

Assessment of homologous recombination deficiency phenotype in breast cancers in adolescents and young adults in the clinical setting

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Abstract

Background Homologous recombination deficiency (HRD), which may be associated with high efficacy of PARP inhibitor- and platinum agent-based therapies, is a prevalent phenotype of breast cancer diagnosed in adolescents and young adults (AYAs; 15–39 years old). HRD score, indicating HRD status, is not routinely assessed in the oncology clinic due to the need for genome-wide analyses. **Methods** Subjects were a Japanese cohort of 46 AYA breast cancer patients, whose HRD scores were calculated from whole-exome sequencing data, and two existing breast cancer cohorts (US and European) for which HRD scores were available. Genetic and clinicopathological factors associated with the HRD-high phenotype, defined as HRD score ≥ 42 , were selected based on the criterion that they be assessable by routine examinations qualifying for insurance reimbursement. A model for prediction of the HRD-high phenotype was constructed and validated using data from the three cohorts. **Results** In the Japanese AYA cohort, as in the US and European cohorts, HRD-high phenotype (13/46, 28.3%) was preferentially observed in cases with any or combination of germline BRCA1/2 mutations, somatic TP53 mutations, triple-negative subtype, and higher tumor grades. Because these four factors can be assessed by routine examination that qualifies for insurance reimbursement, we developed a model based on these factors to judge whether a case is HRD-high, using the US cohort ($n = 744$; Area under the curve [AUC] = 0.85). The predictive power of the model was validated in the Japanese ($n = 46$; AUC = 0.90) and European ($n = 58$; AUC = 0.96) AYA cases. A model developed using the European cohort ($n = 477$; AUC = 0.89) had similar predictive power in Japanese (AUC = 0.89) and US ($n = 54$; AUC = 0.87) AYA cohorts. **Conclusions** The HRD-high phenotype of AYA breast cancer can be deduced based on genomic and pathological factors that are routinely examined in the oncology clinic. The predictive model presented here could increase the fraction of AYA breast cancer patients who could benefit from PARP inhibitor- and platinum agent-based therapies.

Background

Breast cancers diagnosed in adolescents and young adults (AYAs; 15–39 years old) constitute approximately 5% of all breast cancer cases in Asia, Europe, and the US [1, 2]. Because the prognosis of patients with such disease has improved very little over the past decades [3, 4], therapeutic options suitable for AYA breast cancers are urgently required. A fraction of AYA breast cancers express therapeutic targets such as estrogen receptor (ER), progesterone receptor (PgR), and HER2 oncoprotein. However, even in ER-positive cases, AYA breast cancers are more resistant to standard endocrine therapy than breast cancers in older women [3, 4]. Similarly, in HER2-positive cases, AYA cases have significantly worse recurrence-free survival [5]. Relative to non-AYA cancers, more AYA breast cancers do not express the therapeutic targets mentioned above and are thus classified as triple-negative breast cancers (TNBCs), which have a poor prognosis [6, 7].

Breast cancers with germline mutations of the *BRCA1/2* genes, which are prevalent in TNBC cases, respond to therapies based on platinum agents and PARP inhibitors because the cancer cells have defects in homologous recombination (HR) repair of DNA damage caused by these drugs [8–14]. Notably, homologous recombination deficiency (HRD), a phenotype of tumor cells caused not only by germline *BRCA1/2* mutations but also by other alterations of HR repair genes, was recently identified as a biomarker that can more accurately predict the efficacy of platinum/PARP inhibitor therapy than germline *BRCA1/2* mutations [15–23]. Several clinical trials have been conducted to verify the association between the HRD phenotype of tumor cells and the efficacy of such therapies in breast [15–19] and other types of cancers [20, 21]. Genome-wide analytical methods, such as SNP arrays and whole-genome/whole-exome sequencing analyses, have been used to assess the HRD phenotype of tumor cells [24]. The HRD score, a sum of each score that represents loss of heterozygosity, telomeric allelic imbalance, and large-scale state transitions (large-scale chromosomal breaks) in the tumor genome, has been used for this assessment: cases with HRD score ≥ 42 are classified as having the HRD-high phenotype [17]. Specific assays to calculate HRD scores, such as genome-wide SNP array-based MyChoice® HRD (Myriad Genetics) [17], have been developed to identify cases with the HRD-high phenotype. However, assessment of HRD scores is not widely performed in the breast cancer clinic due to the necessity for genome-wide analyses that are not reimbursed by health insurance systems.

