246567	rs1800063	p.T327=	—	0.06	0.06	84.84/103/1	90.56/251/1; ASJ: 1.08%	182.0/18/0	na	na	na	na	na	na	poly	na	na	not listed	**benign/LCS**; inconsistent[a]: ?/?(17), -/-(2), -/.(2); 1-neutral[e]	**B**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
61859 (260/19.3/47.69)	*BRCA1*	c.1418A>T	het	miss	E11	chr17:41246130	rs80357057	p.N473I	IPR025994	—	—	1.648/2/0	2.166/6/0	—	tol	poly	PrD	17.8	2.21	C0	path	3+	undet	not listed	**not yet reviewed**; inconclusive[a]; 3-VUS[e]	*unc.*	MAF within pathogenic range (PM2); not yet reviewed by expert panel, consistently classified as "VUS" in LSDB and NCBI-ClinVar	VUS
39649 (217/18.7/38.25) **58893** (189/18.1/46.03) **66650** (110/16.2/36.36)	*BRCA1*	c.1456T>C	het	miss	E11	chr17:41246092	rs55906931	p.F486L	IPR025994	0.02	0.03	29.66/36/0	27.79/77/0	50.6/5/0	tol	poly	B	0.0	-3.69	C0	poly	0+	B	not listed	**benign/LCS**; inconsistent[a]: -/-(4), -/.(20), ?/.(3), ?/?(1); 1-neutral[e]; class 1[f]; class 1[g]	**B**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
39649 (231/18.8/52.38) **58893** (276/19.4/48.55) **66650** (210/18.5/48.57)	*BRCA1*	c.1648A>C	het	miss	E11	chr17:41245900	rs56012641	p.N550H	—	0.02	0.03	27.27/33/0	25.66/71/0	50.6/5/0	tol	poly	PD	21.8	1.32	C0	poly	2+	undet	DM?/CM025218	**benign/LCS**; inconsistent[a]: -/-(4), -/.(20), ?/.(3), ?/?(1); 1-neutral[e]; class 1[f]; class 1[g]	**B**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
74985 (301/19.7/45.18)	*BRCA1*	c.1881C>G	het	syn	E11	chr17:41245667	rs80356838	p.V627=	—	—	0.02	6.596/8/0	3.972/11/0	—	na	na	na	na	na	na	poly	na	na	not listed	**not yet reviewed**; inconclusive[a]	*unc.*	MAF within pathogenic range (PM2); not yet reviewed by expert panel, consistently classified as "VUS" in NCBI-ClinVar; predicted *de novo* CSDS[2] (PP3)	VUS
70207 (272/19.4/48.53)	*BRCA1*	c.2171C>T	het	miss	E11	chr17:41245377	rs751104940	p.P724L	—	—	—	—	0.8139/2/0	—	tol	poly	PD	23.7	3.41	C0	poly	3+	undet	DM?/CM1715334	**not yet reviewed**	not listed	MAF within pathogenic range (PM2); not yet reviewed by expert panel; CR: OC-affected female suspected of HBOC (Dx 19; FIGO I, mucinous), FH: father diagnosed with gastric cancer; seg n/d [11]	VUS
74844 (477/21.3/49.06)	*BRCA1*	c.2268G>C	het	miss	E11	chr17:41245280	rs80356884	p.R756S	—	—	—	—	1.805/5/0	—	tol	poly	B	8.2	0.56	C0	poly	0+	B	not listed	**not yet reviewed**; probably neutral[a]: -?/.(1); 3-VUS[e]	confl.: LB(1), VUS(5)	MAF within pathogenic range (PM2); not yet reviewed by expert panel, inconsistently classified in LSDB and NCBI-ClinVar (predominantly classified as "VUS"); CR: Portuguese BC-affected female suspected of HBOC, FH: ≥2 BC-affected relatives; seg n/d (classified as "VUS") [12-13]	VUS
73075 (1201/24.5/44.63)	*BRCA1*	c.2584A>G	het	miss	E11	chr17:41244964	rs80356927	p.K862E	—	—	—	9.891/12/0	6.141/17/0	—	tol	poly	PD	15.9	4.09	C0	prob poly	2+	undet	not listed	**benign/LCS**; inconsistent[a]: -/-(1), -/.(2), ?/.(1), ?/?(2); 2-likely neutral[e], class 1[f]	**B**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
75045 (491/21.5/45.82)	*BRCA1*	c.2719G>A ‡	het	miss	E11	chr17:41244829	—	p.E907K	—	—	—	—	—	—	tol	poly	PD	22.6	3.65	C0	poly	3+	undet	not listed	not listed	not listed	novel	VUS
74409 (372/20.5/41.13)	*BRCA1*	c.2733A>G	het	syn	E11	chr17:41244815	rs1800740	p.G911=	—	0.06	0.06	84.87/103/1	90.78/251/1; ASJ: 1.09%	182.0/18/0	na	na	na	na	na	na	poly	na	na	not listed	**likely benign**; inconsistent[a]: ?/?(14), -/-(1), -/.(2); 1-neutral[e]	**LB**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
74742 (392/20.7/45.41)	*BRCA1*	c.2746A>G	het	miss	E11	chr17:41244802	rs398122666	p.N916D	—	—	—	—	—	—	tol	poly	B	1.3	1.44	C0	poly	0+	B	not listed	not listed	unc.	absent in population databases (PM2); insufficient evidence	VUS
68618 (223/18.5/58.74)	*BRCA1*	c.2883C>T	het	syn	E11	chr17:41244665	rs201190540	p.N961=	—	—	0.00	1.649/2/0	1.083/3/0	—	na	na	na	na	na	na	poly	na	na	not listed	**likely benign**; inconsistent[a]: ?/?(1), -/-(1); 3-VUS[e]	**LB**	classified as "likely benign" by expert panel; no evident splicing effect *in silico* (BP7); nonsegregation with disease [14] (BS4); co-occurrence with pathogenic *BRCA1* variant (phase unknown) (UMD database) (BP2)	(LIKELY) BENIGN
72979 (300/19.7/56.67) **74409** (359/20.3/43.45)	*BRCA1*	c.3024G>A	het	miss	E11	chr17:41244524	rs1800704	p.M1008I	—	0.06	0.06	85.81/104/1	91.02/252/1; ASJ: 1.08%	182.0/18/0	tol	poly	B	0.0	-4.11	C0	poly	0+	B	DM?/CM086719	**benign/LCS;** inconsistent[a]: -/-(7), -/.(19), ?/?(1), ?/.(1); 1-neutral[e]; class 1[f]; class 1[g]	**LB**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
75676 (261/19.3/47.13)	*BRCA1*	c.3083G>A	het	miss	E11	chr17:41244465	rs80357459	p.R1028H	—	0.08	—	17.3/21/0	48.77/135/0; LAT: 0.35%	182.0/18/0	tol	poly	PD	10.9	-4.10	C0	poly	0+	B	not listed	**benign/LCS;** inconsistent[a]: -/-(2), -/.(3), -?/.(2), ?/.(1), ?/?(1); 2-likely neutral[e]; class 1[f]; class 1[g]	**B**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
73106 (267/19.5/44.57)	*BRCA1*	c.3302G>A	het	miss	E11	chr17:41244246	rs41293447	p.S1101N	—	—	0.02	15.68/19/0	15.20/42/0	30.4/3/0	tol	poly	B	7.2	-0.43	C0	poly	0+	B	not listed	**benign/LCS;** inconsistent[a]: -/-(2), -/.(7), -?/.(3), ?/?(2), ?/.(2); 1-neutral[e]; class 1[f]; class 1[g]	**B**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
66199 (941/23.7/44.00)	*BRCA1*	c.3597T>A	het	syn	E11	chr17:41243951	—	p.A1199=	—	—	—	—	—	—	na	na	na	na	na	na	poly	na	na	not listed	not listed	*LB*	consistently classified as "likely benign" in NCBI ClinVar (BP6); no evident splicing effect *in silico*[2] (BP7)	(LIKELY) BENIGN
62239 (1030/24.0/46.12)	*BRCA1*	c.3657G>C	het	miss	E11	chr17:41243891	rs80356876	p.E1219D	—	—	0.01	2.473/3/0	2.032/5/0	—	tol	poly	B	24.3	2.29	C0	prob poly	2+	undet	DM?/CM960179	**not yet reviewed**; inconsistent[a]: -/.(1), ?/.(1); 3-VUS[e]	confl.: LB(3), VUS(7)	MAF within pathogenic range (PM2); not yet reviewed by expert panel, inconsistently classified in LSDB and NCBI-ClinVar (predominantly classified as "VUS"); relative HDR activity testing implied low functional impact [15] (BS3?); CR: BC-affected female suspected of HBOC (Dx 50), FH: BC-affected sister (Dx 45), seg n/d [16]; conflicting/insufficient evidence	VUS
66199 (1053/24.1/52.14)	*BRCA1*	c.3666G>C ‡	het	miss	E11	chr17:41243882	—	p.E1222D	—	—	—	—	—	—	tol	poly	B	12.3	-0.92	C0	prob poly	0+	B	not listed	not listed	*unc.*	absent in population databases (PM2); consistently classified as "VUS" in NCBI-ClinVar	VUS
60824 (301/19.7/56.15) **70337** (179/17.9/58.66) **75046** (516/21.5/52.91) **76300** (148/17.1/50.68) **76747** (824/23.2/45.02)	*BRCA1*	c.4039A>G	het	miss	E11	chr17:41243509	rs28897689	p.R1347G	—	0.06	0.67	398.0/483/4	386.5/1070/8; NFE: 0.69%	1000.0/91/0	tol	poly	B	21.2	2.98	C0	prob poly	2+	undet	DM?/CM960181	**benign/LCS**; inconsistent[a]: -/-(11), -/.(14), -?/-(1), ?/.(4), +/.(2); 1-neutral[e]; class 1[f]; class 1[g]	**B**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
73877 (153/17.4/60.78)	*BRCA1*	c.4418T>C Φ	het	miss	E19	chr17:41231356	rs374519494	p.I1473T	—	—	0.00	7.339/8/0	7.361/19/0	—	na	na	na	10.0	0.60	na	poly	na	na	not listed	not listed	LB	MAF within pathogenic range (PM2); insufficient evidence	VUS

Variant Specification										Population Databases					Computational Evidence									Gene/Disease specific Databases			Final Assessment of Variant Pathogenicity	
Carrier ID (quality parameters of the individual variant: Cov./Score/SB)	**Gene**	**Mutation HGVS ¥**	**het/ hom**	**Function**	**Loc.**	**Genomic Pos. (hg19)**	**SNP db_xref**	**Predicted AA Change**	**InterPro domain ID (v.67.0)**	**1000G** [%]	**ESP6500** [%] EurAm	**ExAC v0.3** frequ¶/allele count/# hom	**gnomAD v2.0.2** frequ¶/allele count/# hom	**FLOSSIES** frequ¶ / allele count (Eur)/# hom	**SIFT v6.2.0 †**	**MutTaster v2013†**	**PolyPhen-2 v2.2.2†** HumVar	**CADD Phred**	**GERP+ +_RS**	**Align-GVGD v2007†§**	**UMD predictor**	**Prediction CONSENSUS[/**		**HGMD PRO v2017.3: variant class/acc.**	**Locus specific databases (LSDB, assessed by the LOVD)$**	**NCBI-ClinVar£**	**Rationale for Classification (ACMG evidence category if meeting)**	**final CLASSIFICATION**
65760 (124/16.7/29.84) **71655** (509/21.5/46.56) **72497** (322/20.1/39.13) **73799** (173/17.9/52.02) **74115** (924/23.7/43.51)	*BRCA1*	c.4535G>T	het	miss	E15	chr17:41226488	rs1800744	p.S1512I	—	0.06	0.40	215.1/261/1	232.4/644/1	455.0/44/0	del	poly	B	9.0	3.98	C0	poly	2+	undet	DM?/CM960183	**benign/LCS**; inconsistent[a]: -/-(11), -/.(>30), ?/?(1), ?/.(1); 1-neutral[e]; class 1[f]; class 1[g]	**B**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
74925 (1445/25.2/51.97)	*BRCA1*	c.4883T>C	het	miss	E16	chr17:41223048	rs4986854	p.M1628T	—	0.26	0.03	152.4/185/0	143.6/398/0	70.8/7/0	tol	poly	B	-2.3	0.00	C0	poly	0+	B	DM?/CM065000	**benign/LCS**; inconsistent[a]: -/-(2), -/.(7), ?/.(1); 1-neutral[e]; class 1[f]	**B**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
75672 (377/20.3/55.70)	*BRCA1*	c.5022C>T	het	syn	E17	chr17:41219677	rs786203868	p.I1674=	IPR001357	—	—	—	1.219/3/0	—	na	na	na	na	na	na	poly	na	na	not listed	**likely benign;** inconclusive[a]; 3-VUS[e]	**LB**	classified as "benign/LCS" by expert panel; no evident splicing effect *in silico* (BP7)	(LIKELY) BENIGN
75672 (351/20.2/38.75)	*BRCA1*	c.5468-10C>A	het	nonc	I23	chr17:41197829	rs8176316	nonc	—	0.56	0.02	140.9/171/3	146.6/405/3; AFR: 1.46%	890.0/6/0	na	na	na	na	na	na	na	na	na	DM?/CS086718	**benign/LCS;** inconsistent[a]: -/-(1), -/.(4), -?/-?(1), ?/.(1), ?/?(14); 1-neutral[e]	**B**	classified as "benign/LCS" by expert panel; functional studies show no splicing impact [17] (BS3)	(LIKELY) BENIGN
59806 (327/20.0/44.04) **71052** (238/18.9/42.02)	*BRCA2*	c.68-7T>A	het	nonc	I2	chr13:32893207	rs81002830	nonc	—	0.12	0.15	238.2/282/0	286.5/787/3; NFE:0.24%; ASJ: 1.37%	425.0/41/0	na	na	na	na	na	na	na	na	na	DM?/CS033491	**not yet reviewed**; inconsistent[a]:?/?(13), -/-(1), ?/.(4), -/.(1); 3-VUS[e]; class 1[g]	confl.: B(8), LB(9), VUS(4)	high to very high MAF in population databases/Flossies, gnomAD: 3 homozygous individuals; recent case-control study shows no association with BC risk [18]; nonsegregation with disease [14] (BS4)	(LIKELY) BENIGN
53805 (301/19.7/48.50)	*BRCA2*	c.198A>G	het	syn	E3	chr13:32893344	rs28897700	p.Q66=	—	0.02	0.03	107.2/130/2	99.93/277/5; SAS: 0.77%	20.2/2/0	na	na	na	na	na	na	poly	na	na	DM?/CM173583	**benign/LCS**; inconsistent[a]: -/.(4), ?/.(6), ?/?(>30), ./.(1); 2-likely neutral[e]; class 1[g]	**B**	classified as "benign/LCS" by expert panel, *in silico* predicted *de novo* CSAS[2]	(LIKELY) BENIGN
41390 (170/17.7/42.94)	*BRCA2*	c.201G>A	het	syn	E3	chr13:32893347	—	p.R67=	—	—	—	—	—	—	na	na	na	na	na	na	poly	na	na	not listed	**not yet reviewed**	*LB*	consistently classified as "likely benign" in NCBI-ClinVar (BP6); no evident splicing effect *in silico*[2] (BP7)	(LIKELY) BENIGN
75548 (198/18.2/51.01)	*BRCA2*	c.223G>C	het	miss	E3	chr13:32893369	rs28897701	p.A75P	—	0.02	0.05	16.5/20/0	24.18/67/0	70.8/7/0	tol	path	PrD	20.2	5.59	C0	prob poly	4+	undet	DM?/CM045535	**benign/LCS**; inconsistent[a]: -/-(7), -/.(7), ?/.(3), ?/?(1); 1-neutral[e]; class 1[f]; class 1[g]	**B**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
75387 (463/21.1/43.63)	*BRCA2*	c.339A>G	het	syn	E4	chr13:32899235	rs786203060	p.R113=	—	—	—	—	0.4068/1/0	—	na	na	na	na	na	na	path	na	na	not listed	**likely benign**	**LB**	classified as "likely benign" by expert panel (BP6); no evident splicing effect *in silico* (BP7)	(LIKELY) BENIGN
72585 (253/18.8/100.00)	*BRCA2*	c.631+7A>G	hom	nonc	I7	chr13:32900757	rs431825339	nonc	—	—	—	—	—	—	na	na	na	na	na	na	na	na	na	not listed	**not yet reviewed**; inconclusive[a]; 3-VUS[e]	confl.: LB(1), VUS(4)	absent in population databases (PM2); not yet reviewed by expert panel, inconsistently classified in NCBI ClinVar (predominantly classified as "VUS"); no evident splicing effect *in silico*[2] (BP7); CR: BC-affected NW European female (Dx <50), FH: "low risk" (also carrying *ATM* VUS c.3154-4G>A) [19]; insufficient/conflicting evidence	VUS
59803 (36/12.6/38.89) **71742** (178/18.0/35.96) **73869** (NN/18.0/41.95)	*BRCA2*	c.978C>A	het	miss	E10	chr13:32906593	rs28897706	p.S326R	—	0.02	0.12	80.77/97/1	93.12/241/1	182.0/16/0	tol	poly	B	7.8	-2.86	C0	poly	0+	B	DM?/CM994736	**benign/LCS;** inconsistent[a]: -/-(6), -/.(10), -?/.(1), ?/.(1); 1-neutral[e]; class 1[f]; class 1[g]	**B**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
75150 (413/20.9/44.07)	*BRCA2*	c.1011C>T	het	syn	E10	chr13:32906626	rs41293473	p.N337=	—	0.02	—	3.325/4/0	3.674/10/0	—	na	na	na	na	na	na	poly		na	not listed	**benign/LCS**; 1-neutral[e]; PM[6]	**LB**	classified as "benign/LCS" by expert panel; no evident splicing effect *in silico* (BP7)	(LIKELY) BENIGN
71979 (725/22.7/45.93)	*BRCA2*	c.1151C>T	het	miss	E10	chr13:32906766	rs41293475	p.S384F	—	—	0.15	67.89/82/0	75.43/209/0	192.0/18/0	tol	poly	PD	23.0	3.52	C0	poly	3+	undet	DM?/CM065036	**benign/LCS**; inconsistent[a]: -/-(3), -/.(19), ?/.(1), ?/?(1); 1-neutral[e]; class 1[f]; class 1[g]	**B**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
76771 (1506/25.2/49.73)	*BRCA2*	c.1166C>A	het	miss	E10	chr13:32906781	rs397507263	p.P389Q	—	—	—	49.67/60/2	41.88/103/3 SAS: 0.33%	—	tol	poly	B	1.4	-0.63	C0	poly	0+	B	not listed	**not yet reviewed,** 3-VUS[e]	**LB**	classified as "likely benign" by expert panel	(LIKELY) BENIGN
66137 (422/20.8/45.97)	*BRCA2*	c.1167G>A	het	syn	E10	chr13:32906782	rs397507263	p.P389=	—	—	0.00	4.967/6/0	6.497/18/0	10.1/1/0	na	na	na	na	na	na	poly	na	na	not listed	**likely benign;** inconclusive[a]; 3-VUS[e]	**LB**	classified as "likely benign" by expert panel; no evident splicing effect *in silico* (BP7)	(LIKELY) BENIGN
68840 (449/21.3/47.66)	*BRCA2*	c.1292C>T	het	miss	E10	chr13:32906907	—	p.T431I	—	—	—	—	—	—	tol	poly	B	14.0	0.70	C0	poly	0+	B	not listed	**not yet reviewed**; 3-VUS[e]	*unc.*	absent in population databases (PM2), not yet reviewed by expert panel, consistently classified as "VUS" in LSDB and NCBI ClinVar; CR: Southern German BC-affected, FH: ≥2 BC-cases (including one case with Dx <50 y), seg n/d [20]	VUS
65137 (191/18.2/54.45) **73799** (176/18.0/56.82)	*BRCA2*	c.1788T>C	het	syn	E10	chr13:32907403	rs11571642	p.D596=	—	0.82	0.03	215.5/256/3	263.7/716/11; AFR: 2.47%	1570.0/21/2	na	na	na	na	na	na	poly	na	na	not listed	**benign/LCS**; inconsistent[a]: -/-(1), -/.(5), -?/.(5), ?/?(8); 1-neutral[e]	**B**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
69177 (291/19.6/43.99) **69864** (238/19.1/45.80)	*BRCA2*	c.1792A>G	het	miss	E10	chr13:32907407	rs28897710	p.T598A	—	0.06	0.17	230.9/274/0;	224.9/608/1; NFE: 0.27%; FIN: 0.79%	324.0/30/0	tol	poly	B	0.5	-2.76	C0	poly	0+	B	DM?/CM035689	**not yet reviewed (BIC: no clinical importance)**; neutral[a]: -/-(9), -/.(14); 1-neutral[e], class 1[g]	confl.: B(7), LB(2), VUS(1)	classified as of "no clinical importance" by expert panel (BIC), high to very high MAF in NFE/FIN/Flossies, gnomAD: 2 homozygous individuals; nonsegregation with disease [5] (BS4); co-occurrence with different pathogenic *BRCA2* variants [5] (BP2)	(LIKELY) BENIGN
72876 (159/17.5/37.74)	*BRCA2*	c.2538A>C	het	syn	E11	chr13:32911030	rs11571654	p.S846=	—	0.10	0.02	81.24/98/2	59.11/161/3; SAS: 0.48%	20.2/2/0	na	na	na	na	na	na	poly	na	na	not listed	**benign/LCS**; inconsistent[a]: ?/?(5), -/.(1); 3-VUS[e]	**B**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
72876 (246/18.9/52.85)	*BRCA2*	c.2782G>A	het	miss	E11	chr13:32911274	—	p.V928I	—	—	—	—	—	—	tol	poly	B	8.9	3.50	C0	poly	1+	B	not listed	**not yet reviewed**	not listed	absent in population databases (PM2), not yet reviewed by expert panel; insufficient evidence	VUS
60770 (139/17.1/58.27)	*BRCA2*	c.2883G>A	het	syn	E11	chr13:32911375	rs11571655	p.Q961=	—	0.02	0.17	78.78/95/0	89.88/247/0; NFE: 0.17%	263.0/24/0	na	na	na	na	na	na	poly	na	na	not listed	**benign/LCS**; inconsistent[a]: -/-(2), -/.(4), -?/.(12), ?/? (>>30), ./.(3), ?/.(1); 1-neutral[e]; class 1[g]	**B**	classified as "benign/LCS" by expert panel (BP6); no evident splicing effect *in silico* (BP7)	(LIKELY) BENIGN
64753 (79/15.2/29.11) **72405** (171/17.8/33.33)	*BRCA2*	c.3032C>G	het	miss	E11	chr13:32911524	rs80358548	p.T1011R	IPR002093	—	0.01	2.494/3/0	4.478/11/0	—	del	path	PrD	29.2	5.89	C0	path	6+	dam	DM?/CM035690	**not yet reviewed**; inconsistent[a]: ?/?(2), -?/.(1), ?/.(1); 3-VUS[e]	confl.: LB(4), VUS(4)	MAF within pathogenic range (PM2); deleterious *in silico* prediction consensus (PP3); not yet reviewed by expert panel, conflicting classifications in LSDB and NCBI ClinVar; conflicting functional studies [21-23]; CR: female affected with early-onset BC (Dx 39), no familial cancer history known [24]; HBOC-affected individual from SE Spain (classified as "VUS") [25]; OC-affected Australian female (classified as "VUS") [26]; conflicting evidence	VUS
72158 (40/12.6/42.50)	*BRCA2*	c.3055C>G	het	miss	E11	chr13:32911547	rs55638633	p.L1019V	IPR002093	0.02	0.02	16.63/20/0	11.94/33/0	—	tol	poly	B	11.5	3.07	C0	prob poly	1+	B	DM?/CM035694	**benign/LCS**; inconsistent[a]: -/-(3), -/.(7), -?/.(4), ?/.(1); 1-neutral[e]; class 1[f]	**B**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
77075 (279/19.5/48.03)	*BRCA2*	c.3302A>G	het	miss	E11	chr13:32911794	rs398122761	p.H1101R	—	—	—	—	0.443/1/0	—	tol	poly	B	16.3	1.89	C0	path	1+	B	DM?/CM173958	**not yet reviewed**; inconsistent[a]: ?/?(6), -?/-?(1); 3-VUS[e]	confl.: LB(1), VUS(5)	MAF within pathogenic range (PM2); not yet reviewed by expert panel, conflicting classification in LSDB and NCBI ClinVar (predominantly classified as "VUS"); CR: Cypriot BC-affected female suspected of HBOC [27]	VUS
65523 (284/19.4/51.06)	*BRCA2*	c.3516G>A	het	syn	E11	chr13:32912008	rs1799952	p.S1172=	—	0.16	0.41	296.1/359/0	271.2/750/4; NFE: 0.31%; ASJ: 1.51%	597.0/56/0	na	na	na	na	na	na	poly	na	na	DM?/CM173524	**benign/LCS**; inconsistent[a]: -/-(1), -/.(17), -?/.(1), ?/?(?), ?/.(1); 2-likely neutral[e]; class 1[f], class 1[g]	**B**	classified as "benign/LCS" by expert panel; no evident splicing effect *in silico* (BP7)	(LIKELY) BENIGN
55969 (323/20.0/41.49)	*BRCA2*	c.3711T>C	het	syn	E11	chr13:32912203	rs745588537	p.A1237=	IPR002093	—	—	—	1.092/3/0	—	na	na	na	na	na	na	poly	na	na	not listed	**likely benign;** inconclusive[a]; 3-VUS[e]	**LB**	classified as "likely benign" by expert panel; no evident splicing effect *in silico* (BP7)	(LIKELY) BENIGN
73949 (75/14.9/48.00) **75150** (319/20.0/34.80)	*BRCA2*	c.3869G>A	het	miss	E11	chr13:32912361	rs41293485	p.C1290Y	—	0.34	0.01	100.1/88/1	109.1/243/2; AFR: 1.06%	708.0/3/1	tol	poly	B	15.0	2.52	C0	poly	1+	B	DM?/CM118435	**benign/LCS**; inconsistent[a]: -/-(1), -/.(3), -?/.(2), ?/?(3); 1-neutral[e]	**B**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
71144 (199/18.3/53.27) **74996** (288/19.6/37.15) **76300** (66/14.5/50.00)	*BRCA2*	c.4068G>A	het	syn	E11	chr13:32912560	rs28897724	p.L1356=	—	0.04	0.47	304.9/368/2	292.1/796/3; ASJ: 0.60%; NFE: 0.43%	607.0/57/0	na	na	na	na	na	na	poly	na	na	DM?/CM173521	**benign/LCS;** inconclusive[a]; 1-neutral[e]; class 1[g]	**B**	classified as "likely benign" by expert panel	(LIKELY) BENIGN

