

SUPPLEMENTARY MATERIALS

Supplementary Tables

Supplementary Table 1. Variant calling and pathogenicity assessment pipelines^a

study	gDNA source	analysis	enrichment, SEQ	aligning, calling	annotation, classification	inclusion, exclusion
Akhavanfard et al, 2020 (1)	B, M	WES	Nextera Rapid Capture Exome; HiSeq 2500, 100bp paired-end	aligning: BWA v.0.6.1 recalibrate/deduplicate: GATK v.3.5, SAMtools, Picard calling: GATK Haplotype Caller	annotation: Ingenuity Variant Analysis (IVA) software v.5.4.20190121 (Qiagen, Ingenuity Systems) classification: IVA auto classification following ACMG guidelines	inclusion: IVA auto classification as (likely) pathogenic OR intronic variants ± 20 if MaxEntScan predicted disrupted splicing AND call quality and read depth ≥ 20.0 AND genotype quality ≥ 30.0 . exclusion: MAF $\geq 1.0\%$ in any database (i.e., 1000 Genomes Project (phase3v5b), NHLBI ESP (ESP6500SI-V2), ExAC Frequency (0.3.1), and gnomAD Maximum Frequency (2.0.1); Phred-scaled CADD (v1.3) score < 10 ; tolerant SIFT prediction; ClinVar (likely) benign; exception: established common pathogenic variants
Byrjalsen et al, 2020 (2)	B, BR, F	WGS	HiSeq X, 150bp paired-end, 30x average coverage	aligning: BWA v.0.7.12 deduplicate: biobambam2 v.2.0.27 calling: GATK v.3.8 Haplotype Caller or Sentieon DNaseq	annotation: VarSeq v.2.2.0 classification: interdisciplinary team of experts using variant type, prediction tools (e.g., CADD, PHRED quality score, splice prediction by ADA and Alamut Visual v.2.10, published literature	inclusion: read depth ≥ 8 , genotype quality ≥ 20 , VAF ≥ 0.2 ; within 59 ACMG 'actionable' genes or 314 cancer gene panel exclusion: intronic, UTR, or intergenic; MAF $\geq 1\%$ in any database – except pathogenic variants in known CPS genes (e.g., <i>CHEK2</i>)
Chang et al, 2016 (3)	B	WES	SeqCap Ez Human exome Library v3.0, HiSeq 2500	aligning: BWA v.0.6.2-r126 deduplicate: Picard calling: GATK v.2.4 Haplotype	annotation: NCI-CCR Oncogenomics Annotation Pipeline	inclusion: ≥ 10 total reads; ≥ 3 variant reads; VAF $\geq 10\%$; ESP and 1000 Genomes MAF $< 1\%$