Tumor-profiling multiplex gene panel tests (hereafter, “gene panel tests”), which enable simultaneous examination of tens to hundreds of cancer-related genes, have recently begun to be reimbursed in a tumor-agnostic manner by health insurance in the US, Europe, and Asia, including Japan [25-31]. Gene panel tests are used in routine oncology to detect activating mutations in oncogenes linked to efficacy of molecularly targeted drugs in oncogenes, such as *PIK3CA* and *AKT1* in breast cancer [32, 33]. These tests examine the status of not only the oncogenes but also genes whose aberrations are associated with HRD, such as *BRCA1/2* and *TP53* [15, 16, 34]. Hence, in this study we sought to develop a model for judging whether a given AYA breast cancer case is HRD-high, based on information from gene panel tests and routine clinicopathological examination. The model was constructed and validated using data from our own Japanese AYA cohort and two existing cohorts from the US [35] and Europe [36], which included 70 AYA cases each. The model constructed here, which uses information on *BRCA1/2* and *TP53* status, histological subtype, and tumor grade, robustly identified AYA breast cancer with HRD.

Methods

Preparation of three breast cancer cohorts with HRD scores

A Japanese cohort consisting of 46 AYA breast carcinomas was prepared in a previous study by our group [37]. These cases were sporadic primary breast cancers diagnosed in 2003–2015 in patients aged 27–39. All members of the cohort underwent surgery at the National Cancer Center Hospital, Tokyo, Japan. Clinicopathological information, such as histological subtype and tumor grades, was obtained retrospectively. Tumor grade was classified into three categories (I–III) according to the criteria for the Nottingham histologic score [38]: percentage of tubule formation, degree of nuclear pleomorphism, and accurate mitotic count [38]. Two other cohorts of breast cancer cases, from the US [35] and Europe [36], were also used; each included 70 AYA cases (Table 1). Information regarding clinicopathological characteristics, germline mutations, somatic mutations, methylation status, and HRD score from the US and European cohorts was obtained from the National Cancer Institute Genomic Data Commons, the cBioPortal database, and published supplementary data [34-36, 39-42], as shown in Table S1 in Additional file 1.

Calculation of HRD score

The HRD scores of the 46 Japanese AYA breast cancer cases were calculated based on genome-wide SNP profiles obtained by whole-exome sequencing [37]. The allelic status of each tumor was assessed using the ASCAT (v2.5.2) algorithm [43]. A total HRD score, i.e., the sum of loss of heterozygosity (number of LOH regions longer than 15 Mb), the telomeric allelic imbalance (number of regions of allelic imbalance that extend to one of the subtelomeres but do not cross the centromere), and large-scale state transitions (number of break points between adjacent regions longer than 10 Mb after filtering out regions shorter than 3 Mb), was calculated for each case according to a previously described method [17]. HRD scores of the US and European cohorts were calculated based on SNP array data [34, 36]. HRD scores obtained by whole-exome sequencing and SNP array analysis were consistent with each other (Pearson $r = 0.87$) [24]; therefore, cases with HRD score ≥ 42 were judged as HRD-high for subjects from all three cohorts, according to a threshold commonly used in recent clinical trials [17, 18].

Germline and somatic mutations in 28 cancer-related genes

In the Japanese cohort, 28 cancer-related genes were searched for germline and somatic mutations using existing whole-exome sequencing data [37]. This set of 28 genes consisted of 25 cancer susceptibility genes [44], *APC*, *ATM*, *BARD1*, *BMPR1A*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CDK4*, *CDKN2A*, *CHEK2*, *EPCAM*, *MLH1*, *MSH2*, *MSH6*, *MUTYH*, *NBN*, *PALB2*, *PMS2*,

PTEN, *STK11*, *RAD51C*, *RAD51D*, *SMAD4*, and *TP53*, and three other genes related to HR [45], *CHEK1*, *FAM175A*, and *MRE11A*. For germline mutations, null variants (nonsense, frameshift indel, and splice-site variants) and missense variants classified as “pathogenic” or “likely pathogenic” in the ClinVar database (as of October 19, 2018; <https://www.ncbi.nlm.nih.gov/clinvar/>) were selected. Somatic mutations of the *TP53* gene were annotated according to the criteria of the IARC TP53 database (version R19) [46], and pathogenic mutations including null variants were counted as positive.

