Inherited mutations in 17 breast cancer susceptibility genes among a large triple-negative breast cancer cohort unselected for family history of breast cancer

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SUPPLEMENTARY MATERIALS

SUPPLEMENTARY METHODS

Phenotypic information

DNA samples, and information on age of diagnosis, family history of cancer, and self-reported ethnicity was provided by participating studies. Family history was recorded as all first and second-degree relatives with breast or ovarian cancer. Individual analyses were performed for a family history of breast cancer and a family history of ovarian cancer as well as for a combined family history of these cancers. Information on other cancers in families was not requested for this study.

Pathology Information

Information on the pathology of triple-negative breast cancers (TNBC) (ER, PR, HER2, histological Grade) was obtained from clinical pathology records. A TNBC case was defined as an individual with an ER–negative, PR–negative and HER2–negative (0 or 1 by immunohistochemical staining (IHC)) breast cancer diagnosed after age 18. ER-negative status was defined as <10% of the tumor cells stained for a number of participating studies, because a subset of patients were recruited from Europe (n=6) and the United States (n=6) from 1995-2011 during which time this threshold was used to identify ER-negative tumors. Histologic grade was reported using the Nottingham combined grading system. TNBCs can be defined as basal tumors by EGFR and/or CK5/6 immunohistochemical staining or by gene expression profiling. However, this information was not available in the clinical records of the majority of cases in this study.

Panel-based Mutation Analysis

Germline DNA samples from 1824 TNBC cases were pooled in sets of three for Tru-seq library production. Libraries were bar-coded and pooled in sets of 12 for capture with an Agilent custom capture eArray containing 2X bait coverage of all coding sequences and intron/exon boundaries of coding exons from 122 DNA repair genes including 17 breast cancer predisposition genes. Products from each capture reaction were sequenced in a single lane of a HiSeq 2000 (**Figure S1**), yielding 300 reads on average for each nucleotide in each DNA sample (**Figure S2**). Sanger sequence of pooled samples was conducted to establish the origin of each called variant, such that all reported deleterious mutations were independently validated

Bioinformatics Analysis

Paired end reads (100bp) were aligned to the hg19 reference human genome using Novoalign (Novocraft Technologies, Malaysia). Realignment and recalibration was performed using GATK (VN:1.6-7). Germline variations were called with a combination of GATK UnifiedGenotyper[1] and Samtools(VN 0.1.18)[2]. The minimum quality threshold for calling variants was set at 20. Annotations were defined using SnpEFF (VN: 3.0c)[3] and ANNOVAR[4]. Population allele frequencies were extracted from the Exome Variant Server[5], 1000 Genomes[6] and dbSNP version 137[7]. Deleterious missense mutations were predicted using algorithms available in ANNOVAR[4] (SIFT, PolyPhen2, LRT, MutationTaster, PhyloP, GERP)[8], and AlignGVGD[9]. Thresholds for damaging/deleterious mutations were set as follows: AlignGVGD=C65, PolyPhen2 > 0.957, SIFT < 0.05, LRT > 0.999, MutationTaster > 0.5, PhyloP > 0.95, GERP++ > 4.87, Align-GVGD C65.

Sensitivity analysis

To estimate the sensitivity of the custom capture approach results were compared with those from clinical genetic testing of the *BRCA1* and *BRCA2* genes for a subset of KUMC TNBC samples. The 11 deleterious mutations in the KUMC dataset were identified.

SUPPLEMENTARY RESULTS

Supplementary mutation testing

Previous mutation screening of 79 patients with TNBC from POSH identified 18 mutations (22.8%). When combined with the current study (13%) the overall mutation frequency in POSH was 15.6% (42 of 269). We report the results from the current study.

Missense mutations

A total of 3783 missense mutations in the 17 genes were observed in the 1824 TNBC cases. After exclusion of variants identified in >2% of cases of public controls (Exome variant server, 1000 Genomes Project, HapMap, dbSNP), 448 high quality missense mutations remained (**Table S5**). Variants were annotated for effect by SIFT, Polyphen2, LRT, PhyloP score, Mutation Taster, GERP and also by AlignGVGD. Of the 448 missense variants, 20 were predicted deleterious by all seven methods and 21 were predicted deleterious by all six models when AlignGVGD was not available. In addition, 15 were predicted deleterious by six of seven methods, and 30 were likely deleterious by five of six methods when AlignGVGD was absent. Of the combined 86 variants, 20 were excluded as deleterious because of the frequency of observations in 1000Genomes, HapMap, and ESP6500 databases, leaving 66 candidate deleterious mutations. Of five *BRCA2* variants (p.Ile2627Phe, p.Glu2663Val, p.Asp2723Gly, p.Tyr2726Cys, p.Asn3124Ile) established as deleterious[10,11], only p.Ile2627Phe, which alters splicing of *BRCA2*, was not predicted deleterious by at least six of the seven predictive methods. Of four *BRCA1* missense mutations established as deleterious (p.Leu22Ser, p.Cys61Gly, p.Arg1699Gln, p.Met1775Arg)[12,13], only p.Leu22Ser was not consistently recognized as likely deleterious. Thus a high proportion (80%) of the 66 missense mutations predicted as deleterious in this study, or at least those in *BRCA1* and *BRCA2*, may predispose to TNBC. Alternatively, we noted that eight of 21 *BRCA1* and *BRCA2* missense mutations observed in this study were predicted deleterious. After exclusion of the C61G *BRCA1* mutation, which was observed seven times, seven of 24 patients (29%) with *BRCA1* and *BRCA2* missense mutations were found to carry known deleterious mutations. When applying this to all carriers of predicted deleterious missense mutations we estimate that 25 patients with TNBC, or 18 (1%) when excluding those with known deleterious *BRCA1* and *BRCA2* mutations, may carry predisposing variants. Further family-based and functional studies will be needed to establish the role of missense mutations in these genes in TNBC. However, it is likely that an additional 1% to 3% of TNBC cases may be associated with deleterious missense mutations in the predisposition genes.

