SUPPLEMENTARY MATERIALS

Supplementary Methods

Clinical Cohort Study Population

A total of 54,555 invasive breast cancer patients with available information on pathological characteristics of 56,480 breast tumors among all individuals subjected to clinical germline cancer panel testing between March 2012 and December 2016 at a clinical testing laboratory (Ambry Genetics-Aliso Viejo, CA) were included in this study. Demographic, clinical history, and family history of cancer information was collected from test requisition forms, clinic notes, and pedigrees provided by ordering clinicians at the time of testing. Information was collected on current age, personal history and age at diagnosis of all cancers, ancestry, family history of cancer with cancer type, and age at diagnosis among relatives. Family history was limited to first and second-degree relatives. Families with breast or ovarian cancer in two or more individuals, on the same parental side, were considered positive for family history for each cancer. Estrogen receptor (ER), progesterone receptor (PR), and HER2 status of each breast cancer was provided by ordering clinicians and/or clinical tumor pathology reports.

To assess data quality, a review of a random sample of 1200 (10.0%) breast and ovarian cancer patient intake forms was conducted. Of these, 43.3% with additional clinical history documentation available (clinic notes, pedigrees, detailed letters of medical necessity). Consistent information was observed for breast cancers, with age at breast cancer diagnosis available for 99.5%, ER status for 99.5%, PR status for 98.8%, and HER2 status for 96.3%, suggesting that the intake data for the cohort is of reasonably high quality.

Multigene Panel Testing for the Clinical Cohort

Mutation testing was performed by sequencing of targeted custom capture products from several clinical multigene panels and targeted chromosomal microarray analysis. Genomic deoxyribonucleic acid (gDNA) was isolated from the patient's blood or saliva specimen using a standardized methodology (Qiagen, Valencia, CA). Sequence enrichment was performed by incorporating the gDNA onto microfluidics chip or into microdroplets along with primer pairs or by a bait-capture methodology using long biotinylated oligonucleotide probes (RainDance Technologies, Billerica, MA or Integrated DNA Technologies, San Diego, CA), followed by PCR and then NGS analysis (Illumina, San Diego, CA) of all coding exons plus at least five bases into the 5' and 3' ends of all the introns and untranslated regions (5'UTR and 3'UTR). A targeted chromosomal microarray was used for the detection of gross deletions and duplications for all genes except *PMS2* (Agilent, Santa Clara, CA). Gross deletion/duplication analysis of *PMS2* was performed using MLPA kit# P008-B1 (MRC-Holland, Amsterdam, Netherlands) and Sanger sequencing. Initial data processing and base calling was done using RTA 1.12.4 (HiSeq Control Software 1.4.5; Illumina). Sequence quality filtering at Q20 was executed with the CASAVA software (version 1.8.2; Illumina, Hayward, CA). Sequence fragments were aligned to the reference human genome (GRCh37), and variant calls were generated using CASAVA. Variants were annotated with the Ambry Variant Analyzer, a proprietary alignment and variant annotation software (Ambry Genetics). All variants identified by Ambry Genetics are submitted to the ClinVar public database.

Absolute Risk Estimation for Breast Cancer and pathology subtype from the Clinical Cohort

Absolute risk is the probability an individual with a measured set of risk factors (e.g. mutation status, a Polygenic Risk Score (PRS), and family history of disease) and is disease free at age a will be diagnosed with the disease in the subsequent τ years[1]. Let Z be the set of measured risk factors, then we can express the absolute risk as:

$$R(a,\tau,Z) = \int_a^{a+\tau} h_1(u|Z) \times \exp\left(-\int_a^u \{h_1(v|Z) + h_2(v|Z)\}\,\mathrm{d}v\right)\mathrm{d}u$$

where $h_1(a|Z)$ is the conditional disease-specific hazard at age a and $h_2(a|Z)$ is the competing risks hazard at age a. The competing risks may include other diseases or death. The hazards can be parameterized as:

$$h_1(a|Z) = h_{10}(a)\exp(\beta Z)$$

and

$$h_2(a|Z) = h_{20}(a)\exp(\gamma Z)$$

where $h_{i0}(a)$ is the baseline hazard and β and γ represent the relative risks for each risk factor in Z for the disease of interest and competing risks, respectively. Estimates for β and γ can be estimated from the case-control data. The baseline hazards can be estimated by the following relationship between the baseline hazard and the marginal hazard

$$h_1^*(a) = h_{10}(a)\mathrm{E}(\exp(\beta Z)) \approx \int h_{10}(a)\exp(\beta z)dF(z)$$

where $F(Z)$ denotes the distribution of the risk factors in the population. For each age, we solve for $h_{10}(a)$ by using the expected distribution of the risk factor in the population[2,3].

We estimated the breast cancer subtype absolute risk for a women at age *a* without a diagnosis with any breast cancer, and the risk factor of interest being gene specific mutation carrier status, the odds ratio estimates from the Ambry cases versus gnomAD controls were used as estimates for β, and SEER subtype specific incidence rates[4] were utilized for the estimation of the baseline hazard combined with the gnomAD mutation frequency for the population frequency. The age-specific competing hazards model, $h_2(a|Z)$, involves a mixture of the hazard for all other breast cancer subtypes and death. The hazard function for all cause mortality was estimated using US mortality data subset to the same states as the SEER registries. Confidence bands for the absolute risk curve were estimated by utilizing the upper and lower 95% confidence interval for the gene specific odds ratio and treating the incidence rates from SEER as known rates.

References

1. Gail MH, Brinton LA, Byar DP, et al. Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. *J Natl Cancer Inst.* 1989;81(24):1879-1886.

2. Costantino JP, Gail MH, Pee D, et al. Validation studies for models projecting the risk of invasive and total breast cancer incidence. *J Natl Cancer Inst.* 1999;91(18):1541-1548.

3. Chatterjee N, Shi J, Garcia-Closas M. Developing and evaluating polygenic risk prediction models for stratified disease prevention. *Nat Rev Genet.* 2016;17(7):392-406.