DNA hypermethylation of the *BRCA1* and *RAD51C* genes

DNA methylation assays using the Infinium MethylationEPIC BeadChip Kit (Illumina, San Diego, CA, USA) were performed to obtain genome-wide DNA methylation profiles of 37 tumor (including all 13 HRD-high cases) and 12 adjacent non-tumor (including four HRD-high cases) tissues from the Japanese cohort. After probes with standard deviations greater than 0.05 were removed, 24 probes, including cg04658354 for *BRCA1* and cg14837411 for *RAD51C*, were defined as unmethylated in the 12 non-tumor breast tissues (mean beta value <0.2). A tumor sample was judged as hypermethylated when its beta values for probes of tumor tissues were greater than 0.3. A sample was defined as hypermethylated when it had more than four outlier probes for a specific gene promoter [36]. Based on a previous study [34], hypermethylation of the *BRCA1* and *RAD51C* genes in a US cohort was evaluated based on methylation status at the cg04658354 and cg14837411 loci, respectively. For the European cohort, only the hypermethylation status of *BRCA1* was available [39].

Mutational signature analysis

Mutational signatures of the breast cancer genome from the Japanese cohort samples was obtained by decomposing somatic mutations into four major mutational signatures (catalogue of somatic mutations in cancer (COSMIC) signature 1, 2, 3, 6), defined previously [37], to minimize the Kullback–Leibler divergence. A heat map based on signature profiles was generated using the R command regHeatmap. The Pearson’s correlation coefficient between the percentage of BRCA signatures (COSMIC signature 3) and the HRD score was calculated.

Model for predicting HRD status using factors assessed in clinical setting

A prediction model for judging the HRD-high phenotype (i.e., HRD score ≥ 42) was constructed based on logistic regression of four or five variables: i) presence or absence of pathogenic germline or somatic *BRCA1/2* mutations, ii) presence or absence of non-functional somatic *TP53* mutations, iii) TNBC subtype or not, iv) high tumor grade (grade III) or not, and v) hypermethylation (*BRCA1* and *RAD51C*) or not (Table 1). To construct and validate the model using these four or five factors, only cases with information for all four or five variables were included in the analysis. First, a HRD prediction model was constructed using data from all cases in the US cohort, irrespective of age, as a development cohort (n = 744). Then, the constructed model was applied to the AYA cases of the Japanese (n = 37 or 46) and European (n = 58) cohorts, respectively (i.e., validation cohorts). When all cases of the European cohort (n = 477) were used as the development cohort, AYA cases of the Japanese (n = 37 or 46) and US (n = 54) cohorts were used as validation cohorts. The area under the receiver operating characteristic curve (AUC) was calculated to evaluate the predictive power of each cohort. Cutoff values of the prediction model were defined using Youden’s index where the sum of sensitivity and specificity was maximal. Based on the cutoff value, positive and negative predictive values were calculated in the validation cohorts.

Statistical analyses

Associations among clinicopathological and genetic factors were examined by Fisher's exact test, Chi-squared test, Pearson's correlation, Kruskal–Wallis test, and Mann–Whitney U-test.

Results

Three AYA breast cancer cohorts with HRD scores

The characteristics of three AYA breast cancer cohorts, i.e., Japanese (n = 46), US (n = 70), and European (n = 70) cohorts are shown in Table 1. Pathological stage and histology did not differ significantly among the three cohorts ($P > 0.05$), whereas the European cohort contained more TNBC and high tumor grade cases than other two cohorts ($P < 0.01$) (Table 1). The US and European AYA subjects were members of pan-age cohorts including 1,044 and 560 subjects, respectively.