Association with tumor pathology

Mutations in predisposition genes were not associated with an increased proportion of TNBCs with positive nodal status. However, 15% (32 of 112) of TNBC cases with mutations ($p=2.98x10^{-7}$) and 19% (24 of 125) *BRCA1* carriers ($p=1.7x10^{-9}$) exhibited bilateral or ipsilateral disease compared to 5.3% (65 of 1223) of non-mutant (WT) TNBC cases.

SUPPLEMENTARY REFERENCES

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Supplementary Table 1. The Triple-Negative Breast Cancer Consortium (TNBCC)

Bavarian Breast Cancer Cases and Controls (BBCC): This is a consecutive series of cases with invasive breast cancer recruited at the University Breast Centre, Franconia in Northern Bavaria, Germany from 2002-2006. Cases were between 22-96 years of age. Controls were population-based unaffected women from the same geographical area.

Simultaneous Study of Docetaxel Based Anthracycline Free Adjuvant treatment Evaluation, as well as Life Style Intervention Strategies (SUCCESS C), a prospectively randomized trial for high risk breast cancer patients without metastases. All patients were at least 18 years of age, HER2 negative with an otherwise high risk of recurrence. A total of 3642 patients were recruited from March 2009 to August 2011. Whole blood samples were obtained from 742 TN cases from 3256 total patients.

Dana Farber Cancer Institute (DFCI): Cases were obtained from an unselected series of breast tumor patients from the Dana Farber Cancer Institute. DNA samples from residual bloods from triple negative breast cancer patients were genotyped.

DEMOKRITOS: Cases were enrolled from 1997 until 2010 in several major hospitals covering most geographical areas of Greece, such as Athens metropolitan area, Thessaloniki, Ioannina, Patras, and Crete (Chania), in collaboration with the Hellenic Cooperative Oncology Group (HECOG). Cases had an age range of 20-87 years. Controls were population-based unaffected women of the same age range.

Fox Chase Cancer Center (FCCC): Cases were seen at FCCC and 28-80 years of age at diagnosis. Comprehensive clinical data including histology, staging, treatment and outcomes was provided for all cases. Controls were healthy females with no personal cancer history matched geographically and by gender, race and age. DNA was obtained from peripheral blood samples.

Gene Environment Interaction and Breast Cancer in Germany (GENICA): This is a population-based case-control study of breast cancer in the Greater Bonn area of Germany. Cases were incident breast cancer cases enrolled between 2000 and 2004 (reported from 14 hospitals within the study region), all of which were enrolled within 6 months of diagnosis. Cases were between 23-80 years of age. Controls were selected from population registries from 31 communities in the greater Bonn area and matched to cases in 5-year age classes between 2001 and 2004.

University of Kansas Medical Center (KUMC): Cases were obtained from an unselected series of breast tumors patients from the University of Kansas Medical Center. DNA samples from residual bloods from triple negative breast cancer patients were genotyped.

Helsinki Breast Cancer Study (HEBCS): Cases from this hospital-based case-control study in Southern Finland were consecutive breast cancer cases from the 1) Department of Oncology, Helsinki University Central Hospital 1997-8 and 2000, 2) consecutive cases from the Department of Surgery, Helsinki University Central Hospital 2001 – 2004. Cases were between 22 and 96 years of age. The population allele and genotype frequencies were obtained from the Finnish Genome Centre on 221 healthy population controls in the NordicDB, a Nordic pool and portal for genome-wide control data [14].

Mayo Clinic Breast Cancer Study (MCBCS): This is a clinic-based breast cancer case-control study at the Mayo Clinic. Subjects were enrolled between February 1, 2001 and June 30, 2005. Cases were comprised of Caucasian women with primary invasive breast cancer ascertained with 6 months of diagnosis. Controls were comprised of Caucasian women visiting the Mayo Clinic for general medical exams in the Department of Internal Medicine with no prior history of cancer. Controls were frequency matched to cases on region of residence, race, and 5-year age group.

Ohio State University (OSU): Cases were obtained from an unselected series of breast tumors patients from the Ohio State University Stefanie Spielman Breast Bank. DNA samples isolated from blood of triple negative breast cancer patients were genotyped. Controls were selected from the Columbus Area Control Sample Bank and were frequency matched for age and ethnicity to the cases.

Prospective Study of Outcomes in Sporadic Versus Hereditary Breast Cancer (POSH): Cases from this prospective cohort study in the United Kingdom were aged 40 or younger at breast cancer diagnosis, recruited from oncology clinics across the UK, and diagnosed between January 2000 and December 2007.

Roswell Park Cancer Institute (RPCI): This is a clinic-based case-control study of breast cancer. Cases were newly diagnosed breast cancer patients at RPCI. Data on tumor characteristics, including ER, PR and HER2 status were obtained from the Pathology Resource Network. Healthy controls were identified from family members, friends or visitors of patients with cancer other than breast, employee volunteers, and women recruited from community events.

Sheffield Breast Cancer Study (SBCS): This is a hospital-based case-control study of breast cancer. The study consists of women with pathologically confirmed breast cancer recruited from surgical outpatient clinics at the Royal Hallamshire Hospital, Sheffield, 1998 – 2005 and unselected women attending the Sheffield Mammography Screening Service between Sep 2000 - Aug 2004 if their mammograms showed no evidence of a breast lesion. Cases are a mixture of prevalent and incident disease.