4. National Cancer Institute, DCCPS, Surveillance Research Program, Surveillance Systems Branch. Surveillance, Epidemiology, and End Results (SEER) Program Research Data (1973-2014). https://www.seer.cancer.gov. April 2017 (Based on November 2016 Submission).

Supplementary Table 1: Characteristics of study population and comparison between mutation carriers and non-mutation carriers

Characteristics	All patients (54555)	Mutation carriers (4451)	Non-mutation carriers (50104)	p value*
Mean age (SD), y	49.5 (11.4)	46.7 (11.5)	49.8 (11.4)	<0.001
Race and ethnicity				0.05
Black	4392 (8.1%)	396 (8.9%)	3996 (8.0%)	
Caucasian	34880 (63.9%)	2787 (62.6%)	32093 (64.1%)	
Ashkenazi Jews	2623 (4.3%)	220 (4.9%)	2403 (4.8%)	
Asian	2608 (4.8%)	194 (4.4%)	2414 (4.8%)	
Hispanic	3174 (5.8%)	287 (6.4%)	2887 (5.8%)	
Other/unknown	6878 (12.6%)	567 (12.7%)	6311(12.6%)	
Personal cancer history				
Breast Cancer				
age at diagnosis				<0.001
18-36	6629 (12.2%)	905 (20.4%)	5724 (11.5%)	
37-45	14622 (26.8%)	1239 (27.9%)	13383 (26.8%)	
46-50	10399 (19.1%)	760 (17.1%)	9639 (19.3%)	
51-60	13162 (24.1%)	981 (22.1%)	12181 (24.4%)	
>60	9570 (17.5%)	554 (12.5%)	9016 (18.1%)	
Multiple Breast Cancer	5254 (9.6%)	648 (14.6%)	5474 (10.9%)	<0.001
Ovarian Cancer	701 (1.3%)	130 (2.9%)	571 (1.1%)	<0.001
Pancreatic Cancer	124 (0.2%)	28 (0.6%)	96 (0.2%)	<0.001
Colorectal Cancer	613 (1.1%)	47 (1.1%)	566 (1.1%)	0.66
Uterine/endometrial Cancer	784 (1.4%)	83 (1.9%)	701 (1.4%)	0.01
Family History (1st & 2nd degree)				
Breast Cancer	32390 (59.4%)	2858 (66.9%)	29532 (61.9%)	<0.001
Ovarian Cancer	6486 (11.9%)	681 (15.9%)	5805 (12.2%)	<0.001
Pancreatic Cancer	5036 (9.2%)	471 (11.0%)	4565 (9.6%)	0.002
Colorectal Cancer	12121 (22.2%)	986 (23.1%)	11135 (23.3%)	0.70
Uterine/endometrial Cancer	3698 (6.8%)	281 (6.6%)	3417 (7.2%)	0.15
Tumor subtype†				<0.001
ER+/HER2-	26620 (58.0%)	1861 (50.0%)	24759 (58.7%)	
ER+/HER2+	5979 (13.0%)	424 (11.4%)	5555 (13.2%)	
ER-/HER2+	2701 (5.9%)	173 (4.6%)	2528 (6.0%)	
ER-/HER2-	10621 (23.1%)	1265 (34.0%)	9356 (22.2%)	
TNBC (ER-/PR-/HER2-)	10292 (22.4%)	1229 (33.0%)	9063 (21.5%)	

*: For continuous variable (age), ANOVA p value was provided; for categorical variables, Chi-Square test p value was provided.

†: Includes first primary breast tumors and all synchronous breast tumors (excludes second/asynchronous breast tumors).

Supplementary Table 2. Concordance of clinical pathology subtype for patients with multiple breast tumors

All cases with multiple breast cancer		Second BC pathology				
		ER+/HER2-	ER+/HER2+	ER-/HER2+	TNBC	NA
First BC pathology	ER+/HER2-	1643	88	52	115	500
	ER+/HER2+	96	186	17	20	99
	ER-/HER2+	33	15	62	29	53
	TNBC	175	28	17	466	214
	NA	234	34	21	111	946
Krippendorff's alpha = 0.58						

Mutation carriers with multiple breast cancer*		Second BC pathology				
		ER+/HER2-	ER+/HER2+	ER-/HER2+	TNBC	NA
First BC pathology	ER+/HER2-	46	10	2	9	16
	ER+/HER2+	8	9	1	3	4
	ER-/HER2+	0	0	1	1	0
	TNBC	14	0	0	34	14
	NA	9	2	0	10	31

Non-mutation carriers with multiple breast cancer		Second BC pathology				
		ER+/HER2-	ER+/HER2+	ER-/HER2+	TNBC	NA
First BC pathology	ER+/HER2-	625	34	14	46	171
	ER+/HER2+	36	55	3	7	34
	ER-/HER2+	13	6	22	11	14
	TNBC	69	14	5	136	67
	NA	102	14	10	44	341
Krippendorff's alpha = 0.54						

NA: Not available.
*: number is too small to calculate a Krippendorff alpha value.

Supplementary Table 3: Frequency of mutations in cancer predisposition genes by breast cancer clinical tumor subtype based on ER and HER2 status