HRD scores were calculated for 46 subjects of the Japanese cohort using whole-exome sequencing data (Figure 1A). The fraction of cases with the HRD-high phenotype, defined as HRD score ≥ 42 (13/46, 28.3%), was similar to that of the US cohort but considerably lower than that of the European cohort (Table 1). The calculated HRD scores of the Japanese cohort subjects were strongly correlated with the fraction of cases with the COSMIC mutational signature 3 (Pearson $r = 0.78$), which is predominant in *BRCA1/2*-mutated and/or HRD-high tumors [47] (Figure 1B). Hierarchical clustering analysis revealed that most HRD-high subjects were co-clustered in a group of cases in which the COSMIC mutational signature 3 was predominant (Figure 1C). This result confirmed that the HRD scores of the Japanese cohort samples were tightly linked to *BRCA1/2* and other homologous recombination repair deficiencies.

Genetic and clinicopathological factors associated with the HRD-high phenotype

BRCA1/2 germline mutations, as well as *BRCA1* and *RAD51C* hypermethylation, are well-known alterations associated with HRD [15, 16, 34]. Indeed, both pathogenic *BRCA2* germline mutations were present in HRD-high cases (Figure 1). *BRCA1* and *RAD51C* hypermethylation were also preferentially (4/5, 80%) observed in HRD-high cases. In addition, somatic mutations in the *TP53* gene were more abundant in HRD-high cases than in HRD-low cases (6/13 [46.2%] vs 3/33 [9.1%]; $P < 0.01$ by Fisher's exact test; Table S2 in Additional file 1). In terms of clinicopathological factors, TNBC included many HRD-high cases (5/6, 83.3%) but other categories, such as luminal subtype (6/35, 17.1%), also included HRD-high cases (Figure 2A). In addition, high tumor grades (i.e., grade III) were prevalent in HRD-high cases (11/13, 84.6%) (Table S2 in Additional file 1). The same tendency was also observed in all cases in the US and European cohorts and in AYA cases in these cohorts (Figure S1 in Additional file 2, Table S2 in Additional file 1, and Figure 2B–D).

HRD-high judgement was more prevalent in AYA cases than in non-AYA cases (Figure S2 in Additional file 3), indicating that HRD is a common feature of AYA breast cancers. On the other hand, among the AYA cases, younger age was not associated with the HRD-high phenotype in the Japanese, US, and European AYA subjects (Table S2 in Additional file 1).

Prediction model for the HRD-high phenotype

Five factors prevalent in HRD-high cases, *BRCA1/2* mutation, somatic *TP53* mutation, hypermethylation (*BRCA1* and *RAD51C*), triple-negative subtype, and high tumor grade, were employed as explanatory variables of logistic regression analysis to construct a prediction model for HRD-high cases (HRD scores ≥ 42). Then, the high AUC value of the prediction model using all US cases (n = 744) as a development cohort (0.86) was validated in the Japanese AYA (0.91) and the European AYA (0.95) cohorts (Figure 3A). The high AUC value of the prediction model obtained using all European cases (n

= 477) as a development cohort (0.90) was also validated in the Japanese AYA (0.92) and the US AYA (0.87) cohorts (Figure S3A in Additional file 4). All variables were significant in the logistic regression analysis in both cohorts ($P < 0.05$).

Among the five factors mentioned above, DNA hypermethylation of *BRCA1* and *RAD51C* is not routinely examined in the oncology clinic because this feature of DNA is not assessed by commonly used gene panel tests. Hence, we next constructed a prediction model for HRD-high cases using four factors other than DNA hypermethylation of *BRCA1* and *RAD51C*. Again, a prediction model with comparably high AUC was obtained using all US cases as the development cohort (0.85), and the predictive power of this model was validated in the Japanese AYA (0.90) and the European AYA (0.96) cohorts (Figure 3B, Table S3A in Additional file 1). The AUC value of the prediction model based on all European cases as a development cohort (0.89) was also validated in the Japanese AYA (0.89) and the US AYA (0.87) cohorts (Figure S3B in Additional file 4, Table S3A in Additional file 1). Thus, high predictive power for the HRD-high phenotype was achieved using only the four factors that are routinely assessed in oncological practice. When the cutoff value of the prediction model from the US cohort was tentatively set at 1.0 based on optimal Youden's index (sensitivity: 79.7%; specificity: 80.4%), positive predictive values in the Japanese AYA and European AYA cohorts were 78.6% and 82.5%, respectively, whereas negative predictive values were 93.8% and 94.4%, respectively.