Variant Specification										Population Databases					Computational Evidence									Gene/Disease specific Databases			Final Assessment of Variant Pathogenicity	
Carrier ID (quality parameters of the individual variant: Cov /Score/SB)	**Gene**	**Mutation HGVS ¥**	**het/ hom**	**Function**	**Loc.**	**Genomic Pos. (hg19)**	**SNP db_xref**	**Predicted AA Change**	**InterPro domain ID (v.67.0)**	**1000G** [%]	**ESP6500** [%] EurAm	**ExAC v0.3** frequ¶/allele count/# hom	**gnomAD v2.0.2** frequ¶/allele count/# hom	**FLOSSIES** frequ¶ / allele count (Eur)/# hom	**SIFT v6.2.0 †**	**MutTaster v2013†**	**PolyPhen-2 v2.2.2† HumVar**	**CADD Phred**	**GERP+ +_RS**	**Align-GVGD v2007†§**	**UMD predictor**	**Prediction CONSENSUS[/**		**HGMD PRO v2017.3: variant class/acc.**	**Locus specific databases (LSDB, assessed by the LOVD)$**	**NCBI-ClinVar£**	**Rationale for Classification (ACMG evidence category if meeting)**	**final CLASSIFICATION**
72266 (232/18.8/43.97)	*BRCA2*	c.4241C>T	het	miss	E11	chr13:32912733	rs70953664	p.T1414M	—	0.24	0.00	70.17/84/0	77.57/209/0; AFR: 0.79%	455.0/4/0	tol	poly	B	0.0	-4.98	C0	poly	0+	B	not listed	**benign/LCS**; inconsistent[a]: ?/?(7), -/.(3), -/-(1), -?/.(2); 1-neutral[e]	**B**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
47478 (264/19.1/57.95)	*BRCA2*	c.4284dup	het	fs	E11	chr13:32912771	rs80359439	p.Q1429Sfs*9	IPR002093	—	0.01	—	3.232/1/0	—	na	na	na	na	na	na	na	na	DM/CI011227	**pathogenic;** pathogenic[a]: +/+(4), +/.(17); 5-causal[e]; class 5[f]	**path**	classified as " pathogenic" by expert panel	PATHOGENIC
75475 (486/21.3/48.77)	*BRCA2*	c.4719dup	het	fs	E11	chr13:32913211	—	p.K1574*	—	—	—	—	—	—	na	na	na	na	na	na	na	na	not listed	5-causal[e]	not listed	absent in population database (PM2); predicted LoF-variant (PVS1); known to the UMD database as causal (submission ID 19464) (PP5)	PATHOGENIC
68241 (325/20.1/47.38)	*BRCA2*	c.5130T>C ‡	het	syn	E11	chr13:32913622	rs80359485	p.Y1710=	—	—	—	—	—	—	na	na	na	na	na	na	poly	na	na	not listed	not listed	not listed	novel; no evident splicing effect *in silico*[3] (BP7)	VUS
57429 (248/19.0/52.82) **75035** (191/18.1/54.45) **76367** (80/15.0/43.75)	*BRCA2*	c.5199C>T	het	syn	E11	chr13:32913691	rs28897734	p.S1733=	—	0.16	0.54	450.4/538/3	493.9/1348/6; FIN: 1.07%	971.0/87/0	na	na	na	na	na	na	poly	na	na	DM?/CM173520[]	**benign/LCS,** inconclusive[a]: -/-(4), -/.(3), -?/-(1), ?/.(1); 1-neutral[e]; class 1[g]	**B**	classified as "benign/LCS" by expert panel;	(LIKELY) BENIGN
53539 (189/18.1/53.44)	*BRCA2*	c.5312G>A	het	miss	E11	chr13:32913804	rs80358755	p.G1771D	—	0.02	0.06	30.7/37/1	33.64/93/0; ASJ: 0.11%	30.4/3/0	tol	poly	B	6.2	-0.34	C0	poly	0+	B	DM?/CM041731[]	**benign/LCS;** inconsistent[a]: -/-(5), -/.(12), -?/.(4), ?/.(2); 1-neutral[e], class 1[f]	B	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
75956 (120/16.6/37.50)[@] **75957** (227/18.7/43.61)[@]	*BRCA2*	c.5640T>G	het	miss	E11	chr13:32914132	rs11571657	p.N1880K	—	0.22	0.00	76.29/92/0	94.20/259/1; AFR: 0.90%	526.0/3/0	tol	poly	B	15.4	1.84	C0	poly	0+	B	DM?/CM10169	**not yet reviewed**; inconclusive[a]: ?/?(2), -/-(1), -/.(3), ./.(1); 2-likely neutral[e]	confl.: B(10), LB(5), VUS(1)	very high MAF in AFR, gnomAD: 1 homozygous individual; benign *in silico* prediction consensus (BP4); predominantly classified as "(likely) benign" in LSDB and NCBI ClinVar	(LIKELY) BENIGN
74215 (387/20.5/45.99)	*BRCA2*	c.5652T>C	het	syn	E11	chr13:32914144	rs766067138	p.I1884=	—	—	—	—	0.8173/2/0	—	na	na	na	na	na	na	poly	na	na	not listed	**likely benign**; 3-VUS[e]	**LB**	classified as "likely benign" by expert panel; no evident splicing effect *in silico* (BP7)	(LIKELY) BENIGN
72266 (515/21.5/55.34)	*BRCA2*	c.5704G>A	het	miss	E11	chr13:32914196	rs4987048	p.D1902N	—	0.62	0.01	166.4/201/0	194.9/539/5; AFR: 2.15%	1170.0/7/2	del	poly	B	13.2	3.98	C0	poly	2+	undet	not listed	**benign/LCS**, inconsistent[a]: ?/?(6), -/.(6), -/-(1), -?/.(3); 1-neutral[e]	**B**	classified as benign/LCS by expert panel	(LIKELY) BENIGN
72875 (175/17.8/43.43)	*BRCA2*	c.5986G>A	het	miss	E11	chr13:32914478	rs80358833	p.A1996T	—	0.14	—	52.28/63/0	43.36/120/2; SAS: 0.37%	—	del	path	PD	24.5	3.95	C55	prob path	7+	dam	DM?/CM142734[]	**not yet reviewed**; inconclusive[a]; 3-VUS[e]	confl.: B(2), LB(4), VUS(2)	high MAF in SAS, gnomAD: 2 homozygous individuals; no statistical increased prevalence in affected subjects in a case-control study from a mult-ethnic South Asian cohort [10]	(LIKELY) BENIGN
76300 (60/14.0/60.00)	*BRCA2*	c.6057C>T	het	syn	E11	chr13:32914549	rs147961615	p.N2019=	—	0.24	0.00	49.75/60/0	41.20/114/0; AFR: 0.46%	223.0/6/0	na	na	na	na	na	na	poly	na	na	not listed	**benign/LCS**, inconsistent[a]: ?/?(3), -/.(1); 3-VUS[e]	**B**	classified as "benign/LCS" by expert panel; no evident splicing effect *in silico*[3]	(LIKELY) BENIGN
43268 (269/19.3/49.44)	*BRCA2*	c.6080G>A	het	miss	E11	chr13:32914572	rs431825337	p.R2027K	—	—	—	—	—	—	tol	poly	B	0.0	-4.10	C0	poly	0+	B	not listed	**not yet reviewed**	unc.	absent in population databases (PM2); not yet reviewed by expert panel; insufficient evidence	VUS
67256 (39/12.7/33.33) **71619** (354/20.4/51.98) **72925** (122/16.5/42.62) **73285** (115/16.3/47.83) **75475** (196/18.2/42.86)	*BRCA2*	c.6100C>T	het	miss	E11	chr13:32914592	rs1799954	p.R2034C	—	0.14	0.51	324.6/392/0	306.8/849/2	921.0/85/0	tol	poly	B	22.2	1.77	C0	poly	1+	B	DP/CM994286	**benign/LCS**, inconsistent[a]: -/-(15), -/.(>30), -?/.(1), ?/-(1), ?/.(5); 1-neutral[e]; class 1[f]; class 1[g]	**B**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
72204 (144/17.1/52.08)	*BRCA2*	c.6347A>G	het	miss	E11	chr13:32914839	rs55953736	p.H2116R	—	0.68	0.05	133.0/159/1	136.4/371/4; AFR: 1.15%	799.0/14/1	tol	poly	B	20.8	4.33	C0	poly	2+	undet	DM?/CM022331[]	**benign/LCS**; inconsistent[a]: -/-(3), -/.(10), -?/.(1), ?/.(1); 1-neutral[e]	**B**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
64604 (97/15.8/37.11)	*BRCA2*	c.6853A>G	het	miss	E12	chr13:32918706	rs56272235	p.I2285V	—	—	0.02	28.71/33/0	38.09/104/1	60.7/6/0	tol	poly	B	24.7	4.95	C0	prob poly	2+	undet	FP/CM1110951	**benign/LCS**, inconsistent[a]: -/-(2), -/.(7), -?/.(3), ?/?(1); 2-likely neutral[e], class 1[f]	**B**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
73877 (337/20.1/35.01)	*BRCA2*	c.6953G>A	het	miss	E13	chr13:32920979	rs80358921	p.R2318Q	—	—	—	1.699/2/0	0.817/2/0	10.1/1/0	del	path	PrD	34.0	5.03	C0	path	6+	dam	DM?/CM065024	**not yet reviewed**; inconsistent[a]: ?/?(2), ?/.(2), +?/.(1), 3-VUS[e]; class 2[f]; class 1[g]	confl.: LB(4), VUS(3)	multifactorial likelihood models implicate no pathogenicity [28-29]; co-occurrence with pathogenic BRCA2 variants (phase unknown) [26; UMD database] (BP2); nonsegregation with disease [30] (BS4)	(LIKELY) BENIGN
75895 (189/18.2/32.28)	*BRCA2*	c.7331A>T	het	miss	E14	chr13:65760321	rs431825352	p.D2444V	—	—	—	12.38/15/0	8.957/22/0	—	tol	poly	B	0.8	-0.76	C0	prob path	1+	B	not listed	**not yet reviewed**	*unc.*	not yet reviewed by expert panel, consistently classified as "VUS" in NCBI ClinVar; predicted *de novo* CSDS[2] (PP3); CR: Indian BC- or OC-affected female, FA: "familial" or "sporadic" (classified as "VUS") [31]	VUS
74742 (428/20.9/52.34)	*BRCA2*	c.7464A>C	het	miss	E15	chr13:32930593	rs80358969	p.R2488S	IPR015252	—	—	—	0.406/1/0	—	del	path	PrD	25.1	1.70	C35	path	6+	dam	not listed	**not yet reviewed**; inconclusive[a]	*unc.*	MAF within pathogenic range (PM2); mutational hot spot (DBD domain) (PM1), deleterious *in silico* prediction consensus (PP3); functional study implicates no significant HDR activity impairement [32] (BS3?); conflicting/insufficient evidence	VUS
75817 (215/18.6/40.00)[@] **75818** (162/17.5/46.91)[@]	*BRCA2*	c.7477A>T	het	miss	E15	chr13:32930606	—	p.M2493L	IPR015252	—	—	—	—	—	tol	path	B	22.2	3.08	C0	poly	3+	undet	not listed	not listed	*unc.*	absent from population databases (PM2); mutational hot spot (DBD domain) (PM1); consistently classified as "VUS" in NCBI ClinVar	VUS
76110 (231/18.7/44.59)	*BRCA2*	c.7481G>A	het	miss	E15	chr13:32930610	rs80358973	p.R2494Q	IPR015252	—	—	—	0.812/2/0	—	del	path	B	31.0	4.64	C35	prob path	6+	dam	not listed	**not yet reviewed**; pathogenic[a]: +/.(1); 3-VUS[e]	confl.: LB(2), VUS(2)	MAF within pathogenic range (PM2); mutational hot spot (DBD domain) (PM1); not yet reviewed by expert panel, inconsistently classified in LSDB and NCBI ClinVar (predominantly classified as "VUS"); deleterious *in silico* prediction consensus (PP3); functional study implies no significant HDR activity impairment [32] (BS3?); CR: Italian HBOC family, seg n/d [33]; *in silico* prediction model: classified as "VUS" [34]	VUS
42566 (229/18.7/46.29) **73776** (626/22.1/48.88) **75547** (274/19.3/54.01) **77729** (624/22.2/46.31)	*BRCA2*	c.7544C>T	het	miss	E15	chr13:32930673	rs28897744	p.T2515I	IPR015252	—	0.06	75.31/91/1	61.37/170/2	121.0/12/0	del	poly	PD	27.5	4.64	C0	poly	4+	undet	DM?/CM994287[]	**benign/LCS**; inconsistent[a]: -/-(4); -/.(12), ?/.(4), +/.(1); 2-likely neutral[e]; class 1[f]; class 1[g]	**B**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
80686 (54/13.8/46.3)	*BRCA2*	c.7626G>A	het	syn	E16	chr13:32931887	rs61754138	p.T2542=	IPR015252	0.42	0.00	72.99/88/0	75.54/209/0; AFR: 0.76%	567.0/8/0	na	na	na	na	na	na	poly	na	na	not listed	**benign/LCS**, inconsistent[a]: ?/?(2), -/-(2), -/.(1), -?/.(5); 2-likely neutral[e]	**B**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
67043 (220/18.6/62.27)	*BRCA2*	c.7775A>G ‡	het	miss	E16	chr13:32932036	—	p.D2592G	IPR015252	—	—	—	—	—	del	poly	PD	26.3	2.58	C0	path	5+	dam	not listed	not listed	not listed	absent in population databases (PM2); mutational hot spot (DBD domain) (PM1); insufficient evidence	VUS
72969 (590/22.1/45.08)	*BRCA2*	c.8149G>T	het	miss	E18	chr13:32937488	rs28897747	p.A2717S	IPR015187	0.10	0.22	116.1/141/0	113.0/313/0	212.0/20/0	tol	poly	na	na	na	na	poly	na	na	DM?/CM043984[]	**benign/LCS**, inconsistent[a]: -/-(5), -/.(11), ?/.(3); 1-neutral[e]; class 1[f]; class 1[g]	**B**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
35678 (424/20.9/47.41)	*BRCA2*	c.8182G>A	het	miss	E18	chr13:32937521	rs28897749	p.V2728I	IPR015187	0.06	0.45	203.5/247/1	210.0/581/2; NFE: 0.40%	617.0/58/0	tol	poly	B	0.0	-7.77	C0	poly	0+	B	DM?/CM004715	**not yet reviewed (BIC: no clinical importance),** inconsistent[a]: -/-(9), -/.(11), ?/.(2), ?/?(1); 1-neutral[e]; class 1[g]	**(L)B**	classified as of "no clinical importance" by expert panel (BIC); high to very high MAF in NFE/Flossies; benign *in silico* prediction consensus (BP4); nonsegregation with disease [5] (BS4); co-occurrence with different pathogenic *BRCA2* variants [5] (BP2)	(LIKELY) BENIGN
62240 (370/20.4/43.24)	*BRCA2*	c.8187G>T	het	miss	E18	chr13:32937526	rs80359065	p.K2729N	IPR015187	0.26	—	82.37/100/0	73.08/202/0; EAS: 0.92%	10.1/1/0	del	poly	PrD	21.5	1.54	C0	prob path	4+	undet	DM?/CM021957[]	**benign/LCS**; inconsistent[a]: -/-(1), -/.(5), -?/.(2); ?/.(1), ?/?(8), +/.(1); 3-VUS[e]; class 1[f]	**B**	classified as "benign/LCS" by expert panel; no statistical increased prevalence in affected individuals in a case-control study from a mult-ethnic South Asian cohort [10]	(LIKELY) BENIGN
63408 (478/21.3/52.3)	*BRCA2*	c.8386C>T	het	miss	E19	chr13:32944593	rs146120136	p.P2796S	—	—	0.01	1.647/2/0	1.625/4/0	10.1/1/0	tol	poly	B	14.5	2.02	C0	benign	1+	B	not listed	**not yet reviewed**; 3-VUS[e]	confl.: LB(3), VUS(3)	MAF within pathogenic range (PM2); not yet reviewed by expert panel, conflicting classification in LSDB and NCBI ClinVar; conflicting/insufficient evidence	VUS

Variant Specification									Population Databases					Computational Evidence								Gene/Disease specific Databases			Final Assessment of Variant Pathogenicity	
Carrier ID (quality parameters of the individual variant: Cov./Score/SB)	**Gene**	**Mutation HGVS ¥**	**het/ hom**	**Function**	**Loc.**	**Genomic Pos. (hg19)**	**SNP db_xref**	**Predicted AA Change**	**InterPro domain ID (v.67.0)**	**1000G** [%]	**ESP6500** [%] EurAm	**ExAC v0.3** frequ¶/allele count/# hom	**gnomAD v2.0.2** frequ¶/allele count/# hom	**FLOSSIES** frequ¶ / allele count (Eur)/# hom	**SIFT v6.2.0 †**	**MutTaster v2013†**	**PolyPhen-2 v2.2.2†** HumVar	**CADD Phred**	**GERP+ +_RS**	**Align-GVGD v2007†§**	**UMD predictor**	**Prediction CONSENSUS⌠**		**HGMD PRO v2017.3: variant class/acc.**	**Locus specific databases (LSDB, assessed by the LOVD)$**	**NCBI-ClinVar£**	**Rationale for Classification (ACMG evidence category if meeting)**	**final CLASSIFICATION**
68948 (208/18.5/36.06) **71642** (270/19.2/49.26)	*BRCA2*	c.8567A>C	het	miss	E20	chr13:32945172	rs11571747	p.E2856A	IPR015205	0.02	0.20	88.21/107/0	112.7/312/0	395.0/37/0	tol	path	PrD	26.6	5.28	C0	prob path	5+	undet	not listed	**not yet reviewed (BIC: no clinical importance)**; inconsistent[a]: -/-(8), -/.(5), -/.(5), +/.(1); 1-neutral[a]; class 2[f], class 1[g]	confl.: B(13), LB(10), VUS(1)	classified as of "no clinical importance" by expert panel (BIC); high MAF in NFE/Flossies; predominantly classified as "(likely) benign" in LSDB and NCBI ClinVar; functional study show no deleterious effect [32] (BS3)	(LIKELY) BENIGN
60824 (700/22.6/43.00)	*BRCA2*	c.8723T>G	het	miss	E21	chr13:32950897	rs28897753	p.V2908G	—	—	—	1.667/2/0	1.625/4/0	40.5/4/0	del	path	PrD	26.8	5.32	C35	prob path	7+	dam	not listed	**not yet reviewed**; inconsistent[a]: ?/?(5), -/. (9), ?/.(1); 3-VUS[a]; class 2[f]	confl.:LB(10), VUS(1)	multifactorial likelihood model predicts neutrality [28,35]; functional studies consistently show no deleterious effect [32,36-37] (BS3); predominantly classified as "likely benign" in LSDB and NCBI ClinVar	(LIKELY) BENIGN
74812 (1496/25.2/54.01)	*BRCA2*	c.8844T>G	het	miss	E22	chr13:32953543	—	p.I2948M	—	—	—	—	—	—	del	poly	B	21.5	3.88	C0	poly	3+	undet	not listed	**not yet reviewed**	*unc.*	absent in population databases (PM2); not yet reviewed by expert panel, consistently classified as "VUS" in NCBI ClinVar; insufficient evidence	VUS
53887 (293/19.6/47.44)	*BRCA2*	c.9038C>T	het	miss	E23	chr13:32953971	rs28897755	p.T3013I	—	—	0.06	23.26/28/0	23.53/65/1	132.0/12/0	tol	poly	B	17.0	3.91	C0	poly	1+	B	DM?/CM004193	**not yet reviewed (BIC: no clinical importance)**; inconsistent[a]: -/-(2), -/.(3), ?/.(1), ?/?(1); 1-neutral[a]; class 2[f]; class 1[g]	confl.: B(7), LB(7), VUS(2)	classified as of "no clinical importance" by expert panel (BIC)	(LIKELY) BENIGN
63623 (405/20.7/49.14)	*BRCA2*	c.9155G>A	het	miss	E24	chr13:32954181	rs80359171	p.R3052Q	IPR015188	—	—	3.312/4/0	2.032/5/0	—	del	path	PrD	22.3	5.50	C35	path	7+	dam	DM?/CM082513	**not yet reviewed**; inconsistent[a]: ?/?(4), -/. (4), ?/.(3); 3-VUS[a]; class 2[f]	confl.: LB(4), VUS(4)	mutational hot spot (DBD) (PM1); MAF within pathogenic range (PM2); not yet reviewed by expert panel, conflicting classification in LSDB and NCBI ClinVar; same amino acid change as established pathogenic variant (R3052W) (PM5); deleterious *in silico* prediction consensus (PP3); conflicting functional data [38-39]; multifactorial likelihood analyses/algorithms based on co-segregation/-occurrence analyses and clinical features implicate low clinical significance [28,35,40-41]; conflicting evidence	VUS
74275 (465/21.1/49.89)	*BRCA2*	c.9592T>C	het	miss	E26	chr13:32971125	rs80359229	p.C3198R	—	0.008	0.01	4.118/5/0	5.686/14/0	40.5/4/0	tol	poly	B	6.1	2.96	C0	prob poly	1+	B	not listed	**benign/LCS**; neutral[a]: -/-(2), -/.(1); class 1[g]	**B**	classified as "benign/LCS" by expert panel	(LIKELY) BENIGN
47171 (545/21.9/30.83)	*BRCA2*	c.9965T>G ‡	het	miss	E27	chr13:32972615	—	p.M3322R	—	—	—	—	—	—	tol	poly	B	13.5	1.43	C0	prob path	1+	B	not listed	not listed	not listed	novel	VUS
60577 (215/18.6/41.86) **67256** (64/14.3/48.44) **68134** (413/20.9/52.30) **69162** (394/20.7/51.02) **69486** (237/18.9/48.52) **69938** (332/20.1/46.08) **73879** (237/18.9/45.57) **75036** (95/15.8/50.53) **76029** (131/16.8/42.75) **76111** (211/18.6/51.66) **76382** (334/20.1/42.81) **77074** (121/16.7/37.19)	*BRCA2*	c.9976A>T	het	nons	E27	chr13:32972626	rs11571833	p.K3326*	—	0.44	0.84	701.7/848/8	644.0/1782/13	1240.0/113/1	na	na	na	na	na	na	path	na	na	DP/CM993644	**benign/LCS**, inconsistent[a]: -/-(14), -/.(>>30); ?/-(1); ?/.(7); 1-neutral[a], class 1[f]; class 1[g]	**B**	case-control studies implicate a slight pleiotropic cancer risk elevation [42-44]	HYPOMORPHIC
68367 (567/21.9/32.45)	*BRCA2*	c.10095delCins11	het	fs	E27	chr13:32972746	rs276174803	p.S3366Nfs*4	—	—	—	—	—	80.94/8/0	na	na	na	na	na	na	na	na	na	DM?/CX033308	**not yet reviewed (BIC: no clinical importance);** inconsistent[a]: ?/?(14), -/.(2); 1-neutral[a]	confl.: B(5), LB(2), VUS(2)	classified as of "no clinical importance" by expert panel (BIC); no case-control/GWAS study available	(LIKELY) BENIGN
63314 (711/22.6/49.23)	*BRCA2*	10110G>A	het	syn	E27	chr13:32972746	rs28897762	p.R3370=	—	0.08	0.28	146.9/178/0	139.4/386/0; NFE: 0.18%	344.0/33/0	na	na	na	na	na	na	na	na	na	DM?/CM173607	**benign/LCS**, inconsistent[a]: -/-(4), -/.(12), ?/.(2); 1-neutral[a], class 1[g]	**B**	classified as "benign/LCS" by expert panel; high MAF in NFE/Flossies	(LIKELY) BENIGN
74985 (154/17.5/46.75)	*ATM*	c.162T>C	het	syn	E3	chr11:108098592	rs3218690	p.Y54=	IP021668	0.12	0.26	166.9/202/1	175.5/486/1; NFE: 0.28%	405.0/40/0//0	na	na	na	na	na	na	poly	na	na	not listed	not listed	confl.: B(5), LB(4), VUS(1)	high MAF in NFE/Flossies; gnomAD: 1 homozygous individual; predominantly classified as "(likely) benign" in NCBI ClinVar; no evident splicing effect *in silico* (BP7)	(LIKELY) BENIGN
75579 (661/22.4/50.53)	*ATM*	c.670A>G	het	miss	E7	chr11:108115522	rs145053092	p.K224E	—	—	0.02	5.776/7/0	9.031/25/0	20.2/0/0	del	path	PD	23.9	5.36	C15	prob poly	6+	dam	DM?/CM000650	inconclusive[a]	*unc.*	MAF within pathogenic range (PM2); consistently classified as "VUS" by NCBI ClinVar database; deleterious prediction consensus (PP3); reported with a second deleterious ATM variant in an individual affected with A-T [45] (PM3?); CR: TNBC-affected female (Dx 50), FH: relatives affected with pancreas and cervix carcinoma (classified as "VUS"), seg n/d [46]	VUS
76919 (149/17.3/59.73)	*ATM*	c.978A>G ‡	het	miss	E8	chr11:108117767	—	p.I326M	—	—	—	—	—	—	tol	path	PD	23.1	4.56	C0	prob poly	4+	undet	not listed	not listed	not listed	novel	VUS
65391 (108/16.2/45.37)	*ATM*	c.998C>T	het	miss	E8	chr11:108117787	rs28904919	p.S333F	—	0.14	0.17	128.1/155/0	158.5/439/1; AMR:0.36%; FIN: 0.34%	243.0/24/0	del	path	B	25.1	3.85	C15	prob poly	5+	undet	DM?/CM152767	not listed	*(likely) B*	high MAF in AMR/FIN/Flossies, gnomAD: 1 homozygous individual; no statistical association with BC risk in case-control study/nonsegregation with disease (BS4) [47]; consistently classified as "(likely) benign " in NCBI ClinVar (BP6)	(LIKELY) BENIGN
68424 (61/14.2/47.54)	*ATM*	c.1066-6T>G	het	nonc	I8	chr11:108119654	rs201686625	nonc	—	0.04	0.26	130.1/146/1	134.0/357/2; NFE: 0.23%	344.0/33/0	na	na	na	na	na	na	na	na	na	DM?/CS000846	inconsistent[a]: +/.(1), -/-(1), -?/.(3)	confl.: B(3), LB(4), VUS(6)	high MAF in NFE/Flossies, gnomAD: 2 homozygous individuals; no statistical association with BC risk in case-control studies [47-50]; conflicting segregation data [47,51]; functional *in-vitro* studies imply a "leaky" skipping of exon 11 [51-52], inconsistent functional data for ATM kinase activity [51,53]; reported in presumptive A-T patients in homozygous constellation [52] and *in trans* with a second pathogenic *ATM* variant [53-54] (PM3?); conflicting classification in LSDB and NCBI ClinVar; conflicting evidence, potential hypomorphic effect [55]	VUS
65579 (141/17.1/53.19) **69307** (299/19.7/50.17) **73313** (377/20.5/50.40)	*ATM*	c.1229T>C	het	miss	E9	chr11:108119823	rs56128736	p.V410A	—	0.08	0.22	216.6/262/2	210.2/582/3; NFE: 0.32%; ASJ: 0.40%	587.0/55/0	del	path	B	23.4	5.07	C25	poly	5+	undet	DM?/CM035782	inconclusive[a]	confl.: B(4), LB(2), VUS(2)	high to very high MAF in NFE/ASJ/Latino/Flossies, gnomAD: 3 homozygous individuals; no association with BC in case-control studies [56-57]	(LIKELY) BENIGN
75152 (274/19.5/35.40)	*ATM*	c.1272T>C	het	syn	E10	chr11:108121464	rs35578748	p.P424=	—	—	0.06	30.02/36/0	27.65/76/0	40.5/4/0	na	na	na	na	na	na	poly	na	na	not listed	not listed	confl.: B(2), LB(3), VUS(1)	no evident splicing effect *in silico* [2] (BP7); predominantly classified as "(likely) benign" in NCBI ClinVar; co-occurrence with two pathogenic variants [ClinVar, submission SCV000694176.1] (BP2)	(LIKELY) BENIGN
73949 (115/16.3/42.61)	*ATM*	c.1370G>T	het	miss	E10	chr11:108121562	—	p.R457L	—	—	—	—	0.8125/2/0	—	tol	path	B	24.1	5.04	C0	path	4+	undet	not listed	not listed	*unc.*	MAF within pathogenic range (PM2); consistently classified as "VUS" in NCBI ClinVar; insufficient evidence	VUS
68617 (150/17.2/49.33)	*ATM*	c.1744T>C	het	miss	E11	chr11:108122700	rs2235006	p.F582L	—	0.26	0.09	98.23/119/1	92.83/257/1; AMR: 0.13%; SAS: 0.18%	101.0/10/0	tol	poly	B	10.3	1.13	C0	poly	0+	B	DM?/CM023329	not listed	*(L)B*	high MAF in AMR/SAS/Flossies, gnomAD: 1 homozygous individual; benign *in silico* prediction consensus (BP4); consistently classified as "(likely) benign" in NCBI ClinVar (BP6); functional studies show no deleterious effect [58] (BS3)	(LIKELY) BENIGN
68969 (356/20.2/46.35) 75407 (75/14.8/46.67)	*ATM*	c.1810C>T	het	miss	E12	chr11:108123551	rs2227922	p.P604S	—	0.26	0.29	309.0/345/5	321.4/887/15; ASJ: 3.28%; AFR: 0.67%	860.0/48/1	tol	path	B	24.5	5.54	C0	prob poly	3+	undet	DM?/CM045888	not listed	*(L)B*	very high MAF in AFR/ASJ/Flossies, gnomAD: 15 homozygous individuals; consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN

Variant Specification										Population Databases					Computational Evidence										Gene/Disease specific Databases			Final Assessment of Variant Pathogenicity	
Carrier ID (quality parameters of the individual variant: Cov./Score/SB)	**Gene**	**Mutation HGVS** ¥	**het/ hom**	**Function**	**Loc.**	**Genomic Pos. (hg19)**	**SNP db_xref**	**Predicted AA Change**	**InterPro domain ID (v.67.0)**	**1000G** [%]	**ESP6500** [%] EurAm	**ExAC v0.3** frequ¶/allele count/# hom	**gnomAD v2.0.2** frequ¶/allele count/# hom	**FLOSSIES** frequ¶ / allele count (Eur)/# hom	**SIFT v6.2.0** †	**MutTaster v2013**†	**PolyPhen-2 v2.2.2**† HumVar	**CADD Phred**	**GERP+ +_RS**	**Align-GVGD v2007**†§	**UMD predictor**	**Prediction CONSENSUS**ſ		**HGMD PRO v2017.3: variant class/acc.**	**Locus specific databases (LSDB, assessed by the LOVD)**$	**NCBI-ClinVar**£	**Rationale for Classification (ACMG evidence category if meeting)**	**final CLASSIFICATION**
76678 (253/19.2/50.2)	*ATM*	c.1837G>T	het	miss	E12	chr11:108123578	rs200124136	p.V613L	—	—	—	4.265/5/0	2.442/6/0	10.1/1/0	tol	path	PD	24.0	5.66	C0	prob path	5+	undet	DM?/CM0910486	not listed	*unc.*	MAF within pathogenic range (PM2); *in silico* predicted CSDS deactivation[2] (PP3); consistently classified as "VUS" in NCBI ClinVar; reported in 1/4112 BC cases (vs 0/2399 controls) in case-control-study [56]; insufficient evidence	VUS
63722 (513/21.5/46.78)	*ATM*	c.2040C>T	het	syn	E13	chr11:108124682	rs587780855	p.F680=	—	—	—	—	5.286/13/0	10.1/1/0	na	na	na	na	na	na	poly	na	na	not listed	not listed	*(L)B*	no evident splicing effect *in silico* (BP7); consistently classified as "likely benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
73106 (207/18.4/46.38)	*ATM*	c.2275A>G	het	miss	E15	chr11:108128232	rs148705269	p.S759G	—	—	0.03	2.475/3/0	2.889/8/0	20.2/2/0	tol	poly	B	na	3.73	C0	prob path	2+	undet	not listed	not listed	*unc.*	MAF within pathogenic range (PM2); *in silico* predicted *de novo* CSAS[2] (PP3); consistently classified as "VUS" in NCBI ClinVar; insufficient evidence	VUS
62277 (399/20.7/42.86)	*ATM*	c.2805G>C	het	syn	E18	chr11:108139303	rs55934812	p.T935=	—	0.04	0.06	28.01/34/1	29.23/81/1	20.2/0/0	na	na	na	na	na	na	poly	na	na	not listed	not listed	confl.: B(1), LB(3), VUS(1)	MAF somewhat high, gnomAD: 1 homozygous individual; no evident splicing effect *in silico* (BP7); (consistently) classified as "(likely) benign" in NCBI ClinVar (SCV000694235.1: "VUS-possibly benign"); no association with BC in case-control study (ranked as "neutral") [59]	(LIKELY) BENIGN
60720 (185/18.1/44.32)	*ATM*	c.3154-4G>A	het	nonc	I21	chr11:108143445	rs199543313	nonc	—	0.02	0.03	22.38/27/0	19.5/54/0	70.8/7/0	na	na	na	na	na	na	na	na	na	not listed	not listed	confl.: B(1), LB(3), VUS(1)	no evident splicing effect *in silico*[2] (BP7), not yet confirmed by functional studies; inconsistently classified in NCBI ClinVar; CR: individual affected with pancreatic cancer (classified as "VUS") [60]; CRC-affected patient suspected of LS (classified as "VUS") [61]; insufficient evidence	VUS
76725 (289/19.6/60.9)	*ATM*	c.3240C>A	het	miss	E22	chr11:108143535	rs149911447	p.D1080E	—	0.04	0.00	2.474/3/0	2.439/6/0	—	del	poly	PrD	23.5	2.04	C35	prob path	6+	dam	not listed	not listed	*unc.*	MAF within pathogenic range (PM2); deleterious *in silico* prediction consensus (PP3); consistently classified as "VUS" in NCBI ClinVar; CR: BC-affected individual (classified as "VUS"), seg n/d [62]; male adolescent (Dx 17) affected with concurrent megakaryoblastic leukemia and mediastinal germ cell tumor [63]	VUS
73535 (374/20.5/39.04)	*ATM*	c.3772C>A	het	miss	E26	chr11:108154979	rs587782741	p.H1258N	—	—	—	—	—	—	del	path	B	25.3	5.68	C0	prob path	5+	undet	not listed	not listed	*unc.*	absent in population databases (PM2), consistently classified as "VUS" in NCBI ClinVar; insufficient evidence	VUS
62670 (792/23.1/49.12) **64885** (239/18.8/40.59) **73106** (328/19.9/41.77) **73325** (482/21.3/35.27)	*ATM*	c.3925G>A	het	miss	E26	chr11:108155132	rs149711770	p.A1309T	—	0.02	0.15	61.83/75/0	68.96/191/0; NFE 0.12%	243.0/22/0	tol	path	B	21.8	3.41	C0	prob poly	3+	undet	not listed	not listed	confl.: LB(6), VUS(3)	high MAF in NFE/Flossies, no association with BC in large meta-analysis case-control study [56]	(LIKELY) BENIGN
62041 (511/21.5/38.55)	*ATM*	c.4060C>A	het	miss	E27	chr11:108158393	rs145119475	p.P1354T	—	—	0.03	20.66/25/0	18.22/50/0	60.7/6/0	tol	poly	B	19.2	4.13	C0	prob path	2+	B	not listed	not listed	confl.: LB(1), VUS(3)	predominatly classified as "VUS" in NCBI ClinVar; no association with BC in case-control study (1/2531 BC affected patients *vs.* 1/2245 controls in large meta-analysis [56]); insufficient evidence	VUS
71230 (53/13.8/49.06)	*ATM*	c.4244A>G	het	miss	E29	chr11:108160336	—	p.Y1415C	—	—	—	—	—	—	tol	path	B	28.0	3.99	C45	path	5+	undet	not listed	not listed	unc.	absent from population databases (PM2); insufficient evidence	VUS
63408 (1149/24.3/44.73) **72969** (1552/25.4/49.87)	*ATM*	c.4473C>T	het	syn	E30	chr11:108163382	rs4988008	p.F1491=	—	0.10	0.29	138.4/168/0	120.2/333/0; NFE 0.21%; ASJ: 0.22%	314.0/29/0	na	na	na	na	na	na	poly	na	na	not listed	probably neutral[a]: -?/.(2) (likely benign)	confl.: B(5), LB(3), VUS(1)	high MAF in NFE/Flossies; no evident splicing effect *in silico* (BP6); predominantly classified as "(likely) benign" in LSDB and NCBI ClinVar	(LIKELY) BENIGN
68134 (57/13.9/50.88)	*ATM*	c.4768C>T	het	miss	E31	chr11:108164196	rs35962982	p.L1590F	—	—	0.01	20.7/25/0	18.46/51/0	30.4/3/0	del	path	PrD	33.0	5.31	C15	path	7+	dam	DM?/CM177886	not listed	*unc.*	deleterious *in silico* prediction consensus (PP3); consistently classified as "VUS" in NCBI ClinVar; CR: affected individual with early-onset sigmoid CRC (Dx 44), MMR proficient (classified as "VUS") [64]; CRC-affected patient suspected of LS (classified as "VUS") [61]; insufficient evidence	VUS
65523 (137/17/45.99)	*ATM*	c.4794C>G ‡	het	syn	E32	chr11:108165671	—	p.L1598=	—	—	—	—	—	—	na	na	na	na	na	na	prob path	na	na	not listed	not listed	not listed	novel, no evident splicing effect *in silico*[2] (BP7)	VUS
74232 (32/11.9/50)	*ATM*	c.5009C>T	het	miss	E34	chr11:108170444	rs375131360	p.A1670V	—	—	0.01	—	1.220/3/0	—	tol	path	PrD	33.0	5.47	C0	path	5+	undet	not listed	not listed	*unc.*	MAF within pathogenic range (PM2); consistently classfied as "VUS" in NCBI ClinVar; NCBI ClinVar submission SCV000261480.3 (Invitae, Apr, 2017): alterated RNA splicing suggested *in silico*[2]; insufficient evidence	VUS
72497 (121/16.4/44.63)	*ATM*	c.5071A>C	het	miss	E34	chr11:108170506	rs1800059	p.S1691R	—	0.02	0.29	201.9/245/1	178.0/493/3; NFE 0.29%	364.0/32/0	tol	poly	B	13.7	2.65	C0	poly	1+	B	DM?/CM980141	inconsistent[a]: ?/.(1), -?/.(4)	*(L)B*	high MAF in NFE/Flossies; benign *in silico* prediction consensus (BP4); consistently classified as "(likely) benign" in NCBI ClinVar (BP6); functional studies implicate no impaired kinase activity [65] (BS3)	(LIKELY) BENIGN
63408 (504/21.4/49.60)	*ATM*	c.5189G>A	het	miss	E35	chr11:108172386	rs373789346	p.R1730Q	—	—	0.01	3.297/4/0	1.806/5/0	10.1/1/0	tol	path	PrD	26.2	4.90	C0	path	5+	undet	not listed	not listed	*unc.*	MAF within pathogenic range (PM2), consistently classified as "VUS" in NCBI ClinVar; insufficient evidence	VUS
74657 (476/21.3/50.00)	*ATM*	c.5271A>G ‡	het	syn	E35	chr11:108172468	—	p.T1757=	—	—	—	—	—	—	na	na	na	na	na	na	poly	na	na	not listed	not listed	*LB*	no evident splicing *in silico*[2] (BP7); consistently classified as "likely benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
59803 (241/18.9/54.36) **60769** (584/21.9/52.74) **61859** (413/20.6/51.09) **67161** (396/20.6/47.73) **71694** (178/17.9/34.27) **72168** (273/19.3/46.89)	*ATM*	c.5558A>T	het	miss	E37	chr11:108175463	rs1801673	p.D1853V	—	0.18	0.69	518.6/627/2	491.5/1360/6; NFE: 0.69%; ASJ: 0.75%	1010.0/97/1	del	path	B	19.3	5.52	C15	path	5+	undet	DP/CM083593	neutral[a]: -/.(1)	*(L)B*	very high MAF in NFE/ASJ/Flossies, gnomAD: 6 homozygous individuals; consistently classified as "(likely) benign" in NCBI ClinVar (BP6); significantly decreased risk for contralateral BC in [66]	(LIKELY) BENIGN
70829 (66/14.5/31.82) **72926** (484/21.3/48.97)	*ATM*	c.5793T>C	het	syn	E39	chr11:108180917	rs3092910	p.A1931=	—	0.62	0.78	520.9/629/1	505.7/1400/6; EAS: 1.13%	1470.0/101/0	na	na	na	na	na	na	poly	na	na	not listed	probably neutral[a]: -?/.(2)	*(L)B*	very high MAF in EAS/Flossies, gnomAD: 6 homozygous individuals; consistently classified as "(likely) benign" in NCBI ClinVar	(LIKELY) BENIGN
66396 (318/20.0/45.91) **71741** (225/18.7/41.78)	*ATM*	c.5890A>G	het	miss	E39	chr11:108181014	rs201963507	p.K1964E	IPR003151	—	—	10.74/13/0	9.031/25/1	10.1/1/0	tol	path	B	20.0	4.57	C0	prob poly	3+	undet	DM?/CM0910514	not listed	confl.: LB(1), VUS(5)	mutational hot spot (PIK-related kinase, FAT) (PM1); predominantly classified as "VUS" in NCBI ClinVar; CR: HBOC-affected female [67]; CRC-affected patient suspected of LS (classified as "VUS") [61]; two BC-affected females [56,68]; insufficient evidence	VUS
60546 (154/17.4/39.61) **68948** (276/19.5/44.57) **73281** (205/18.3/47.32)	*ATM*	c.6067G>A	het	miss	E41	chr11:108186610	rs11212587	p.G2023R	IPR003151	0.12	0.31	15.75/191/0	14.32/397/1; NFE 0.24%	263.0/24/0	del	path	PrD	22.0	5.33	C25	path	7+	dam	DM?/CM092585	inconclusive[a]	confl.: B(2), LB(4), VUS(1)	high MAF in NFE/Flossies, gnomAD: 1 homozygous individual; co-occurrence with pathogenic *ATM* and *BRCA1* variants (BP2) (ClinVar submission SCV000694314.1); very recently classified as "poly-morphism" in the literature [69]	(LIKELY) BENIGN
76725 (277/19.5/42.60)	*ATM*	c.6088A>G	het	miss	E41	chr11:108186631	rs145847315	p.I2030V	IPR003151	0.66	0.00	160.8/195/1	160.2/444/6; AFR: 1.74%	1150.0/7/2	tol	poly	B	18.8	4.41	C0	poly	1+	B	DM?/CM014793	not listed	*(L)B*	very high MAF in AFR/Flossies, gnomAD: 6 homozygous individuals; benign *in silico* prediction consensus (BP4); consistently classified as "benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
68424 (211/18.4/49.76)	*ATM*	c.6114C>T	het	syn	E42	chr11:108186756	rs774993357	p.H2038=	IPR003151	—	—	—	3.656/9/0	—	na	na	na	na	na	na	poly	na	na	not listed	not listed	*(L)B*	no evident splicing effect *in silico* (BP7), consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
77365 (569/21.9/46.75)	*ATM*	c.6282A>C‡	het	miss	E43	chr11:108188183	—	p.E2094D	IPR003151	—	—	—	—	—	tol	path	PD	11.5	-2.98	C0	poly	2+	undet	not listed	not listed	not listed	novel	VUS

Variant Specification									Population Databases					Computational Evidence								Gene/Disease specific Databases			Final Assessment of Variant Pathogenicity	
Carrier ID (quality parameters of the individual variant: Cov./Score/SB)	**Gene**	**Mutation HGVS ¥**	**het/ hom**	**Function**	**Loc.**	**Genomic Pos. (hg19)**	**SNP db_xref**	**Predicted AA Change**	**InterPro domain ID (v.67.0)**	**1000G** [%]	**ESP6500** [%] EurAm	**ExAC v0.3** frequ¶/allele count/# hom	**gnomAD v2.0.2** frequ¶/allele count/# hom	**FLOSSIES** frequ¶ / allele count (Eur)/# hom	**SIFT v6.2.0 †**	**MutTaster v2013†**	**PolyPhen-2 v2.2.2†** HumVar	**CADD Phred**	**GERP+ +_RS**	**Align-GVGD v2007†§**	**UMD predictor**	**Prediction CONSENSUS∫**		**HGMD PRO v2017.3: variant class/acc.**	**Locus specific databases (LSDB, assessed by the LOVD)$**	**NCBI-ClinVar£**	**Rationale for Classification (ACMG evidence category if meeting)**	**final CLASSIFICATION**
62542 (91/15.5/39.56)	*ATM*	c.6385T>G	het	miss	E44	chr11:108190718	—	p.Y2129D	IPR003151	—	—	—	—	—	del	path	PrD	22.1	5.59	C55	path	7+	dam	not listed	not listed	confl.: LP(1), VUS(1)	absent in population databases (PM2); mutational hot spot (PIK-related kinase/FAT domain) (PM1); detected *in trans* with a pathogenic variant in an A-T patient (ClinVar Submission SCV000537715.1, HUG Geneva) (PM3); deleterious *in silico* prediction consensus (PP3)	LIKELY PATHOGENIC
67514 (374/20.5/45.99)	*ATM*	c.6466G>T ‡	het	nons	E45	chr11:108192041	—	p.E2156*	IPR003151	—	—	—	—	—	na	na	na	na	na	na	path	na	na	not listed	not listed	not listed	predicted LoF-Variant (PVS1); absent in population databases (PM2)	LIKELY PATHOGENIC
65390 (178/17.9/43.26)	*ATM*	c.6860G>C	het	miss	E47	chr11:108196837	rs1800061	p.G2287A	IPR003151	—	0.01	19.79/24/0	27.08/75/0	40.5/4/0	tol	path	B	17.1	3.62	C0	poly	2+	undet	DM?/CM016182	not listed	confl.: LB(4), VUS(1)	functional data show no deleterious effect [70], tumor analysis in one BC patient shows loss of variant allele [71] (BS3); (consistently) classified as "likely benign"in NCBI ClinVar (submission SCV000694336.1: "VUS-possibly benign") (BP6)	(LIKELY) BENIGN
4162 (1111/24.2/47.61) 61748 (621/22.2/50.56)	*ATM*	c.7475T>G	het	miss	E50	chr11:108201108	rs56399857	p.L2492R	IPR003151	—	0.01	5.77/7/0	10.11/28/0	50.6/5/0	del	path	PrD	22.0	4.83	C45	path	7+	dam	not listed	not listed	*unc.*	MAF in pathogenic range (PM2); mutational hot spot (PIK-related kinase/FAT domain) (PM1); consistently classified as "VUS" in NCBI ClinVar; deleterious *in silico* prediction consensus (PP3); CR: CRC-affected patient supected of LS (classified as "VUS") [61]	VUS
67185 (317/19.9/51.10) 75407 (93/15.6/64.52)	*ATM*	c.7521C>T	hom	syn	E51	chr11:108202176	rs751234924	p.D2507=	IPR003151	—	—	—	7.599/21/0; EAS: 0.060%	10.1/1/0	na	na	na	na	na	na	poly	na	na	not listed	neutral[a]: -/.(1)	*LB*	no evident splicing effect *in silico* (BP7); consistently classified as "benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
70207 (161/17.6/96.27)	*ATM*	c.7602C>T	het	syn	E51	chr11:108202257	rs562264493	p.G2534=	IPR003151	0.04	—	9.108/11/0	6.504/16/0; SAS: 0.052%	—	na	na	na	na	na	na	prob path	na	na	not listed	not listed	*LB*	no evident splicing effect *in silico* (BP7), consistently classified as "likely benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
66198 (717/22.7/40.31) **72970** (831/23.1/54.27)	*ATM*	c.8495G>A	het	miss	E58	chr11:108216546	rs529296539	p.R2832H	IPR000403	0.04	—	16.49/20/1	11.19/31/0	—	tol	path	B	22.2	5.45	C0	path	4+	undet	not listed	not listed	*unc.*	mutation hot spot (PI3/PI4 kinase, catalytic domain) (PM1); novel missense change at an amino acid residue where a different pathogenic missense change has been seen before (R2832C) (PM5); consistently classified as "VUS" in NCBI ClinVar; CR: individuals affected with BC and lung cancer [15]	VUS
71978 (396/20.6/51.52)	*ATM*	c.8592C>T	het	syn	E59	chr11:108218013	rs56025670	p.Y2864=	IPR000403	0.04	0.06	41.74/50/0	44.99/124/0	111.0/11/0	na	na	na	na	na	na	poly	na	na	not listed	not listed	*(L)B*	no evident splicing *in silico* (BP7); consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
52069 (352/20.2/48.86)	*ATM*	c.8730C>G	het	syn	E60	chr11:108224551	rs551041839	p.L2910=	IPR000403	0.12	—	75.78/92/1	62.14/153/1; SAS: 0.49%	—	na	na	na	na	na	na	poly	na	na	not listed	not listed	*(L)B*	high to very high MAF in SAS, gnomAD: 1 homozygous individual; no evident splicing *in silico* (BP7); consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
70581 (517/21.5/46.03)	*ATM*	c.9086G>A	het	miss	E63	chr11:108236150	rs201199629	p.G3029D	IPR000403	—	0.05	14.83/18/0	14.07/39/0	101.0/8/0	del	path	B	15.4	4.18	C0	path	4+	undet	DM?/CM177897	not listed	*unc.*	mutation hot spot (PI3/PI4 kinase, catalytic domain) (PM1); consistently classified as "VUS" in NCBI ClinVar; observed in BC-patients and controls (1/2531 cases *vs* . 2/2245 controls [56]; CR: male affected with MMR proficient rectal cancer (Dx 39) [64]; CRC-affected patient (classified as "VUS") [72]; insufficient evidence	VUS
71642 (917/23.5/41.11)	*ATM*	c.9111_9112 delinsAA ‡	het	Indel	E63	chr11:108236175	—	p.Q3038K	IPR000403	—	—	—	—	—	tol	path	PrD	22.1	5.09	C0	na	4+	undet	not listed	not listed	not listed	absent in population databases (PM2); mutation hotspot (PI3/PI4 kinase, catalytic domain) (PM1); *in silico* predicted CSAS deactivation[2] (PP3)	VUS
62926 (519/21.6/46.24) **71230** (148/17.2/47.30)	*BRIP1*	c.139C>G	het	miss	E3	chr17:59937223	rs28903098	p.P47A	IPR014001 IPR006554 IPR014013	—	0.03	23.90/29/1	24.90/69/1	50.6/5/0	del	path	PrD	24.1	5.26	C25	path	7+	dam	DM/CM014756	inconclusive[b]	confl.: P(1), VUS(8)	case-control studies implicate a slight pleiotropic cancer risk elevation [73-75]	HYPOMORPHIC
71655 (245/19.0/53.06) **73906** (173/17.7/50.87) **74232** (82/15.2/46.34) **74983** (336/20.0/46.73)	*BRIP1*	c.517C>T	het	miss	E6	chr17:59924572	rs4988345	p.R173C	IPR014001 IPR006554 IPR014013	0.16	0.49	270.3/328/1	266.0/737/2; NFE 0.46%	900.0/84/0	del	path	PrD	28.0	5.26	C55	path	7+	dam	DM?/CM035889	inconsistent[b]: ?/?(4), +/?(2)	*(L)B*	high to very high MAF in NFE/Flossies, gnomAD: two homozygous individuals; consistently classified as "(likely) benign" in NCBI ClinVar database (BP6); no association with BC and OC in large case-control studies [76-77]	(LIKELY) BENIGN
47478 (571/21.9/50.60) **52150** (633/22.2/43.60) **61860** (274/19.4/46.35) **68184** (495/21.3/51.72) **71430** (436/20.9/52.29) **71978** (418/20.8/50.24) **72168** (221/18.6/49.32) **74662** (310/19.8/46.13) **74742** (415/20.8/50.60) **75035** (354/20.2/57.63) 75745 (804/22.1/46.64)	*BRIP1*	c.577G>A	het	miss	E6	chr17:59924512	rs4988346	p.V193I	IPR014001 IPR006554 IPR014013	0.14	0.56	396.4/481/5	355.8/986/9; NFE 0.51%	749.0/71/0	tol	poly	B	0.0	-8.22	C0	poly	0+	B	not listed	inconsistent[b]: ?/?(5), +/?(1)	*B*	very high MAF in NFE/Flossies, gnomAD: 9 homozygous individuals; consistently classified as "benign" in NCBI ClinVar (BP6); benign *in silico* prediction consensus (BP4)	(LIKELY) BENIGN
68914 (275/19.2/53.09) **66416** (524/21.6/44.66) **68948** (283/19.4/40.64)	*BRIP1*	c.584T>C	het	miss	E6	chr17:59924505	rs4988347	p.L195P	IPR014001 IPR006554 IPR014013	0.08	0.19	159.9/194/1	193.8/537/2; NFE: 0.20%; FIN: 0.88%	304.0/27/0	tol	path	B	9.3	-0.01	C0	poly	1+	B	not listed	probably neutral[a]: -?/.(2); inconsistent[b]: ?/?(4), -?/?(1), +/?(1)	*(L)B*	high to very high MAF in NFE/FIN/Flossies, gnomAD: 2 homozygous individuals; consistently classified as "(likely) benign" in NCBI ClinVar; benign *in silico* prediction consensus (BP4)	(LIKELY) BENIGN
67161 (665/22.4/49.47) **64566** (188/18.0/46.81)	*BRIP1*	c.890A>G	het	miss	E7	chr17:59885856	rs28997570	p.K297R	IPR014001 IPR006554 IPR014013	0.04	0.16	110.4/134/0	133.0/285/0; NFE 0.17%	202.0/18/0	tol	poly	B	19.0	5.29	C0	prob path	2+	undet	not listed	inconsistent[b]: +/? (1), ?/?(1)	*(L)B*	high MAF in NFE/Flossies; consistently classified as "likely benign" in NCBI ClinVar (BP6); no association witch BC and OC in large case-control studies [77-78]	(LIKELY) BENIGN
65579 (168/17.8/53.57)	*BRIP1*	c.1356C>T	het	syn	E10	chr17:59871075	rs730881640	p.N452=	—	—	—	—	6.594/16/0; LAT: 0.036%	—	na	na	na	na	na	na	poly	na	na	not listed	not listed	*(L)B*	no evident splicing effect *in silico* (BP7); consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
52150 (396/20.7/43.18)	*BRIP1*	c.1357G>A	het	miss	E10	chr17:59871074	rs587780227	p.A453T	—	—	—	—	2.061/5/0	—	tol	poly	B	17.1	-0.40	C0	poly	0+	B	not listed	not listed	*unc.*	MAF within pathogenic range/absent in ExAC population database (PM2); consistently classified as "VUS" in NCBI ClinVar; insufficient evidence	VUS
62976 (348/20.2/57.18) **76380** (338/20.0/59.47)	*BRIP1*	c.1629-3T>C	het	nonc	I11	chr17:59858369	rs587780828	nonc	—	—	—	4.989/6/0	5.418/15/0	20.2/2/0	na	na	na	na	na	na	na	na	na	na	not listed	not listed	confl.: B(1), LB(1), VUS(3)	MAF within pathogenic range (PM2); no evident splicing effect *in silico*[2] (BP7), not yet confirmed by functional splicing studies; conflicting classification in NCBI ClinVar; insufficient evidence	VUS
4162 (358/20.2/59.50) **60770** (283/19.5/56.54) **62002** (259/19.3/43.63) 65761 (429/21.0/43.82)	*BRIP1*	c.2097+7G>A	het	nonc	I14	chr17:59853755	rs4988352	nonc	—	0.22	0.33	253.6/307/1	232.5/644/2; NFE: 0.41%	—	na	na	na	na	na	na	na	na	na	not listed	inconclusive[b]	confl.: B(2), LB(4), VUS(2)	high MAF in NFE, gnomAD: 2 homozygous individuals; no evident splicing effect *in silico* (BP7)	(LIKELY) BENIGN
73281 (375/20.4/48.27)	*BRIP1*	c.2097+8A>C	het	nonc	I14	chr17:59853754	rs730881642	nonc	—	—	—	—	0.4064/1/0	—	na	na	na	na	na	na	na	na	na	not listed	not listed	*(L)B*	no evident splicing effect *in silico*[2] (BP7); consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
68160 (410/20.8/42.20)	*BRIP1*	c.2684_2687del	het	fs	E19	chr17:59763415	rs760551339	p.S895*	—	—	—	—	1.444/4/0	—	na	na	na	na	na	na	na	na	na	DM/CD159813	not listed	*(likely) path*	predicted LoF-Variant (PVS1); MAF in population database within pathogenic range (PM2), known to HGMD and ClinVar databases as (likely) pathogenic (PP5); CR: reported in two HBOC-affected females [79-80]	LIKELY PATHOGENIC
75672 (356/20.4/36.24)	*BRIP1*	c.3042T>C	het	syn	E20	chr17:59761365	rs188258913	p.G1014=	—	0.02	0.01	4.12/5/0	3.969/11/0	—	na	na	na	na	na	na	poly	na	na	not listed	not listed	*LB*	MAF within pathogenic range (PM2); *in silico* predicted CSDS deactivation[2] (PP3); consistently classified as "likely benign" in NCBI ClinVar (BP6); conflicting/ insufficient evidence	VUS
75956 (132/17/65.15)	*BRIP1*	c.3459T>C	het	syn	E20	chr17:59760948	rs4987050	p.D1153=	—	0.02	0.07	79.42/96/0	72.84/201/0; AMR: 0.10%; ASJ: 0.14%	111.0/11/0	na	na	na	na	na	na	poly	na	na	not listed	not listed	confl.: B(2), LB(6), VUS(2)	high MAF in AMR/ASJ/Flossies; no evident splicing effect *in silico* (BP7); predominantly classified as "(likely) benign" in NCBI ClinVar	(LIKELY) BENIGN