Supplementary Table 2. List of likely deleterious mutations

Gene ID	HGVS Mutation nomenclature	HGVS protein level	#Mutations
ATM	c.1215delT	p.Asn405fs	1
ATM	c.4451delT	p.Met1484fs	1
BARD1	c.1525_1528dupATAG	p.Val510fs	1
BARD1	c.1932_1933delAT	p.Cys645X	1
BARD1	c.1935_1954dupTGAACAGGAAGAAAAGTATG	p.Cys645X	3
BARD1	c.2002-2A>C		1
BARD1	c.2300_2301delTG	p.Val767fs	1
BARD1	c.824T>A	p.Leu275X	1
BARD1	c.896insG	p.Pro300fs	1
BRCA1	BART dup exons 18-19		1
BRCA1	c.1016delA	p.Lys339fs	1
BRCA1	c.1059G>A	p.Trp353X	1
BRCA1	c.1116G>A	p.Trp372X	1
BRCA1	c.1299_1302dupCAGT	p.Asp435fs	1
BRCA1	c.135-1G>T		2
BRCA1	c.1500_1504delTAAAT	p.Leu502fs	2
BRCA1	c.1601_1602delAG	p.Gln534fs	1
BRCA1	c.1621C>T	p.Gln541X	1
BRCA1	[#]c.181T>G	p.Cys61Gly	7
BRCA1	c.1823_1826delAGAA	p.Lys608fs	1
BRCA1	c.1874_1877dupTAGT	p.Val627fs	2
BRCA1	c.1916T>A	p.Leu639X	1
BRCA1	c.1950_1953delAAAG	plLys653fs	1
BRCA1	c.1953dupG	p.Lys652fs	1
BRCA1	c.1961delA	p.Lys654fs	1
BRCA1	c.1961dupA	p.Tyr655fs	1
BRCA1	c.2035A>T	p.Lys679X	1
BRCA1	c.2071delA	p.Arg691fs	1
BRCA1	c.2077delG	p.Asp693fs	1
BRCA1	c.213-11T>G		3
BRCA1	c.2309C>A	p.Ser770X	1
BRCA1	c.2411_2412delAG	p.Gln804fs	1
BRCA1	c.2457delC	p.Asp821fs	1
BRCA1	c.2475delC	p.Asp825fs	1
BRCA1	c.2676_2679delAAAG	p.Lys893fs	1
BRCA1	c.2679_2682delGAAA	p.Ly893fs	1
BRCA1	c.2681_2682delAA	p.Lys894fs	4
BRCA1	c.2722G>T	p.Glu908X	3
BRCA1	c.2934T>G	p.Tyr978X	1
BRCA1	c.3005delA	p.Asn1002fs	1
BRCA1	c.302-2_302-2delA		1
BRCA1	c.3084_3094delTAATAACATTA	p.Asn1029fs	1
BRCA1	c.329dupA	p.Glu111fs	4
BRCA1	c.3400G>T	p.Glu1134X	1
BRCA1	c.3436_3439delTGTT	p.Cys1146X	1
BRCA1	c.3481_3491delGAAGATACTAG	p.Glu1161fs	2
BRCA1	c.3485delA	p.Asp1162fs	1
BRCA1	c.3607C>T	p.Arg1203X	1
BRCA1	c.3661G>T	p.Glu1221X	1
BRCA1	c.3700_3704delGTAAA	p.Val1234fs	2
BRCA1	c.3748G>T	p.Glu1250X	1
BRCA1	c.3756_3759delGTCT	p.Ser1253fs	4
BRCA1	c.3770_3771delAG	p.Glu1257fs	1
BRCA1	c.3869_3870delAA	p.Arg1290fs	1

BRCA1	c.3916_3917delTT	p.Leu1306fs	1
BRCA1	c.4035delA	p.Glu1346fs	1
BRCA1	c.4065_4068delTCAA	p.Asn1355fs	5
BRCA1	c.4165_4166delAG	p.Ser1389X	2
BRCA1	c.4183C>T	p.Gln1395X	1
BRCA1	c.4251_4252delGT	p.Leu1418fs	1
BRCA1	c.4258C>T	p.Gln1420X	1
BRCA1	c.427G>T	p.Glu143X	3
BRCA1	c.4327C>T	p.Arg1443X	3
BRCA1	c.4357+1G>A		2
BRCA1	c.4524G>A	p.Trp1508X	2
BRCA1	c.4675+1G>A		1
BRCA1	c.4676-1G>A		1
BRCA1	c.4689C>G	p.Tyr1563X	2
BRCA1	c.4932_4933dupAA	p.Arg1645fs	1
BRCA1	c.5030_5033delCTAA	p.Thr1677fs	1
BRCA1	c.5074+1G>A		1
BRCA1	#c.5096G>A	p.Arg1699Gln	1
BRCA1	c.5137delG	p.Val1713X	1
BRCA1	c.5177_5180delGAAA	p.Arg1726fs	1
BRCA1	c.520delC	p.Gln174fs	1
BRCA1	*c.5266dupC	p.Gln1756fs	19
BRCA1	#c.5324T>G	p.Met1775Arg	1
BRCA1	c.5328delC	p.Thr1777fs	1
BRCA1	c.5386delT	p.Ser1796fs	1
BRCA1	c.5431C>T	p.Gln1811X	2
BRCA1	c.547+2T>A		1
BRCA1	c.5503C>T	p.Arg1835X	3
BRCA1	#c.65T>C	p.Leu22Ser	1
BRCA1	*c.68_69delAG	p.Glu23fs	18
BRCA1	c.697_698delGT	p.Val233fs	1
BRCA1	c.929delA	p.Gln310fs	1
BRCA1	c.962G>A	p.Trp321X	3
BRCA1	exon 22del510bp		1
BRCA2	c.1364delC	p.Ser455X	1
BRCA2	c.145G>T	p.Glu49X	1
BRCA2	c.1813dupA	p.Ile605fs	5
BRCA2	c.2339C>G	p.Ser780X	1
BRCA2	c.2836_2837delGA	p.Asp946fs	1
BRCA2	c.3002C>G	p.Ser1001X	1
BRCA2	c.3199delA	p.Thr1067fs	1
BRCA2	c.3847_3848delGT	p.Val1283fs	1
BRCA2	c.4588A>T	p.Lys1530X	1
BRCA2	c.4729G>T	p.Glu1577X	1
BRCA2	c.5071A>T	p.Lys1691X	1
BRCA2	c.5073dupA	p.Trp1692fs	1
BRCA2	c.516+2T>A		1
BRCA2	c.517-1_517-1delG		1
BRCA2	c.5641_5644delAAAT	p.Lys1881fs	1
BRCA2	c.5806_5807delAT	p.Met1936fs	1
BRCA2	c.5857G>T	p.Glu1953X	1
BRCA2	*c.5946delT	p.Ser1982fs	6
BRCA2	c.6275_6276delTT	p.Leu2092fs	3
BRCA2	c.6405_6409delCTTAA	p.Asn2135fs	1
BRCA2	c.6486_6489delACAA	p.Lys2162fs	1
BRCA2	c.6941delC	p.Thr2314fs	1