Discussion

Due to the high rate of germline *BRCA1/2* mutations, as well as the high fraction of TNBC [6, 48-50], HRD has been considered to be a major phenotype of AYA breast cancer. Indeed, this study confirmed that HRD-high cases are a major fraction not only of European and US cases (25–50%) [34, 36], but also of Japanese cases (28%). Therefore, PARP inhibitor- and platinum agent-based therapies for these patients could improve the current poor prognosis of AYA breast cancer patients. Notably, as shown in Figure 1A, six (46.2%) of the 13 HRD-high cases in the Japanese cohorts were negative for both *BRCA1/2* germline mutation and TNBC; this fraction is much higher than among the other two cohorts (3/16 [18.8%] and 6/40 [15.0%], respectively; see Figure S1 in Additional file 2). In routine oncological practice, these cases are considered not actively subjected to HRD examination. By contrast, our prediction model is simple and employs only four factors that can be assessed by gene panel tests and pathological examinations that qualify for insurance reimbursement from health care systems. In the near future, gene panel tests will be used routinely in the breast cancer clinic because new therapeutic regimens require testing for mutations in oncogenes such as *PIK3CA* and *AKT1* [32, 33]. Thus, our prediction model will give physicians another option for usage of gene panel test data, i.e., select patients who may carry the HRD-high phenotype for whom it is worthwhile to perform HRD examination and/or to consider subsequent PARP inhibitor- and platinum agent-based therapies.

The HRD-high phenotype is associated with the therapeutic effects of PARP inhibitors and platinum agents in ovarian cancer [20]. In breast cancer, however, only germline mutations of the *BRCA1/2* genes, rather than the HRD-high phenotype, have been proposed as strong predictive factors for such therapeutic effects [10]. Theoretically, HRD makes cancer cells susceptible to these agents irrespective of tumor type; therefore, the predictive power of the HRD-high phenotype for the efficacy of PARP inhibitory therapy is now being investigated in a clinical trial (NCT02401347) [19]. The prediction model proposed in our study would help to validate the significance of the HRD-high phenotype using real-world data accumulated routinely in the oncology clinic. Notably, distinguishing between germline and somatic *BRCA1/2* mutations did not significantly affect the prediction power (Table S3B in Additional file 1), therefore, the results of gene panel test data that report *BRCA1/2* mutations without informing germline/somatic status such as FoundationOne® CDx test, can be used for prediction.

HRD is caused by alterations in the genes involved in homologous recombination repair, such as inactivating mutations of the *BRCA1* and *BRCA2* genes and hypermethylation of the *BRCA1* and *RAD51C* genes [15, 16, 34]. In fact, 6/13 (46.2%)

Japanese, 8/16 (50.0%) US, and 22/40 (55.0%) European AYA cases harbored these alterations. However, the molecular alterations responsible for HRD in the remaining cases (approximately 50%) remain unknown. To address this issue, we examined gross germline rearrangements, such as exonic deletions and duplications, in the *BRCA1*, *BRCA2*, *TP53*, *PTEN*, *ATM*, and *CHEK2* genes in the 45 Japanese cases by multiplex ligation-dependent probe amplification analysis. In addition, we searched for genetic and epigenetic alterations in 25 genes (other than *BRCA1*, *BRCA2*, and *RAD51C*) involved in hereditary cancers and/or HR. However, neither of these analyses yielded positive results, indicating that other unknown mechanisms caused HRD in these cases.

In this study, we established a prediction model for the HRD-high phenotype of AYA breast cancer patients based on gene panel tests and clinicopathological findings. However, this study has a limitation, in that the numbers of AYA patients in all three cohorts is small, potentially leading to an overestimation of predictive power. Further studies that include a larger number of cases, as well as prospective studies, are needed to firmly conclude that a HRD-high phenotype of AYA breast cancer can be deduced based on genomic and pathological factors that are routinely examined in the oncology clinic.