				Caller for SNV, bam2mpg and Platypus for indels		- and rare filter w/oT (ExAC, CG69); CADD score w/oT; within 49 ACMG 'actionable genes' OR within 48 HGMD 'disease causing genes' OR ClinVar classification (likely) pathogenic OR nonsense/frameshift/splicing variant within 11 cancer genes
Fiala et al, 2021 (4)	B	panel NGS	custom-made capture-based NGS panel (MSK-IMPACT), SeqCap EZ library custom oligo system, HiSeq 2500, 100bp paired-end	aligning: BWA v.0.7.5a deduplicate: Picard recalibrate: IndelRealigner, BaseRecalibrator (GATK v2.7.227) calling: SNV: MuTect v.1.1.4 and Haplotype caller (GATK v.2.3.9), indels: IndelDetector (GATK v.2.3.9)	annotation: ANNOVAR v.527 classification: classification by multidisciplinary board following ACMG guidelines	inclusion: quality score ≥ 20 ; exonic SNV and indels: coverage depth ≥ 20 , VAF $\geq 20\%$; all other: coverage depth ≥ 20 , VAF $\geq 20\%$ exclusion: MAF $> 1\%$ in 1000 Genomes and NHLBI ESP
Gröbner et al, 2018 (5)	ns	WES, WGS and lcWGS	cohort analysis including data from various sources, Illumina sequencing technology, 100bp paired-end	aligning: BWA-MEM v.0.7.8 duplicate removal: Picard tools calling: SAMtools v.0.1.17-based approach using mpileup and bcftools (SNV), SAMtools mpileup, Platypus v.0.7.4 (indels), Pindel v.0.2.4h	annotation: ANNOVAR classification: manually scored in ACMG categories using given annotations and additional data from online databases, especially gene-specific databases	inclusion: 162 candidate genes, $\geq 15\times$ coverage, VAF ≥ 0.2 , phred-based quality score ≥ 10 , dbSNP variants with 'precious' annotation were not filtered; variants with phred-based quality score ≥ 20 and Mutation Assessor categories 'medium' and 'high', or no available annotation exclusion: reported in 1000 Genomes rel. Nov. 2010 & dbSNPv14.1; MAF ≥ 0.01 in NHLBI ESP, 1000 Genomes, or ExAC; ClinVar annotation as 'benign', 'likely benign' or 'uncertain significance'
Kim et al, 2021 (6)	B	WGS/WES	SeqCap EZ Human Exome Library, paired-end	aligning: Novoalign 3 software (v.3.00.05) deduplicate: Picard v.1.126 recalibrate: BaseRecalibrator, RealignerTargetCreator and IndelRealigner (GATK v.3.1) calling: UnifiedGenotyper and HaplotypeCaller (GATK v.3.1), FreeBayes variant caller (v.9.9.2); subsequently, integration in Ensemble's variant calling pipeline v.0.2.2	annotation: in-house pipeline adding data from SnpEff/SnpSift, ANNOVAR, UCSC GoldenPath database, ESP6500 dataset, dbNSFP, MSigDB database, dbSNPv137, and 1000 Genomes, etc. classification: following ACMG guidelines	inclusion: 130 cancer susceptibility genes exclusion: read depth < 10 , ABHet < 0.2 or > 0.8 or other quality control issues in CScorefilter; MAF $\geq 1\%$ in ESP, 1000 Genomes, gnomAD and ExAC, both w/o TCGA
Li et al, 2020 (7)	B	WES	HGSC VCRome2.1, paired-end, NovaSeq	aligning: BWA-MEM v.0.7.15-r1140 recalibrate: GATK v.3.4-0	annotation: ANNOVAR v.2018.04.16 classification: (likely)	inclusion: 70 cancer predisposition genes, MAF < 0.01 in 1000 Genome Phase

				calling: xAtlas v.0.2.1	pathogenic variants in ClinVar v.20190305 with ≥ 1 stars OR novel, rare, potential loss-of-function variants (i.e., splicing variants, frameshift insertion or deletion variants, nonsense single-nucleotide variants, or stoploss variants)	3, gnomAD v.2.1.1 and ExAC v.0.3 exclusion: read depth <15, quality score <15; allelic ratio <0.25 or >0.75; xAtlas 'non-PASS'
Mirabello et al, 2020 (8)	B, M	WES, targeted NGS	SeqCap EZ Human Exome Library, Exome+UTR, HiSeq 2500 (125bp paired-end), HiSeq 4000 (150bp paired-end) replication: targeted sequencing, HiSeq 2500 (125bp paired-end), SureSelect All Exon V5+UTRs, HiSeq 2000 (100bp paired-end)	aligning: Novoalign software v.3.00.05 recalibrate: GATK v.3.1 calling: UnifiedGenotyper and HaplotypeCaller (GATK v.3.1) and FreeBayes variant caller v.9.9.2; subsequently, integration by Ensembl's variant calling pipeline (bcbio v.0.2.2)	classification: ACMG categories were assigned as follows (1) ClinVar classification by ClinVar-badged laboratories. (2) remaining variants were classified using InterVar v.2.1.2, default setting. (3) VUS resulting from step 2 were classified as likely pathogenic, if they were high impact (i.e., frameshift indels, stop gain/loss, or known splice sites) AND listed as disease-causing in HGMD v.2018.1. (4) remaining VUS that are either high-impact OR HGMD disease-causing were evaluated by in silico tools and if 'deleterious' (i.e., MetaSVM: damaging, REVEL: ≥ 0.5 and CADD ≥ 20) they were manually curated for likely pathogenicity. (5) (l)p variants from step 1-4 with VAF >0.005 and >0.001 for AR and AD genes, respectively, were down-graded as VUS. (6) finally, manual review of all (l)p variants	exclusion: poor quality scores w/oT, read depth <5, allele fraction <0.25, MAF >1% in 1000 Genomes, NHLBI ESP, or ExAC
Mody et al, 2015 (9)	B, M	WES	SureSelect Human All Exon V4, HiSeq 2000 and HiSeq 2500, paired-end	aligning: Novoalign Multithreaded v.2.08.02 processing: SAMtools v.0.1.18, Novosort threaded v.1.00.01, Picard v.1.74 calling: VarScan2 v.2.3.2 utilizing files created by SAMtool mpileup	annotation: ANNOVAR classification: manual classification based on published literature, public databases (e.g., ClinVar, HGMD, LOVD, and variant specific such as IARC TP53); variants with conflicting classification or not previously reported were considered as VUS	inclusion: variants passing quality filtering w/oT regarding coverage, variant read support, variant frequency, P-value, base quality, homopolymer, and strandedness exclusion: synonymous variants