	ER+/HER2-			ER+/HER2+			ER-/HER2+			ER-/HER2-			Het*
Gene	No. mutation	No. tested	Freq (%)	No. mutation	No. tested	Freq (%)	No. mutation	No. tested	Freq (%)	No. mutation	No. tested	Freq (%)	p value
ATM	179	15882	1.1	59	3507	1.7	17	1586	1.1	15	6304	0.2	< 0.001
BARD1	24	14778	0.2	7	3280	0.2	5	1480	0.3	53	5838	0.9	< 0.001
BRCA1	185	20275	0.9	32	4562	0.7	37	2030	1.8	546	8160	6.7	< 0.001
BRCA2	424	20275	2.1	76	4562	1. 7	26	2030	1.3	221	8160	2.7	< 0.001
BRIP1	43	14825	0.3	8	3285	0.2	1	1484	0.1	27	5848	0.5	0.05
CDH1	22	19795	0.1	2	4464	0.0	1	1985	0.1	4	7973	0.1	0.31
CHEK2	305	15864	1.9	81	3502	2.3	17	1583	1.1	25	6287	0.4	< 0.001
MSH6	24	9146	0.3	6	1885	0.3	1	851	0.1	9	3330	0.3	0.79
NBN	40	14778	0.3	7	3280	0.2	2	1480	0.1	12	5838	0.2	0.81
NF1	16	14554	0.1	7	3219	0.2	2	1456	0.1	11	5749	0.2	0.39
PALB2	155	16844	0.9	26	3733	0.7	8	1667	0.5	95	6706	1.4	< 0.001
PTEN	17	20314	0.1	2	4572	0.0	2	2034	0.1	3	8165	0.0	0.43
RAD51C	24	14825	0.26	3	3285	0.19	4	1484	0.3	26	5848	0.4	0.002
RAD51D	8	14601	0.1	1	3224	0.0	1	1460	0.1	15	5759	0.3	0.001
TP53	25	20356	0.1	19	4581	0.4	13	2037	0.6	9	8186	0.1	<0.001
Total			8.6			8. 9			7.7			14.4	

ER: estrogen receptor; HER2: human epidermal growth factor 2-neu; Freq: mutation frequency. *: Het: Heterogeneity analysis using General Linear Regression analysis adjusted for age at diagnosis and race and ethnicity.

Supplementary Table 4: Frequency of mutations in cancer predisposition genes by breast cancer clinical subtype in non-Hispanic whites

	ER+/HER2-			ER+/HER2+			ER-/HER2+			TNBC			Het*
Gene	No. mutation	No. tested	Freq (%)	No. mutation	No. tested	Freq (%)	No. mutation	No. tested	Freq (%)	No. mutation	No. tested	Freq (%)	p value
ATM	120	10542	1.1	36	2177	1.7	13	950	1.4	10	3571	0.3	< 0.001
BARD1	14	9881	0.1	7	2045	0.3	3	895	0.3	29	3308	0.9	< 0.001
BRCA1	102	13442	0.8	18	2823	0.6	23	1209	1.9	273	4561	6.0	< 0.001
BRCA2	243	13442	1.8	39	2823	1.4	13	1209	1.1	117	4561	2.6	< 0.001
BRIP1	31	9916	0.3	5	2049	0.2	1	897	0.1	16	3316	0.5	0.23
CDH1	14	13110	0.1	2	2756	0.1	1	1182	0.1	1	4448	0.0	0.39
CHEK2	235	10533	2.2	65	2174	3.0	13	949	1.4	15	3558	0.4	< 0.001
MSH6	15	6143	0.2	5	1214	0.4	1	525	0.2	7	1905	0.4	0.67
NBN	31	9881	0.3	5	2045	0.2	1	895	0.1	10	3308	0.3	0.70
NF1	9	9713	0.1	5	2002	0.3	1	874	0.1	11	3246	0.3	0.04
PALB2	90	11145	0.8	9	2304	0.4	5	991	0.5	54	3775	1.4	< 0.001
PTEN	12	13477	0.1	1	2830	0.0	1	1213	0.1	0	4565	0.0	0.05
RAD51C	16	9916	0.2	2	2049	0.1	4	897	0. 5	13	3316	0.4	0.03
RAD51D	4	9748	0.0	1	2006	0.1	1	876	0.1	7	3254	0.2	0.05
TP53	14	13502	0.1	12	2837	0.4	6	1215	0.5	6	4580	0.1	0.02
Total			8.4			9.2			8.3			13.8	

ER: estrogen receptor; HER2: human epidermal growth factor 2-neu; Freq: mutation frequency. *: Het: Heterogeneity analysis using General Linear Regression analysis adjusted for age at diagnosis.

Supplementary Table 5. Frequency of mutations in cancer predisposition genes by clinical pathology tumor subtype and heterogeneity analysis subset to single primary breast cancer cases

	Overall			ER+/HER2-			ER+/HER2+			ER-/HER2+			TNBC			Het*
Gene	No. mutation	No. tested	Freq (%)	No. mutation	No. tested	Freq (%)	No. mutation	No. tested	Freq (%)	No. mutation	No. tested	Freq (%)	No. mutation	No. tested	Freq (%)	p value
ATM	312	29231	1.1	156	13917	1.1	51	3190	1.6	13	1447	0.9	12	5564	0.2	< 0.001
BARD1	81	27199	0.3	20	12927	0.2	3	2981	0.1	5	1348	0.4	47	5150	0.9	< 0.001
BRCA1	776	37880	2.1	149	17826	0.8	28	4157	0. 7	31	1858	1. 7	439	7186	6.1	< 0.001
BRCA2	833	37880	2.2	373	17826	2.1	68	4157	1.6	24	1858	1.3	185	7186	2.6	< 0.001
BRIP1	87	27269	0.3	40	12972	0.3	8	2986	0.3	1	1352	0.1	24	5160	0.5	0.12
CDH1	32	37007	0.1	20	17386	0.1	2	4067	0.1	1	1814	0.1	3	7028	0.0	0.31
CHEK2	457	29178	1.6	258	13899	1.9	67	3185	2.1	17	1444	1.2	23	5551	0.4	< 0.001
MSH6	40	16464	0.2	20	8083	0.3	4	1716	0.2	1	783	0.1	9	2936	0.3	0.88
NBN	63	27199	0.2	31	12927	0.2	5	2981	0.2	2	1348	0.2	11	5150	0.2	0.88
NF1	35	26744	0.1	13	12745	0.1	7	2928	0.2	2	1326	0.2	8	5075	0.2	0.47
PALB2	313	31069	1.0	136	14778	0.9	20	3395	0.6	4	1520	0.3	86	5922	1. 5	< 0.001
PTEN	20	37946	0.1	11	17854	0.1	1	4166	0.0	2	1861	0.1	3	7192	0.0	0.49
RAD51C	60	27269	0.2	21	12972	0.2	2	2986	0.1	4	1352	0.3	25	5160	0.5	0.001
RAD51D	26	26814	0.1	6	12790	0.1	1	2933	0.0	1	1330	0.1	13	5085	0.3	0.005
TP53	62	38040	0.2	16	17895	0.1	16	4175	0.4	11	1864	0.6	8	7209	0.1	0.001
Total			9.7			8.4			8.2			7.3			13.8	

ER: estrogen receptor; HER2: human epidermal growth factor 2-neu; TNBC: ER-PR-HER2- (PR: progesterone receptor); Freq: mutation frequency.
*: Het: Heterogeneity analysis using Generalized Linear regression test adjusted for age at diagnosis and race and ethnicity.