Conclusions

The present prediction model provides a tool for identifying the AYA breast cancer patients who would benefit from PARP- and/or platinum-based therapies in the clinical setting.

Abbreviations

AUC: area under the receiver operating characteristic curve; AYA: adolescents and young adults; COSMIC: catalogue of somatic mutations in cancer; ER: estrogen receptor; HRD: homologous recombination deficiency; MLPA: multiplex ligation-dependent probe amplification; PgR: progesterone receptor; SNP: single-nucleotide polymorphism; TNBC: triple-negative breast cancer

Declarations

Ethics approval and consent to participate

This project received institutional review board approval from the National Cancer Center (2015-159, 2015-278). Written informed consent was obtained from the patients.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated during the current study are not publicly available due to ongoing analysis but are available from the corresponding author on reasonable request. The availability of the datasets analyzed during the current study is described in Table S1 in Additional file 1.

Competing interests

The authors have no conflicts of interest to declare.

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Authors' contributions

TK had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. TW, TH, HT, MT, and TK contributed to the study concept and design. KS, YS, TW, MU, MY, EA, and YK contributed to the acquisition of the data. TW, TH, HT, MT, KS, KT, EA, TY, YK, and TK contributed to the analysis and interpretation of the data. TW drafted the manuscript. TK critically revised the manuscript for important intellectual content. All authors read and approved the final version of the manuscript.

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Tables

Table 1 Characteristics of three AYA breast cancer cohorts

	Japan (this study)		US cohort ^a		European cohort ^b		P-value
	(N = 46)		(N = 70)		(N = 70)		
	N	%	N	%	N	%	
Age ^c							0.05
Mean (±SD)	36.2	(±2.8)	34.9	(±3.7)	34.6	(±3.4)	
Stage^d							
0	2	4.3%	0	0%	0	0%	0.06
I	15	32.6%	11	15.7%	7	10.0%	
II	22	47.8%	36	51.4%	21	30.0%	
III	7	15.2%	22	31.4%	5	7.1%	
IV	0	0%	1	1.4%	0	0%	
Unknown	-		0	0%	37	52.9%	
Histology ^d							
DCIS	2	4.3%	0	0%	0	0%	0.12
IDC	42	91.3%	64	91.4%	62	88.6%	
ILC	1	2.2%	1	1.4%	0	0.0%	
Special type	1	2.2%	1	1.4%	4	5.7%	
Unknown	-		4	5.7%	4	5.7%	
Subtype ^d							
Luminal	35	76.1%	50	71.4%	26	37.1%	<0.01
Luminal HER2	3	6.5%	6	8.6%	8	11.4%	
HER2	2	4.3%	2	2.9%	5	7.1%	
TNBC	6	13.0%	12	17.1%	31	44.3%	
Tumor grade ^d							
Low (Grade I, II)	28	60.9%	25	35.7%	17	24.3%	<0.01
High (Grade III)	18	39.1%	29	41.4%	41	58.6%	
No data	-		16	22.9%	12	17.1%	
Genomic features							
HRD-high	13	28.3%	16	22.9%	40	57.1%	
Germline mutation	3	6.5%	9	12.9%	21	30.0%	
(BRCA1/2 mutation)							
Somatic mutation	0	0%	2 ^e	2.9%	1 ^e	1.4%	
(BRCA1/2 mutation)							
Methylation	5	10.9%	1	1.4%	1	1.4%	
(BRCA1/RAD51C)							
Somatic mutation	9	19.6%	21	30.0%	36	51.4%	
(TP53)							

SD, standard deviation; DCIS, ductal carcinoma in situ; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma.

^a Cancer Genome Atlas Network, *Nature*, 2012 [35].

^b Nik-Zainal et al., *Nature*, 2016 [36].

^c Kruskal-Wallis test.

^d Fisher's exact test.

^e One case overlapped with a case harboring a germline mutation.