Newman et al., 2021 (10)	ns	WES, WGS	WGS, TruSeq DNA PCR-free sample preparation; WES, Nextera Rapid Capture Exome, TruSeq Exome, HiSeq 2500/4000	aligning: BWA v.0.5.9 deduplicate: Picard v.1.65 calling: Bambino, CREST, CONSERING; manual review of exonic variants	annotation: Medial Ceremony/PeCanPIE pipeline classification: ACMG 2015 guidelines; adapted – and overruled, if applicable – by an expert committee applying clinical, biological, and functional insights from laboratory studies	inclusion: WGS, 45x in ≥40% of coding exons or manually reviewed and 30x in ≥80%; WES, 45x in ≥65% or manually reviewed and 30x in ≥80%; RNA-seq, 45x in ≥15% or manually reviewed and 30x in ≥20%; cross-validation re WES and WGS exclusion: overlap with variant list, variants found >10 in 6,500 non-cancer individuals in the NHLBI ESP
Oberg et al, 2016 (11)	B, M	WES, NGS panel	SureSelectXT All Exon V5+UTRs, HiSeq 2500, 125bp paired-end; Custom SureSelectXT library targeting 467 genes, HiSeq 2500, 125bp paired-end	aligning/calling: NextGene v.2.3.4 using a modified Burrows-Wheeler transform (BWT) alignment	classification: manual classification by multi-disciplinary board; classification based on known well-characterized hot spot variants, unambiguous loss-of-function variants in tumor suppressor genes (i.e., nonsense/frame-shifts leading to loss of functional domains); variants with experimental data supporting pathogenicity	inclusion: quality score ≥20, ≥3 reads; VAF ≥5%; variants in cancer predisposition genes, relevant to pharmacogenomics, or variants relevant to patient care; ACMG secondary findings gene list; MAF <1% in 1000 Genomes (phase 1, version 3)
Parsons et al, 2016 (12)	B	WES	VCRome v.2.1, HiSeq, paired-end	aligning: BWA calling: Atlas-SNP and Atlas-indel	annotation/filtering: <i>in-house</i> solution, i.e., CASSANDRA, utilizing ANNOVAR annotation, in silico prediction (e.g., SIFT, PolyPhen, etc.) classification: ACMG guidelines	inclusion: coverage >20; exclusion: quality score w/oT, MAF >1% and >5% for non-HGMD-listed variants and HGMD-listed variants, respectively, regarding 1000 Genomes and NHLBI ESP; synonymous variants; intronic >±5bp from exon boundary
Stedingk et al, 2021 (13)	B	NGS panel	Fluidigm Juno target-enriched custom panel addressing 22 cancer predisposing genes, HiSeq 2500, 150bp paired-end	aligning: Novoalign v.3.02.13 quality control: Picard calling: HaplotypeCaller (GATK v.3.7), Freebayes v.1.1.0-3-g961e5f3, VarDict v.1.5.0	annotation: HaplotypeCaller (GATK v.3.7), Freebayes v.1.1.0-3-g961e5f3, VarDict v.1.5.0, VEP classification: following ACMG guidelines & ClinGen expert-approved gene-specific expert panel criteria	exclusion: DP <30, QUAL <200 (HaplotypeCaller and Freebayes) or QUAL <150 (VarDict), QD <5.0, GQ <50, AB < 0.2 for het calls, AB <0.9 (FreebayesB and VarDict), or AB <0.1 and AB <0.9 (HaplotypeCaller) for hom calls, SNV: MQ <30, MQRankSum <(-12.5), BaseQRankSum <(-12.5) (HaplotypeCaller and Freebayes) or BaseQRankSum <(-6.0) (VarDict); MAF >1% in gnomAD v.2.1 (non-cancer)