Variant Specification										Population Databases					Computational Evidence									Gene/Disease specific Databases			Final Assessment of Variant Pathogenicity	
Carrier ID (quality parameters of the individual variant: Cov./Score/SB)	**Gene**	**Mutation HGVS ¥**	**het/ hom**	**Function**	**Loc.**	**Genomic Pos. (hg19)**	**SNP db_xref**	**Predicted AA Change**	**InterPro domain ID (v.67.0)**	**1000G** [%]	**ESP6500** [%] EurAm	**ExAC v0.3** frequ¶/allele count/# hom	**gnomAD v2.0.2** frequ¶/allele count/# hom	**FLOSSIES** frequ¶ / allele count (Eur)/# hom	**SIFT v6.2.0 ŧ**	**MutTaster v2013†**	**PolyPhen-2 v2.2.2ŧ** HumVar	**CADD Phred**	**GERP+ +_RS**	**Align-GVGD v2007†§**	**UMD predictor**	**Prediction CONSENSUS⌈**		**HGMD PRO v2017.3: variant class/acc.**	**Locus specific databases (LSDB, assessed by the LOVD)$**	**NCBI-ClinVar£**	**Rationale for Classification (ACMG evidence category if meeting)**	**final CLASSIFICATION**
66309 (181/17.9/56.35)	*CDH1*	c.69G>A	het	syn	E2	chr16:68772220	rs786202657	p.Q23=	—	—	—	—	—	—	na	na	na	na	na	na	poly	na	na	not listed	not listed	confl.: LB(2), VUS(1)	absent in population database (PM2); *in silico* predicted CSDS deactivation[2] (PP3); conflicting classification in NCBI ClinVar; insufficient/conflicting evidence	VUS
73285 (110/16.1/50.00) **73879** (188/18.1/35.11)	*CDH1*	c.88C>A	het	miss	E2	chr16:68772239	rs139866691	p.P30T	IPR014868	0.04	0.16	93.8/19/0	129.9/232/0; NFE: 0.25%	243.0/24/0	tol	poly	PD	14.8	3.81	C0	poly	2+	undet	DM?/CM130987	inconclusive[a]; inconsistent[b]: ?/+?(1), +/?(1)	confl.: B(2), LB(8), VUS(2)	high MAF in NFE/Flossies; benign *in silico* prediction consensus (BP4); co-occurrence with multiple pathogenic variants [61,81] (BP2); functional studies implicate a possible deleterious effect but clinical relevance remains unclear [82]	(LIKELY) BENIGN
72970 (123/16.5/55.28)	*CDH1*	c.150C>A	het	syn	E2	chr16:68772301	rs786201262	p.R50=	IPR014868	—	—	—	0.6805/1/0	—	na	na	na	na	na	na	poly	na	na	not listed	not listed	confl.: LB(3), VUS(1)	absent in ExAC population database/MAF within pathogenic range (PM2); *in silico* predicted CSAS deactivation[2] (PP3); inconsistent predictions in NCBI ClinVar; insufficient evidence	VUS
80688 (150/17.3/39.33)	*CDH1*	c.261G>T ‡	het	miss	E3	chr16:68835670	—	p.R87S	IPR014868	—	—	—	—	—	del	path	B	21.7	-0.13	C0	prob path	4+	undet	not listed	not listed	not listed	novel	VUS
53539 (68/14.5/55.88)	*CDH1*	c.303C>T	het	syn	E3	chr16:68835712	rs150789339	p.Y101=	IPR014868	0.04	0.01	4.119/5/0	7.582/21/0; AFR: 0.07%	40.5/0/0	na	na	na	na	na	na	poly	na	na	not listed	not listed	*(L)B*	no evident splicing effect *in silico* (BP7); consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
76771 (1019/24.0/50.44)	*CDH1*	c.322A>G	het	miss	E3	chr16:68835731	rs587778172	p.R108G	IPR014868	—	—	2.472/3/0	2.033/5/0	—	tol	poly	B	13.2	1.66	C0	path	1+	B	not listed	not listed	unc.	MAF within pathogenic range (PM2); *in silico* predicted CSAS deactivation[2] (PP3); insufficient evidence	VUS
41653 (74/14.8/47.30) **64721** (295/19.6/62.71) **68567** (282/19.6/53.55) **72824** (236/19.0/48.31) **72981** (215/18.6/49.30) **80686** (84/15.3/54.76)	*CDH1*	c.345G>A	het	syn	E3	chr16:68835754	rs1801023	p.T115=	IPR014868	0.26	0.42	391.9/475/4	358.0/991/5; ASJ: 1.08%	587.0/54/1	na	na	na	na	na	na	poly	na	na	not listed	inconsistent[a]: ?/.(2), -/.(2); probably neutral[b]: ?/-?(1)	*(L)B*	very high MAF in population databases/Flossies; no evident splicing effect *in silico* (BP7); consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
71620 (179/18/48.04)	*CDH1*	c.671G>A	het	miss	E5	chr16:68842735	rs201511530	p.R224H	IPR014868	—	—	3.311/4/0	3.257/8/0	10.1/1/0	tol	poly	B	4.8	-2.90	C0	poly	0+	B	not listed	inconclusive[a]	*unc.*	MAF within pathogenic range (PM2); consistently classified as "VUS" in NCBI ClinVar; CR: patient affected with prostate cancer [15]; insufficient evidence	VUS
64721 (216/18.6/53.24)	*CDH1*	c.892G>A	het	miss	E7	chr16:68845646	rs142822590	p.A298T	IPR002126	0.04	0.02	53.54/65/0	41.13/114/0; SAS: 0.13%	60.7/6/0	del	path	PrD	22.0	5.19	C0	path	6+	dam	DM/CM041745	inconsistent[a]: +/.(1), -/.(1); inconsistent[b]: ?/+?(2), +/?(1)	confl.: B(1), LB(1), VUS(4)	(somewhat) high MAF in SAS/Flossies; deleterious in silico prediction consensus (PP3); conflicting functional and structural analyses [83-88]; inconsistent classification in LSDB and NCBI ClinVar; CR: patient affected with diffuse gastric cancer (Dx 36), FH: 2 relatives with gastric cancer (D/O 32; Dx 33), seg n/d [85]; patient affected with concurrent bilateral BC and endometrial adenocarcinoma (concomitant pathogenic TP53 variant) [89]	VUS
47171 (467/21.3/20.34) **74993** (326/20.0/42.94)	*CDH1*	c.957T>A	het	syn	E7	chr16:68845711	rs549252135	p.I319=	IPR002126	—	—	8.239/1/0	1.804/5/0	—	na	na	na	na	na	na	poly	na	na	not listed	not listed	*LB*	MAF within pathogenic range (PM2); *in silico* predicted CSAS deactivation[2] (PP3); consistently classified as "likely benign" in NCBI ClinVar; conflicting/insufficient evidence	VUS
67601 (326/20.0/43.56)	*CDH1*	c.1273G>A	het	miss	E9	chr16:68847351	rs570930882	p.V425I	IPR002126	0.04	—	1.647/2/0	3.246/9/0	—	tol	poly	B	8.7	4.09	C0	poly	1+	B	DM?/CM1516921	not listed	confl.: B(1), VUS(2)	MAF within pathogenic range (PM2), inconsistent classification in NCBI ClinVar (predominantly classified as "VUS"); insufficient evidence	VUS
73799 (290/19.5/46.9)	*CDH1*	c.1297G>A	het	miss	E9	chr16:68847375	rs199886166	p.D433N	IPR002126	—	—	2.471/3/0	4.873/12/0	—	tol	poly	B	15.0	2.84	C0	poly	1+	B	DM?/CM11011	inconclusive[a]	confl.: LB(1), VUS(6)	MAF within pathogenic range (PM2); inconsistently classified in NCBI ClinVar (predominantly classified as "VUS"); CR: BC-affected female (Dx 41), FH: lung cancer/mouth cancer mat, intestinal cancer pat, seg n/d [90]; females affected with BC [62] and gastric adenocarcinoma (Dx 70) [91]; classified as "VUS" in [92]; insufficient/conflicting evidence	VUS
41653 (107/15.9/47.66) **53539** (106/16.2/40.57) **70233** (108/16.1/46.30) **70375** (186/18.0/41.94) **73877** (138/17.1/44.20) **75407** (324/20.0/40.12)	*CDH1*	c.1680G>C	het	syn	E11	chr16:68853297	rs35741240	p.T560=	IPR002126	0.18	0.29	372.3/452/3	333.7/925/4; NFE: 0.45%; SAS: 0.69%	617.0/57/0	na	na	na	na	na	na	poly	na	na	not listed	inconsistent[a]: -/.(2), -?/.(3); probably neutral[b]: ?/-?(1)	*(L)B*	high to very high MAF in NFE/SAS/Flossies, gnomAD: 4 homozygous individuals; no evident splicing effect *in silico* (BP7); consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
65300 (459/21.3/53.16) **69644** (382/20.5/51.05) **72013** (204/18.3/44.12) **72981** (492/21.4/44.51) **74997** (482/21.3/52.70) **76028** (402/20.8/49.50)	*CDH1*	c.1774G>A	het	miss	E12	chr16:68855966	rs35187787	p.A592T	IPR002126	0.12	0.63	321.2/390/1	322.1/893/4; NFE: 0.48%; SAS: 0.33%	759.0/65/0	del	na	B	15.5	5.56	C0	poly	2+	undet	DM?/CM994192	probably neutral[a]: -?/.(2); probably pathogenic[b]: ?/+?(2), +/?(1)	*(L)B*	high to very high MAF in NFE/SAS/Flossies, gnomAD: 4 homozygous individuals; consistently classified as "(likely) benign" in NCBI ClinVar	(LIKELY) BENIGN
74846 (427/21.0/38.41)	*CDH1*	c.2165-12C>G	het	nonc	I13	chr16:68862065	rs760834250	nonc	—	—	—	0.8236/1/0	0.4064/1/0	—	na	na	na	na	na	na	na	na	na	not listed	not listed	LB	MAF within pathogenic range (PM2); *in silico* predicted deactivation of canonical SAS[2] (PP3); insufficient evidence	VUS
70337 (136/16.9/55.88)	*CDH1*	c.2177T>C	het	miss	E14	chr16:68862089	—	p.L726P	—	—	—	—	—	—	del	path	PrD	22.5	6.07	C25	path	7+	dam	not listed	not listed	not listed	novel	VUS
67197 (206/18.5/50.00) **71694** (175/17.9/58.29) **70976** (410/20.8/40.00) **72168** (252/19.0/51.98)	*CDH1*	c.2292C>T	het	syn	E14	chr16:68862204	rs61747636	p.D764=	IPR000233	0.18	0.28	154.1/187/0	166.7/462/0; NFE: 0.23%; SAS: 0.27%	344.0/30/0	na	na	na	na	na	na	poly	na	na	not listed	inconsistent[a]: ?/.(3), -?/.(4); inconclusive[b]: -?/+?(1)	*(L)B*	high MAF in NFE/SAS/Flossies; no evident splicing effect *in silico* (BP7); consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
70207 (197/18.2/44.67)	*CDH1*	c.2399G>A	het	miss	E15	chr16:68863660	rs370345996	p.R800H	IPR000233	—	0.01	1.657/2/0	1.626/4/0	10.1/1/0	del	path	PrD	34.0	6.05	C0	path	6+	dam	not listed	not listed	unc.	MAF within pathogenic range (PM2); deleterious *in silico* prediction consensus (PP3); consistently classified as "VUS" in NCBI ClinVar; CR: patient affected with intestinal-type gastric cancer (Dx 32), FH: gastric cancer (Dx 46), seg n/d (classified as "VUS") [81]; patient affected with "advanced cancer" (classified as "VUS") [93]	VUS
62239 (298/19.7/56.04)	*CDH1*	c.2439+10C>T	het	nonc	I15	chr16:68863710	rs35236080	nonc	—	—	0.16	55.78/67/0	51.41/142/1; NFE: 0.10%	—	na	na	na	na	na	na	na	na	na	not listed	probably neutral[a]:-?/.(2)	*(L)B*	high MAF in NFE, gnomAD: 1 homozygous individual; consistently classified as "likely benign" in NCBI ClinVar (BP6); *in silico* predicted *de novo* CSDS[2] (PP3), no functional splicing studies published yet	(LIKELY) BENIGN
75549 (212/18.6/48.11)	*CDH1*	c.2440-6C>G	het	nonc	I15	chr16:68867187	rs139757930	nonc	—	0.08	0.30	179.8/218/1	163.4/453/1; NFE: 0.17%; ASJ: 0.72%	—	na	na	na	na	na	na	na	na	na	DM?/CS071158	pathogenic[c] +/.(1); inconclusive[b]: +/-?(1)	*(L)B*	high to very high MAF in NFE/ASJ, gnomAD: 1 homozygous individual; functional studies show no consistent splicing effect [46,94-95]; consistently classified as "(likely) benign" in NCBI ClinVar	(LIKELY) BENIGN
66151 (183/18.0/44.26)	*CDH1*	c.2520C>T	het	syn	E16	chr16:68867273	rs140328601	p.S840=	IPR000233	0.02	0.01	32.95/40/0	37.87/105/0; AFR: 0.17%; AMR: 0.11%	70.8/2/0	na	na	na	na	na	na	poly	na	na	not listed	inconclusive[a]	*(L)B*	high MAF in AFR/AMR; no evident splicing effect *in silico* (BP7); consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
68859 (265/19.3/40.75)	*CHEK2*	c.319+7C>A	het	nonc	I2	chr22:29130384	—	nonc	—	—	—	—	0.4067/1/0	—	na	na	na	na	na	na	na	na	na	not listed	not listed	*(L)B*	no evident splicing *in silico*[2] (BP7); consistently classified as "likely benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
67184 (552/21.8/50.91) **75475** (457/21.2/50.55)	*CHEK2*	c.320-5T>A	het	nonc	I2	chr22:29121360	rs121908700	nonc	—	0.08	0.05	52.06/63/0	54.61/151/1; ASJ: 0.39%	10.1/0/0	na	na	na	na	na	na	na	na	na	DM?/CS086685	VUS[a]: ?/. (1)	confl.: B(1), LB(3), VUS(3)	case-control study implicate slight elevated BC risk [96]; functional studies implicate leaky splicing[2] [46]	HYPOMORPHIC

Variant Specification										Population Databases					Computational Evidence									Gene/Disease specific Databases			Final Assessment of Variant Pathogenicity	
Carrier ID (quality parameters of the individual variant: Cov./Score/SB)	**Gene**	**Mutation HGVS ¥**	**het/ hom**	**Function**	**Loc.**	**Genomic Pos. (hg19)**	**SNP db_xref**	**Predicted AA Change**	**InterPro domain ID (v.67.0)**	**1000G** [%]	**ESP6500** [%] EurAm	**ExAC v0.3** frequ¶/allele count/# hom	**gnomAD v2.0.2** frequ¶/allele count/# hom	**FLOSSIES** frequ¶ / allele count (Eur)/# hom	**SIFT v6.2.0 †**	**MutTaster v2013†**	**PolyPhen-2 v2.2.2†** HumVar	**CADD Phred**	**GERP+ +_RS**	**Align-GVGD v2007†§**	**UMD predictor**	**Prediction CONSENSUS[/**		**HGMD PRO v2017.3: variant class/acc.**	**Locus specific databases (LSDB, assessed by the LOVD)$**	**NCBI-ClinVar£**	**Rationale for Classification (ACMG evidence category if meeting)**	**final CLASSIFICATION**
73184 (661/22.4/44.33) **73281** (427/20.9/50.59)	*CHEK2*	c.349A>G	het	miss	E3	chr22:29121326	rs28909982	p.R117G	IPR000253	—	0.01	13.2/16/0	11.19/31/0	20.2/2/0	del	path	PrD	26.2	4.81	C65	path	7+	dam	DM/CM023898	inconclusive[a]; inconsistent[b]: +?/?(3), ?/?(1)	confl.: LP(5), P(2), VUS(1)	established recurrent pathogenic variant associated with BC [97]	PATHOGENIC
68159 (291/19.5/50.52)	*CHEK2*	c.538C>T	het	miss	E4	chr22:29121019	rs77130927	p.R180C	IPR000253	0.20	0.13	136.0/165/0	94.17/261/0	91.1/9/0	del	path	B	23.6	5.87	C0	prob poly	4+	undet	DM/CM030417	inconclusive[e]; inconsistent[b]: +/?(1), ?/?(1)	confl.: B(2), LB(2), VUS(5)	case-control studies implicate slight BC risk elevation [97]	HYPOMORPHIC
64604 (121/16.6/46.28)	*CHEK2*	c.715G>A	het	miss	E6	chr22:29107974	rs121908702	p.E239K	IPR000719	—	0.02	8.242/10/0	8.662/24/0	20.2/2/0	tol	path	PrD	20.5	5.27	C0	prob path	5+	undet	DM/CM030421	inconsistent[b]: ?/?(1), +?/?(1)	*unc.*	mutational hot spot (protein kinase domain) (PM1); functional studies show a potential deleterious impact [98-99]; consistently classified as "VUS" in NCBI ClinVar; no association with increased risk for BC, OC, and prostate cancer in case-control study [97]; conflicting evidence	VUS
70337 (88/15.5/36.36)	*CHEK2*	c.846+4_846+7 del	het	nonc	I7	chr22:29105987	rs764884641	nonc	—	—	—	2.026/2/0	2.93/8/0	—	na	na	na	na	na	na	na	na	na	DM/CD119707	not listed	*unc.*	MAF within pathogenic range (PM2); *in silico* predicted deactivation of canonical SAS[2] (PP3); consistently classified as "VUS" in NCBI ClinVar; CR: patient with individual and familial BC history (considered as "deleterious"), seg n/d [100]	VUS
73325 (330/20.0/46.67) **74925** (659/22.5/46.89)	*CHEK2*	c.1336A>G	het	miss	E12	chr22:29091154	rs121908705	p.N446D	IPR000719	—	—	5.788/7/0	6.863/19/0	20.2/2/0	tol	poly	B	15.7	4.86	C0	prob poly	1+	B	DM?/CM1511948	not listed	*unc.*	MAF within pathogenic range (PM2); mutational hot spot (protein kinase domain) (PM1); consistently classified as "VUS" in NCBI ClinVar; CR: BC-affected female (Dx 59), FH: BC [46]; rectal cancer affected female (Dx 38), MMR proficient [64]; patients affected with (early-onset) BC and NHL [101-103]	VUS
72814 (415/20.8/40.00)	*CHEK2*	c.1421G>A	het	miss	E13	chr22:29090060	rs121908706	p.R474H	IPR000719	—	0.02	7.05/8/0	7.304/19/0	10.1/1/0	del	path	PrD	35.0	5.46	C25	prob path	7+	dam	DM/CM1511949	not listed	*unc.*	MAF within pathogenic range (PM2); mutational hotspot (protein kinase domain) (PM1); deleterious *in silico* prediction consensus (PP3); consistently classified as "VUS" in NCBI ClinVar; CR: two TNBC-affected females [104]; patients affected with NHL and stomach cancer [103,105]	VUS
68618 (71/14.8/42.25)	*CHEK2*	c.1525C>T	het	miss	E14	chr22:29085140	rs587780179	p.P509S	—	—	—	—	8.294/19/0; NFE: 0.02%	30.35/3/0	poly	tol	B	3.1	-2.34	C0	poly	0+	B	not listed	probably neutral[a]: -?/?(2); probably neutral[b]: -?/?(1);	*unc.*	MAF absent from ExAC database (PM2); consistently classified as "VUS" in NCBI ClinVar; co-occurrence with deleterious *BARD1* variant [106] (BP2?); CR: male ASJ affected with prostate cancer [107]; two CRC-affected individuals suspected of LS [61]; conflicting/insufficient evidence	VUS
75150 (571/22.1/51.66)	*CHEK2*	c.1556G>T	het	miss	E15	chr22:29083961	rs587780180	p.R519L	—	—	—	23.15/25/0	20.41/53/0	10.1/1/0	del	path	PD	23.5	-2.75	C0	path	5+	undet	not listed	inconclusive[e]	*unc.*	consistently classified as "VUS" in NCBI ClinVar; CR: one early-onset BC case (Dx <45) [101]; insufficient evidence	VUS
74656 (433/20.8/48.04)	*CHEK2*	c.*2dup	het	nonc	3'UTR	chr22:29083883	rs749257861	nonc	—	—	0.02	—	—	—	na	na	na	na	na	na	na	na	na	not listed	not listed	unc.	3'UTR; insufficient evidence	VUS
75407 (391/20.6/58.82)	*MSH2*	c.128A>G	het	miss	E1	chr2:47630458	rs17217723	p.Y43C	IPR007695	—	0.01	2.778/2/0	7.024/18/0	na	del	path	PrD	17.0	5.49	C55	path	6+	dam	DM/CM068366	**3-VUS;** 3-VUS[e]	**unc.**	classified as "class 3-VUS" by expert panel; MAF within pathogenic range (PM2); deleterious *in silico* prediction consensus (PP3)	VUS
70798 (288/19.6/48.96) **71654** (114/16.4/42.11)	*MSH2*	c.339G>A	het	syn	E2	chr2:47635667	rs35898375	p.K113=	IPR007695	0.10	0.52	273.1/331/2	283.3/785/3	na	na	na	na	na	na	na	poly	na	na	not listed	**1-benign;** (probably) neutral[a]: -/.(4), -?/.(1); neutral[d]: -/-; 1-neutral[e]	**B**	classified as "class 1-benign" by expert panel; no evident splicing effect *in silico* (BP7)	(LIKELY) BENIGN
73282 (653/22.4/45.02)	*MSH2*	c.435T>G	het	miss	E3	chr2:47637301	rs63750124	p.I145M	—	0.02	0.06	33.77/41/0	29.58/82/0	na	tol	poly	B	16.9	3.36	C0	poly	1+	B	DM?/CM011416	**3-VUS;** inconclusive[a]; inconclusive[d]; 3-VUS[e]	**unc.**	classified as "class 3-VUS" by expert panel	VUS
73949 (198/18.3/51.52)	*MSH2*	c.775C>T	het	miss	E4	chr2:47639682	rs587781294	p.P259S	IPR007860	—	—	0.8276/1/0	0.4066/1/0	na	tol	path	PD	22.9	4.65	C0	path	5+	undet	DM?/CM117423	not listed	*unc.*	MAF within pathogenic range (PM2); mutational hot spot (connector domain) (PM1); consistently classified as "VUS" in NCBI ClinVar; CR: two LS families (classified as "VUS") seg n/d [108]; patient meeting modified Amsterdam criteria [109]; BC-affected female [62]	VUS
74925 (397/20.7/34.01)	*MSH2*	c.984C>T	het	syn	E6	chr2:47643476	rs4987189	p.A328=	IPR007696	0.78	0.14	543.3/659/14	458.2/1270/26; SAS: 3.01%	na	na	na	na	na	na	na	poly	na	na	not listed	**2-likely benign;** (probably) neutral[a]: -/.(3), -?/.(3); probably neutral[d]: -?/-?; 1-neutral[e]	**LB**	classified as "class 2-likely benign" by expert panel; very high MAF in population databases, gnomAD: 26 homozygous individuals	(LIKELY) BENIGN
70798 (1628/25.5/54.30)	*MSH2*	c.1131A>G	het	syn	E7	chr2:47656935	rs181852377	p.Q377=	IPR007696	0.02	0.01	7.424/9/0	4.873/12/0	na	na	na	na	na	na	na	poly	na	na	not listed	not listed	*(L)B*	no evident splicing *in silico*[2] (BP7); consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
68068 (54/13.7/50.00) **62001** (60/14.2/50.00) **72157** (51/13.7/43.14)	*MSH2*	c.1387-8G>T	het	nonc	I8	chr2:47690162	rs187525243	nonc	—	0.10	0.19	194.4/235/0	204.0/565/2; NFE: 0.19%; ASJ: 0.95%	na	na	na	na	na	na	na	na	na	na	not listed	**2-likely benign;** (probably) neutral[a]: -?/.(3), -/.(1); probably neutral[d]: -?/-?; 1-neutral[e]	**LB**	classified as "class 2- likely benign" by expert panel; high to very high MAF in population databases; functional studies show no splicing effect [110] (BS3)	(LIKELY) BENIGN
72266 (599/22.1/50.58)	*MSH2*	c.1489A>G	het	miss	E9	chr2:47690272	rs755501968	p.I497V	IPR007696 IPR007861	—	—	1.65/2/0	0.8125/2/0	na	tol	poly	B	0.1	-6.90	C0	prob poly	0+	B	not listed	not listed	confl.: LB(1), VUS(2)	MAF within pathogenic range (PM2); *in silico* predicted *de novo* CSDS[2] (PP3); conflicting classification in NCBI ClinVar; insufficient/conflicting evidence	VUS
69413 (96/15.7/59.38) **73463** (34/12.2/29.41)	*MSH2*	c.1662-9G>A	het	nonc	I10	chr2:47698095	rs17218356	nonc	—	0.68	0.03	157.3/187/2	168.3/465/4; AFR: 1.61%	na	na	na	na	na	na	na	na	na	na	not listed	**1-benign;** neutral[a]: -/.(2); neutral[d]: -/-; 1-neutral[e]	**B**	classified as "class 1-benign" by expert panel; very high MAF in AFR, gnomAD: 4 homozygous individuals	(LIKELY) BENIGN
35678 (120/16.5/44.17) **55035** (187/18.1/51.34) **73800** (218/18.6/50.46) **77428** (246/19.0/41.46)	*MSH2*	c.1666T>C	het	syn	E11	chr2:47698108	rs61756466	p.L556=	IPR007696 IPR007861	0.22	0.80	452.4/544/3	430.5/1189/6; NFE: 0.70%	na	na	na	na	na	na	na	poly	na	na	DM?/CM036021	**1-benign;** (probably) neutral[a]: -/.(2),-?/.(2); neutral[d]: -/-; 1-neutral[e]	**B**	classified as "class 1-benign" by expert panel; very high MAF in NFE, gnomAD: 6 homozygous individuals; no evident splicing effect *in silico* (BP7);	(LIKELY) BENIGN
60824 (175/17.9/42.29) **74116** (263/19.2/40.68)	*MSH2*	c.1681G>A	het	miss	E11	chr2:47698123	rs63750328	p.E561K	IPR007696 IPR007861	—	—	1.657/2/0	1.631/4/0	na	tol	path	B	25.1	5.86	C0	prob path	4+	undet	DM?/CM068371	**3-VUS;** 3-VUS[e]	**unc.**	classified as "class 3-VUS" by expert panel; MAF within pathogenic range (PM2); mutational hot spot (clamp) (PM1)	VUS
62001 (67/14.3/40.30) **68068** (72/14.6/40.28) **72157** (30/11.6/50.00)	*MSH2*	c.1737A>G	het	syn	E11	chr2:47698179	rs61756467	p.K579=	IPR007696	0.10	0.19	193.0/233/0	204.1/565/2	na	na	na	na	na	na	na	poly	na	na	not listed	**2-likely benign;** neutral[a]: -/.(1); neutral[d]: -?/-?; 1-neutral[e]	**LB**	classified as "class 2-likely benign" by expert panel; high MAF in population databases, gnomAD: 2 homozygous individuals; functional studies show no splicing effect [110-111] (BS3)	(LIKELY) BENIGN
65761 (542/21.7/54.80)	*MSH2*	c.1787A>G	het	miss	E12	chr2:47702191	rs41295288	p.N596S	IPR007696	—	0.02	25.58/31/0	29.58/82/0	na	tol	path	B	21.0	5.61	C0	path	4+	undet	DM?/CM994603	**3-VUS**; probably neutral[a]: -?/.(1); inconclusive[d]; 3-VUS[e]	**unc.**	classified as "class 3-VUS" by expert panel; mutational hot spot (lever domain) (PM1)	VUS
76918 (188/18.2/48.40)	*MSH2*	c.2043A>T	het	miss	E13	chr2:47703543	rs730881763	p.Q681H	IPR000432	—	—	0.8242/1/0	0.4061/1/0	na	del	path	PrD	15.7	-9.68	C15	path	5+	undet	not listed	not listed	*unc.*	MAF within pathogenic range (PM2); mutational hot spot (ATPase domain) (PM1); consistently classified as "VUS" in NCBI ClinVar database; insufficient evidence	VUS
75894 (139/17.1/65.47)	*MSH2*	c.2205C>T	het	syn	E13	chr2:47703705	rs533553381	p.I735=	IPR000432	0.26	—	152.4/185/2	133.1/369/5; SAS: 1.17%	na	na	na	na	na	na	na	poly	na	na	not listed	**inconclusive: ./?(1)**	confl.: B(6), LB(3), VUS(1)	very high MAF in SAS, gnomAD: 5 homozygous individuals; no evident splicing effect *in silico* (BP7); predominantly classified as "(likely) benign" in NCBI ClinVar	(LIKELY) BENIGN
75711 (262/19.3/44.66)	*MSH2*	c.2595C>T	het	syn	E15	chr2:47707971	rs547695133	p.I865=	—	0.04	—	12.35/15/0	11.78/29/0	na	na	na	na	na	na	na	poly	na	na	not listed	not listed	*LB*	no evident splicing effect *in silico*[2] (BP7); consistently classified as "likely benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
68067 (178/17.8/48.88)	*MLH1*	c.221A>T	het	miss	E3	chr3:37042459	rs751894165	p.D74V	IPR002099 IPR003594	—	—	0.8246/1/0	0.4062/1/0	na	del	path	B	23.5	3.46	C0	path	5+	undet	not listed	not listed	*unc.*	MAF within pathogenic range (PM2); mutational hot spot (histidine kinase, ATPase domain) (PM1); not yet reviewed by expert panel, consistently classified as "VUS" in NCBI ClinVar; insufficient evidence	VUS
41652 (370/20.3/45.68)	*MLH1*	c.277A>G	het	miss	E3	chr3:37042515	rs78852840	p.S93G	IPR002099 IPR003594	—	0.01	3.297/4/0	2.165/6/0	na	del	poly	B	16.1	0.36	C0	path	2+	undet	DM?/CM981289	**3-VUS;** inconclusive[a]; inconclusive[d]; 3-VUS[e]	**unc.**	classified as "class 3-VUS" by expert panel; MAF within pathogenic range (PM2); mutational hot spot (histidine kinase; ATPase domain) (PM1); functional studies show no obvious deleterious effect[2] [112-114]	VUS