Figures

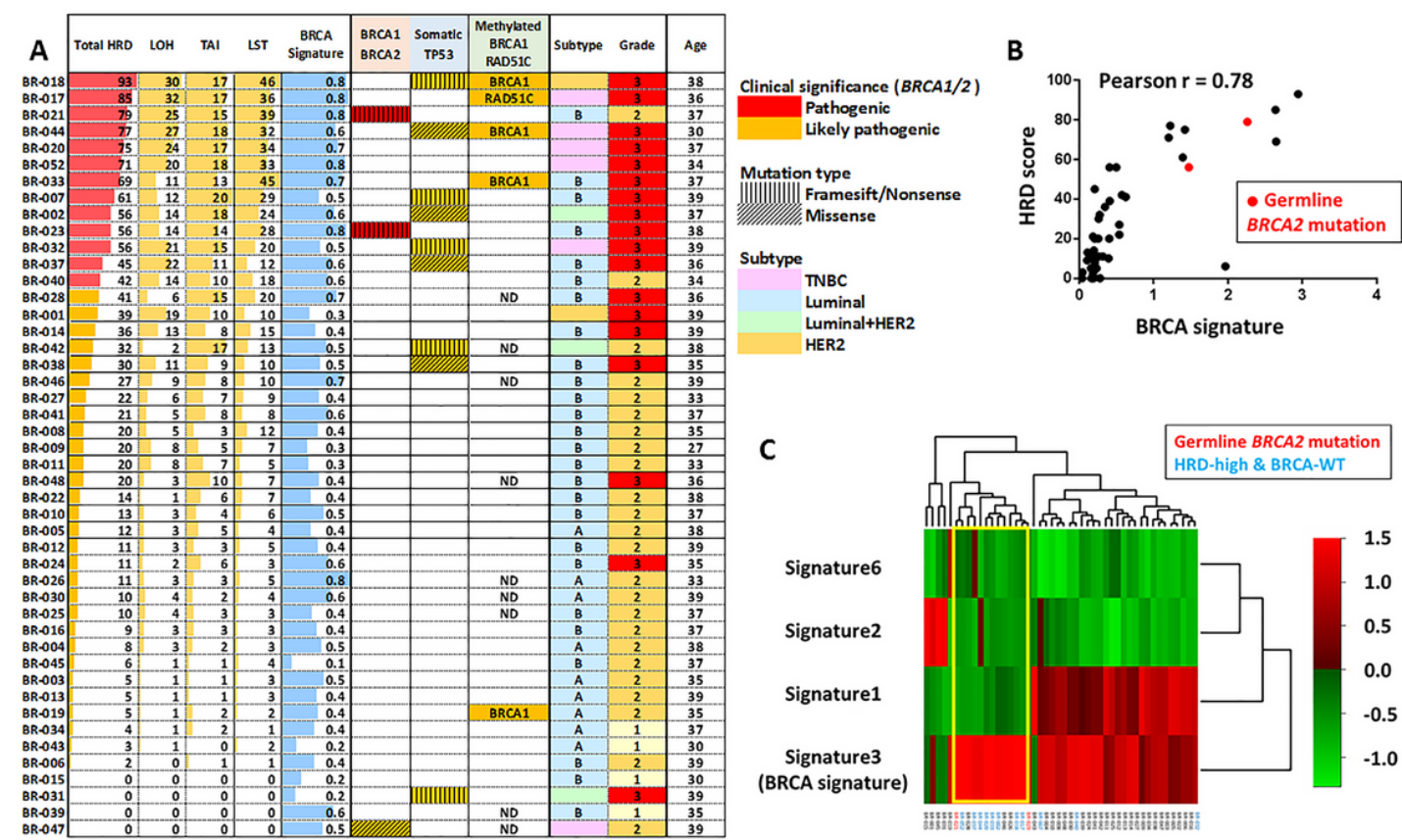


Figure 1

Homologous recombination deficiency (HRD)-high phenotype in Japanese AYA breast cancer cases. A HRD scores and other factors in each case. LOH, loss of heterozygosity; TAI, telomeric allelic imbalance; LST, large-scale state transitions. B Correlation between HRD score and the proportion of the BRCA1/2 deficiency-associated mutational signature after correction for the number of single-nucleotide variants per Mb. C Hierarchical clustering performed on mutational signatures in 46 breast cancers.