Supplementary Table 6: Associations between gene-specific mutations and breast cancer with ER status and HER2 status*

	ER+ vs. ER-			HER2+ vs. HER2-			Interaction of ER & HER2 status		
Gene	OR	95% C.I.	p value	OR	95% C.I.	p value	OR	95% C.I.	p value
ATM	4.87	2.97-8.61	< 0.001	4.07	2.00-8.35	< 0.001	0.34	0.16-0.74	0.006
BARD1	0.18	0.11-0.29	< 0.001	0.37	0.13-0.83	0.03	3.53	1.02-13.05	0.05
BRCA1	0.13	0.11-0.16	< 0.001	0.21	0.15-0.29	< 0.001	2.69	1.61-4.47	< 0.001
BRCA2	0.80	0.68-0.94	0.007	0.42	0.28-0.63	< 0.001	1.61	1.01-2.64	0.05
BRIP1	0.62	0.39-1.02	0.05	0.15	0.008-0.70	0.06	5.86	0.99-111.96	0.10
CDH1	2.06	0.79-7.08	0.19	1.05	0.05-7.12	0.96	0.43	0.03-10.65	0.53
CHEK2	4.89	3.32-7.56	< 0.001	2.67	1.42-4.93	0.002	0.44	0.23-0.87	0.02
MSH6	0.96	0.46-2.19	0.92	0.45	0.024-2.39	0.45	2.83	0.40-57.44	0.37
NBN	1.30	0.70-2.59	0.43	0.68	0.11-2.49	0.61	1.22	0.25-8.97	0.82
NF1	0.58	0.27-1.30	0.17	0.69	0.11-2.58	0.63	2.69	0.53-20.44	0.27
PALB2	0.66	0.51-0.86	0.002	0.33	0.15-0.64	0.003	2.19	0.99-5.36	0.07
PTEN	2.38	0.80-10.20	0.17	2.41	0.32-14.62	0.34	0.18	0.02-1.99	0.15
RAD51C	0.36	0.20-0.63	< 0.001	0.62	0.18-1.60	0.38	0.95	0.17-4.75	0.95
RAD51D	0.21	0.08-0.48	< 0.001	0.27	0.02-1.35	0.21	2.22	0.08-61.34	0.59
TP53	1.31	0.63-2.98	0.49	4.64	2.0-11.30	< 0.001	0.46	0.16-1.29	0.14

*: Logistic regression analysis for main and interaction effect of ER and HER2 status, adjusted for age at diagnosis of breast cancer.

OR: odds ratio; 95% CI: 95% confidence interval

Supplementary Table 7: Pairwise enrichment of gene-specific mutations between clinical pathology subtypes*

	ER+/HER2+ vs ER+/HER2-		ER-/HER2+ vs ER+/HER2-		ER-/HER2+ vs ER+/HER2+		TNBC vs ER+/HER2-		TNBC vs ER-/HER2+		TNBC vs ER+/HER2+	
Gene	OR (95%CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
ATM	1.40 (1.04-1.89)	0.03	0.85 (0.51-1.42)	0.53	0.61 (0.35-1.06)	0.08	0.19 (0.11-0.33)	<0.001	0.22 (0.11-0.46)	<0.001	0.13 (0.07-0.25)	<0.001
BARD1	1.31 (0.56-3.06)	0.53	2.06 (0.78-5.43)	0.14	1.57 (0.50-4.96)	0.44	5.68 (3.49-9.27)	<0.001	2.76 (1.10-6.92)	0.03	4.33 (1.96-9.57)	<0.001
BRCA1	0.58 (0.40-0.85)	0.005	1.60 (1.12-2.28)	0.01	2.75 (1.71-4.44)	<0.001	7.68 (6.46-9.13)	<0.001	4.82 (3.43-6.77)	<0.001	13.25 (9.24-19.02)	<0.001
BRCA2	0.68 (0.53-0.87)	0.002	0.53 (0.35-0.78)	0.002	0.78 (0.50-1.22)	0.27	1.21 (1.02-1.43)	0.03	2.30 (1.53-3.47)	<0.001	1.79 (1.37-2.33)	<0.001
BRIP1	0.87 (0.41-1.86)	0.72	0.24 (0.03-1.74)	0.16	0.28 (0.03-2.20)	0.22	1.55 (0.94-2.55)	0.08	6.52 (4.52-9.41)	<0.001	1.78 (0.80-3.97)	0.16
CHEK2	1.19 (0.93-1.53)	0.16	0.58 (0.35-0.94)	0.03	0.48 (0.29-0.82)	0.007	0.22 (0.14-0.33)	<0.001	0.38 (0.20-0.70)	0.002	0.18 (0.11-0.29)	<0.001
PALB2	0.72 (0.47-1.09)	0.12	0.49 (0.24-0.99)	0.05	0.68 (0.31-1.50)	0.34	1.46 (1.12-1.90)	0.005	3.01 (1.46-6.21)	0.003	2.04 (1.31-3.17)	0.001
RAD51C	0.61 (0.19-2.02)	0.42	1.76 (0.61-5.09)	0.30	2.92 (0.65-13.05)	0.16	2.82 (1.60-4.95)	<0.001	1.61 (0.56-4.63)	0.37	4.70 (1.42-15.58)	0.01
RAD51D	0.61 (0.08-4.90)	0.64	1.35 (0.17-10.93)	0.78	2.21 (0.71-6.82)	0.17	4.77 (1.97-11.58)	0.001	3.50 (1.48-8.27)	0.004	7.79 (3.30-18.39)	<0.001
TP53	2.26 (1.23-4.15)	0.008	3.68 (1.86-7.28)	<0.001	1.63 (0.80-3.31)	0.18	0.84 (0.39-1.82)	0.66	0.23 (0.10-0.54)	<0.001	0.37 (0.17-0.83)	0.02