Variant Specification										Population Databases					Computational Evidence										Gene/Disease specific Databases			Final Assessment of Variant Pathogenicity	
Carrier ID (quality parameters of the individual variant: Cov./Score/SB)	**Gene**	**Mutation HGVS ¥**	**het/ hom**	**Function**	**Loc.**	**Genomic Pos. (hg19)**	**SNP db_xref**	**Predicted AA Change**	**InterPro domain ID (v.67.0)**	**1000G** [%]	**ESP6500** [%] EurAm	**ExAC v0.3** frequ¶/allele count/# hom	**gnomAD v2.0.2** frequ¶/allele count/# hom	**FLOSSIES** frequ¶ / allele count (Eur)/# hom	**SIFT v6.2.0 †**	**MutTaster v2013†**	**PolyPhen-2 v2.2.2†** HumVar	**CADD Phred**	**GERP+ +_RS**	**Align-GVGD v2007†§**	**UMD predictor**	**Prediction CONSENSUS[/**		**HGMD PRO v2017.3: variant class/acc.**	**Locus specific databases (LSDB, assessed by the LOVD)$**	**NCBI-ClinVar£**	**Rationale for Classification (ACMG evidence category if meeting)**	**final CLASSIFICATION**
72876 (111/16.3/56.76)	*MLH1*	c.303T>G	het	syn	E3	chr3:37042541	rs4647220	p.G101=	IPR002099 IPR003594	0.64	—	273.5/331/5	227.7/631/11; SAS: 2.00%	na	na	na	na	na	na	na	poly	na	na	not listed	**2-likely benign**; probably neutral[a]: -?/-?; probably neutral[d]: ?/-?; 3-VUS[e]	**LB**	classified as "class 2-likely benign" by expert panel; very high MAF in SAS, gnomAD: 11 homozygous individuals	(LIKELY) BENIGN
73215 (229/18.7/48.47)	*MLH1*	c.652T>C	het	miss	E8	chr3:37053565	rs750650349	p.S218P	IPR002099 IPR013507	—	—	0.8244/1/0	0.4067/1/0	na	tol	path	PD	22.0	5.76	C0	path	5+	undet	not listed	not listed	*unc.*	MAF within pathogenic range (PM2); consistently classified as "VUS" in NCBI ClinVar; insufficient evidence	VUS
4162 (233/18.9/47.21)	*MLH1*	c.702G>A	het	syn	E9	chr3:37055947	rs35908749	p.E234=	IPR013507	0.02	0.07	38.79/46/0	39.72/110/0	na	na	na	na	na	na	na	prob poly	na	na	not listed	**2-likely benign**; probably neutral[a]: -?/-?; probably neutral[d]: ?/-?; 1-neutral[e]	**LB**	classified as "class 2-likely benign" by expert panel; functional studies showed no impact on mRNA processing or stability [115] (BS3)	(LIKELY) BENIGN
68159 (208/18.4/56.73)	*MLH1*	c.790+10A>G	het	nonc	I9	chr3:37056045	rs182733777	nonc	—	0.02	0.22	194.4/220/1	186.2/512/2; NFE: 0.36%	na	na	na	na	na	na	na	na	na	na	DM?/CS086116	**1-benign**; neutral[a]: -/-; neutral[d]: -/-; 1-neutral[e]	**B**	classified as "class 1-benign" by expert panel; high MAF in NFE, gnomAD: 2 homozygous individuals; no evident splicing effect *in silico* (*BP7*)	(LIKELY) BENIGN
63624 (304/19.8/57.89)	*MLH1*	c.1217G>A	het	miss	E12	chr3:37067306	rs41294980	p.S406N	—	0.04	0.20	93.08/113/0	89.96/249/0; NFE: 0.14%	na	tol	poly	B	12.8	2.94	C0	poly	1+	B	DM?/CM014585	**1-benign**; neutral[a]: -/-; neutral[d]: -/-; 1-neutral[e]	**B**	classified as "class 1-benign" by expert panel; high MAF in NFE	(LIKELY) BENIGN
66137 (134/16.8/52.24)	*MLH1*	c.1401C>T	het	syn	E12	chr3:37067490	rs587778910	p.S467=	—	—	—	—	0.8128/2/0	na	na	na	na	na	na	na	prob path	na	na	not listed	**3-VUS**; inconclusive[a]	**unc.**	classified as "class 3-VUS" by expert panel; absent from ExAC population database/MAF within pathogenic range (PM2); no evident splicing effect *in silico* [2] (BP7)	VUS
73326 (170/17.7/61.18)	*MLH1*	c.1587C>T	het	syn	E14	chr3:37081705	rs767089159	p.S529=	IPR032189	—	—	0.8244/1/0	0.4066/1/0	na	na	na	na	na	na	na	poly	na	na	not listed	not listed	*LB*	no evident splice effect *in silico* [2] (BP7); consistently classified as "likely benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
67184 (848/23.2/55.31)	*MLH1*	c.1732G>A	het	miss	E16	chr3:37089010	rs863224635	p.E578K	IPR032189	—	—	—	0.4065/1/0	na	tol	path	B	23.1	5.56	C0	path	4+	undet	not listed	not listed	*unc.*	absent from ExAC population database/MAF withinin pathogenic range (PM2); consistently classified as "VUS" in NCBI ClinVar database; no significant splice effect *in silico* [2] (last exonic base at splice junction) (BP7); insufficient/conflicting evidence	VUS
63315 (403/20.7/48.64) **71229** (200/18.2/47.00)	*MLH1*	c.1852_1853 delinsGC	het	miss	E16	chr3:37089130	rs63750449	p.K618A	IPR032189	—	—	na	na	na	del	na	PrD	na	na	C35	na	3+	undet	DM?/CX973308	**1-benign**; neutral[a]: -/-(1), -/.(1); neutral[d]: -/-; 1-neutral[e]	**B**	classified as "class 1-benign" by expert panel; most recent large case-control study implicate no association with CRC risk [116]	(LIKELY) BENIGN
67184 (403/20.6/39.95)	*MLH1*	c.1935C>G	het	miss	E17	chr3:37090046	rs863224636	p.N645K	IPR032189	—	—	—	—	na	tol	path	B	20.5	2.06	C0	path	4+	undet	not listed	not listed	unc.	absent from population databases (PM2); mutational hot spot (interaction domain) (PM1); *in silico* predicted *de novo* CSDS[2] (PP3); insufficient evidence	VUS
74409 (224/18.7/55.8)	*MLH1*	c.2103+9G>A	het	nonc	I18	chr3:37090517	—	nonc	—	—	—	—	—	na	na	na	na	na	na	na	na	na	na	not listed	not listed	LB	absent from population databases (PM2); no evident splicing effect *in silico* [2] (BP7); insufficient evidence	VUS
71655 (266/19.2/53.01) **72979** (187/18.1/43.32)	*MLH1*	c.2146G>A	het	miss	E19	chr3:37092019	rs35831931	p.V716M	IPR032189	0.04	0.21	122.1/148/0	126.4/350/1; NFE 0.20%; FIN: 0.16%	na	del	path	PD	22.1	5.41	C0	prob poly	5+	undet	DM?/CM981295	**1-benign;** (probably) neutral[a]: -?/.(2), -/.(3), -/-(1); neutral[d]: -/-; 1-neutral[e]	**B**	classified as "class 1-benign" by expert panel; high MAF in NFE, gnomAD: 1 homozygous individual	(LIKELY) BENIGN
71136 (124/16.7/37.9)	*MSH6*	c.73G>T	het	miss	E1	chr2:48010445	rs267608026	p.A25S	—	—	0.02	15.06/17/0	13.75/37/0	na	tol	poly	B	10.1	0.56	C0	poly	0+	B	DM?/CM0910272	**3-VUS**; inconclusive[a]; 1-neutral[e]	**unc.**	classified as "class 3-VUS" by expert panel; no significant association with CRC in case-control study [117]	VUS
66151 (223/18.7/44.39)	*MSH6*	c.178T>C	het	syn	E1	chr2:48010550	rs35819209	p.L60=	—	—	—	22.94/5/0	6.366/10/0	na	na	na	na	na	na	na	poly	na	na	not listed	**not classified: -?/.(2)**; inconclusive[a]	*(likely) B*	no evident splicing effect *in silico* (BP7); consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
65761 (92/15.7/44.57)	*MSH6*	c.660A>C	het	miss	E4	chr2:48025782	rs1800938	p.E220D	—	—	—	15.06/18/0	11.64/32/0	na	tol	path	B	18.2	3.44	C0	prob poly	2+	undet	not listed	**2-likely benign;** probably neutral[a]: -?/-?; 1-neutral[e]	**LB**	classified as "class 2-likely benign" by expert panel	(LIKELY) BENIGN
74115 (414/20.8/50.00) **76747** (376/20.4/51.06)	*MSH6*	c.663A>C	het	miss	E4	chr2:48025785	rs41557217	p.E221D	—	—	0.13	62.68/75/0	68.70/189/0; NFE: 0.11%	na	tol	poly	B	16.0	-0.72	C0	prob poly	0+	B	not listed	**3-VUS**; inconclusive[a]; 3-VUS[e]	**unc.**	classified as "class 3-VUS" by expert panel	VUS
75045 (348/20.3/44.25)	*MSH6*	c.942C>T	het	syn	E4	chr2:48026064	—	p.S314=	—	—	—	—	—	na	na	na	na	na	na	na	poly	na	na	not listed	not listed	LB	absent from population database (PM2); no evident splicing effect *in silico* [2] (BP7); insufficient evidence	VUS
74215 (309/19.8/50.16)	*MSH6*	c.998C>T	het	miss	E4	chr2:48026120	rs587781983	p.T333I	—	—	—	—	0.3228/1/0	na	del	path	B	22.0	4.41	C0	path	5+	undet	not listed	3-VUS[e]	*unc.*	absent from ExAC population database/MAF within pathogenic range (PM2); consistently classified as "VUS" in LSDB and NCBI ClinVar; CR: LS-affected patient (concomitant to a pathogenic *MLH1* variant) (classified as "VUS") [118]; insufficient evidence	VUS
75035 (376/20.5/48.4)	*MSH6*	c.1144C>T	het	miss	E4	chr2:48026266	rs587779207	p.H382Y	—	—	—	1.649/2/0	1.446/4/0	na	tol	poly	B	16.0	1.14	C0	prob poly	0+	B	not listed	**3-VUS**; inconclusive[a]	**unc.**	classified as "class 3-VUS" by expert panel; MAF within pathogenic range (PM2)	VUS
60770 (409/20.8/48.9)	*MSH6*	c.1186C>G	het	miss	E4	chr2:48026308	rs2020908	p.L396V	—	0.20	0.65	518.5/629/1	561.9/1556/9; NFE: 0.73%; FIN: 2.05%	na	tol	path	B	17.6	1.71	C0	poly	1+	B	FP/CM101608	**1-benign**; neutral[a]: -/.(6), -/-(1); neutral[d]: -/-; 1-neutral[e]	**B**	classified as "1-benign" by expert panel; very high MAF in NFE/FIN, gnomAD: 9 homozygous individuals; *in silico* predicted *de novo* CSAS[2] (PP3)	(LIKELY) BENIGN
67044 (186/18.0/40.86)	*MSH6*	c.1331T>C ‡	het	miss	E4	chr2:48026453	—	p.V444A	IPR007695	—	—	—	—	na	del	path	PD	21.2	5.15	C25	prob poly	6+	dam	not listed	not listed	not listed	novel	VUS
62976 (156/17.5/37.18)	*MSH6*	c.1449G>T	het	syn	E4	chr2:48026571	rs35590297	p.V483=	IPR007695	0.04	0.13	28.02/34/0	33.23/92/0	na	na	na	na	na	na	na	poly	na	na	not listed	**not classified: -?/- (1)**; inconsistent[a]: -?/.(4), ?/.(1); 3-VUS[e]	*(likely) B*	no evident splicing effect *in silico* (BP7); consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
41652 (100/15.8/55.00)	*MSH6*	c.1768C>T ‡	het	miss	E4	chr2:48026890	—	p.P590S	IPR007860	—	—	—	—	na	tol	path	B	18.3	3.30	C0	path	3+	undet	not listed	not listed	not listed	novel; no evident splicing effect *in silico* [2] (BP7)	VUS
53539 (62/14.3/41.94)	*MSH6*	c.1907C>G	het	miss	E4	chr2:48027029	—	p.T636S	IPR007860	—	—	—	—	na	tol	path	B	16.9	3.52	C0	prob poly	2+	undet	not listed	not listed	unc.	absent from population databases (PM2); insufficient evidence	VUS
53804 (174/17.8/48.28) **68185** (332/20.1/45.18) **72585** (149/17.2/48.32)[@] **73183** (227/18.8/41.85) **75600** (139/17.1/43.17)[@] **75739** (220/18.7/50.00)	*MSH6*	c.2633T>C	het	miss	E4	chr2:48027755	rs2020912	p.V878A	IPR007696	0.40	0.77	527.8/635/4	495.1/1369/10; NFE: 0.70%; ASJ: 1.01%	na	tol	path	B	7.8	1.99	C0	poly	1+	B	DM?/CM003461	**1-benign;** (probably) neutral[a]: -?/.(2), -/.(5), -/-(1); neutral[d]: -/-; 1-neutral[e]	**B**	classified as "class 1-benign" by expert panel; very high MAF in NFE, gnomAD: 10 homozygous individuals	(LIKELY) BENIGN
67514 (294/19.7/52.72)	*MSH6*	c.2883A>G	het	syn	E4	chr2:48028005	—	p.R961=	IPR007696 IPR007861	—	—	—	—	na	na	na	na	na	na	na	poly	na	na	not listed	not listed	*LB*	no evident splicing effect *in silico* [2] (BP7); consistently classified as "likely benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
75894 (243/18.9/43.21)	*MSH6*	c.3151G>A	het	miss	E4	chr2:48028273	rs576269342	p.V1051I	IPR007696	0.10	—	89.72/107/0	89.86/218/1; SAS: 0.69%	na	tol	path	B	17.9	4.18	C0	prob path	3+	undet	not listed	not listed	confl.: B(2), LB(1), VUS(2)	MAF very high in SAS, gnomAD: 1 homozygous individual; classified as "likely benign" by recent ClinVar submissions (SCV000166225.4; Invitae, Dec 2017/SCV000211312.12; GeneDx, Jan, 2018)	(LIKELY) BENIGN
73776 (641/22.3/51.48) **73878** (723/22.7/47.99) **76367** (264/19.3/43.56)	*MSH6*	c.3246G>T	het	syn	E4	chr2:48030632	rs3136351	p.P1082=	IPR007696	0.14	0.53	345.3/419/0	332.7/922/2; NFE: 0.56%	na	na	na	na	na	na	na	poly	na	na	not listed	**2- likely benign**; (probably) neutral[a]: -?/.(3), -/.(2), -?/-?(1); probably neutral[d]: -?/-?; 1-neutral[e]	**LB**	classified as "class 2-likely benign" by expert panel; very high MAF in NFE, gnomAD: 2 homozygous individuals; functional *ex vivo* studies show no splicing effect (BS3) (UMD database)	(LIKELY) BENIGN
74742 (321/20.0/57.32)	*MSH6*	c.3758T>A	het	miss	E8	chr2:48033454	rs202066386	p.V1253E	IPR000432	0.02	0.02	18.97/23/0	14.80/41/0	na	del	path	PrD	22.7	5.50	C65	path	7+	dam	DM?/CM148415	inconclusive[a]; 3-VUS[e]	*unc.*	deleterious *in silico* prediction consensus (PP3); consistently classified as "VUS" in NCBI ClinVar; CR: reported in a CRC-affected patient, MSS/IHC-proficient/*BRAF* V600E wild-type [119]; CRC-patient suspected of LS (classified as "VUS") [61]; patients affected with BC and renal cell cancer [15]; insufficient evidence	VUS
75579 (326/20.0/46.75)	*MSH6*	c.4001+12_4001+15 del	het	nonc	I9	chr2:48033792	rs267608132	nonc	—	—	0.13	—	122.1/330/0	na	na	na	na	na	na	na	na	na	na	not listed	**3-VUS**; inconsistent[a]: -/.(1), ?/?(1); 1-neutral[e]	**unc.**	classified as "class 3-VUS" by expert panel; no evident splicing *in silico* [2] (BP7)	VUS
70861 (293/19.6/34.47)	*MSH6*	c.4001+4_4001+8 dup	het	nonc	I9	chr2:48033793	rs587782853	nonc	—	—	—	3.575/4/0	3.712/10/0	na	na	na	na	na	na	na	na	na	na	not listed	inconsistent[a]: +?/?(1), -?/-(1) (**clinical class: likely benign**); 3-VUS[e]	confl.: B(1), LB(3), VUS(2)	clinical class in InSiGHT database: "likely benign" (BP6); no evident splicing *in silico* [2] (BP7);	(LIKELY) BENIGN