BRCA2	c.7024C>T	p.Gln2342X	1
BRCA2	c.7480C>T	p.Arg2494X	1
BRCA2	c.774_775delAA	p.Glu260fs	1
BRCA2	c.7878G>A	p.Trp2626X	1
BRCA2	#c.7879A>T	p.Ile2627Phe	1
BRCA2	c.7913_7917delTTCCT	p.Phe2638X	1
BRCA2	#c.7988A>T	p.Glu2663Val	1
BRCA2	#c.8168A>G	p.Asp2723Gly	1
BRCA2	#c.8177A>G	p.Tyr2726Cys	1
BRCA2	c.8537_8538delAG	p.Glu2846fs	1
BRCA2	c.8761_8762delTT	p.Phe2921fs	1
BRCA2	c.9196C>T	p.Gln3066X	1
BRCA2	c.9257-1G>C		1
BRCA2	#c.9371A>T	p.Asn3124Ile	1
BRCA2	c.9382C>T	p.Arg3128X	1
BRCA2	c.9672dupA	p.Tyr3225fs	1
BRIP1	c.1341-1G>C		1
BRIP1	c.2038_2039dupTT	p.Leu660fs	1
BRIP1	c.2108delAinsTCC	p.Lys703fs	1
BRIP1	c.2392C>T	p.Arg798X	3
BRIP1	c.2765T>G	p.Leu922X	1
BRIP1	c.440dupA	p.Tyr147X	1
MRE11A	c.1514G>T	p.Glu506X	1
MRE11A	c.1726C>T	p.Arg576X	1
NBN	c.657_661delACAAA	p.Lys219fs	1
PALB2	c.123delT	p.Glu42fs	1
PALB2	c.156delA	p.Glu53fs	1
PALB2	c.1592delT	p.Leu531fs	3
PALB2	c.172_175delTTGT	p.Leu58fs	2
PALB2	c.2257C>T	p.Arg753X	1
PALB2	c.2470dupT	p.Cys824fs	1
PALB2	c.3116delA	p.Asn1039fs	1
PALB2	c.3323delA	p.Tyr1108fs	2
PALB2	c.3361delG	p.Gly1121fs	1
PALB2	c.3456dupA	p.Pro1153fs	1
PALB2	c.3549C>G	p.Tyr1183X	1
PALB2	c.49-2A>T		1
PALB2	c.509_510delGA	p.Arg170fs	2
PALB2	c.688G>T	p.Glu230X	1
PALB2	c.93dupA	p.Leu32fs	1
PALB2	c.956_962delCTAATTT	p.Ser319X	1
PTEN	c.790delA	p.Met264fs	1
RAD50	c.1875C>G	p.Tyr625X	2
RAD50	c.2165dupA	p.Glu723fs	1
RAD50	c.326_329delCAGA	p.Thr109fs	1
RAD50	c.687delT	p.Ser229fs	1
RAD50	c.832C>T	p.Arg278X	1
RAD51C	c.706-2A>G		5
RAD51C	c.955C>T	p.Arg319X	1
RAD51D	c.144+1G>T		1
RAD51D	c.478C>T	p.Gln160X	1
RAD51D	c.564delT	p.Val189fs	1
RAD51D	c.623dupT	p.Thr209fs	1
RAD51D	c.748delC	p.His250fs	2
RAD51D	c.898C>T	p.Arg300X	1
TP53	#c.743G>A	p.Arg248Gln	1

XRCC2	c.377T>A	p.Leu126X	1
XRCC2	c.96delT	p.Phe32fs	2
		Total	271

* BRCA1 and BRCA2 Ashkenazi Jewish founder mutations; #known deleterious missense mutations

Supplementary Table 3. Gene-specific deleterious mutations by study

Gene	BBCC n=270	DEMOKRITOS n=223	DFCI n=252	FCCC n=108	GENICA n=48	HEBCS n=87	KUMC n=87	MCBCS n=186	OSU n=205	POSH n=190	RPCI n=75	SBCS n=30
ATM	1	1	0	0	0	0	0	0	0	0	0	0
BRCA1	21	11	40	13	2	3	11	10	22	16	6	0
BRCA2	9	6	8	5	0	1	2	3	4	5	3	3
BARD1	1	3	0	1	0	1	0	1	1	0	1	0
BRIP1	1	0	1	0	0	0	0	1	3	1	1	0
CDH1	0	0	0	0	0	0	0	0	0	0	0	0
CHEK2	0	0	0	0	0	0	0	0	0	0	0	0
MRE11A	0	0	1	0	0	0	0	0	1	0	0	0
NBN	1	0	0	0	0	0	0	0	0	0	0	0
PALB2	2	2	5	2	0	3	0	2	4	0	1	0
PTEN	0	1	0	0	0	0	0	0	0	0	0	0
RAD50	0	1	2	0	0	1	0	0	1	0	1	0
RAD51C	2	1	2	0	0	0	0	1	0	0	0	0
RAD51D	2	0	1	1	0	0	1	0	0	2	0	0
STK11	0	0	0	0	0	0	0	0	0	0	0	0
TP53	0	0	1	0	0	0	0	0	0	0	0	0
XRCC2	0	0	0	0	0	0	0	1	2	0	0	0
Total	40	26	61	22	2	9	14	19	38	24	13	3
% Mutations	15%	12%	24%	20%	4%	10%	16%	10%	19%	13%	17%	10%

Supplementary Table 4. TNBC cases with two independent deleterious mutations.

