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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

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

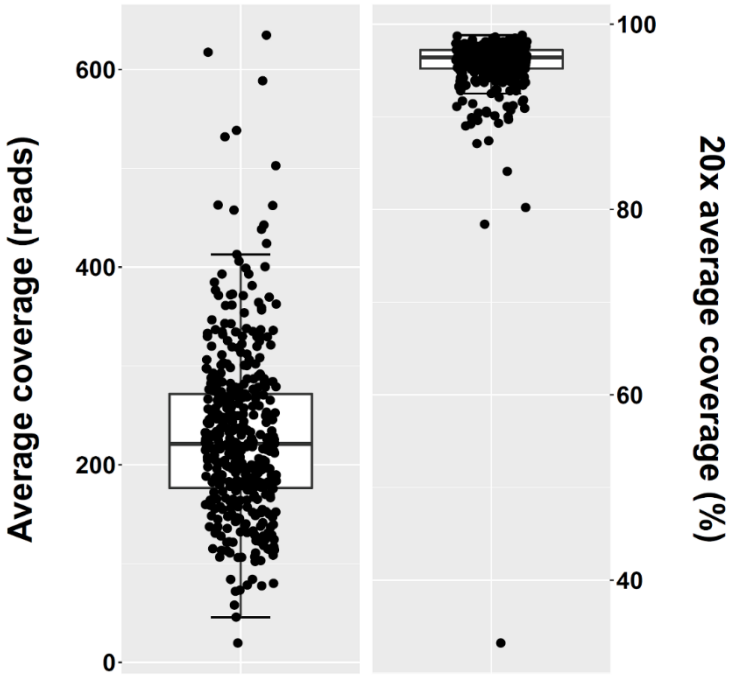

Figure S1: Box plot of the coverage data of all individual WES analyses. Each dot represents one individual WES data set. The dots representing the four WES data sets excluded due to insufficient data quality (<85% 20x average coverage) are indicated for completeness. For one WES data set, overall coverage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

Appendix 5

References for supplementary data appendices

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