*: Polytomous regression analysis adjusted for age at diagnosis and racial/ethnic group.
OR: odds ratio; 95% CI: 95% confidence interval

Supplementary Table 8: Enrichment analysis of gene-specific mutations between each clinical pathologic subtype in non-Hispanic whites*

	ER+/HER2+ vs ER+/HER2-		ER-/HER2+ vs ER+/HER2-		ER-/HER2+ vs ER+/HER2+		TNBC vs ER+/HER2-		TNBC vs ER-/HER2+		TNBC vs ER+/HER2+	
Gene	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
ATM	1.36 (0.93-2.00)	0.11	1.18 (0.67-2.09)	0.57	0.84 (0.44-1.59)	0.59	0.24 (0.13-0.46)	<0.001	0.21 (0.09-0.48)	<0.001	0.18 (0.09-0.36)	<0.001
BARD1	2.45 (0.98-6.11)	0.05	2.41 (0.70-8.39)	0.17	0.98 (0.25-3.79)	0.98	6.26 (3.3-11.88)	<0.001	2.63 (0.80-8.65)	0.11	2.57 (1.12-5.89)	0.03
BRCA1	0.64 (0.39-1.06)	0.09	2.09 (1.33-3.30)	0.002	3.22 (1.73-5.99)	<0.001	7.88 (6.26-9.93)	<0.001	3.85 (2.49-5.93)	<0.001	12.25 (7.57-19.82)	<0.001
BRCA2	0.65 (0.46-0.92)	0.02	0.52 (0.30-0.92)	0.02	0.80 (0.43-1.50)	0.49	1.38 (1.10-1.73)	0.005	2.64 (1.48-4.71)	0.001	2.11 (1.46-3.05)	<0.001
BRIP1	0.80 (0.31-2.07)	0.65	0.36 (0.05-2.66)	0.32	0.45 (0.05-3.88)	0.47	1.56 (0.85-2.86)	0.15	4.30 (0.57-32.51)	0.16	1.95 (0.71-5.34)	0.20
CHEK2	1.26 (0.95-1.67)	0.10	0.58 (0.33-1.02)	0.06	0.46 (0.25-0.84)	0.01	0.19 (0.11-0.31)	<0.001	0.32 (0.15-0.67)	0.003	0.15 (0.08-0.26)	<0.001
PALB2	0.47 (0.24-0.94)	0.03	0.63 (0.26-1.54)	0.31	1.30 (0.43-3.89)	0.64	1.77 (1.26-2.48)	0.001	2.90 (1.16-7.28)	0.02	3.78 (1.86-7.67)	<0.001
RAD51C	0.65 (0.15-2.86)	0.57	2.93 (0.97-8.83)	0.06	4.47 (0.82-24.49)	0.08	2.51 (1.20-5.21)	0.01	0.85 (0.28-2.61)	0.77	3.79 (0.85-16.86)	0.08
RAD51D	1.29 (0.14-11.72)	0.82	2.93 (0.32-26.48)	0.34	2.27 (0.14-36.37)	0.56	5.39 (1.57-18.47)	0.007	1.85 (0.23-15.11)	0.57	4.18 (0.51-34.20)	0.18
TP53	2.85 (1.30-6.24)	0.009	3.57 (1.36-9.38)	0.01	1.25 (0.47-3.35)	0.66	1.15 (0.44-2.99)	0.78	0.32 (0.10-1.00)	0.05	0.41 (0.15-1.09)	0.07

*: Polytomous regression analysis adjusted for age at diagnosis.
OR: odds ratio; 95% CI: 95% confidence interval

Supplementary Table 9: Case-control associations between gene mutations in breast cancer subtypes and reference controls in non-Hispanic whites*

Gene	ER+/HER2-							ER+/HER2+						
	No. mut	No. tested	Freq (%)	OR	95% C.I.	p value	Adj p value	No. mut	No. tested	Freq (%)	OR	95% C.I.	p value	Adj p value
ATM	115	10542	1.1	2.64	2.10-3.31	<0.001	<0.001	35	2177	1.6	3.90	2.71-5.59	<0.001	<0.001
BARD1	14	9881	0.1	1.39	0.75-2.53	0.25	0.68	7	2045	0.3	3.35	1.51-7.26	0.007	0.07
BRCA1	88	13442	0.7	2.77	2.10-3.63	<0.001	<0.001	16	2823	0.6	2.40	1.41-4.00	0.003	0.03
BRCA2	216	13442	1.6	5.19	4.24-6.36	<0.001	<0.001	36	2823	1.3	4.11	2.85-5.90	<0.001	<0.001
BRIP1	29	9916	0.3	1.37	0.90-2.08	0.14	0.54	5	2049	0.2	1.14	0.44-2.74	0.63	1.00
CDH1	14	13110	0.1	7.29	2.99-18.12	<0.001	<0.001	2	2756	0.1	4.95	0.76-24.58	0.08	0.48
CHEK2	205	10533	2.0	2.30	1.95-2.71	<0.001	<0.001	63	2174	2.9	3.44	2.61-4.48	<0.001	<0.001
MSH6	13	6143	0.2	2.08	1.10-3.78	0.03	0.15	4	1214	0.3	3.24	1.07-8.93	0.04	0.31
NBN	31	9881	0.3	2.01	1.32-3.05	0.002	0.01	5	2045	0.2	1.56	0.60-3.83	0.26	1.00
NF1	8	9713	0.1	2.29	0.94-5.32	0.06	0.29	5	2002	0.3	6.94	2.49-18.42	0.002	0.02
PALB2	83	11145	0.7	4.34	3.22-5.87	<0.001	<0.001	9	2304	0.4	2.27	1.11-4.48	0.04	0.31
PTEN	12	13477	0.1	16.43	4.71-68.17	<0.001	<0.001	1	2830	0.0	6.52	0.25-59.13	0.18	0.91
RAD51C	13	9916	0.1	1.43	0.76-2.64	0.23	0.68	2	2049	0.1	1.07	0.18-4.04	0.71	1.00
RAD51D	4	9748	0.0	0.99	0.31-2.82	1.00	1.00	1	2006	0.1	1.21	0.06-7.10	0.57	1.00
TP53	11	13502	0.1	2.67	1.22-5.97	0.02	0.10	10	2837	0.4	11.58	4.98-26.08	<0.001	<0.001
Total Freq (%)			7.5							8.7				