Waszak et al, 2018 (14)	B	WES, WGS	ns	alignment/calling: aligned: BWA-MEM, following standards defined by the Pan-Cancer Analysis of Whole Genomes (PCAWG) Project calling: freebayes v1.1.0 (snv, mnv, indel); delly (SV); DNACopy v.1.5 (CNV)	annotation: VEP v.81; damaging classification based on frameshift, stop gain, start lost, canonical splice site, exon or gene deletions. ClinVar-known damaging non-canonical splice site variants; for TP53 data of the IARC TP53 database and functional assays were also regarded	exclusion: MAF >0.1% in ExAC, 1000 Genomes, or NHLBI ESP; ClinVar classification as (likely) benign
Wagener et al, 2021 (15)	B, F	WES	SureSelect Human All Exon V5+UTR, HiSeq 2500 (100bp paired-end) or NextSeq 550 (150bp paired-end)	aligning: BWA-MEM v.0.7.12, Samtools v.1.2 deduplicate: Picard v.2.0.1 calling: GATK v.4.1.4.1 and VarScan2 v.2.3.9 (SNV, indels), platypus v.0.8.1 (indels)	annotation: VEP v.98.3 classification: manually-reviewed automated classification by CharGer and/or CPSR pipeline following ACMG guidelines	inclusion: variants in 295 pre-selected (candidate) cancer predisposition genes exclusion: VAF <10% in index (Trio-WES); MAF >1% in gnomAD, non-cancer; variants present in ≥5% of cases in present cohort
Wong et al., 2020 (16)	B, F	WGS	TruSeq Nano DNA HT Sample Prep Kit or KAPA PCR-Free v.2.1, HiSeq X, 150bp paired-end	aligning: BWA-MEM v.0.17.10-r789 deduplicate/recalibration: Novosort v.1.03.01, Indel Realignment (GATK v.3.3) calling: HaplotypeCaller (GATK), GenotypeVCFs, and VQSR	annotation: VEP v.87 and Seave for subsequent filtration and prioritization classification: manual by national variant curation team following principles of ACMG guidelines; previous annotation as (likely) pathogenic in ClinVar, novel loss-of-function variants in tumor suppressor genes	inclusion: variants in 161 cancer predisposition genes, MAF <1% in ExAC, gnomAD, MGRB, NHLBI ESP, and 1000 Genomes
Zhang et al, 2015 (17)	B, B ^R	WES, WGS	TruSeq Expanded Exome, HiSeq 2000, 101bp paired-end	aligning: BWA v.0.5.5 deduplicate: Picard v.1.29 calling: Samtools svn rev 454, variation detection module of Bambino	annotation: VEP with postprocessing for enhanced splice variant calling classification: automated classification following 3-tier 'medal ceremony' based on mutation databases (e.g., IARC TP53, ClinVar, HGMD, LOVD), mutation type, population frequency, tumor suppressor status, and predicted functional impact; in case of indicated clinical implication, manual classification regarding ACMG criteria	inclusion: MAF <0.1% in NHLBI ESP exclusion: in-frame indels reported in dbSNP; indels present in 1000 Genomes or with multiple submissions in dbSNP