ID	study	famhist	bilateral	grade	nodes	stage	agedx	Gene ID	position	cDNA	Protein
TN07831	MCBCS	0	1	3	0	3	68	BRCA1	41276044	c.68_69delAG	p.Glu23ValfsX17
TN07831	MCBCS	0	1	3	0	3	68	BRCA2	32914437	c.5946delT	p.Ser1982ArgfsX22
TN06850	BBCC	0	0	3	1	3	49	BRCA2	32936733	c.7879A>T	p.Ile2627Phe
TN06850	BBCC	0	0	3	1	3	49	PALB2	23641004	c.2470dupT	p.Cys824LeufsX2
TN03008	MCBCS	1	1	2	0	1	38	BRCA1	41245594	c.1950_1953delAAAG	p.Lys652GlufsX21
TN03008	MCBCS	1	1	2	0	1	38	BRIP1	59793412	c.2392C>T	p.Arg798X
TN01532	FCCC	0	0	3	1	2	39	PALB2	23647179	c.688G>T	p.Glu230X
TN01532	FCCC	0	0	3	1	2	39	RAD51D	33433416	c.564delT	p.Val189TrpfsX5

Supplementary Table 5. Phenotypic categories for TNBC cases with likely deleterious mutations

		ATM	BARD1	BRCA1	BRCA2	BRIP1	MRE11A	NBN	PALB2	PTEN	RAD50	RAD51C	RAD51D	TP53	XRCC2	WT
Bilateral	unilateral	0	6	101	34	5	2	0	16	1	5	4	4	0	2	1,158
	bilateral	1	1	18	3	1	0	0	0	0	0	0	1	0	0	57
	ipsilateral	0	0	6	0	0	0	0	0	0	0	0	0	1	0	8
Grade	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	18
	2	1	2	6	4	2	0	0	2	0	0	0	1	0	0	196
	3	1	5	105	35	4	2	1	9	1	3	6	5	0	0	940
Stage	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	6
	1	0	4	59	11	4	1	1	4	0	2	3	2	1	0	410
	2	1	1	43	18	2	1	0	6	0	2	2	1	0	1	502
	3	0	3	7	6	0	0	0	6	0	0	2	0	0	1	137
	4	0	0	1	2	0	0	0	1	0	0	0	1	0	0	42
Node Status	Negative	1	4	79	23	5	2	1	6	0	3	4	3	1	0	650
	Positive	0	4	31	14	2	0	0	12	1	1	2	1	0	2	496
Breast Ca. History	No	2	3	66	24	5	1	0	10	0	3	4	3	0	2	873
(1st or 2nd Degree)	Yes	0	2	66	16	3	1	1	5	1	2	1	3	1	1	413
Ovarian Ca. History	No	2	5	108	35	8	2	1	15	1	5	5	5	1	3	1254
(1st or 2nd Degree)	Yes	0	0	24	5	0	0	0	0	0	0	0	1	0	0	32