Gene	ER-/HER2+							TNBC						
	No. mut	No. tested	Freq (%)	OR	95% C.I.	p value	Adj p value	No. mut	No. tested	Freq (%)	OR	95% C.I.	p value	Adj p value
ATM	12	950	1.3	3.06	1.69-5.41	<0.001	0.01	10	3571	0.3	0.67	0.35-1.25	0.28	0.55
BARD1	2	895	0.2	2.19	0.38-8.23	0.24	1.00	27	3308	0.8	8.02	5.00-12.74	<0.001	<0.001
BRCA1	17	1209	1.4	5.97	3.46-10.01	<0.001	<0.001	236	4561	5.2	22.39	18.06-27.8	<0.001	<0.001
BRCA2	12	1209	1.0	3.20	1.76-5.72	0.001	0.009	107	4561	2.4	7.61	5.95-9.73	<0.001	<0.001
BRIP1	1	897	0.1	0.52	0.03-2.90	1.00	1.00	15	3316	0. 5	2.12	1.18-3.65	0.01	0.06
CDH1	1	1182	0.1	5.78	0.26-36.83	0.18	1.00	1	4448	0.0	1.53	0.07-9.78	0.51	0.55
CHEK2	12	949	1.3	1.49	0.83-2.65	0.16	1.00	14	3558	0.4	0.46	0.27-0.79	0.002	0.02
MSH6	1	525	0.2	1.87	0.09-10.86	0.42	1.00	7	1905	0.4	3.62	1.63-7.83	0.005	0.04
NBN	1	895	0.1	0.71	0.04-4.02	1.00	1.00	10	3308	0.3	1.93	0.98-3.68	0.07	0.21
NF1	1	874	0.1	3.18	0.16-19.32	0.28	1.00	10	3246	0.3	8.57	3.69-18.35	<0.001	<0.001
PALB2	5	991	0.5	2.94	1.13-7.15	0.03	0.36	50	3775	1.3	7.74	5.48-10.97	<0.001	<0.001
PTEN	1	1213	0.1	15.22	0.59-138.00	0.08	0.75	0	4565	0.0	N/A	N/A	N/A	N/A
RAD51C	3	897	0.3	3.66	0.97-11.41	0.05	0.54	9	3316	0.3	2.97	1.42-6.08	0.006	0.04
RAD51D	1	876	0.1	2.76	0.14-16.28	0.31	1.00	6	3254	0.2	4.46	1.78-11.17	0.004	0.04
TP53	5	1215	0.4	13.52	4.77-38.21	<0.001	0.001	5	4580	0.1	3.58	1.26-10.11	0.02	0.09
Total Freq (%)			7.2							12.3				

Mut: mutation; Freq: frequency; OR: odds ratio; 95% C.I.: 95% confidence interval; N/A: not applicable

*: Fisher's exact test comparing mutation frequency by gene in breast cancer cases with gnomAD reference controls. Adj p-value: significance of association adjusted for multiple testing with false discovery rate (FDR) =0.1.

Supplementary Table 10. Influence of PR status on associations between gene mutations and breast cancer subtypes in case-control analyses*