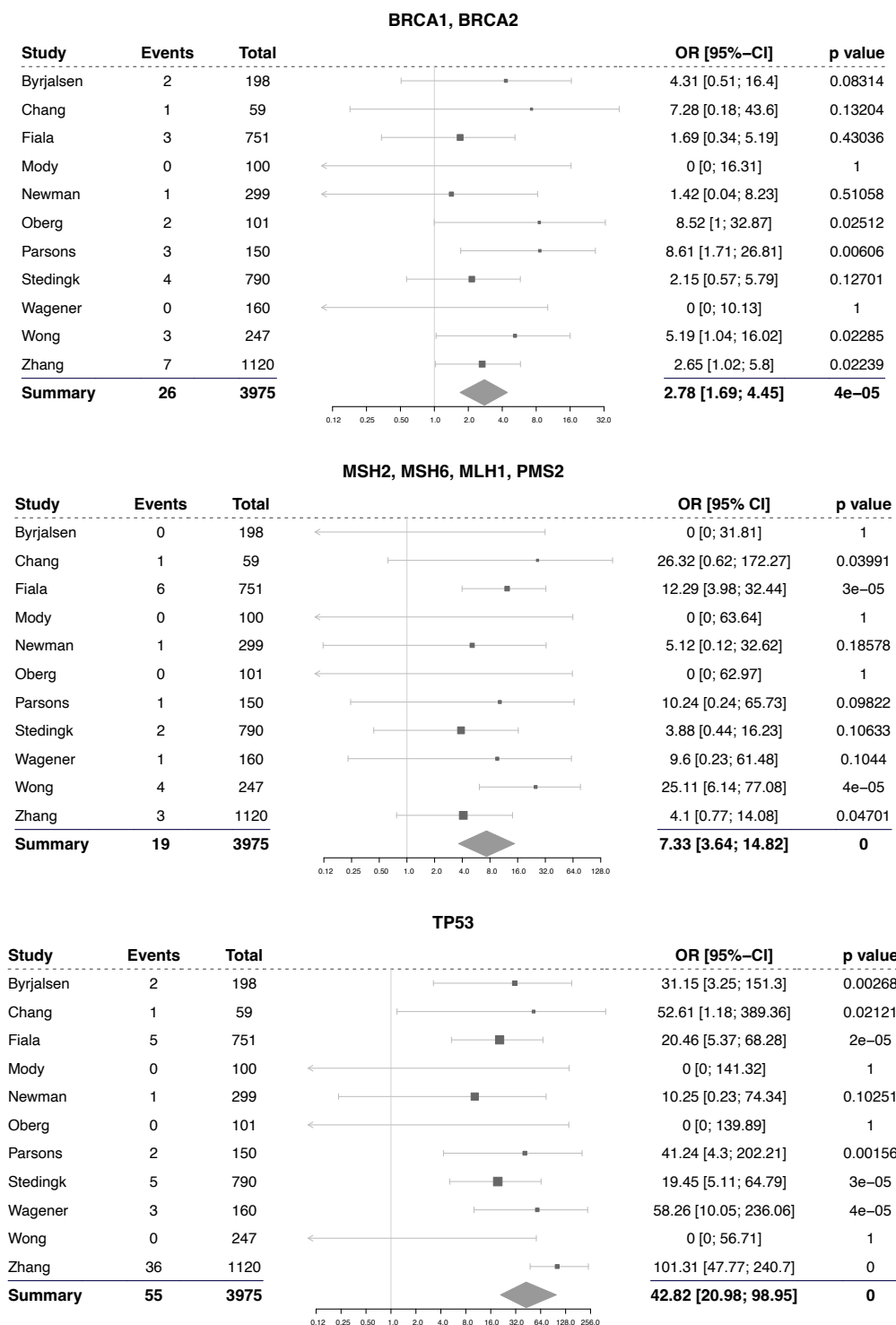
^aAB, allele balance; AD, depth per allele by sample; B, blood; BR, blood in remission; BaseQRankSum, base quality rank sum test; DP, coverage; F, fibroblast; gDNA, germline DNA; GQ, genotype quality; M, buccal mucosa; MQ, root mean square mapping quality; MQRankSum, mapping quality rank sum test; MSK-IMPACT, Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPAC); NGS, next generation sequencing; ns, not specified; QD, quality by depth; RNA-seq, RNA sequencing; SNV, single nucleotide variant; VAF, variant allele frequency; VEP, Ensembl's Variant Effect Predictor; VUS, variants of uncertain significance; w/oT, without given threshold for filtering.