Supplementary Table 6. Predicted deleterious missense mutations

Gene	Chr	Position	REF	ALT	#	Mutation	rsID	A-GVGD Class	A-GVGD Result	SIFT	POLY-PHEN2	POLY-PHEN2	LRT	PHYLO-P	PHYLO-P	MT	MT	GERP_GT2	GERP_GT2	#Del
ATM	chr11	108218084	T	C	1	I2888T	.	Class C65	del	del	0.999	del	del	0.997	del	0.990	del	5.52	del	7
ATM	chr11	108224555	A	G	2	R2912G	.	Class C65	del	del	1	del	del	0.998	del	0.948	del	5.42	del	7
ATM	chr11	108180934	A	G	1	N1937S	.	Class C15	ben	del	0.991	del	del	0.999	del	0.550	del	5.7	del	6
BARD1	chr2	215593443	A	G	1	I764T	.	.	Unk	del	0.99	del	del	0.970	del	0.920	del	5.63	del	6
BARD1	chr2	215593566	T	C	1	H723R	.	.	Unk	del	1	del	del	0.084	ben	0.989	del	5.81	del	5
BRCA1	chr17	41209139	A	G	1	V1736A	rs45553935	Class C65	del	del	0.975	del	del	0.999	del	0.878	del	5.07	del	7
BRCA1	chr17	41215389	C	A	1	W1718C	rs80357239	Class C65	del	del	1	del	del	0.999	del	0.999	del	5.46	del	7
BRCA1	chr17	41256941	A	G	1	L82P	.	Class C65	del	del	0.969	del	del	0.999	del	0.998	del	5.3	del	7
BRCA1	chr17	41258504	A	C	7	C61G	rs28897672	Class C65	del	del	1	del	del	0.998	del	0.989	del	5.17	del	7
BRCA1	chr17	41203088	A	C	1	M1775R	rs41293463	Class C45	ben	del	1	del	del	0.999	del	0.998	del	5.21	del	6
BRCA1	chr17	41215947	C	T	1	R1699Q	rs41293459	Class C35	ben	del	1	del	del	0.999	del	0.902	del	5.83	del	6
BRCA1	chr17	41219642	T	C	1	H1686R	.	Class C25	ben	del	1	del	del	0.998	del	0.913	del	5.24	del	6
BRCA1	chr17	41244952	G	A	3	R866C	rs41286300	Class C65	del	del	1	del	ben	1.000	del	0.982	del	5.15	del	6
BRCA2	chr13	32937327	A	T	1	E2663V	rs80359031	Class C65	del	del	0.999	del	del	0.998	del	0.920	del	4.93	del	7
BRCA2	chr13	32937507	A	G	1	D2723G	rs41293513	Class C65	del	del	1	del	del	0.999	del	0.895	del	5.49	del	7
BRCA2	chr13	32937516	A	G	1	Y2726C	rs80359064	Class C65	del	del	1	del	del	0.999	del	0.954	del	5.49	del	7
BRCA2	chr13	32937642	T	A	1	L2768H	.	Class C65	del	del	0.998	del	del	0.998	del	0.882	del	5.68	del	7
BRCA2	chr13	32944642	G	A	2	G2812E	rs80359091	Class C65	del	del	0.999	del	del	0.999	del	0.947	del	5.19	del	7
BRCA2	chr13	32954031	C	T	1	T3033I	.	Class C65	del	del	0.999	del	del	0.999	del	0.838	del	5.66	del	7
BRCA2	chr13	32968940	A	T	1	N3124I	rs28897759	Class C65	del	del	1	del	del	0.999	del	0.927	del	5.89	del	7
BRCA2	chr13	32972522	C	G	2	S3291C	rs200210279	Class C65	del	del	0.991	del	del	0.999	del	0.819	del	5.74	del	7
BRCA2	chr13	32911818	C	T	1	A1109V	rs41293479	Class C0	ben	del	0.98	del	del	0.999	del	0.813	del	5.75	del	6
BRCA2	chr13	32913062	T	G	1	F1524V	rs56386506	Class C45	ben	del	0.999	del	del	0.998	del	0.960	del	5.74	del	6
BRCA2	chr13	32915190	C	A	1	A2233D	rs41293501	Class C15	ben	del	0.995	del	del	0.999	del	0.958	del	5.71	del	6
BRCA2	chr13	32920979	G	A	1	R2318Q	rs80358921	Class C0	ben	del	0.999	del	del	0.999	del	0.712	del	5.03	del	6
BRCA2	chr13	32930688	G	T	1	R2520L	.	Class C65	del	del	1	del	del	0.994	del	0.977	del	4.63	ben	6
BRIP1	chr17	59770799	T	C	1	Y856C	.	.	Unk	del	0.999	del	del	0.998	del	0.954	del	5.74	del	6
BRIP1	chr17	59821942	T	A	1	K703I	.	.	Unk	del	0.992	del	del	0.962	del	0.837	del	5.63	del	6
BRIP1	chr17	59858343	G	T	1	A551E	.	.	Unk	del	0.981	del	del	0.999	del	0.680	del	5.17	del	6
BRIP1	chr17	59870990	C	A	1	G481C	.	.	Unk	del	0.999	del	del	0.998	del	0.979	del	5.24	del	6
BRIP1	chr17	59937223	G	C	3	P47A	rs28903098	.	Unk	del	0.999	del	del	1.000	del	0.925	del	5.26	del	6
BRIP1	chr17	59853875	C	T	1	A662T	.	.	Unk	del	0.98	del	del	0.999	del	0.453	ben	5.58	del	5
BRIP1	chr17	59853913	C	G	1	G649A	.	.	Unk	tol	0.995	del	del	0.999	del	0.635	del	5.69	del	5
BRIP1	chr17	59876594	G	A	2	R403W	.	.	Unk	del	1	del	del	0.963	del	0.996	del	3.73	ben	5
BRIP1	chr17	59878714	A	G	1	L347P	.	.	Unk	del	0.998	del	ben	0.998	del	0.966	del	5	del	5
CHEK2	chr22	29121058	C	T	1	G167R	rs72552322	Class C65	del	del	0.999	del	del	0.999	del	1.000	del	5.87	del	7
CHEK2	chr22	29090060	C	T	2	R474H	rs121908706	Class C25	ben	del	1	del	del	0.998	del	1.000	del	5.46	del	6
CHEK2	chr22	29091220	A	G	1	Y424H	rs139366548	Class C0	ben	del	0.998	del	del	0.999	del	0.999	del	5.81	del	6
MRE11A	chr11	94192612	G	A	4	R488C	.	.	Unk	del	0.993	del	del	1.000	del	0.994	del	5.89	del	6
MRE11A	chr11	94204759	G	A	1	P276S	.	.	Unk	del	0.992	del	del	1.000	del	0.997	del	5.45	del	6