	ER+/PR+/HER2+							ER+/PR-/HER2+						
Gene	No. mut	No. tested	Freq (%)	OR	95% C.I.	p value	Adj p value	No. mut	No. tested	Freq (%)	OR	95% C.I.	p value	Adj p value
ATM	44	2761	1.6	4.66	3.38-6.35	<0.001	<0.001	12	640	1.9	5.49	3.04-9.79	<0.001	<0.001
BARD1	5	2570	0.2	2.45	0.94-5.95	0.06	0.48	0	608	0.0	0.00	0.00-8.1	1.00	1.00
BRCA1	22	3610	0.6	2.65	1.68-4.12	<0.001	0.001	7	811	0.9	3.76	1.75-7.93	0.003	0.02
BRCA2	60	3610	1.7	5.22	3.94-6.85	<0.001	<0.001	11	811	1.4	4.25	2.19-7.69	<0.001	0.001
BRIP1	7	2574	0.3	1.51	0.70-3.2	0.24	0.73	0	609	0.0	N/A	N/A	N/A	N/A
CDH1	2	3542	0.1	4.55	0.74-18.36	0.08	0.50	0	785	0.0	N/A	N/A	N/A	N/A
CHEK2	65	2759	2.4	3.53	2.72-4.56	<0.001	<0.001	9	637	1.4	2.11	1.04-4.01	0.05	0.18
MSH6	3	1444	0.2	2.12	0.57-6.55	0.17	0.70	1	383	0.3	2.67	0.14-14.87	0.32	0.95
NBN	6	2570	0.2	1.95	0.84-4.38	0.14	0.69	1	608	0.2	1.37	0.07-7.59	0.52	1.00
NF1	7	2522	0.3	8.73	3.83-19.11	<0.001	<0.001	0	597	0.0	N/A	N/A	N/A	N/A
PALB2	18	2945	0.6	3.66	2.23-5.95	<0.001	<0.001	4	676	0.6	3.54	1.20-9.44	0.03	0.15
PTEN	2	3619	0.1	5.63	0.90-25.05	0.06	0.48	0	811	0.0	N/A	N/A	N/A	N/A
RAD51C	2	2574	0.1	0.92	0.16-3.52	1.00	1.00	0	609	0.0	N/A	N/A	N/A	N/A
RAD51D	1	2526	0.0	0.78	0.04-4.49	1.00	1.00	0	598	0.0	N/A	N/A	N/A	N/A
TP53	12	3625	0.3	13.59	6.61-26.71	<0.001	<0.001	4	814	0.5	20.2	6.50-57.96	<0.001	0.001
Total Freq (%)			8.6							7.0				
	ER+/PR+/HER2-							**ER+/PR-/HER2-**						
Gene	No. mut	No. tested	Freq (%)	OR	95% C.I.	p value	Adj p value	No. mut	No. tested	Freq (%)	OR	95% C.I.	p value	Adj p value
ATM	145	14073	1.0	3	2.48-3.63	<0.001	<0.001	19	1495	1.3	3.71	2.26-5.89	<0.001	<0.001
BARD1	21	13100	0.2	2.02	1.24-3.24	0.007	0.03	2	1372	0.2	1.83	0.32-6.63	0.30	0.92
BRCA1	113	17967	0.6	2.74	2.19-3.41	<0.001	<0.001	47	1915	2.5	10.80	7.86-14.74	<0.001	<0.001
BRCA2	335	17967	1.9	5.86	5.05-6.79	<0.001	<0.001	42	1915	2.2	6.91	4.99-9.56	<0.001	<0.001
BRIP1	36	13136	0.3	1.52	1.06-2.17	0.03	0.08	3	1381	0.2	1.21	0.33-3.64	0.74	1.00
CDH1	18	17542	0.1	8.27	4.18-17.33	<0.001	<0.001	1	1867	0.1	4.32	0.21-28.5	0.22	0.92
CHEK2	237	14057	1.7	2.52	2.18-2.91	0.001	<0.001	22	1494	1.5	2.20	1.42-3.34	0.001	0.01
MSH6	20	8080	0.3	2.53	1.53-4.04	<0.001	0.003	0	896	0.0	N/A	N/A	N/A	N/A
NBN	35	13100	0.3	2.23	1.52-3.26	<0.001	0.001	5	1372	0.4	3.04	1.18-7.24	0.03	0.22
NF1	12	12895	0.1	2.93	1.47-5.63	0.003	0.01	3	1356	0.2	6.96	1.82-21.02	0.011	0.10
PALB2	117	14931	0.8	4.69	3.73-5.90	<0.001	<0.001	25	1584	1.6	9.49	6.13-14.43	<0.001	<0.001
PTEN	13	18004	0.1	7.36	3.15-16.16	<0.001	<0.001	1	1918	0.1	5.31	0.25-33.09	0.18	0.92
RAD51C	17	13136	0.1	1.53	0.89-2.55	0.12	0.24	3	1381	0.2	2.57	0.69-7.57	0.12	0.70
RAD51D	7	12931	0.1	1.07	0.48-2.29	0.84	0.84	1	1365	0.1	1.45	0.07-8.32	0.50	1.00
TP53	16	18039	0.1	3.64	1.95-6.82	<0.001	0.001	2	1924	0.1	4.26	0.73-16.01	0.09	0.61
Total Freq (%)			7.5							10.4				
	ER-/PR+/HER2+							**ER-/PR-/HER2+**						
Gene	No. mut	No. tested	Freq (%)	OR	95% C.I.	p value	Adj p value	No. mut	No. tested	Freq (%)	OR	95% C.I.	p value	Adj p value
ATM	1	78	1.3	3.74	0.19-20.76	0.24	0.24	15	1475	1.0	2.96	1.71-4.93	<0.001	0.004
BARD1	0	72	0.0	N/A	N/A	N/A	N/A	4	1378	0.3	3.65	1.22-9.60	0.03	0.24
BRCA1	0	107	0.0	N/A	N/A	N/A	N/A	28	1883	1.5	6.50	4.35-9.56	<0.001	<0.001
BRCA2	0	107	0.0	N/A	N/A	N/A	N/A	25	1883	1.3	4.16	2.74-6.26	<0.001	<0.001

BRIP1	0	72	0.0	N/A	N/A	N/A	N/A	1	1382	0.1	0.40	0.02-2.37	0.53	1.00
CDH1	0	103	0.0	N/A	N/A	N/A	N/A	1	1843	0.1	4.37	0.21-28.88	0.22	1.00
CHEK2	1	78	1.3	1.91	0.10-10.57	0.41	0.41	13	1473	0.9	1.31	0.73-2.29	0.33	1.00
MSH6	0	45	0.0	N/A	N/A	N/A	N/A	1	791	0.1	1.29	0.07-7.17	0.54	1.00
NBN	0	72	0.0	N/A	N/A	N/A	N/A	2	1378	0.2	1.21	0.21-4.58	0.68	1.00
NF1	0	71	0.0	N/A	N/A	N/A	N/A	2	1356	0.2	4.64	0.80-18.15	0.07	0.59
PALB2	0	82	0.0	N/A	N/A	N/A	N/A	8	1550	0.5	3.09	1.42-6.16	0.006	0.06
PTEN	0	107	0.0	N/A	N/A	N/A	N/A	2	1887	0.1	10.8	1.73-48.07	0.02	0.19
RAD51C	0	72	0.0	N/A	N/A	N/A	N/A	3	1382	0.2	2.57	0.69-7.56	0.12	0.82
RAD51D	0	71	0.0	N/A	N/A	N/A	N/A	1	1360	0.1	1.46	0.07-8.35	0.50	1.00
TP53	0	107	0.0	N/A	N/A	N/A	N/A	12	1890	0.6	26.10	12.68-51.35	<0.001	<0.001
Total Freq (%)			2.6							7.1				