Supplementary Table 2. Details on cancer types and variants in genes of interest. All cases from the main burden test with a ClinVar pathogenic/likely pathogenic variant in a gene of interest are included.^a

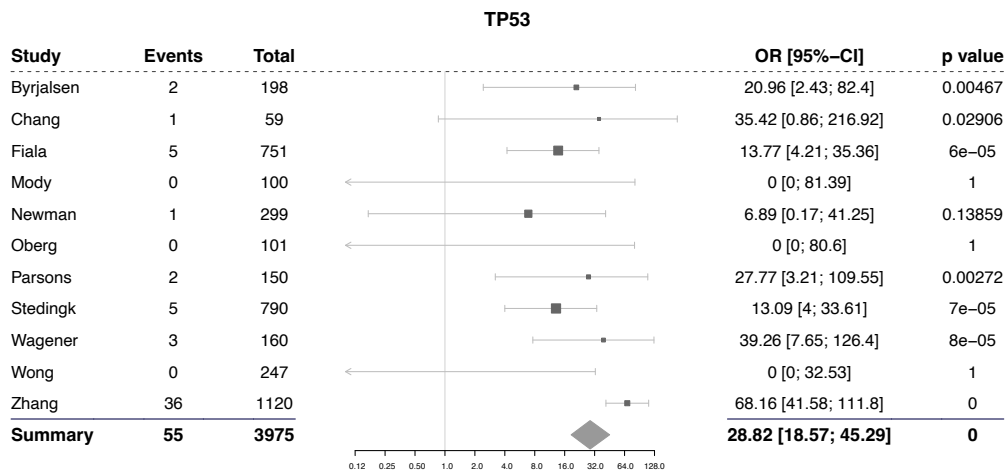
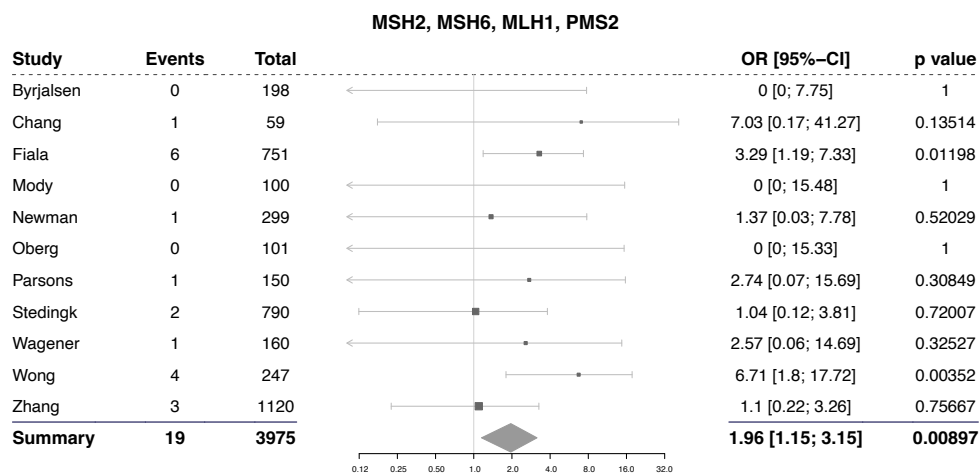
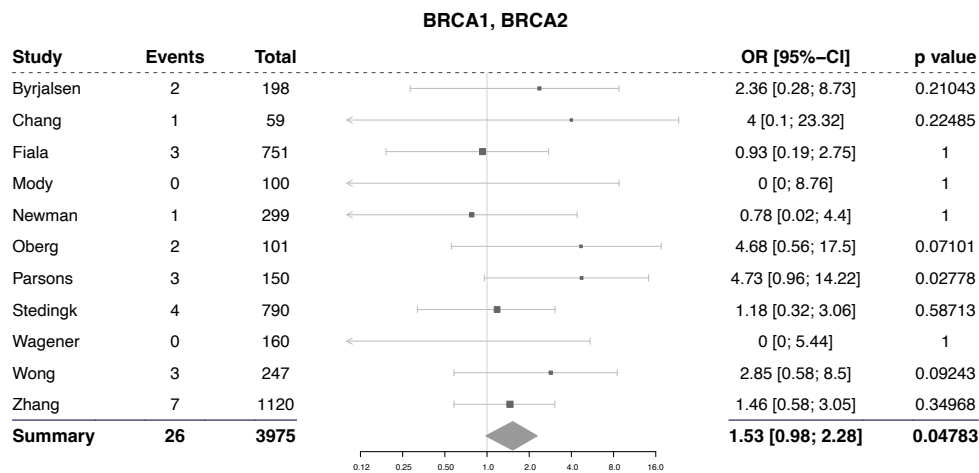
This supplementary table is available online for separate download as a .xls file.

