Supplementary Table 2. *In silico* predictions and functional assays results for *BRCA1, BRCA2, CHEK2* and *TP53* missense variants classified as loss-of-function.

Gene (RefSeq)	Nucleotide change	Predicted Protein change	Domain	SIFT	Align GVGD	Poly Phen2	phastCons	PhyloP	Mutation Taster	Functional assay Results	ClinVar Classification
BRCA1 (NM_007294.3)	c.181T>G	p.Cys61Gly	RING-finger	Damaging	C65	Benign	1	2.861	Disease causing (0.992)	**Defective impact on protein function** (Homology directed recombination assay & Protein binding ability assay)[1], **Mutant** (E3 Ub-ligase activity assay)[2]	Pathogenic
BRCA1 (NM_007294.3)	c.5212G>A	p.Gly1738Arg	C-terminal BRCT region	Damaging	C65	Possibly damaging	1	2.959	Disease causing (0.999)	**Deleterious** (protease-based assay) [3], **Mutant** (peptide binding ability assay), **Strong functional effect** (Transcription activation)[4]	Pathogenic
BRCA1 (NM_007294.3)	c.5246C>G	p.Pro1749Arg	C-terminal BRCT region	Damaging	C65	Benign	1	2.959	Disease causing (0.999)	**Deleterious**[3] (protease-based assay), **Strong functional effect** (Transcription activation) [4]	Pathogenic
BRCA1 (NM_007294.3)	c.5425G>T	p.Val1809Phe	C-terminal BRCT region	Damaging	C65	Benign	1	3.01	Disease causing (0.999)	**Deleterious**[3] (protease-based assay), **Strong functional effect** (Transcription activation)[4]	Likely Pathogenic
BRCA1 (NM_007294.3)	c.5497G>A	p.Val1833Met	C-terminal BRCT region	Damaging	C15	Probably damaging	1	3.088	Disease causing (0.999)	**Deleterious** (Transcription activation property of *BRCA1* in human cells)[5], **Deleterious-Destabilizing** (Thermodynamic stability prediction-biophysical assay)[6]	Likely Pathogenic
BRCA2 (NM_000059.3)	c.7879A>T	p.Ile2627Phe	BRCA-2-helical	Damaging	C15	Probably damaging	1	1.777	Disease causing (0.999)	**As mutant control/Inactivated** (Centrosome amplification assay)[7], **Predicted Pathogenic** (Homology directed repair assay) [8-10]	Pathogenic
CDKN2A (NM_000077.4)	c.71G>C	p.Arg24Pro	ANK	Tolerated	-	Probably damaging	0.085	0.647	Disease causing (0.999)	N/A	Pathogenic/Likely Pathogenic
CHEK2 (NM_007194.3)	c.475T>C	p.Tyr159His	FHA	Damaging	C65	Probably damaging	1	3.51	Disease causing (0.999)	N/A	Unknown Significance
CHEK2 (NM_007194.3)	c.499G>A	p.Gly167Arg	FHA	Damaging	C65	Probably damaging	1	4.205	Disease causing (0.999)	**Damaging** (DNA repair assay)[9]	Likely Pathogenic
CHEK2 (NM_007194.3)	c.548T>C	p.Leu183Ser	FHA	Damaging	C65	Probably damaging	0.988	3.51	Disease causing (0.999)	**Damaging** (DNA repair assay)**	N/A
CHEK2 (NM_007194.3)	c.549G>C	p.Leu183Phe	FHA	Damaging	C15	Probably damaging	0.995	1.26	Disease causing (0.999)	**Damaging** (DNA repair assay)**	Unknown Significance

TP53 (NM_000546.5)	c.578A>T	p.His193Leu	DNA-binding domain	Damaging	C65	Probably damaging	0.998	3.095	Disease causing (0.999)	**Non-Functional** (Transcriptional activity) [11]	Likely Pathogenic
TP53 (NM_000546.5)	c.847C>T	p.Arg283Cys	DNA-binding domain	Damaging	C55	Benign	0.188	2.044	Disease causing (0.890)	**Partially Functional** (Transcriptional activity) [11]	Unknown Significance
TP53 (NM_000546.5)	c.1021T>G	p.Phe341Val	TP53-tetramerization motif	Damaging	C0	Benign	0.932	0.027	Disease causing (0.986)	**Partially Functional** (Transcriptional activity) [11]	N/A

Notes: *Missense variants identified during the study have been evaluated and classified. Of these, seventeen variants in five genes (BRCA1, BRCA2, CHEK2, TP53 and CDKN2A) have been classified as loss-of-function. These were,* ***BRCA1****: c.181T>G, c.5212G>A, c.5246C>G, c.5425G>T, c.5497G>A and c.5467G>A,* ***BRCA2****: c.7879A>T, c.7976G>A and c.7006C>T,* ***CHEK2****: c.475T>C, c.499G>A, c.548T>C and c.549G>C,* ***TP53****: c.578A>T, c.847C>T and c.1021T>G,* ***CDKN2A****: c.71G>C. Of these,* ***BRCA1****, c.5467G>A,* ***BRCA2****, c.7976 G>A and c.7007G>A, involve base changes in the last nucleotide of an exon and were previously shown to cause aberrant splicing.*

Most of the BRCA1 variants reported herein are either recurrent or have a founder effect among Greek individuals, while there are multiple families with complete segregation of the missense variant along with breast and/or ovarian cancer. CHEK2 missense variants, lying in the forkhead-associated domain (FHA), have been also identified in multiple Greek families/individuals with strong family history for breast cancer and have been categorized as damaging by a Saccharomyces cerevisiae yeast functional assay (Delimitsou et al, under revision). CDKN2A c.71G>C variant has shown an impaired binding ability to CDK4, has been reported in melanoma/pancreatic families and is classified as pathogenic on ClinVar.

Supplementary References

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** *Delimitsou et al, under revision*