MRE11A	chr11	94211916	C	T	2	A177T	rs142996063	.	Unk	del	0.997	del	del	0.999	del	0.999	del	5.54	del	6
MRE11A	chr11	94192594	C	T	2	E494K	rs104895016	.	Unk	del	0.303	ben	del	0.999	del	0.988	del	5.89	del	5
NBN	chr8	90949269	G	A	1	A740V	.	.	Unk	del	0.993	del	del	0.991	del	0.197	ben	5.22	del	5
PALB2	chr16	23635372	A	C	2	L931R	.	.	Unk	del	0.996	del	del	0.999	del	0.918	del	5.81	del	6
PALB2	chr16	23637693	T	C	1	D871G	.	.	Unk	del	0.999	del	del	0.998	del	0.962	del	5.93	del	6
PALB2	chr16	23637686	A	C	1	S873R	.	.	Unk	del	0.999	del	del	0.973	del	0.964	del	3.31	ben	5
PTEN	chr10	89685278	A	G	1	D58G	.	.	Unk	del	0.981	del	del	0.998	del	1.000	del	5.46	del	6
RAD50	chr5	131893071	G	T	1	D19Y	.	.	Unk	del	0.986	del	del	1.000	del	0.999	del	5.72	del	6
RAD50	chr5	131895049	A	G	1	H68R	.	.	Unk	del	0.999	del	del	0.998	del	0.998	del	4.98	del	6
RAD50	chr5	131911482	C	T	1	T76I	.	.	Unk	del	0.983	del	del	0.999	del	0.989	del	5.75	del	6
RAD50	chr5	131911500	T	A	1	I82N	.	.	Unk	del	0.998	del	del	0.998	del	0.998	del	5.75	del	6
RAD50	chr5	131915097	A	T	1	N152Y	.	.	Unk	del	0.978	del	del	0.999	del	0.997	del	5.69	del	6
RAD50	chr5	131911515	G	A	1	R87H	.	.	Unk	del	0.935	ben	del	1.000	del	0.902	del	5.75	del	5
RAD50	chr5	131923282	T	G	1	L262R	rs201728859	.	Unk	del	0.831	ben	del	0.998	del	0.990	del	5.77	del	5
RAD50	chr5	131939181	G	C	1	Q799H	rs61749630	.	Unk	del	0.746	ben	del	0.999	del	0.729	del	5.16	del	5
RAD50	chr5	131944882	G	A	1	G968E	rs199895166	.	Unk	del	0.03	ben	del	0.985	del	0.977	del	4.98	del	5
RAD50	chr5	131953931	A	T	1	R1112W	.	.	Unk	del	0.993	del	del	0.999	del	0.994	del	4.16	ben	5
RAD50	chr5	131972857	T	C	1	I1147T	.	.	Unk	del	0.881	ben	del	0.999	del	1.000	del	6.17	del	5
RAD50	chr5	131976470	T	C	1	I1242T	.	.	Unk	del	0.953	ben	del	0.999	del	1.000	del	6.16	del	5
RAD51D	chr17	33430511	G	A	1	A210V	.	.	Unk	del	0.783	ben	del	1.000	del	1.000	del	5.05	del	5
RAD51D	chr17	33430520	G	A	4	S207V	.	.	Unk	del	0.789	ben	del	1.000	del	1.000	del	5.05	del	5
STK11	chr19	1226569	C	T	1	R409W	.	.	Unk	del	NA	del	del	NA	del	NA	del	NA	del	6
STK11	chr19	1206943	A	G	1	M11V	.	.	Unk	tol	NA	del	del	NA	del	NA	del	NA	del	5
STK11	chr19	1220466	G	A	2	G187S	.	.	Unk	tol	NA	del	del	NA	del	NA	del	NA	del	5
STK11	chr19	1220595	G	A	1	A205T	.	.	Unk	tol	NA	del	del	NA	del	NA	del	5.56	del	5
TP53	chr17	7574027	C	G	1	G334R	.	Class C65	del	del	1	del	del	0.999	del	1.000	del	5.43	del	7

Del: deleterious; ben: benign; Unk: unknown; tol: tolerated; #: number; MT: Mutation Taster