^a For the validation cohort, detailed sequence information is provided. Variants identified in controls are also shown.

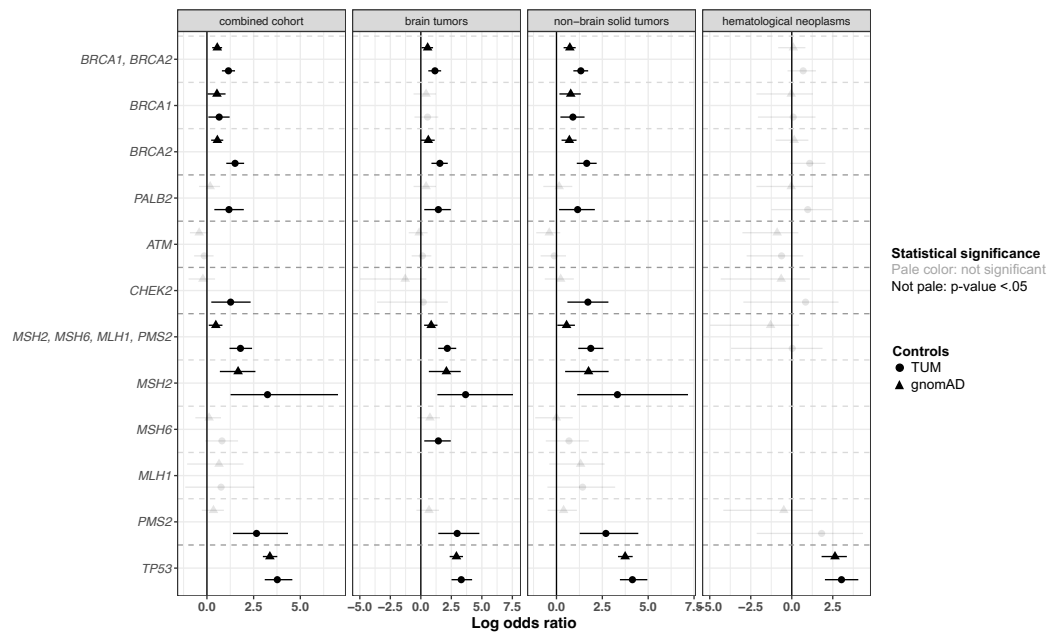
Supplementary Figures



Supplementary Figure 1. Forest plots showing occurrence rates for heterozygous PVs in *BRCA1/2* (A), mismatch repair (MMR) genes (B), and *TP53* (C) across 11 studies included in this meta-analysis. Control group 1.



Supplementary Figure 2. Forest plots showing occurrence rates for heterozygous PVs in *BRCA1/2* (A), mismatch repair (MMR) genes (B), and *TP53* (C) across 11 studies included in this meta-analysis. Control group 2.



Supplementary Figure 3. Burden test results of the supplementary analysis including 17 studies. Results are shown for all patients combined, brain tumors, non-brain solid tumors, and hematologic malignancies. Both control groups are included. TUM, Technical University of Munich; gnomAD, Genome Aggregation Database.

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