Variant Specification										Population Databases					Computational Evidence									Gene/Disease specific Databases			Final Assessment of Variant Pathogenicity	
Carrier ID (quality parameters of the individual variant: Cov./Score/SB)	**Gene**	**Mutation HGVS ¥**	**het/ hom**	**Function**	**Loc.**	**Genomic Pos. (hg19)**	**SNP db_xref**	**Predicted AA Change**	**InterPro domain ID (v.67.0)**	**1000G** [%]	**ESP6500** [%] EurAm	**ExAC v0.3** frequ¶/allele count/# hom	**gnomAD v2.0.2** frequ¶/allele count/# hom	**FLOSSIES** frequ¶ / allele count (Eur)/# hom	**SIFT v6.2.0 †**	**MutTaster v2013†**	**PolyPhen-2 v2.2.2†** HumVar	**CADD Phred**	**GERP+ +_RS**	**Align-GVGD v2007†§**	**UMD predictor**	**Prediction CONSENSUS⌈**		**HGMD PRO v2017.3: variant class/acc.**	**Locus specific databases (LSDB, assessed by the LOVD)$**	**NCBI-ClinVar£**	**Rationale for Classification (ACMG evidence category if meeting)**	**final CLASSIFICATION**
71230 (39/12.8/20.51)	*MSH6*	c.4002-8A>T	het	nonc	I9	chr2:48033910	rs778957100	nonc	—	—	—	—	—	na	na	na	na	na	na	na	na	na	na	not listed	not listed	*LB*	no evident splicing effect *in silico*[2] (BP7); consistently classified as "likely benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
75152 (286/19.8/53.85)	*MSH6*	c.4026G>A ‡	het	syn	E10	chr2:48033942	—	p.R1342=	—	—	—	—	—	na	na	na	na	na	na	na	poly	na	na	not listed	not listed	not listed	novel; *in silico* predicted CSAS deactivation[2] (PP3)	VUS
47172 (667/22.5/43.18)	*MSH6*	c.4068_4071dup	het	fs	E10	chr2:48033982	rs55740729	p.K1358Dfs*2	—	0.80	—	232.7/281/5	229.5/635/11; EAS: 3.2%	na	na	na	na	na	na	na	na	na	na	DM?/CI148414	**2-likely benign;** probably neutral[a]: -?/-?	**LB**	classified as "class 2-likley benign" by expert panel; very high MAF in EAS, gnomAD: 11 homozygous individuals; functional study show no deleterious effect (termination codon only two codons before normal stop) [120] (BS3)	(LIKELY) BENIGN
75580 (1306/24.8/37.60)	*MSH6*	c.4068G>A	het	syn	E10	chr2:48033984	rs192740549	p.L1356=	—	0.02	0.01	1.656/2/0	2.170/6/0	na	na	na	na	na	na	na	poly	na	na	not listed	not listed	*LB*	no evident splicing effect *in silico*[2] (BP7); consistently classified as "likely benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
72678 (244/18.8/45.90)	*PMS2*	c.-7T>C	het	nonc	5'UTR	chr7:6048657	rs199660792	nonc	—	—	0.01	13.45/16/0	15.57/43/0	na	na	na	na	na	na	na	na	na	na	DM?/CR1711499	not listed	confl.: LB(2), VUS(3)	5'-UTR; conflicting classification in NCBI ClinVar; CR: detected in 3 CRC-affected patients (classified as "VUS") [72]; insufficient evidence	VUS
64721 (75/14.8/56.00)	*PMS2*	c.23+10G>C	het	nonc	I1	chr7:6048618	rs192027828	nonc	—	0.10	0.31	189.3/226/2	179.4/496/2; NFE: 0.29%	na	na	na	na	na	na	na	na	na	na	not listed	**not classified: -?/.(1);** (probably) neutral[a]: -/.(2), -?/.(4)	*(likely) B*	high MAF in NFE, gnomAD: 2 homozygous individuals; no evident splicing effect *in silico* (BP7); consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
74658 (608/22.2/41.94)	*PMS2*	c.376C>G	het	miss	E5	chr7:6042245	rs774135207	p.H126D	IPR002099 IPR003594	—	—	—	—	na	tol	path	PrD	14.7	5.82	C15	path	5+	undet	not listed	not listed	not listed	absent from population databases (PM2); insufficient evidence	VUS
66138 (163/17.5/53.37)	*PMS2*	c.988+11T>C	het	nonc	I9	chr7:6031593	rs139969671	nonc	—	0.04	—	58.24/68/1	58.95/163/1; FIN: 0.47%	na	na	na	na	na	na	na	na	na	na	not listed	not listed	*B*	high to very high MAF in FIN, gnomAD: 1 homozygous individual; no evident splicing effect *in silico*[2] (BP7); consistently classified as "benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
63328 (490/21.4/48.57)	*PMS2*	c.1032G>A	het	syn	E10	chr7:6029543	—	p.L344L	IPR013507	—	—	—	—	na	na	na	na	na	na	na	poly	na	na	not listed	not listed	*LB*	no evident splicing *in silico*[2] (BP7); consistently classified as "benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
68968 (329/20.1/45.59) **70860** (334/20.1/50.00)	*PMS2*	c.1243G>A	het	miss	E11	chr7:6027153	rs138387687	p.V415M	—	0.02	0.02	24.1/29/0	15.88/44/0	na	tol	poly	B	9.6	1.60	C0	poly	0+	B	not listed	**not classified: -?/.(1)**; inconclusive[a]	confl.: LB(1), VUS(3)	not classified by InSiGHT, inconsistent classification in NCBI ClinVar (predominantly classified as "VUS"); CR: patients affected with pancreatic cancer [121] and pediatric acute megakaryoblastic leukemia [122]; HBOC-suspected female (classified as "VUS") [123]; OC-affected female, no LOH in tumour tissue [15]; insufficient evidence	VUS
71052 (53/13.5/64.15)	*PMS2*	c.1437C>G	het	miss	E11	chr7:6026959	rs63750685	p.H479Q	—	0.28	0.28	—	462.1/1281/8; NFE: 0.39%; FIN: 1.12%	na	tol	poly	B	2.1	2.17	C0	poly	1+	B	DM?/CM1612932	**3-VUS;** inconsistent[a]: ?/?(1), -/.(4), ?/.(2)	**unc.**	classified as "class 3-VUS" by expert panel	VUS
71742 (168/17.8/50.00) **74214** (275/19.3/65.82)	*PMS2*	c.1569C>G	het	syn	E11	chr7:6026827	rs141458772	p.S523=	—	0.08	0.29	508.8/612/15	621.5/1721/31; FIN: 4.13%; NFE: 0.42%	na	na	na	na	na	na	na	poly	na	na	not listed	**2-likely benign;** probably neutral[a]: ?/-?(1), -?/-?(1), -?/.(5)	**LB**	classified as "likely benign" by expert panel; no evident splicing effect *in silico* (BP7)	(LIKELY) BENIGN
73328 (308/19.8/44.48)	*PMS2*	c.1595A>G	het	miss	E11	chr7:6026801	—	p.H532R	—	—	—	—	—	na	tol	poly	B	0.0	-6.67	C0	poly	0+	B	not listed	not listed	*unc.*	absent from population databases (PM2); consistently classified as "VUS" by NCBI ClinVar database; insufficient evidence	VUS
70460 (482/21.4/42.74) **71145** (234/18.9/33.76) **76027** (560/21.9/51.43)	*PMS2*	c.1688G>T	het	miss	E11	chr7:6026708	rs63750668	p.R563L	—	0.30	1.00	581.3/703/2	638.0/1767/3; NFE: 0.91%	na	tol	poly	B	12.7	-0.27	C0	poly	0+	B	DM?/CM061908	**2-likely benign;** inconsistent[a]: -?/.(4), -/.(1), ?/.(1), +/.(1)	**LB**	classified as "class 2-likely benign" by expert panel; very high MAF in NFE, gnomAD: 3 homozygous individuals	(LIKELY) BENIGN
68068 (438/21.0/41.78)	*PMS2*	c.1711C>A	het	miss	E11	chr7:6026685	rs63750055	p.L571I	—	0.56	0.08	243.7/295/3	274.2/760/10; AFR: 2.11%	na	tol	poly	B	0.0	-3.33	C0	poly	0+	B	not listed	**2-likely benign**; inconsistent[a]: -?/.(4), -/.(1), ?/.(1), +/.(1)	**LB**	classified as "class 2-likely benign" by expert panel; very high MAF in AFR, gnomAD: 10 homozygous individuals	(LIKELY) BENIGN
75817 (234/18.9/46.58)	*PMS2*	c.1864A>G	het	miss	E11	chr7:6026532	rs370853512	p.M622V	—	0.10	0.01	37.9/46/0	35.74/88/0; SAS: 0.28%	na	tol	poly	B	1.8	2.02	C0	prob path	2+	undet	DM?/CM1111596	not listed	confl.: LB(2), VUS(2)	*in silico* predicted *de novo* CSDS[2] (PP3); functional studies implicate a possible damaging effect (concomitant to a pathogenic *PMS2* variant of unknown phase in two siblings with childhood gliosarcomas) [124]; CR: CRC-affected patient suspected of LS [61]; conflicting classification in NCBI ClinVar; conflicting/insufficient evidence	VUS
55969 (233/18.3/66.95)	*PMS2*	c.2012C>T	het	miss	E12	chr7:6022617	rs587780046	p.T671M	—	—	—	—	17.43/34/0	na	tol	poly	B	26.3	3.71	C0	path	3+	undet	DM?/CM1613756	not listed	confl.: B(1), VUS(7)	predominantly classified as "VUS" in NCBI ClinVar; CR: two patients affected with pancreatic cancer [121,125]; CRC-affected patient (classified as "VUS") [72]; insufficient evidence	VUS
53804 (132/16.7/63.64)[®] **53805** (184/17.8/69.57)[®]	*PMS2*	c.2068A>C	het	miss	E12	chr7:6022561	rs587781909	p.K690Q	IPR014790	—	—	—	4.096/10/0	na	del	path	B	24.0	4.74	C0	prob path	5+	undet	not listed	not listed	*unc.*	absent from ExAC population database (PM2); consistently classified as "VUS" in NCBI ClinVar; CR: patient affected with endometrial cancer [126]	VUS
73484 (132/17.1/67.42)	*PMS2*	c.2149G>A	het	miss	E12	chr7:6022480	rs201671325	p.V717M	IPR014790	0.02	0.02	90.54/73/0	69.80/184/1; ASI: 0.71%	na	del	path	PD	20.6	3.82	C0	prob poly	5+	undet	DM?/CM144762	**not classified: ?/?(1), ?/.(1)**; inconclusive[a]	confl.: B(1), LB(3), VUS(7)	very high MAF in ASJ, gnomAD: 1 homozygous individual; functional studies and segregation studies implicate no deleterious effect (BS3, BS4) [127]; multiple co-occurrences with pathogenic LS associated gene variants [64,72,128] (BP2)	(LIKELY) BENIGN
52069 (179/17.9/54.19)[®] **69938** (268/19.3/52.99)[®]	*PMS2*	c.2350G>A	het	miss	E14	chr7:6017314	rs143340522	p.D784N	IPR014790	—	0.00	141.6/170/7	113.5/278/9; AFR: 0.70%; SAS: 0.49%	na	tol	path	PD	32.0	5.75	C0	poly	4+	undet	not listed	not listed	confl.: LB(1), VUS(4)	very high MAF in SAS/AFR, gnomAD: 9 homozygous individuals; ClinVar classifications inconcruent to provided summary evidence; (strong pseudogene homology)	(LIKELY) BENIGN
74410 (358/20.3/34.36)	*PMS2*	c.2356C>A	het	miss	E14	chr7:6017308	rs576055272	p.L786M	IPR014790	0.18	—	127.2/152/7	117.2/287/10; SAS: 0.87%	na	tol	path	PD	22.9	2.71	C0	prob poly	4+	undet	not listed	not listed	confl.: B(2), LB(2), VUS(1)	very high MAF in SAS, gnomAD: 10 homozygous individuals; predominantly classified as "(likely) benign" in NCBI ClinVar database; (strong pseudogene homology);	(LIKELY) BENIGN
66416 (67/14.7/49.25) **73791** (65/14.2/55.38)	*EPCAM*	c.5C>T	het	miss	E1	chr2:47596649	rs201402370	p.A2V	—	0.04	0.17	613.3/109/2	218.2/397/3; NFE: 0.18%; ASI: 2.39%	na	tol	poly	B	22.2	3.97	C0	prob poly	2+	undet	not listed	not listed	*(likely) B*	high to very high MAF in ASJ/NFE including 3 homozygous individuals; consistently classified as "(likely) benign" in NCBI ClinVar database; *in silico* predicted CSAS activation[2]	(LIKELY) BENIGN
68134 (357/20.3/45.38) **71430** (164/17.7/63.41) **76028** (191/18.1/58.12)	*EPCAM*	c.267G>C	het	miss	E3	chr2:47601029	rs146480420	p.Q89H	IPR000716	0.10	0.58	257.9/313/2	270.2/749/5; NFE: 0.46%	na	tol	path	PD	13.9	1.39	C0	prob poly	2+	undet	not listed	inconsistent[a]: +/.(1), -?/.(1); inconclusive[b]	confl.: B(2), LB(3), VUS(1)	very high MAF in NFE, gnomAD: 5 homozygous individuals; predominantly classified as "(likely) benign" in NCBI ClinVar	(LIKELY) BENIGN
62976 (152/17.5/40.79)	*EPCAM*	c.345G>A	het	miss	E3	chr2:47601107	rs115212523	p.M115I	IPR000716	0.10	—	101.3/123/0	103.2/286/1; AFR: 0.94%	na	del	poly	B	14.76	-4.2	C0	poly	1+	B	not listed	inconclusive[b]	not listed	very high MAF in AFR, gnomAD: 1 homozygous individual; benign *in silico* prediction consensus (BP4)	(LIKELY) BENIGN
72982 (263/19.2/46.01)	*EPCAM*	c.401C>G ‡	het	miss	E3	chr2:47601163	—	p.T134S	IPR000716	—	—	—	—	na	tol	poly	B	15.1	0.63	C0	prob poly	0+	B	not listed	not listed	not listed	novel	VUS
76087 (253/19.2/46.64)	*EPCAM*	c.493G>A	het	miss	E5	chr2:47604154	rs146685071	p.A165T	—	0.02	0.02	7.429/9/0	5.282/13/0	na	tol	path	B	15.8	4.98	C0	poly	2+	undet	not listed	inconclusive[b]	LB	no evident splicing effect *in silico* (penultimate exonic nucleotide before splice junction) (BP7); insufficient evidence	VUS
62001 (166/17.6/48.19) **72157** (149/17.3/42.28)	*EPCAM*	c.831A>G	het	miss	E7	chr2:47607081	rs115283528	p.I277M	—	0.08	0.22	234.4/249/0	205.3/566/1; ASI: 0.84%; NFE: 0.20%	na	del	poly	B	11.9	-0.51	C0	poly	1+	B	not listed	VUS[a]: ?/.(1)	confl.: B(1), LB(1), VUS(1)	high to very high MAF in NFE/SAS/ASJ, gnomAD: 1 homozygous individual; benign *in silico* prediction consensus (BP4)	(LIKELY) BENIGN
68241 (247/19.1/46.15)	*EPCAM*	c.904-9C>G ‡	het	nonc	I8	chr2:47613702	rs377687304	nonc	—	—	—	—	—	na	na	na	na	na	na	na	na	na	na	not listed	not listed	not listed	novel; no evident splicing effect *in silico*[2] (BP7)	VUS
60577 (79/15.1/40.51)	*NBN*	c.37+5G>A	het	nonc	I1	chr8:90996748	rs116735828	nonc	—	0.90	0.10	268.7/302/7	277.4/755/12; AFR: 2.34%	1430.0/22/1	na	na	na	na	na	na	na	na	na	DM/CS1410391	not listed	*(likely) B*	very high MAF in AFR/Flossies database, gnomAD: 12 homozygous individuals; no evident splicing effect *in silico* (BP7); consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN

Variant Specification										Population Databases					Computational Evidence									Gene/Disease specific Databases			Final Assessment of Variant Pathogenicity	
Carrier ID (quality parameters of the individual variant: Cov./Score/SB)	**Gene**	**Mutation HGVS ¥**	**het/ hom**	**Function**	**Loc.**	**Genomic Pos. (hg19)**	**SNP db_xref**	**Predicted AA Change**	**InterPro domain ID (v.67.0)**	**1000G** [%]	**ESP6500** [%] EurAm	**ExAC v0.3** frequ¶/allele count/# hom	**gnomAD v2.0.2** frequ¶/allele count/# hom	**FLOSSIES** frequ¶ / allele count (Eur)/# hom	**SIFT v6.2.0 †**	**MutTaster v2013†**	**PolyPhen-2 v2.2.2†** HumVar	**CADD Phred**	**GERP+ +_RS**	**Align-GVGD v2007†§**	**UMD predictor**	**Prediction CONSENSUS⌈**		**HGMD PRO v2017.3: variant class/acc.**	**Locus specific databases (LSDB, assessed by the LOVD)$**	**NCBI-ClinVar£**	**Rationale for Classification (ACMG evidence category if meeting)**	**final CLASSIFICATION**
73325 (277/19.4/43.68) **73877** (201/18.3/35.82) **78686** (119/16.3/57.14)	*NBN*	c.283G>A	het	miss	E3	chr8:90993640	rs61753720	p.D95N	IPR000253	0.08	0.26	185.8/225/0	172.1/477/1; ASJ: 0.39%; NFE: 0.29%	476.0/43/0	tol	path	PrD	25.8	4.83	C0	poly	4+	undet	DM/066927	inconclusive[a]; inconsistent[b]: +?/+? (1), ?/+?(1), ?/?(1)	confl.: B(3), LB(3), VUS(1)	high MAF in NFE/ASJ, gnomAD: 1 homozygous individual; no association with BC and cancer in meta-analyses [129-130]	(LIKELY) BENIGN
64604 (129/16.7/57.36)	*NBN*	c.353_355delCTT	het	In-Frame	E4	chr8:90993087	rs730881841	p.S118del	IPR001357	—	—	3.302/4/0	2.890/8/0	—	na	na	na	na	na	na	na	na	na	not listed	not listed	*unc.*	MAF within pathogenic range (PM2); protein length changing variant (PM4); consistently classified as "VUS" in NCBI ClinVar; insufficient evidence	VUS
74742 (449/21.0/47.66) **75388** (507/21.4/46.35)	*NBN*	c.381T>C	het	syn	E4	chr8:90993061	rs61754795	p.A127=	IPR001357	0.22	0.45	321.6/390/4	309.8/858/2; SAS: 0.78%; NFE: 0.39%	607.0/54/0	na	na	na	na	na	na	poly	na	na	not listed	not listed	*(L)B*	high to very high MAF in SAS/NFE, gnomAD: 4 homozygous individuals; no evident splicing effect *in silico* (BP7); consis-tently classified as "(likely) benign" in NCBI ClinVar (BP6); no association with BC in case-control study [129]	(LIKELY) BENIGN
57429 (161/17.5/49.07) **77074** (123/16.6/71.54) **77745** (197/18.2/39.09)	*NBN*	c.511A>G	het	miss	E5	chr8:90990521	rs61754966	p.I171V	IPR001357	0.04	0.16	139.4/169/0	150.9/418/0; NFE: 0.26%	293.0/28/0	del	path	PrD	16.8	4.81	C0	prob path	5+	undet	DM/CM011800	inconclusive[a]; probably pathogenic[b]: +?/+?(3), +?/+(2)	confl. or risk factor: B(1), P(1), VUS(8)	case-control studies implictate a pleomorphic cancer risk elevation [130]	HYPOMORPHIC
66310 (300/19.6/46.67)	*NBN*	c.643C>T	het	miss	E6	chr8:90983460	rs34767364	p.R215W	—	0.10	0.37	295.3/355/3	242.5/671/3; NFE: 0.40%	405.0/37/0	tol	poly	PrD	25.9	4.96	C0	path	4+	undet	DM/CM044022	inconclusive[a]; probably pathogenic[b]: +?/+?(4)	confl.: B(2), LB(2), P(2), VUS(5)	case-control studies implicate a pleomorphic cancer risk elevation [130]	HYPOMORPHIC
62542 (121/16.6/49.59)	*NBN*	c.1231T>C ‡	het	miss	E10	chr8:90967677	—	p.S411P	—	—	—	—	—	—	tol	poly	PD	11.3	3.99	C0	poly	2+	undet	not listed	not listed	not listed	novel	VUS
65137 (271/19.3/53.51)	*NBN*	c.1317A>G	het	miss	E10	chr8:90967591	rs28538230	p.I439M	—	0.04	0.00	38.73/47/0	34.65/96/0; AFR:0.40%	243.0/1/0	tol	poly	B	0.0	-1.03	C0	poly	0+	B	not listed	inconclusive[b]	*LB*	high MAF in AFR/Flossies; consistently classified as "likely benign" in NCBI ClinVar (BP6); *in silico* predicted *de novo* CSDS[2] (PP3)	(LIKELY) BENIGN
68840 (791/23.0/49.81)	*NBN*	c.1526C>A	het	miss	E11	chr8:90965791	—	p.S509Y	—	—	—	—	—	—	tol	poly	B	12.5	3.46	C0	poly	1+	undet	not listed	not listed	*unc.*	absent from population database (PM2); consistently classified as "VUS" in NCBI ClinVar database; insufficient evidence	VUS
73800 (277/19.4/53.43)	*NBN*	c.2202A>G	het	miss	E11	chr8:90949286	rs200452212	p.A734=	—	—	0.02	18.37/21/0	15.97/44/0; NFE: 0.031%	10.12/1/0	na	na	na	na	na	na	na	na	na	not listed	not listed	B(1), LB(3), VUS(1)	no evident splicing effect *in silico* (BP7); (consistently) classified as "(likely) benign" in NCBI ClinVar (ClinVar submission SCV000697963.1 : classification "VUS-likely benign") (BP6); CR: patient affected with larynx cancer [131]	(LIKELY) BENIGN
73184 (205/18.4/44.88)	*NF1*	c.475A>G	het	miss	E4	chr17:29490390	rs371192107	p.T159A	—	—	0.01	3.306/4/0	2.439/6/0	na	tol	path	B	23.5	5.84	C0	path	4+	undet	not listed	not listed	*unc.*	MAF within pathogenic range (PM2); consistently classified as "VUS" in NCBI ClinVar; insufficient evidence	VUS
62926 (124/16.5/45.97) **63408** (259/19.2/48.26) **65391** (72/14.6/59.72) **69307** (322/19.8/50.93) **76382** (352/20.1/43.18) **73215** (125/16.6/43.20)	*NF1*	c.528T>A	het	miss	E5	chr17:29496957	rs112306990	p.D176E	—	0.08	0.49	328.6/397/3	368.6/1021/7; FIN: 1.07%; NFE: 0.50%	na	tol	path	PD	18.1	3.38	C0	poly	3+	undet	not listed	neutral[a]: -/-(2), -/.(3)	*(L)B*	very high MAF in NFE/FIN, gnomAD: 7 homozygous individuals; consistently classified as "(likely) benign" in NCBI ClinVar (BP6); nonsegregation with disease [132] (BS4); *in silico* predicted CSAS deactivation[2]	(LIKELY) BENIGN
72585 (87/15.5/50.57)	*NF1*	c.696A>G	het	syn	E7	chr17:29508769	rs368691517	p.T232=	—	—	0.01	7.426/9/0	6.094/15/0	na	na	na	na	na	na	na	poly	na	na	not listed	not listed	*LB*	no evident splicing *in silico*[2] (BP7); consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
60545 (522/21.7/40.23) **68567** (414/20.8/47.58) **75407** (162/17.6/43.83)	*NF1*	c.731-6A>C	het	nonc	I7	chr17:29509520	rs369366499	nonc	—	—	0.05	35.9/42/0	32.15/89/0	na	na	na	na	na	na	na	na	na	na	not listed	neutral[a]: -/-(1)	*(L)B*	functional studies show no splicing effect [133] (BS3); consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
74925 (1076/24.2/46.47)	*NF1*	c.825C>T ‡	het	syn	E8	chr17:29509620	—	p.I275=	—	—	—	—	—	na	na	na	na	na	na	na	poly	na	na	not listed	not listed	not listed	novel; no evident splicing effect *in silico*[2] (BP7)	VUS
43268 (385/20.5/99.22) **53804** (309/19.7/47.25) **64997** (759/22.9/47.96) **68241** (452/21.1/48.23) **73313** (911/23.5/45.77) **74925** (952/23.8/53.89)	*NF1*	c.846G>A	het	syn	E8	chr17:29509641	rs138840528	p.Q282=	—	0.62	0.26	605.2/706/23	501.2/1387/29; SAS: 3.25%	na	na	na	na	na	na	na	poly	na	na	not listed	(probably) neutral[a]: -/-(1), -?/.(1), -/.(1)	*(L)B*	very high MAF in SAS, gnomAD: 29 homozygous individuals; no evident splicing effect *in silico* (BP7); consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
65761 (36/12.3/36.11)	*NF1*	c.*11C>T Ψ	het	nonc	3'UTR	chr17:29549019	rs369651443	nonc	—	0.02	—	179.1/43/0	142.0/232/0; NFE: 0.26%	na	na	na	na	na	na	na	na	na	na	not listed	not listed	not listed	high MAF in NFE; 3'UTR	(LIKELY) BENIGN
42566 (203/18.2/49.75)	*NF1*	c.1900A>G	het	miss	E17	chr17:29552167	rs745906742	p.I634V	—	—	—	3.299/4/0	2.439/6/0	na	tol	poly	B	0.4	0.96	C0	poly	0+	B	not listed	not listed	confl.: LB(1), VUS(1)	MAF within pathogenic range (PM2); benign *in silico* prediction consensus (BP4); conflicting classification in NCBI ClinVar; insufficient/conflicting evidence	VUS
73808 (651/22.3/48.85)	*NF1*	c.1901T>C	het	miss	E17	chr17:29552168	rs527563505	p.I634T	—	0.06	—	17.32/21/0	14.08/39/0; SAS: 0.12%	na	del	poly	B	17.8	4.14	C25	poly	3+	undet	not listed	not listed	*LB*	high MAF in SAS; consistently classified as "likely benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
75676 (473/21.3/38.27)	*NF1*	c.2985G>C	het	syn	E22	chr17:29556987	rs17881467	p.L995=	—	0.26	0.00	118.9/143/0	124.8/345/1; AFR: 1.34%	na	na	na	na	na	na	na	poly	na	na	not listed	neutral[a]: -/-(1)	*(L)B*	very high MAF in AFR, gnomAD: 1 homozygous individual; consistently classified as "(likely) benign" in NCBI ClinVar (BP6); no evident splicing effect *in silico*[2] (BP7)	(LIKELY) BENIGN
78686 (192/18.2/53.12)	*NF1*	c.3468C>T	het	syn	E26	chr17:29559871	rs147955381	p.N1156=	—	0.10	0.13	70.92/86/0	68.21/189/0; NFE: 0.12%	na	na	na	na	na	na	na	poly	na	na	not listed	(probably) neutral[a]: -/-(1), -?/.(1), -/.(1)	*(L)B*	high MAF in NFE; no evident splicing effect *in silico* (BP7); consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
72013 (191/18.2/46.07)	*NF1*	c.3867C>T	het	syn	E28	chr17:29562787	rs138186428	p.F1289=	IPR001936	0.02	0.16	147.5/179/3	116.4/322/3; NFE: 0.20%	na	na	na	na	na	na	na	poly	na	na	DM?/CM077210	neutral[a]: -/-(1)	confl.: B(3), LB(4), VUS(1)	high MAF in NFE, gnomAD: 3 homozygous individuals; no evident splicing effect *in silico* (BP7), functional analysis implicate no NMD effect [134]; co-occurrence with a pathogenic *NF1* variant (BP2) [135]	(LIKELY) BENIGN
72092 (378/20.4/46.3)	*NF1*	c.4174-8_4174-6del	het	nonc	I31	chr17:29585352	rs751729752	nonc	—	—	0.29	55.28/67/0	43.34/120/0	na	na	na	na	na	na	na	na	na	na	DM/CD040916	not listed	*(L)B*	no evident splicing effect *in silico* (BP7); consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
55035 (459/21.1/53.59) **59803** (689/22.6/48.04) **73484** (540/21.7/53.33) **80688** (105/16.0/39.05)	*NF1*	c.4577+11C>G	het	nonc	I34	chr17:29587544	rs190614908	nonc	—	0.10	0.09	88.99/108/0	108.7/301/0; ASJ: 0.70%	na	na	na	na	na	na	na	na	na	na	not listed	not listed	*LB*	very high MAF in ASJ; no evident splicing effect *in silico* (BP7); consistently classified as "(likely) benign" in NCBI ClinVar (BP6);	(LIKELY) BENIGN
72266 (719/22.7/46.59)	*NF1*	c.4577+12C>T	het	nonc	I34	chr17:29587545	rs17878332	nonc	—	0.24	0	92.29/112/1	112.4/311/2; AFR: 1.22%	na	na	na	na	na	na	na	na	na	na	not listed	not listed	*(L)B*	very high MAF in AFR, gnomAD: 2 homozygous individuals; no evident splicing effect *in silico* (BP7); consistently classified as "(likely benign)" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
75387 (550/21.8/48)	*NF1*	c.4749A>G	het	syn	E36	chr17:29592271	rs144091165	p.E1583=	IPR001251	0.02	—	22.39/27/0	22.74/63/0	na	na	na	na	na	na	na	poly	na	na	not listed	not listed	confl.: LB(3), VUS(4)	no evident splicing effect in silico (BP7); conflicting classification in NCBI ClinVar; CR: subject with individual or familial history of NF1, seg n/d [136]; insufficient evidence	VUS
71620 (316/19.8/50.00) **75424** (389/20.6/45.50)	*NF1*	c.5235G>A	het	syn	E37	chr17:29653237	rs17887014	p.K1745=	—	0.20	0.49	340.9/408/1	324.6/889/3; SAS: 0.29%; NFE: 0.52%	na	na	na	na	na	na	na	poly	na	na	not listed	not listed	*(L)B*	very high MAF in SAS/NFE, gnomAD: 3 homozygous individuals; functional studies show no obvious splicing effect [132] (BS3); consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
62541 (119/16.5/52.10)	*NF1*	c.6033A>G	het	syn	E41	chr17:29663377	rs147995863	p.L2011=	—	—	0.00	4.946/6/0	6.134/17/0	na	na	na	na	na	na	na	poly	na	na	not listed	not listed	*LB*	no evident splicing *in silico*[2] (BP6); consistently classified as "likely benign" in NCBI ClinVar (BP7)	(LIKELY) BENIGN
75045 (560/21.8/53.93)	*NF1*	c.6345G>A	het	syn	E42	chr17:29663850	rs779475182	p.P2115=	—	—	—	0.8238/1/0	1.626/4/0	na	na	na	na	na	na	na	poly	na	na	not listed	not listed	*LB*	MAF within pathogenic range (PM2); *in silico* predicted CSDS activation[2] (PP3); consistently classified as "likely benign" in NCBI ClinVar; conflicting evidence	VUS
75818 (164/17.7/42.68)	*NF1*	c.6940G>T ‡	het	miss	E47	chr17:29667541	—	p.A2314S	—	—	—	—	—	na	tol	path	B	22.3	6.08	C0	path	4+	undet	not listed	not listed	not listed	novel	VUS