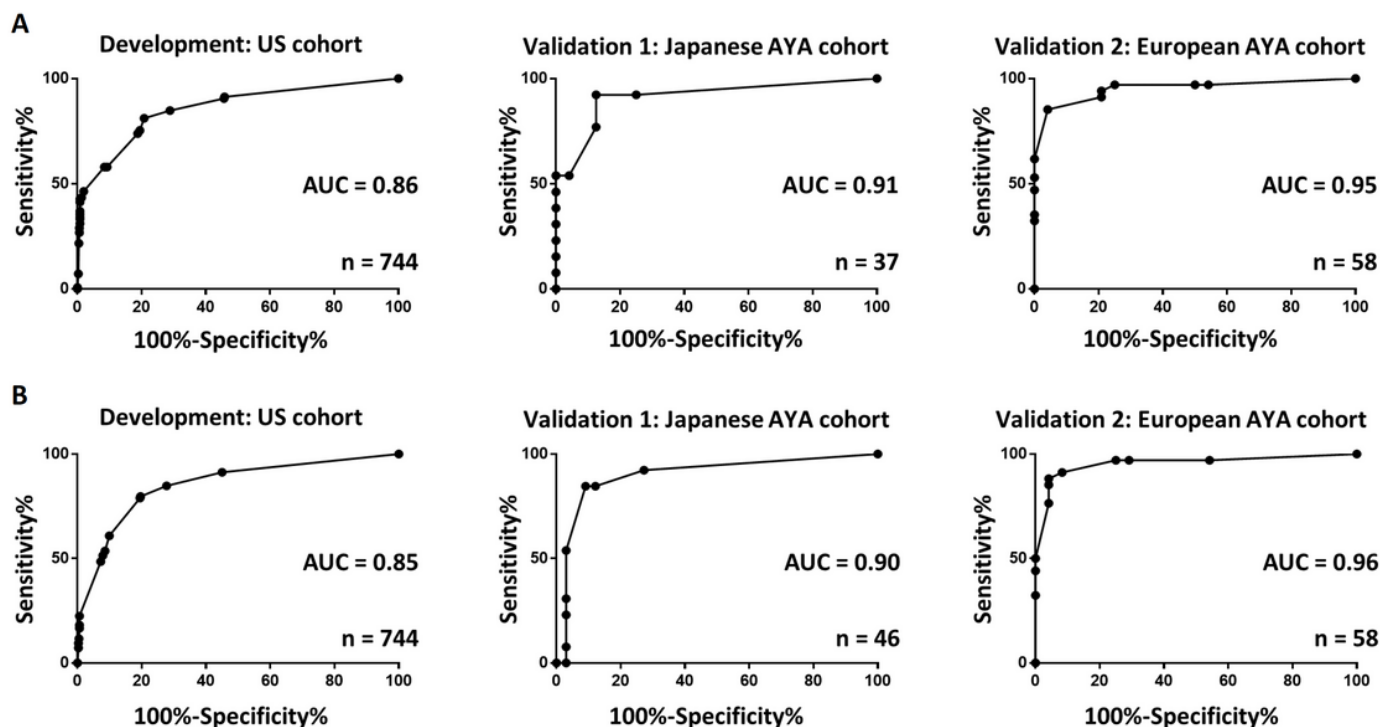


Figure 3

Prediction of HRD (development cohort: US cohort; validation cohort: Japanese AYA and European AYA cohorts). A Receiver operating characteristic (ROC) curves on the basis of BRCA1/2 mutation, somatic TP53 mutation, BRCA1/RAD51C hypermethylation, subtype (TNBC), and high grade (Grade III).

$y = \text{BRCA}[\text{if_positive_3.3}] + \text{methylation}[\text{if_positive_2.6}] + \text{TP53}[\text{if_positive_1.0}] + \text{Subtype}[\text{if_TNBC_0.87}] + \text{Grade}[\text{if_GradeIII_0.97}]$

B Receiver operating characteristic (ROC) curves generated on the basis of BRCA1/2 mutation, somatic TP53 mutation, subtype (TNBC), and high grade (Grade III).

$y = \text{BRCA}[\text{if_positive_3.1}] + \text{TP53}[\text{if_positive_1.0}] + \text{Subtype}[\text{if_TNBC_1.2}] + \text{Grade}[\text{if_GradeIII_0.99}]$

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