	ER-/PR+/HER2-							**ER-/PR-/HER2-**						
Gene	No. mut	No. tested	Freq (%)	OR	95% C.I.	p value	Adj p value	No. mut	No. tested	Freq (%)	OR	95% C.I.	p value	Adj p value
ATM	2	158	1.3	3.69	0.65-13.85	0.10	0.31	13	6117	0.2	0.62	0.34-1.06	0.09	0.18
BARD1	0	142	0.0	N/A	N/A	N/A	N/A	48	5670	0.9	10.70	7.49-15.26	<0.001	<0.001
BRCA1	13	228	5.7	25.47	13.80-45.05	<0.001	<0.001	459	7896	5.8	26.00	22.34-30.19	<0.001	<0.001
BRCA2	8	228	3.5	11.12	5.12-22.13	<0.001	<0.001	199	7896	2.5	7.95	6.68-9.44	<0.001	<0.001
BRIP1	1	142	0.7	3.92	0.20-21.72	0.23	0.45	24	5680	0.4	2.35	1.51-3.60	<0.001	0.002
CDH1	0	222	0.0	N/A	N/A	N/A	N/A	4	7715	0.1	4.18	1.28-12.63	0.02	0.10
CHEK2	1	157	0.6	0.95	0.05-5.56	1.00	1.00	22	6101	0.4	0.54	0.35-0.82	0.002	0.01
MSH6	0	78	0.0	N/A	N/A	N/A	NA	9	3233	0.3	2.84	1.39-5.53	0.006	0.04
NBN	0	142	0.0	N/A	N/A	N/A	NA	12	5670	0.2	1.77	0.97-3.18	0.08	0.18
NF1	0	139	0.0	N/A	N/A	N/A	NA	10	5585	0.2	5.63	2.68-11.17	<0.001	<0.001
PALB2	2	176	1.1	6.82	1.20-25.86	0.04	0.18	84	6501	1.3	7.76	5.98-10.02	<0.001	<0.001
PTEN	0	227	0.0	N/A	N/A	N/A	N/A	3	7901	0.0	3.87	0.93-13.49	0.06	0.18
RAD51C	0	142	0.0	N/A	N/A	N/A	N/A	19	5680	0.3	3.96	2.40-6.51	<0.001	<0.001
RAD51D	1	139	0.7	14.28	0.72-83.06	0.07	0.28	12	5595	0.2	4.25	2.24-7.82	<0.001	0.001
TP53	0	228	0.0	N/A	N/A	N/A	N/A	6	7921	0.1	3.11	1.26-7.31	0.02	0.10
Total Freq (%)			13.7							12.9				

Mut: mutation; Freq: frequency; OR: odds ratio; 95% C.I.: 95% confidence interval; N/A: not applicable

*: Logistic regression by comparing each gene mutation frequency with gnomAD reference controls, weighted by race and ethnicity. Adj p-value: significance of association adjusted for multiple testing with false discovery rate (FDR) =0.1.

Supplementary Table 11. Lifetime absolute risk estimation by subtype for each predisposition gene in non-Hispanic whites*

		OR (95% CI)					Absolute risk at age 85				
Gene	Case Freq	HR+/HER2-	HR+/HER2+	HR-/HER2+	TNBC	BC	HR+/HER2-	HR+/HER2+	HR-/HER2+	TNBC	BC
ATM	0.5%	2.77 (2.22-3.45)	4.02 (2.79-5.69)	3.3 (1.82-5.84)	0.67 (0.35-1.25)	2.73 (2.28-3.28)	0.23	0.04	0.01	0.01	0.28
BARD1	0.1%	1.47 (0.82-2.62)	3.28 (1.48-7.09)	3.55 (0.94-10.95)	8.61 (5.42-13.46)	2.83 (1.95-4.12)	0.13	0.03	0.01	0.09	0.29
BRCA1	0.8%	3.45 (2.68-4.46)	2.63 (1.57-4.31)	8.72 (5.48-13.56)	26 (21.11-32.20)	7.98 (6.58-9.68)	0.23	0.02	0.03	0.22	0.57
BRCA2	0.9%	5.92 (4.87-7.21)	4.34 (3.03-6.18)	3.73 (2.02-6.64)	8.33 (6.56-10.60)	6.55 (5.52-7.79)	0.40	0.04	0.01	0.07	0.52
CHEK2	0.9%	2.65 (2.26-3.10)	3.57 (2.76-4.63)	1.34 (0.71-2.51)	0.49 (0.29-0.82)	2.31 (2.02-2.64)	0.23	0.04	0.01	0.01	0.24
PALB2	0.4%	4.7 (3.51-6.29)	2.22 (1.08-4.38)	3.17 (1.22-7.72)	8.36 (5.95-11.69)	5.28 (4.14-6.78)	0.34	0.02	0.01	0.07	0.45
RAD51C	0.1%	1.75 (0.96-3.10)	1.04 (0.18-3.95)	5.27 (1.74-13.90)	4.29 (2.26-7.91)	2.28 (1.50-3.44)	0.15	0.01	0.02	0.04	0.21
RAD51D	0.0%	1.23 (0.45-3.14)	1.18 (0.06-6.93)	2.98 (0.15-17.59)	5.21 (2.15-12.18)	1.84 (0.93-3.55)	0.11	0.01	0.01	0.06	0.20
TP53	0.1%	3.36 (1.65-6.97)	13.55 (6.28-29.42)	17.47 (6.76-45.40)	4.3 (1.66-11.15)	5.57 (3.15-9.82)	0.24	0.12	0.06	0.04	0.42
SEER estimates†							0.10	0.01	0.004	0.01	0.12

*: Limited to patients with available ER and/or PR and HER2 tumor status: HR+ (ER+ and/or PR+), HR- (ER- and PR-).

†: SEER estimates: SEER 2010-2015 breast cancer incidence rates.

OR: odds ratio; BC: overall breast cancer; 95% CI: 95% confidence interval