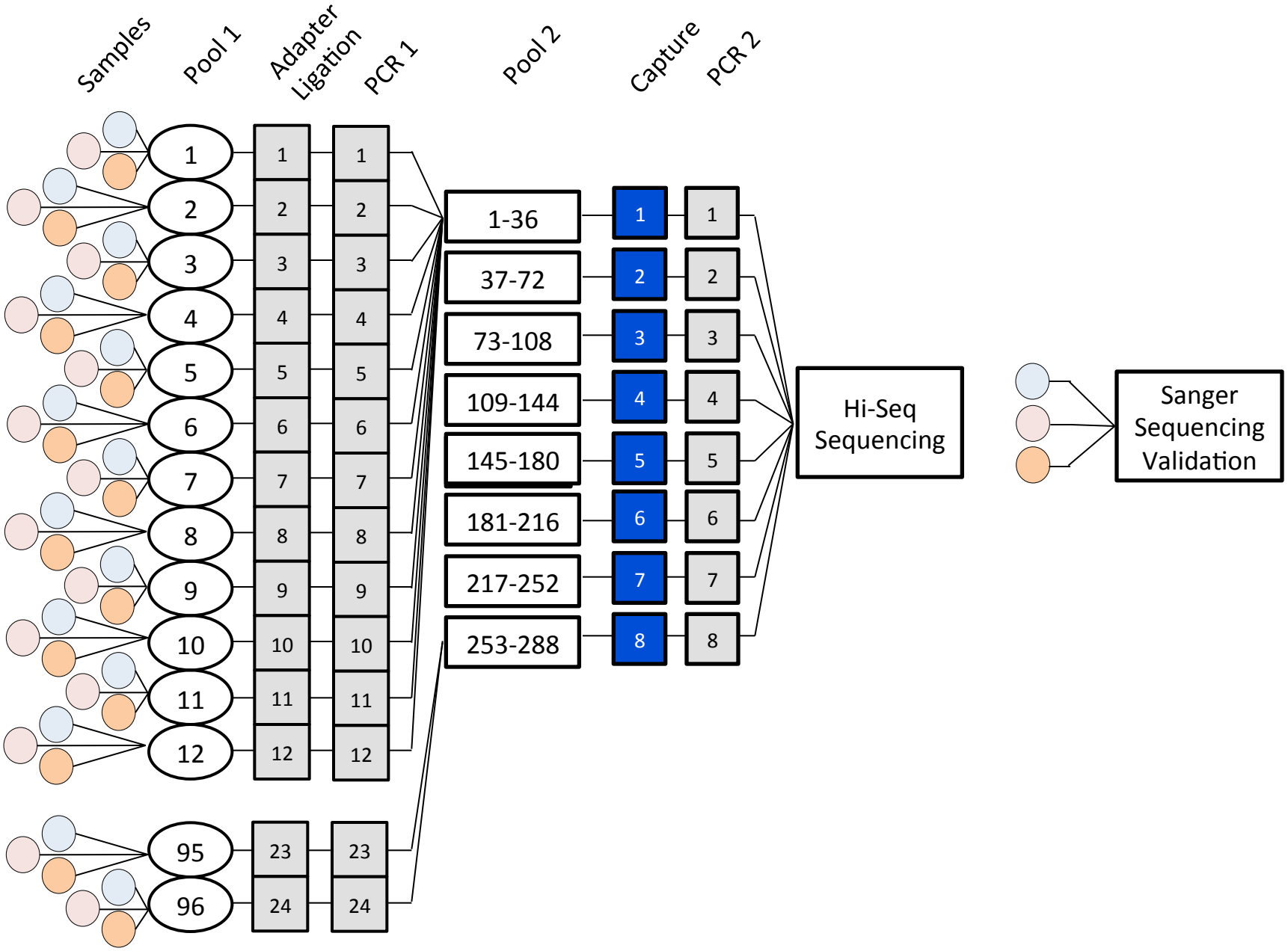
Supplementary Figure Legends

Supplemental Figure 1. Schematic of mutation screening protocol. DNA samples were pooled in sets of three for library production, pooled again in batches of 12 libraries for capture and sequencing. All likely deleterious mutations were Sanger validated in individual samples.

Supplemental Figure 2. Quality control of sequencing. A) Average coverage of exons with read depth on the x-axis and number of exons on the y-axis. B) Frequency of alternate alleles in pooled samples with four or six possible alleles.

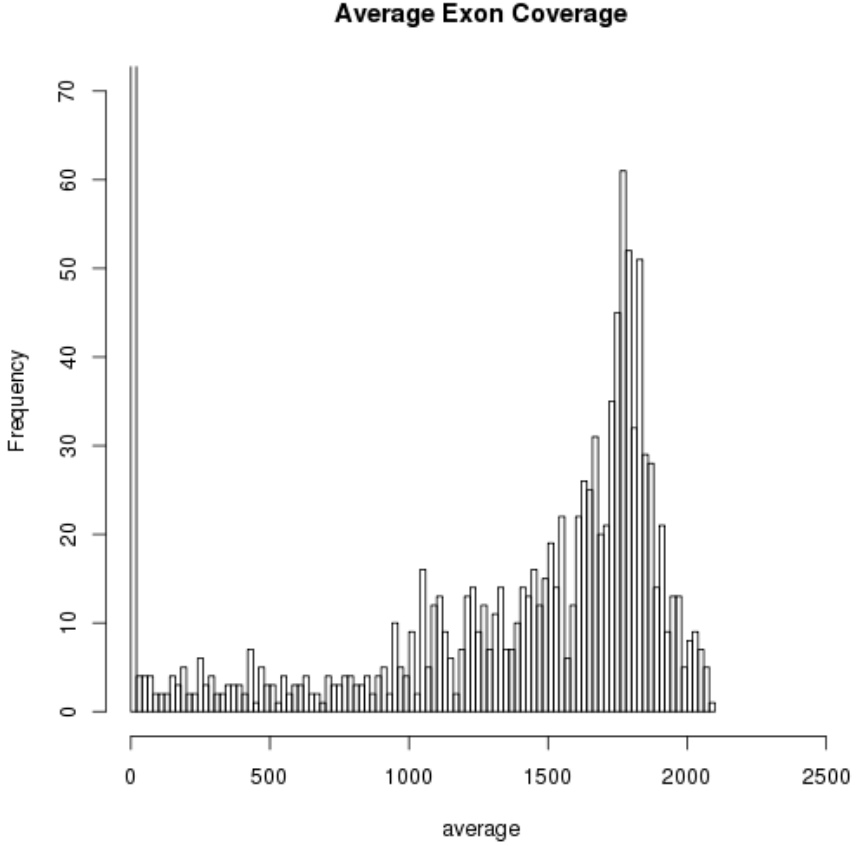
Supplemental Figure 3. Frequency of likely deleterious mutations in 14 genes by study.

Supplemental Figure 1

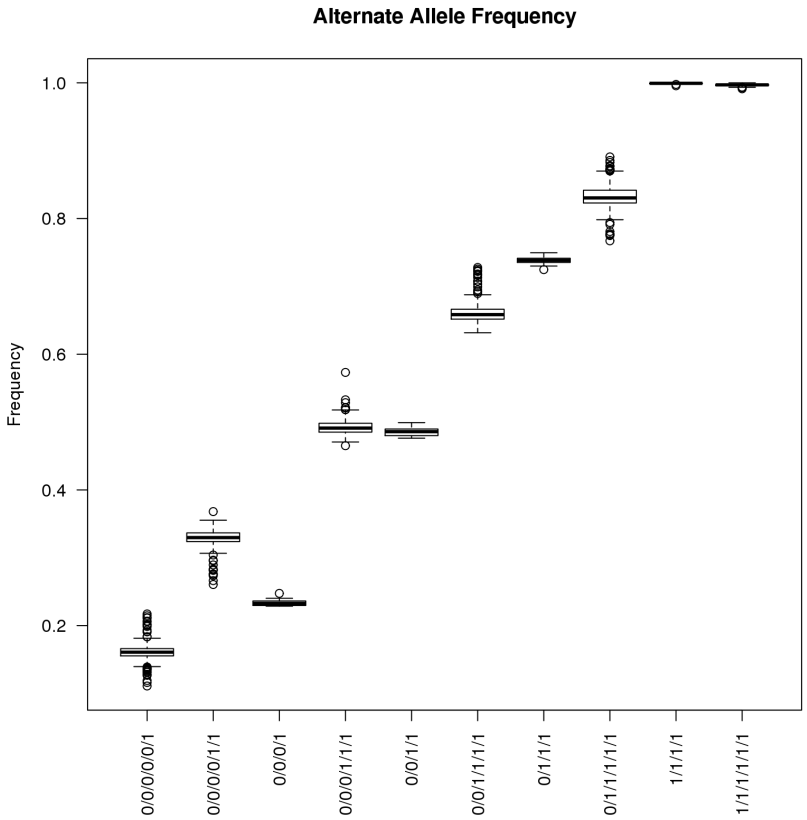


Supplemental Figure 2

A



B



Supplemental Figure 3