Variant Specification										Population Databases					Computational Evidence									Gene/Disease specific Databases			Final Assessment of Variant Pathogenicity	
Carrier ID (quality parameters of the individual variant: Cov./Score/SB)	**Gene**	**Mutation HGVS ¥**	**het/ hom**	**Function**	**Loc.**	**Genomic Pos. (hg19)**	**SNP db_xref**	**Predicted AA Change**	**InterPro domain ID (v.67.0)**	**1000G** [%]	**ESP6500** [%] EurAm	**ExAC v0.3** frequ¶/allele count/# hom	**gnomAD v2.0.2** frequ¶/allele count/# hom	**FLOSSIES** frequ¶ / allele count (Eur)/# hom	**SIFT v6.2.0 †**	**MutTaster v2013†**	**PolyPhen-2 v2.2.2†** HumVar	**CADD Phred**	**GERP+ +_RS**	**Align-GVGD v2007†§**	**UMD predictor**	**Prediction CONSENSUS∫**		**HGMD PRO v2017.3: variant class/acc.**	**Locus specific databases (LSDB, assessed by the LOVD)$**	**NCBI-ClinVar£**	**Rationale for Classification (ACMG evidence category if meeting)**	**final CLASSIFICATION**
62805 (439/21.0/53.30)	*NF1*	c.6942C>T ‡	het	syn	E47	chr17:29667543	—	p.A2314=	—	—	—	—	—	na	na	na	na	na	na	na	poly	na	na	not listed	not listed	not listed	novel; no evident splicing effect *in silico*[2] (BP6)	VUS
55035 (325/20.1/43.08) **59803** (76/15.0/40.79) **73484** (245/19.0/47.76) **80688** (91/15.7/34.07)	*NF1*	c.7584A>G	het	syn	E51	chr17:29679401	rs55865524	p.Q2528=	—	0.10	0.09	91.14/110/0	110.1/305/0; AMR 0.19%; ASJ: 0.70%	na	na	na	na	na	na	na	poly	na	na	not listed	not listed	confl.: B(1), LB(4), VUS(1)	high to very MAF in AMR/ASJ; no evident splicing effect *in silico* (BP6)	(LIKELY) BENIGN
39649 (337/20.1/46.59) **58893** (338/20.1/51.48)	*NF1*	c.7623G>A	het	syn	E52	chr17:29683485	—	p.R2541=	—	—	—	—	1.794/2/0	na	na	na	na	na	na	na	poly	na	na	not listed	not listed	*LB*	no evident splicing effect *in silico*[2] (BP6); consistently classified as "likely benign" in NCBI ClinVar (BP7)	(LIKELY) BENIGN
74410 (551/21.7/39.02)	*NF1*	c.8041A>G	het	miss	E55	chr17:29685568	rs146315101	p.I2681V	—	—	0.02	17.33/21/0	17.68/49/0; ASJ: 0.11%	na	tol	path	B	10.7	3.44	C0	prob path	3+	undet	not listed	inconclusive[a]	*LB*	*in silico* predicted *de novo* CSAS[2] (PP3); consistently classified as "likely benign" in NCBI ClinVar (BP6); conflicting evidence	VUS
75711 (329/20.0/41.03)	*NF1*	c.8151G>A	het	syn	E56	chr17:29686024	rs2285895	p.P2717=	—	0.52	—	166.0/198/2	127.3/352/6; EAS: 1.59%	na	na	na	na	na	na	na	poly	na	na	not listed	not listed	*(L)B*	very high MAF in EAS, gnomAD: 6 homozygous individuals; no evident splicing effect *in silico* (BP7); consistently classified as "likely benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
74409 (271/19.4/51.66)	*NF1*	c.8499T>C	het	syn	E58	chr17:29701152	rs142636150	p.N2833=	—	0.06	0.05	32.16/39/1	27.79/77/1; SAS: 0.12%	na	na	na	na	na	na	na	poly	na	na	not listed	not listed	confl.: B(1), LB(5), VUS(4)	high MAF in SAS, gnomAD: 1 homozygous individual; no evident splicing effect *in silico*[2] (BP7); CR: subject with individual or familial history of NF1 (classified as "benign") [137]	(LIKELY) BENIGN
75895 (165/17.6/47.88)	*NF1*	c.*4T>C	het	nonc	3'UTR	chr17:29701177	rs201044568	nonc	—	0.58	—	285.4/346/6	224.8/623/5; SAS: 1.99%	na	na	na	na	na	na	na	na	na	na	not listed	not listed	*(L)B*	very high MAF in SAS, gnomAD: 5 homozygous individuals; consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
71979 (827/23.2/48.13)	*PALB2*	c.656A>G	het	miss	E4	chr16:23647211	rs45594034	p.D219G	—	0.02	0.01	17.32/21/0	19.12/53/0	30.4/2/0	tol	poly	B	0.0	-5.08	C0	poly	0+	B	DM?/CM1314596	probably neutral[a]: -?/-?(7); inconclusive[b]	confl.: LB(2), VUS(4)	inconsistent classification in NCBI ClinVar (predominantly classified as "VUS"); CR: reported in multiple individuals with a personal and/or familiy history of BC [19,62,138-145]; occurrence in a control subject and concomitant with a pathogenic *BRCA2* variant [62,78,146]	VUS
70976 (810/23.2/48.64)	*PALB2*	c.833_834delinsAT	het	miss	E4	chr16:23647034	rs587778582	p.L278H	—	—	—	—	—	—	tol	na	PD	na	na	C0	na	na	na	not listed	not listed	*unc.*	absent from population database (PM2); consistently classified as "VUS" in NCBI ClinVar database; insuffient evidence	VUS
75817 (195/18.2/42.56)	*PALB2*	c.899C>T	het	miss	E4	chr16:23646968	rs528541334	p.T300I	—	0.12	—	52.75/64/2	39.69/110/1; SAS: 0.34%	—	tol	poly	B	5.2	1.88	C0	poly	0+	B	DM?/CM108903	inconsistent[a]: ?/?(1), -?/-?(1)	confl.: B(1), LB(1), VUS(4);	high MAF in SAS, gnomAD: 1 homozygous individual; benign *in silico* prediction consensus (BP4); *in silico* predicted CSAS deactivation[2]	(LIKELY) BENIGN
73791 (251/19.1/39.04) **76367** (165/17.6/52.73)	*PALB2*	c.1194G>A	het	syn	E4	chr16:23646673	rs61755173	p.V398=	—	0.08	0.23	72.62/88/0	76.64/212/0; NFE: 0.13%	182/17/0	na	na	na	na	na	na	poly	na	na	not listed	(probably) neutral[a]: -?/.(3), -?/-?(1), ?/-?(1), -/.(1)	*(L)B*	high MAF in NFE/Flossies database; no evident splicing effect *in silico* (BP7); consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
44325 (267/19.3/46.07)	*PALB2*	c.1470C>T	het	syn	E4	chr16:23646397	rs45612837	p.P490=	—	0.02	0.00	28.83/35/0	27.06/75/0	91.1/9/0	na	na	na	na	na	na	poly	na	na	not listed	(probably) neutral[a]: -?/.(3), -?/-?(1), ?/-?(1), -/.(1); inconsistent[b]: -?/?(1), ?/?(1)	*(L)B*	no evident splicing effect *in silico* (BP6); consistently classified as "likely benign" in NCBI ClinVar (BP7)	(LIKELY) BENIGN
69414 (435/20.9/49.89)	*PALB2*	c.1544A>G	het	miss	E4	chr16:23646323	rs515726072	p.K515R	—	—	—	4.942/6/0	8.296/23/0	20.2/2/0	tol	poly	B	15.5	3.30	C0	poly	1+	B	DM?/CM1612928	probably neutral[a]: -?/?(1)	confl.: LB(1), VUS(6)	MAF within pathogenic range (PM2); predominantly classified as "VUS" in NCBI ClinVar; CR: BC-affected females [147,141]; BC-affected females suspected of HBOC [145,148] (classified as "VUS" in [148]); CR: BC-affected female (Dx 60), FA: BC-affected relative (classified as "VUS") [46]; BC- or OC-affected Polish/West-Ukrainian female, supected of HBOC [149]	VUS
61860 (505/21.5/44.16) **75580** (849/23.3/45.47)	*PALB2*	c.1572A>G	het	syn	E4	chr16:23646295	rs45472400	p.S524=	—	0.22	0.45	312.2/379/1	324.3/899/2; SAS: 0.36%; NFE: 0.49%	—	na	na	na	na	na	na	poly	na	na	not listed	(probably) neutral[a]: -/-(15), -?-(2), ?/-(1), -?/.(3); probaly neutral[b]: -?/?(1), ?/-?(1)	*(L)B*	high MAF in SAS/NFE, gnomAD: 2 homozygous individuals; no evident splicing *in silico* (BP7); consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
76919 (171/17.7/53.22)	*PALB2*	c.2235A>G	het	syn	E5	chr16:23641240	rs765854776	p.K745=	—	—	—	1.647/2/0	0.8121/2/0	—	na	na	na	na	na	na	poly	na	na	not listed	not listed	*LB*	no evident splicing effect *in silico*[2] (BP7); consistently classified as "likely benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
64604 (312/19.8/50.32) **67044** (291/19.6/48.45) **67184** (345/20.1/50.72) **69858** (342/20.2/39.47) **73284** (950/23.6/47.89)	*PALB2*	c.2590C>T	het	miss	E7	chr16:23637715	rs45568339	p.P864S	IPR031920	0.12	0.31	266.1/323/2	272.3/755/2; ASJ: 1.11%; NFE: 0.39%	678.0/62/1	tol	poly	B	20.9	2.82	C0	prob poly	2+	undet	DP/CM105610	inconsistent[a]: -?/-?(14), ?/-?(3), -?/.(3), ./.(2); inconclusive[b]	*(L)B*	high to very high MAF in ASJ/NFE/Flossies, gnomAD: 2 homozygous individuals; consistently classified as "(likely) benign" in NCBI ClinVar (BP6); no association with BC in case-control study [145]	(LIKELY) BENIGN
74116 (550/21.8/40.73)	*PALB2*	c.2656T>A	het	miss	E7	chr16:23637649	—	p.C886S	IPR031920	—	—	—	—	—	del	path	PrD	20.2	5.93	C65	prob poly	6+	dam	not listed	not listed	*unc.*	absent from population databases (PM2); deleterious *in silico* prediction consensus (PP3); consistently classified as "VUS" in NCBI ClinVar; insufficient evidence	VUS
60577 (278/19.4/40.65)	*PALB2*	c.2794G>A	het	miss	E8	chr16:23635370	rs45624036	p.V932M	IPR031920	0.10	0.59	597.2/708/3	520.8/1441/4; FIN: 1.66%; NFE: 0.64%	799.0/70/0	tol	path	PrD	25.8	4.85	C0	poly	4+	undet	DM?/CM112100	inconsistent[a]: -?/-(12), ?/-?(1), -/-(1), ?/-(2), ./.(2), -?/.(2), -/.(1); inconclusive[b]	*(likely) B*	very high MAF in population databases/Flossies, gnomAD: 4 homozygous individuals in gnomAD database; consistently classified as "(likely) benign" in NCBI ClinVar (BP6); *in silico* predicted CSDS deactivation[2]	(LIKELY) BENIGN
73791 (360/20.2/46.67) **76028** (238/18.9/44.54) **76367** (188/18.0/60.11)	*PALB2*	c.2816T>G	het	miss	E8	chr16:23635348	rs45478192	p.L939W	IPR031920	0.08	0.59	96.98/114/0	97.94/271/0; ASJ: 0.18%; NFE: 0.17%	223.0/21/0	del	path	PrD	29.9	5.81	C55	path	7+	dam	DM?/CM105609	inconsistent[a]: -?/-?(12), ?/-?(3), ./.(1), ?/.(3); inconclusive[b]	confl.: B(4), LB(4), VUS(5)	high MAF in NFE/ASJ/Flossies; no association with BC, prostate cancer, and OC in large case-control study [97]	(LIKELY) BENIGN
47939 (221/18.6/39.37)	*PALB2*	c.3059A>G	het	miss	E10	chr16:23632737	rs776221283	p.Q1020R	IPR031920	—	—	1.648/2/0	1.218/3/0	—	del	path	PD	20.2	5.31	C0	path	6+	dam	not listed	not listed	unc.	MAF within pathogenic range (PM2); deleterious *in silico* prediction consensus (PP3); *in silico* predicted *de novo* CSDS[2]; insufficient evidence	VUS
73907 (206/18.5/50.97)	*PALB2*	c.3128G>C	het	miss	E11	chr16:23625398	rs377713277	p.G1043A	IPR031920	—	0.01	1.797/2/0	2.035/5/0	—	del	path	PrD	21.8	5.95	C55	prob path	7+	dam	DM?/CM113829	inconsistent[a]: ?/?(2), -?/?(2); inconclusive[b]	*unc.*	MAF within pathogenic range (PM2); deleterious *in silico* prediction consensus (PP3); consistently classified as "VUS" in NCBI ClinVar; CR: early-onset BC-affected female (Dx <40), FH: grandfather pat affected with pancreatic cancer, seg n/d [150]; BC-affected female [141] (suspected of HBOC) [145]; female affected with BC and other cancer (Dx 40), FH: "low", seg n/d [19]; female affected with serous OC [78]; 51 yo female control without family history of cancer [144]	VUS
75388 (510/21.6/47.45)	*PALB2*	c.3251C>T	het	miss	E12	chr16:23619284	rs62625271	p.S1084L	IPR031920	—	—	7.414/9/0	9.018/25/0	40.5/4/0	tol	poly	B	12.0	0.40	C0	poly	0+	B	not listed	inconsistent[a]: -/.(1), ?/?(1), ./.(1)	*unc.*	consistently classified as "VUS" in NCBI ClinVar; CR: detected in individuals affected by TNBC and serous OC as well as unaffected individuals, seg n/d [78,151]; insufficient evidence	VUS
60824 (563/21.9/39.79)	*PALB2*	c.3296C>G	het	miss	E12	chr16:23619239	rs142132127	p.T1099R	IPR031920	0.02	—	6.59/8/0	6.132/17/0	10.1/1/0	del	path	PrD	20.7	6.14	C15	path	7+	dam	DM?/CM1512729	probably neutral[a]: -?/?(1); inconclusive[b]	*unc.*	MAF in ExAC database within pathogenic range (PM2); consistently classified as "VUS" in NCBI ClinVar; deleterious *in silico* prediction consensus (PP3); CR: individuals affected by BC and serous OC, seg n/d [62,78]	VUS

Variant Specification										Population Databases					Computational Evidence									Gene/Disease specific Databases			Final Assessment of Variant Pathogenicity	
Carrier ID (quality parameters of the individual variant: Cov./Score/SB)	**Gene**	**Mutation HGVS ¥**	**het/ hom**	**Function**	**Loc.**	**Genomic Pos. (hg19)**	**SNP db_xref**	**Predicted AA Change**	**InterPro domain ID (v.67.0)**	**1000G** [%]	**ESP6500** [%] EurAm	**ExAC v0.3** frequ¶/allele count/# hom	**gnomAD v2.0.2** frequ¶/allele count/# hom	**FLOSSIES** frequ¶ / allele count (Eur)/# hom	**SIFT v6.2.0 †**	**MutTaster v2013†**	**PolyPhen-2 v2.2.2†** HumVar	**CADD Phred**	**GERP+ +_RS**	**Align-GVGD v2007†§**	**UMD predictor**	**Prediction CONSENSUS[/**		**HGMD PRO v2017.3: variant class/acc.**	**Locus specific databases (LSDB, assessed by the LOVD)$**	**NCBI-ClinVar£**	**Rationale for Classification (ACMG evidence category if meeting)**	**final CLASSIFICATION**
75957 (407/20.6/43.00)	*PALB2*	c.3428T>A	het	miss	E13	chr16:23614913	rs62625284	p.L1143H	IPR031920	—	0.01	20.61/25/0	15.51/43/0	—	tol	path	PD	20.2	5.77	C0	path	5+	undet	not listed	inconsistent[a]: ?/?(3), ?/-?(1), -?/?(1), ./.(1)	*unc.*	consistently classified as "VUS" in NCBI ClinVar; *in silico* predicted CSAS deactivation[2] (PP3); CR: BC-affected individuals suspected of HBOC [152-155]; BC-affected (Dx 67), FH: 1st-relative affected with BC (Dx 54), three ≥3rd-relatives affected with BC (Dx 21; Dx 50s; Dx 80s) [156]; female affected with serous OC [78]; BC-affected females [19; 144-145], FH: "low" in [19]; individual affected with pancreatic cancer (classified as "VUS") [60]	VUS
63672 (345/20.2/44.93) 74555 (263/19.2/47.15)	*PALB2*	c.3495G>A	het	syn	E13	chr16:23614846	rs45439097	p.S1165=	IPR031920	—	0.13	65.9/80/0	69.27/192/0	465.0/35/0	na	na	na	na	na	na	poly	na	na	not listed	probably neutral[a]: -/-?(3), –?/-?(2), ?/-?(1); probably neutral[b]: -?/?(1)	*(L)B*	high MAF in AFR/NFE/Flossies database; consistently classified as "(likely) benign" in NCBI ClinVar (BP6); *in silico* predicted *de novo* CSDS[2], not yet confirmed by functional studies	(LIKELY) BENIGN
47172 (288/19.6/44.44)	*PTEN*	c.-9C>G	het	nonc	5'UTR	chr10:89624218	rs11202592	nonc	—	1.10	0.02	362.4/440/12	360.7/1000/24; EAS: 4.79%	—	na	na	na	na	na	na	na	na	na	DP/CR033149	inconclusive[a]; inconclusive[b]: -?/+?(1)	*(L)B*	very high MAF in EAS, gnomAD: 24 homozygous individuals; consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
60720 (109/16.2/33.03) **73799** (100/15.8/37.00)	*PTEN*	c.132C>T	het	syn	E2	chr10:89653834	rs150651961	p.G44=	IPR029023 IPR03595	0.02	0.22	156.9/190/1	167.3/463/3; ASJ: 0.33%; NFE: 0.25%	344.0/34/0	na	na	na	na	na	na	poly	na	na	not listed	inconclusive[a]: -?/.(2), -/.(1), ?/.(1)	*(L)B*	high MAF in NFE/FIN/ASJ/Flossies, gnomAD: 3 homozygous individuals; consistently classified as "likely benign" in NCBI ClinVar (BP6); *in silico* predicted CSDS deactivation[2]	(LIKELY) BENIGN
74818 (441/21.0/46.03)	*RAD51C*	c.106G>A	het	miss	E1	chr17:56770110	rs773998134	p.E36K	—	—	—	1.649/2/0	1.625/4/0	—	tol	path	B	22.5	3.69	C0	prob poly	3+	undet	not listed	not listed	*unc.*	MAF within pathogenic range (PM2); consistently classified as "VUS" in NCBI ClinVar; CR: detected in affected females with BC/OC [157-160] (HBOC-suspected in [158]; high risk jewish patient in [159]; high risk, Claus score>70% in [160]); insufficient evidence	VUS
78686 (146/17.3/40.41)	*RAD51C*	c.145+12T>G	het	nonc	I1	chr17:56770161	rs377297129	nonc	—	—	0.03	13.22/16/0	14.09/39/0	—	na	na	na	na	na	na	na	na	na	not listed	inconclusive[a]	*(L)B*	no evident splicing effect *in silico*[2] (BP7); consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
68134 (318/19.9/43.08)	*RAD51C*	c.335G>C	het	miss	E2	chr17:56772481	rs370212314	p.G112A	IPR033925 IPR020588	—	0.01	0.8272/1/0	0.4064/1/0	—	del	path	PrD	21.8	5.41	C55	prob poly	6+	dam	not listed	not listed	*unc.*	MAF within pathogenic range (PM2); deleterious *in silico* prediction consensus (PP3); consistently classified as "VUS" in NCBI ClinVar database; CR: Czech subject with individual and/or family history of cancer [161]	VUS
73135 (367/20.4/44.96) **75579** (896/23.5/50.00) **77116** (1696/25.8/60.32)	*RAD51C*	c.376G>A	het	miss	E2	chr17:56772522	rs61758784	p.A126T	IPR033925 IPR020588	0.20	0.64	352.9/422/0	346.2/955/3; NFE: 0.54%; ASJ: 0.43%	1100.0/101/0	tol	path	B	21.7	3.35	C0	prob path	4+	undet	not listed	(probably) neutral[a]: -?/.(6), ?/.(3), -?/.(2), -/.(1); inconclusive[b]	*(L)B*	very high MAF in NFE/Flossies, gnomAD: 3 homozygous individuals; consistently classified as "(likely) benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
76918 (328/19.9/42.38)	*RAD51C*	c.433C>A‡	het	miss	E3	chr17:56774082	—	p.P145T	IPR033925 IPR020588	—	—	—	—	—	del	path	PrD	22.3	4.69	C35	path	7+	dam	not listed	not listed	not listed	novel	VUS
60577 (381/20.4/48.29) **68968** (687/22.5/45.85) **70861** (732/22.7/45.22)	*RAD51C*	c.790G>A	het	miss	E5	chr17:56787304	rs147241704	p.G264S	IPR033925 IPR020588	—	0.35	185.3/225/2	174.3/483/2; NFE: 0.34%	546.0/50/0	tol	del	B	25.0	6.00	C0	prob path	4+	undet	DFP/CM123214	inconsistent[a]: ?/.(8), -?/.(5); inconsistent[b]	confl.: B(3), LB(2), VUS(5)	case-control studies imply a potential slight OC risk elevation [159,162]; functional studies implicate a partially defective HDR activity [162-164]	HYPOMORPHIC
72824 (155/17.1/100.00) **73906** (143/17.0/46.85) **74984** (175/17.8/45.71	*RAD51C*	c.859A>G	het	miss	E6	chr17:56798128	rs28363317	p.T287A	IPR033925	0.40	1.02	546.3/658/3	556.7/1542/6; NFE: 0.96%	1390.0/121/0	tol	path	PrD	25.5	5.07	C0	prob poly	4+	dam	DM?/CM1010198	inconsistent[a]: -?/.(6), ?/.(3); inconclusive[b]	*(likely) B*	very high MAF in NFE/Flossies, gnomAD: 6 homozygous individuals; consistently classified as "(likely) benign" in NCBI ClinVar (BP6); no association with BC and OC in case-control study [162]	(LIKELY) BENIGN
75152 (365/20.2/55.89)	*RAD51C*	c.1008A>G	het	syn	E8	chr17:56809887	—	p.T336=	IPR033925	—	—	—	—	—	na	na	na	na	na	na	prob path	na	na	not listed	not listed	*LB*	no evident splicing effect *in silico*[2] (BP7); consistently classified as "likely benign in NCBI ClinVar database (BP6)	(LIKELY) BENIGN
74846 (735/22.8/40.95)	*RAD51D*	c.212C>T	het	miss	E3	chr17:33443989	rs572710839	p.S71L	—	—	—	4.215/5/0	4.068/10/0	—	tol	poly	B	0.3	-5.90	C0	poly	0+	B	not listed	not listed	not listed	MAF within pathogenic range (PM2); benign *in silico* prediction consensus (PP3); insufficient evidence	VUS
71417 (364/20.4/49.73)	*RAD51D*	c.225C>T‡	het	syn	E3	chr17:33443976	—	p.V75=	IPR033925	—	—	—	—	—	na	na	na	na	na	na	poly	na	na	not listed	not listed	not listed	novel; no evident splicing effect *in silico*[2] (BP7)	VUS
72092 (151/17.2/50.99)	*RAD51D*	c.286G>A	het	miss	E3	chr17:33443915	rs147933658	p.A96T	IPR033925 IPR020588	0.02	0.03	66.59/27/0	32.90/81/0	—	tol	poly	B	4.8	-1.42	C0	poly	0+	B	not listed	not listed	*(L)B*	benign *in silico* prediction consensus (BP4); consistently classified as "likely benign" in NCBI ClinVar (BP6)	(LIKELY) BENIGN
67161 (225/18.7/48.44)	*RAD51D*	c.853G>A	het	miss	E9	chr17:33428330	rs140285068	p.G285R	IPR033925	—	0.02	4.122/5/0	4.874/12/0	—	del	path	PrD	22.3	4.92	C65	path	7+	dam	not listed	not listed	*unc.*	MAF within pathogenic range (PM2); deleterious *in silico* prediction consensus (PP3); consistently classified as "VUS" in NCBI ClinVar database; CR: reported in individual affected with BC and in a HBOC family, but with incomplete segregation and in control subjects [165-168]; two TNBC-affected females [169]; ClinVar Submission SCV000287725.3 (Invitae; Feb, 2017): alterated RNA splicing suggested *in silico* , not yet confirmed in the literature[2]	VUS
67601 (255/19.1/52.55)	*STK11*	c.31A>G	het	miss	E1	chr19:1206943	rs753834428	p.M11V	—	—	—	—	0.446/1/0	—	tol	poly	B	19.0	0.15	C0	poly	0+	B	DM?/CM1516526	inconclusive[a]	*unc.*	MAF within pathogenic range (PM2); conistently classified as "VUS" in NCBI ClinVar database; CR: TNBC-affected female [104]; insufficient evidence	VUS
69177 (72/14.5/68.06)	*STK11*	c.42G>A	het	syn	E1	chr19:1206954	rs758769888	p.E14=	—	—	—	8.398/5/0	3.808/10/0	—	na	na	na	na	na	na	poly	na	na	not listed	not listed	*LB*	no evident splicing effect *in silico*[2] (BP7); consistently classified as "likely benign" in NCBI ClinVar database (BP6)	(LIKELY) BENIGN
78685 (66/14.7/51.52)	*STK11*	c.277G>A	het	miss	E1	chr19:1207189	—	p.A93T	IPR000719	—	—	—	3.233/1/0	—	tol	path	B	22.4	3.90	C0	path	4+	undet	not listed	not listed	not listed	MAF within pathogenic range (PM2); insufficient evidence	VUS
69176 (156/17.2/51.92) **69177** (89/15.5/41.57)	*STK11*	c.310A>T	het	miss	E2	chr19:1218435	rs587782783	p.R104W	IPR000719	—	—	—	3.232/1/0	10.1/0/0	del	path	PrD	13.2	1.90	C25	path	5+	undet	not listed	not listed	*unc.*	MAF within pathogenic range (PM2); *in silico* predicted *de novo* CSDS[2] (PP3); consistently classified as "VUS" in NCBI ClinVar; insufficient evidence	VUS
68914 (410/20.7/44.15)	*STK11*	c.1039G>A	het	miss	E8	chr19:1223102	rs369744528	p.A347T	—	—	0.01	7.384/7/0	6.619/16/0	—	tol	poly	B	12.2	3.68	C0	poly	1+	B	not listed	not listed	confl.: LB(1), VUS(5)	MAF within pathogenic range (PM2); predominantly classified as "VUS" in NCBI ClinVar database; benign *in silico* prediction consensus (PP3); insufficient/conflicting evidence	VUS
77365 (85/15.3/50.59)	STK11	c.1225C>T	het	miss	E9	chr19:1226569	rs368466538	p.R409W	—	—	0.01	14.7/6/0	8.421/16/0	—	del	path	PD	12.9	-2.87	C0	poly	3+	undet	DM?/CM1516525	not listed	unc.	MAF within pathogenic range (PM2); consistently classified as "VUS" in NCBI ClinVar; CR: identified in TNBC-patient [104]; male BC-patient (Dx 49), FH: "low" [19]; 3 BC-affected females meeting NCCN criteria (classified as "VUS") [170]	VUS
74847 (60/14.1/50)	*STK11*	c.1225C>G	het	miss	E9	chr19:1226569	rs368466538	p.R409G	—	—	—	—	—	—	tol	poly	B	12.4	-2.87	C0	poly	0+	B	not listed	not listed	*unc.*	absent in population databases (PM2); consistently classified as "VUS" in NCBI ClinVar; insufficient evidence	VUS
68948 (57/13.7/57.89)	*STK11*	c.1265G>T ‡	het	miss	E9	chr19:1226609	—	p.S422I	—	—	—	—	—	—	tol	path	B	20.5	2.18	C0	path	4+	undet	not listed	not listed	not listed	novel	VUS
63671 (34/12.4/55.88) **76086** (33/12.1/75.76)	*STK11*	c.*8C>T	het	nonc	3'UTR	chr19:1226654	rs587782259	nonc	—	—	—	11.34/1/0	30.27/42/0	80.9/8/0	na	na	na	na	na	na	—	na	na	not listed	not listed	confl.: B(1), LB(1), VUS(2)	MAF within pathogenic range in ExAC database (PM2); conflicting classification in NCBI ClinVar; insufficient evidence	VUS
75710 (158/17.5/48.1)	*TP53*	c.1096T>G	het	miss	E10	chr17:7573931	rs17881470	p.S366A	—	0.02	—	5.56/6/0	5.730/14/0	—	tol	poly	B	13.1	1.77	C0	prob poly	0+	B	DM/CM078492	**validated polymorphism**	confl.: LB(3); VUS(1)	classified as "validated polymorphism" by expert panel; benign *in silico* prediction consensus (BP4); no consistent functional impairment [171-172] (BS3)	(LIKELY) BENIGN

Variant Specification										Population Databases					Computational Evidence								Gene/Disease specific Databases			Final Assessment of Variant Pathogenicity	
Carrier ID (quality parameters of the individual variant: Cov /Score/SB)	**Gene**	**Mutation HGVS ¥**	**het/ hom**	**Function**	**Loc.**	**Genomic Pos. (hg19)**	**SNP db_xref**	**Predicted AA Change**	**InterPro domain ID (v.67.0)**	**1000G** [%]	**ESP6500** [%] EurAm	**ExAC v0.3** frequ¶/allele count/# hom	**gnomAD v2.0.2** frequ¶/allele count/# hom	**FLOSSIES** frequ¶ / allele count /#hom	**SIFT v6.2.0 †**	**MutTaster v2013†**	**PolyPhen-2 v2.2.2†** HumVar	**CADD Phred**	**GERP+ +_RS**	**Align-GVGD v2007†§**	**UMD predictor**	**Prediction CONSENSUS∫**	**HGMD PRO v2017.3: variant class/acc.**	**Locus specific databases (LSDB, assessed by the LOVD)$**	**NCBI-ClinVar£**	**Rationale for Classification (ACMG evidence category if meeting)**	**final CLASSIFICATION**

Appendix 2

Legend to table S2: Synopsis of all detected secondary variants including annotations and criteria for pathogenicity assessment.

[1]NM_007294.3:c.4358-2725T>C;
[2]see table S3;
@variant shared by consanguineous carriers;
‡novel variant, unreported in LSDBs and reference populations;
Φreferring to NM_007300.3
Ψreferring to NM_001128147.2
¥ Reference transcripts: *BRCA1*, NM_007294.3; *BRCA2*, NM_000059.3; *ATM*, NM_000051.3; *BRIP1*, NM_032043.2; *CDH1*, NM_004360.3; *CHEK2,* NM_007194.3; *MSH2*, NM_000251.1; *MLH1*, NM_000249.3; *MSH6*, NM_000179.2; *PMS2,* NM_000535.5; *EPCAM*, NM_002354.2; *NBN*, NM_002485.4; *NF1*, NM_001042492.2; *PALB2*, NM_024675.3; *PTEN,* NM_000314.4; *RAD51C*, NM_058216.1; *RAD51D*, NM_001152571.1; *STK11*, NM_000455.4; *TP53,* NM_000546.4
¶E-05 notation;
†assessed by Alamut Visual v.2.10;
ʃprediction consensus is considered as "damaging" if at least 6 out of the 7 used *in-silico* predictions indicate a deleterious functional effect (SIFT: "deleterious"; MutationTaster: "pathogenic"; PolyPhen-2 "possibly damaging" or "probably damaging" for the HumVar algorithm; CADD Phred Score ≥20; GERP++_RS score >2; Align-GVGD ≥C15; UMD predictor: "probably pathogenic" or "pathogenic"). Accordingly, the prediction consensus is considered as "benign" if ≥6/7 *in-silico* predictions are benign; in any other cases, "indetermined" is used.
§Align-GVGD: C0, C15, C25, C35, C45, C55, and C65, with C65 being supposed to show the most likely deleterious functional effect;
ŞLOVD annotated pathogenicity interpretations are indicated in the format "reported in the literature/concluded by curators" with figures in brackets corresponding to the number of identical pathogenicity tags ("+" = affects function,"'+?" = probably affects function,"-" = does not affect function,"-?" = probably does not affect function, "?" = effect unknown, "." = effect not classified). Following LOVD overall classificators adapted from Plon *et al* [174] were used: class 1 = not pathogenic or of no clinical significance/neutral/benign/polymorphism (PM), class 2 = likely not pathogenic or of little clinical significance (LCS)/probably neutral/likely benign, class 3 = uncertain/VUS (UV), class 4 = likely pathogenic, class 5-pathogenic/causal. The significance classificator "inconsistent" is used if mixed pathogenicity tags are given.
£"one star" NCBI ClinVar classificators (review status "criteria provided, single submitter") are normal font; "two-star" variants ("criteria provided, multiple submitters, no conflicts") are italicized; "three-star" variants (reviewed by expert panel) are bolded.
Abbreviations
= count; AA = amino acid; acc. = accession; ACMG = American College of Medical Genetics and Genomics; Afr. Am. = African American; AFR = African/African American; AMR = American (Latino); ASJ = Ashkenazi Jewish; A-T = Ataxia-teleangiectasia (MIM #208900); B = benign; BC = breast cancer; BIC = Breast Cancer Information Core; conflicting = confl.; CR = case report (clinical observation); CRC = colorectal cancer; CSAS = cryptic splice-acceptor site; CSDS = cryptic splice-donor site; D/O (age of death) of diagnosis (years); dam = damaging; DBD = DNA-binding domain; del = deleterious; DFP = disease-associated polymorphism with supporting functional evidence; DM = disease-causing mutation; DM? = questionable disease-causing mutation; DP = disease-associated polymorphism; Dx = (age at) first diagnosis (years); E = exon; EAS = East Asian; ENIGMA = Evidence-based Network for the Interpretation of Germline Mutant Alleles; Eur. Am. = European American; FH = family history; FIN = Finnish; FP = in-vitro/laboratory functional polymorphism; frameshift = fs; frequ = frequency; GWAS = genome-wide association study; HBOC = hereditary breast and ovarian cancer; HDR = homology directed repair; het = heterozygous, HGVS = Human Genome Variation Society; hom = homozygous or homozygotes; HUG = Hôpitaux Universitaires de Genève; I = intron; IHC = immunohistochemistry; Indel = insertion-deletion; indet. = indetermined; LCS = low clinical significance; LoF = loss-of-function; LOH = loss-of-heterozygosity; LOVD = Leiden Open Variation Database; LS = Lynch syndrome; LSDB = locus-specific database; MAF = minor allele frequency; mat = maternal; missense = miss; MMR = DNA mismatch repair; MSI = microsatellite instable; MSS = microsatellite stable; n/d = not done; na = not applicable; NFE = Non-Finnish European; NGS = next-generation sequencing; NHL = Non-Hodgkin lymphoma; NMD = nonsense-mediated mRNA decay; noncoding = nonc; nonsense = nons; NW = North Western; OC = ovarian cancer; pat = paternal; path = pathogenic; poly = polymorphism; PD = possibly damaging; PrD = probably damaging; SAS = South Asian; SE = South Eastern; seg = segregation; SNP = single-nucleotide polymporphism; SR = supporting reads; synonymous = syn; TNBC = triple-negative breast cancer; tol = tolerated; uncertain = unc.; VUS = variant of unclear (clinical) significance
Web Resources
Align-Grantham Variation Grantham Deviation (GVGD) (http://agvgd.hci.utah.edu/); ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/); Combined Annotation Dependent Depletion (CADD) Score (http://cadd.gs.washington.edu/score/); Database of Single Nucleotide Polymorphism (dbSNP) (https://www.ncbi.nlm.nih.gov/SNP/); Exome Aggregation Consortium (ExAC) Browser (http://exac.broadinstite.org/); Fabulous Ladies Over Seventy (FLOSSIES) database (https://whi.color.com/), Genomic Evolutionary Rate Profiling (GERP++)_ Rejected Substitution (RS) (http://mendel.standford.edu/SidowLab/downloads/gerp/); Genome Aggregation Database (gnomAD) (http://gnomad.broadinstitute.org/); Human Gene Mutation Database (HGMD) (https://www.hgmd.org); Human Genome Variation Society (HGVS) (http://varnomen.hgvs.org/); MutationTaster (MutatTaster) (http://www.mutationtaster.org/); NHLBI Exome Sequencing Project (ESP) Exome Variant Server (http://evs.gs.washington.edu/EVS/); Polymorphism Phenotyping (PolyPhen)-2 algorithm (http://genetics.bwh.harvard.edu/pph2); Sorting Intolerant From Tolerant (SIFT) algorithm (http://sift.jcvi.org/); 1000 Genomes (1000G) Project (http://www.1000genomes.org); Universal Mutation Database (UMD) predictor (http://umd-predictor.eu/)
Locus specific (mutation) databases (LSDBs): BRCA Exchange Database (http://brcaexchange.org)*; NHGRI Breast Cancer Information Core (BIC) database (https://research.nhgri.nih.gov/bic/)*, International Society for Gastrointestinal Hereditary Tumours (InSight) databases (http://www.insight-database.org/genes/)*; International Agency for Research on Cancer (IARC) TP53 Database (http://p53.iarc.fr/)*; [a]Global Variome shared LOVD (https://databases.lovd.nl/shared/genes/); [b]Zhejiang University Center for Genetic and Genomic Medicine (ZJU-CGGM) database (http://www.genomed.org/lovd2/home.php); [c]Mismatch Repair Genes Variant Database (http://www.med.mun.ca/mmrvariants/); [d]Leiden Open Variation Database (LOVD) - human mismatch repair genes (http://HCI-LOVD.hci.utah.edu/home.php); [e]Universal Mutation Databases (UMD) (INSERM, University of Aix-Marseille) (http://www.umd.be/); [f]ARUP BRCA Mutation Database (Huntsman Cancer Institute, University of Utah, US) (http://www.arup.utah.edu/database/), [g]Kathleen Cuningham Foundation Consortium for Research into Familial Breast Cancer (kConFab) database (http://www.kconfab.org). *pathogenicity classifications reviewed by expert panels are bolded, respectively.

Appendix 3

Table S3: Synopsis of the *in-silico* predictions of potentially splice-affecting variants.

Variant	Loc. *	Effect on proximal canonical splice site (SS)	Effect on predicted proximal cryptic splice acceptor sites (CSAS) or donor sites (CSDS)	predicted proximal CSAS/CSDS *de novo*	PREDICTION CONSENUS¥	relevant references/database submissions
Wildtype (WT)->Mutant (±% WT)†						
BRCA1 (NM_007294.3)						
c.135-12del	I3	SSF 82.9->80.1 (-3.4%) MES 8.2->7.6 (-7.3%) NNS 1.0 (±0%) HSF 77.8 (±0%)			negative	none
c.693G>A p.(Thr231=)	E11	SSF 87.3 (±0%) MES 8.9 (±0%) NNS 0.9 (±0%) HSF 85.1 (±0%)	CSAS (c.691): HSF 72.2 (±0%)	CSAS (c.695): HSF +84.9, MES +2.7	negative	***BRCA1*Δ11 increased** (RT-PCR, PBL/Puromycin [1], minigene splicing assay [2]), clinical relevance unclear
c.1881C>G p.(Val627=)	E11			CSDS (c.1878): SSF +71.9 MES +5.8 NNS +0.7 HSF 77.9->81.8 (+5.0%)	***de novo* CSDS**	none

c.3597T>A p.(Ala1199=)	E11		CSAS (c.3601): HSF 77.3 (±0%) MES 4.0->3.6 (-10%) HSF 86.0->86.1 (-0.1%)		negative	none
BRCA2 (NM_000059.3)						
c.198A>G p.(Gln66=)	E3			CSAS (c.199): SSF +74.5 MES +6.3 HSF +80.7	***de novo* CSAS**	none
c.201G>A p.(Arg67=)	E3		CSAS (c.201): MES 2.3->0.0 HSF 77.0->73.9 (-4.0%) CSAS (c.220): SSF 74.4 (±0%) MES 0.7->2.0 (+186%) HSF 83.3 (±0%)		negative	none
c.631+7A>G	I7	SSF 78.2 (±0%) MES 6.8 (±0%) NNS 0.4->0.0 HSF 83.4 (±0%)			negative	none
c.5130T>C p.(Tyr170=)	E11			CSDS (c.5130): MES +1.3 HSF 76.5->76.3 (-0.3%)	negative	none

c.6057C>T p.(Asn2019=)	E11		CSDS (c.6053): SSF +70.1 MES 2.9->1.2 (-58.6%) HSF 74.5->74.2 (-0.4%)		negative	none
c.7331A>T p.(Asp2444Val)	E14			CSDS (c.7330): SSF +74.5 NNS +0.5 HSF +82.3	***de novo* CSDS**	none
c.8386C>T p.(Pro2796Ser)	E19		CSAS (c.8397): SSF 76.9->81.0 (+5.3%) MES 7.0->7.2 (+2.9%) NNS 0.8 (±0%) HSF 79.8->81.6 (+2.3%)		negative	none
c.9965T>G p.(Met3322Arg)	E27		CSAS (c.9963): MES 5.5->4.2 (-23.6%) NNS 0.6->0.0 HSF 87.6->87.0 (-0.7%)		negative	none
ATM (NM_000051.3)						
c.1272T>C p.(Pro424=)	E10		CSAS (c.1272): SSF 75.4 (±0%)	CSAS (c.1265): MES +0.4 (±0%) HSF +65.5 (±0%)	negative	none
c.1837G>T p.(Val613Leu)	E12		CSDS (c.1837): SSF 74.5->0.0 MES 7.2->0.0 NNS 0.9 ->0.0 HSF 83.2->0.0		**CSDS deactivated**	none

c.2275A>G p.(Ser759Gly)	E15			CSAS (c.2275): SSF +71.5 MES +7.2 HSF 65.3->75.9 (+16.2%)	***de novo* CSAS**	none
c.3154-4G>A	I21	SSF 88.0 (±0%) MES 8.3 (±0%) NNS 1.0 (±0%) HSF 88.1->88.2 (+0.1%)	CSAS (c.3154-4): MES 0.7->0.0 HSF 73.7->0.0		negative	none
c.4794C>G p.(Leu1598=)	E32		CSDS (c.4799): SSF 84.4->84.1 (-0.4%) MES 3.8->2.5 (-34.2%) HSF 88.8->86.0 (-3.2%)		negative	none
c.5009C>T p.(Ala1670Val)	E34 (4 bps to SAS)	SSF 80.5 (±0%) MES 2.5 (±0%) HSF 80.8 (±0%)	CSAS (c.5008): SSF 79.1 (±0%) HSF 77.3->78.2 (+1.2%)		negative	ClinVar submission SCV000261480.3 (Invitae, Apr, 2017): splicing defect suggested *in silico*, not yet confirmed in the literature
c.5271A>G p.(Thr1757=)	E35		CSAS (c.5296): SSF 78.2 (±0%) MES 4.1 (±0%) HSF 85.7 (±0%)		negative	none
c.9111_9112delinsAA p.(Gln3038Lys)	E63		CSAS (c.9112): SSF 83.1->0.0 MES 2.3->0.0 HSF 86.2->0.0		**CSAS deactivated**	none

BRIP1 (NM_032043.2)						
c.1629-3T>C	I11	SSF 84.4->90.6 (+7.3%) MES 6.9->6.8 (-1.4%) NNS 0.9->0.9 (±0%) HSF 82.0->89.7 (+9.4%)			negative	none
c.2097+8A>C	I14	SSF 94.7 (±0%) MES 10.5 (±0%) NNS 1.0 (±0%) HSF 97.1 (±0%)			negative	none
c.3042T>C p.(Gly1014=)	E20		CSDS (c.3041): SSF 74.0->0.0 MES 4.8->0.0 NNS 0.6->0.0 HSF 80.7->0.0		**CSDS deactivated**	none
CDH1 (NM_004360.3)						
c.69G>A p.(Gln23=)	E2		CSDS (c.70): SSF 84.4->0.0 MES 7.9->0.0 NNS 0.7->0.0 HSF 86.4->0.0		**CSDS deactivated**	ClinVar submission SCV000545385.1 (Invitae; Apr, 2017): splicing defect suggested *in silico*, not yet confirmed in the literature

c.150C>A p.(Arg50=)	E2		CSAS (c.162): SSF 71.4->0.0 MES 2.5->0.0 HSF 82.0->79.9 (-2.6%)		**CSAS deactivated**	none
c.322A>G p.(Arg108Gly)	E3		CSAS (c.324): SSF 77.3->0.0 MES 5.6->0.0 HSF 89.8->0.0		**CSAS deactivated**	none
c.957T>A p.(Ile319=)	E7		CSAS (c.963): SSF 82.8->76.8 (-7.2%) MES 6.0->5.3 (-11.7%) HSF 84.4->81.6 (-3.3%)		**CSAS deactivated**	none
c.2165-12C>G	I13	SSF 71.5->0.0 MES 6.4->5.0 (-21.9%) HSF 84.1->82.2 (-2.3%)			**canonical SAS deactivated**	none
c.2439+10C>T	I15			CSDS (c.2439+5): SSF +71.3 MES +1.8 HSF 72.9->75.1 (+3.0%)	***de novo* CSDS**	none
CHEK2 (NM_007194.3)						
c.319+7C>A	I2	SSF 82.0->82.0 (±0%) MES 8.8->8.8 (±0%) NNS 1.0->1.0 (±0%) HSF 90.9->90.9 (±0%)			negative	none

c.320-5T>A	I3	SSF 92.9->86.5 (-6.9%) MES 7.7->6.0 (-22.1%) NNS 0.9->0.6 (-33.3%) HSF 88.5->84.9 (4.1%)			**canonical SAS deactivated**	partial expression of **in-frame Δ3,4 transcript** in one individual with BC/OC (RT-PCR blood analysis) [46]
c.846+4_846+7del	I8	SSF 87.4->0.0 MES 8.3->0.0 NNS 1.0->0.0 HSF 89.1->65.7 (-26.3%)			**canonical SDS deactivated**	ClinVar submissions SCV000545385.1 (Invitae; Apr, 2017) & SCV000329279.5 (GeneDx, Jun, 2017): splicing defect suggested *in silico* (natural SDS destroyed), not yet confirmed in the literature
MSH2 (NM_000251.1)						
c.1131A>G p.(Gln377=)	E7		CSDS (c.1157): SSF 79.77 (±0%) MES 7.47 (±0%) NNS 0.8 7 (±0%) HSF 85.37 (±0%)		negative	none
c.1489A>G p.(Ile497Val)	E9			CSDS (c.1489): SSF +75.2 MES +7.8 NNS +1.0 HSF +82.7	***de novo* CSDS**	ClinVar submission SCV000567758.3 (GeneDx, Mar, 2016): splicing defect suggested *in silico* (CSDS creation upstream of the natural splice donor), not yet confirmed in the literature
c.2595C>T p.(Ile865=)	E15		CSDS (c.2606): SSF 0.1->0.0 HSF 77.9->79.7 (+2.3%)		negative	none

MLH1 (NM_000249.3)						
c.277A>G p.(Ser93Gly)	E3			CSDS (c.277): MES +4.6 NNS +0.5 HSF +74.1	***de novo* CSDS**	no aberant splicing in minigene assay (not proven by using patient RNA) (overall classification as VUS) [178]. MMR proficiency in *in-vitro* approaches [179-182, 113].
c.1401C>T p.(Ser467=)	E12		CSDS (c.1401): SSF 73.6->73.1 (-0.7%) HSF 85.9->85.8 (-0.1%)		negative	none
c.1587C>T p.(Ser529=)	E14		CSDS (c.1612): SSF 70.5 (±0%) MES 4.4 (±0%) HSF 81.1 (±0%)		negative	none
c.1732G>A p.(Glu578Lys)	E16 (SAS, 1st exonic bp)	SSF 86.2 (±0%) MES 9.3->7.8 (-16.1%) NNS 1.0 (±0%) HSF 84.6->81.4 (-3.8%)			negative	none
c.1935C>G p.(Asn645Lys)	E17			CSDS (c.1935): SSF +77.7 MES +7.4 NNS +0.7 HSF +80.6	***de novo* CSDS**	ClinVar submission SCV000254363.3 (Invitae; May, 2017): splicing defect suggested *in silico*, not yet confirmed in the literature

c.2103+9G>A	I18	SSF 72.0 (±0%) MES 8.7 (±0%) NNS 0.9 (±0%) HSF 77.1 (±0%)			negative	none
MSH6 (NM_000179.2)						
c.942C>T p.(Ser314=)	E4		CSDS (c.936): SSF 74.4 (±0%) MES 4.3 (±0%) HSF 82.7 (±0%)		negative	none
c.1186C>G p.(Leu396Val)	E4			CSAS (c.1187): SSF +76.5 MES +3.9 HSF +83.7	***de novo* CSAS**	none
c.1768C>T p.(Pro590Ser)	E4		CSDS (c.1775): SSF 72.6->74.1 (+2.1%) MES 3.2->2.4 (-25.0%) HSF 85.0->84.6 (-0.5%)		negative	none
c.2883A>G p.(Arg961=)	E4		CSDS (c.2867): MES 1.0 (±0%) HSF 71.3 (±0%)		negative	none
c.4001+4_4001+8dup	I9				n/d via †	none
c.4001+12_4001+15del	I9				n/d via †	none

c.4002-8A>T	I9	SSF 89.5->89.6 (+0.1%) MES 10.1->11.0 (+8.9%) NNS 0.9->1.0 (+11.1%) HSF 82.5->84.5 (+2.4%)			**canonical SAS activated**	none
c.4026G>A p.(Arg1342=)	E10		CSAS (c.4026): SSF 71.9->0.0 MES 2.2->0.0 HSF 79.7->0.0		**CSAS deactivated**	none
c.4068G>A p.(Leu1356=)	E10		CSAS (c.4075): SSF 73.5->73.8 (+0.4%) MES 3.3->3.0 (-9.1%) HSF 77.3->77.4 (+0.1%)		negative	none
PMS2 (NM_000535.5)						
c.988+11T>C	I9	SSF 100.0 (±0%) MES 10.9 (±0%) NNS 1.0 (±0%) HSF 100.0 (±0%)			negative	none
c.1032G>A p.(Leu344=)	E10		CSAS (c.1040): SSF 72.0 (±0%) HSF 80.1 (±0%)		negative	none
c.1864A>G p.(Met622Val)	E11			CSDS (c.1864): MES +7.0 NNS +0.6 HSF 81.2	***de novo* CSDS**	ClinVar submission SCV000566510.3 (GeneDx, Aug, 2017): splicing defect suggested *in silico* (creation of a CSDS upstream of the natural splice donor), not yet confirmed in the literature

EPCAM (NM_002354.2)						
c.5C>T p.(Ala2Val)	E1		CSAS (c.16): SSF 76.8->80.9 (+5.3%) MES 5.2->6.2 (+19.2%) NNS 0.6->0.7 (+16.7%) HSF 89.2->91.1 (+2.1%)	CSDS (c.5): MES 0.6 HSF 70.1	**CSAS activated**	none
c.904-9C>G	I8	SSF 92.5->92.4 (-0.1%) MES 12.5->11.6 (-7.2%) NNS 1.0 (±0%) HSF 90.4->88.5 (-2.1%)			negative	none
NBN (NM_002485.4)						
c.1317A>G p.(Ile439Met)	E10			CSDS (c.1313): SSF +72.4 MES +4.7 HSF 66.1->78.3 (+18.5%)	***de novo* CSDS**	none
NF1 (NM_001042492.2)						
c.528T>A p.(Asp176Glu)			CSAS (c.533): SSF 74.7->0.0 MES 4.6->3.5 (-31.4%) HSF 77.3->73.7 (-4.9%)		**CSAS deactivated**	none
c.696A>G p.(Thr232=)	E7		CSAS (c.709): MES 2.0->2.3 (-15%) HSF 77.8 (±0%)		negative	none

c.825C>T p.(Ile275=)	E8		CSAS (c.836): SSF 85.5->89.6 (+4.8%) MES 7.9->7.8 (-1.3%) NNS 0.8->0.9 (+12.5%) HSF 87.8->89.7 (+2.2%)		negative	none
c.2985G>C p.(Leu995=)	E22		CSDS (c.2986): SSF 76.6->0.0 MES 3.7->0.0 HSF 88.3->77.3 (-12.5%)		negative	none
c.6033A>G p.(Leu2011=)	E41		CSAS (c.6035->c.6039): SSF 70.9->72.6 (+2.4%) MES 3.9->4.4 (+12.8%) HSF 74.5->80.7 (8.3%)		negative	none
c.6345G>A p.(Pro2115=)	E42		CSDS (c.6341): SSF 73.6->0.0 MES 5.1->0.0 NNS 0.7->0.0 HSF 84.0->71.8 (-14.5%)		**CSDS deactivated**	none
c.6942C>T p.(Ala2314=)	E47		CSAS (c.6941): MES 1.3->1.9 (+46.2) HSF 76.2->77.1 (+1.2%)		negative	none

c.7623G>A p.(Arg2541=)	E52	SSF 90.3 (±0%) MES 9.2 (±0%) NNS 1.0 (±0%) HSF 87.4 (±0%)			negative	none
c.8041A>G p.(Ile 2681Val)	E55			CSAS (c.8042): SSF +81.6 MES +3.5 HSF +82.6	***de novo* CSAS**	none
c.8499T>C p.(Asn2833=)	E58		CSDS (c.8495): MES 2.1->4.0 (+90.5%) HSF 73.4->73.7 (+0.4%)		negative	none
PALB2 (NM_024675.3)						
c.899C>T p.(Thr300Ile)	E4		CSAS (c.903): SSF 82.1->76.8 (-6.5%) MES 7.1->6.0 (-15.5%) NNS 0.5 (±0%) HSF 91.6->83.9 (-8.4%)		**CSAS deactivated**	none
c.2235A>G p.(Lys745=)	E5		CSAS (c.2236): HSF 68.6->76.6 (+11.7%)	CSDS (c.2236): SSF 76.9	negative	none
c.2794G>A p.(Val932Met)	E8		CSDS (c.2796): SSF +72.8 MES 1.3->2.6 (+100.0%) HSF 74.3->79.2 (+6.6%)		**CSDS activated**	none

c.3059A>G p.(Gln1020Arg)	E10			CSDS (c.3055): SSF +70.9 MES +1.6 HSF 69.9->82.1 (+17.5%)	***de novo* CSDS**	none
c.3428T>A p.(Leu1143His)	E13		CSAS (c.3439): SSF 78.4->71.6 (-8.8%) MES 3.7->2.1 (-28.6%) HSF 84.3->80.4 (-4.6%)		**CSAS deactivated**	none
c.3495G>A p.(Ser1165=)	E13			CSDS (c.3497): SSF +72.0 MES 4.2->8.7 (+107.1%) NNS +0.8 HSF 72.2->77.1 (+6.8%) CSAS (c.3497): MES +3.1 HSF +79.1 CSDS (c.3492): SSF +76.3 MES +2.9 HSF 79.0->87.3 (+10.5%)	***de novo* CSDS**	none
PTEN (NM_000314.4)						
c.132C>T p.(Gly44=)	E2		CSDS (c.131): SSF +72.0 MES 4.2->8.7 (+107.1%) NNS +0.8 HSF 72.2->77.1 (+6.8%)		**CSDS activated**	none

RAD51C (NM_058216.1)						
c.145+12T>G	I1	SSF 82.0 (±0%) MES 10.2 (±0%) NNS 1.0 (±0%) HSF 85.1 (±0%)			negative	none
c.1008A>G p.(Thr336=)	E8			CSDS (c.1009): MES +5.9 HSF +71.2	negative	none
RAD51D (NM_001142571.1)						
c.225C>T p.(Val75=)	E3		CSDS (c.222): SSF 73.1->0.0 MES 0.4->0.0 HSF 86.4 (±0%)		negative	none
c.853G>A p.(Gly285Arg)	E9			CSAS (c.855): MES +3.5 HSF +86.0	negative	ClinVar submission SCV000287725.3 (Invitae; Feb, 2017): splicing defect suggested *in silico*, not yet confirmed in the literature
STK11 (NM_000455.4)						
c.42G>A p.(Glu14=)	E1			CSDS (c.44): SSF +80.1	negative	none
c.310A>T p.(Arg104Trp)	E2			CSDS (c.308): SSF +70.1 MES +0.8 HSF +76.8	***de novo* CSDS**	none

†prediction algorithms (score range; cut off ±% WT): SpliceSiteFinder (SSF)-like (0-100; 5%); MaxEntScan (MES) (0-16; 10%); NNSplice (NNS) (0-1; 5%); Human Splicing Finder (HSF) (0-100; 2%); cut-off values according to Tang *et al* [175] and Baert *et al* [176] (assessed by Alamut Visual v.2.10);

*juxta-splice junction consensus region (11 bases at the SDS [+3 to -8], 14 bases at the SAS [-12 to +2] according to Cartegni *et al* [177];

¥Splice consensus prediction is considered positive if at least three algorithms scored above the relative cut-off values established for the score differences between the wild-type and variant sequence;

CSAS = cryptic splice acceptor sites; CSDS = cryptic splice donor sites; E = exon; I = intron; Loc. = localization; MMR = DNA mismatch repair; PBL = peripheral blood lymphocytes; RT-PCR = reverse transcription polymerase chain reaction; SAS = splice acceptor site; SDS = splice donor site; SS = splice site; WT = wild-type

Appendix 4

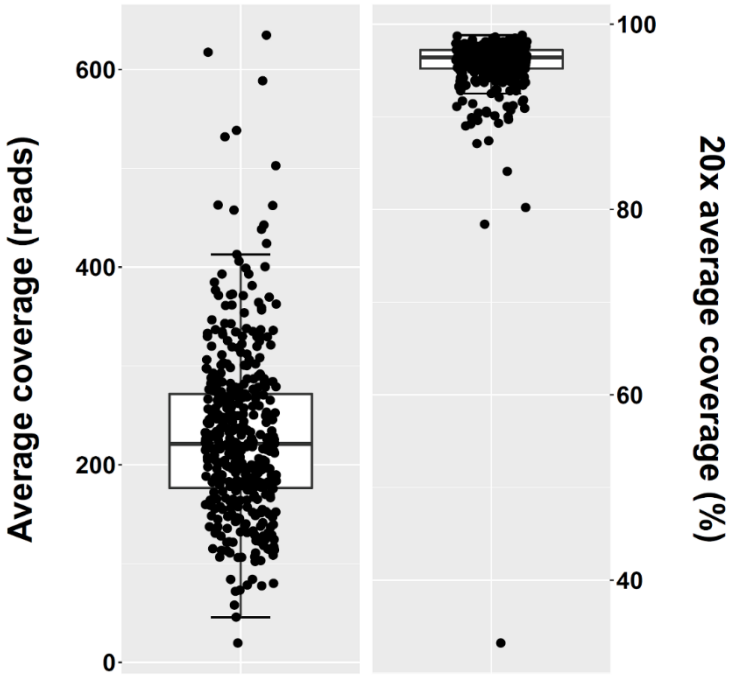

Figure S1: Box plot of the coverage data of all individual WES analyses. Each dot represents one individual WES data set. The dots representing the four WES data sets excluded due to insufficient data quality (<85% 20x average coverage) are indicated for completeness. For one WES data set, overall coverage data were not available, but all targeted genes were sufficiently covered.

Appendix 5

References for supplementary data appendices

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