

Immune infiltration in breast cancers with germline alterations in DNA repair genes: effects of age and ancestry.

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Introduction: Approximately 10% of breast cancers (BCs) harbor germline alterations in homologous recombination deficiency/DNA damage response (gHRD/DDR) genes. *BRCA1/2* alterations have been associated with increased immune infiltration, but data on other HRD/DDR genes are limited. Here, we sought to define associations between gHRD/DDR genotype and tumor infiltrating lymphocytes (TILs) extent, and determine whether age and ancestry modify these associations. **Methods:** We analyzed 204 BCs harboring germline pathogenic/likely pathogenic variants (gP/LPV) across 20 HRD/DDR genes with paired tumor/normal targeted sequencing and genetic ancestry. TILs were assessed by the International TILs Working Group criteria. Allelic status was assessed using FACETS. Mutational signatures were inferred with SigMA. **Results:** The HRD/DDR genes most frequently affected by gP/LPV were *BRCA2* (n=50), *BRCA1* (n=37), *CHEK2* (n=32), *ATM* (n=20), and *FANCA* (n=15). Genetic ancestry was predominantly European (n=105) and Ashkenazi Jewish (ASJ; n=33), followed by African and Asian (n=17, each). ASJ patients were enriched for g*BRCA1/2* variants ($P=0.01$). g*BRCA1* patients were younger at diagnosis than other gHRD/DDR carriers ($P<0.01$), and their BCs showed higher TILs, followed by g*BRCA2*, g*ATM*, and g*CHEK2*. Most g*BRCA1* (76%) and g*BRCA2* (72%) BCs had a dominant HRD signature (SBS3), whereas clock/aging was predominant in g*ATM* (62%) and g*CHEK2* (60%) BCs. While TILs declined with age overall, gene-specific analyses showed that this association was restricted to g*ATM* ($\rho=-0.59$; $P=0.006$) and g*CHEK2* ($\rho=-0.54$; $P=0.001$) BCs, and was absent in g*BRCA1*, g*BRCA2* or g*FANCA* BCs. The age-related TIL decline was confined to non-SBS3 BCs ($\rho=-0.57$; $P=0.001$), even within g*BRCA1/2* BCs ($P=0.034$). TILs were higher in ASJ and African BCs vs European BCs (both, $P<0.01$), which remained significant after adjusting for

gHRD/DDR gene, age, and ER/HER2 status ($P=0.006$). **Conclusions:** Immune infiltration in gHRD/DDR BCs varies by genotype, ancestry and age. Age-related TIL decline was restricted to *ATM*- and *CHEK2*-associated BCs, and more broadly to tumors lacking an HRD signature, even within *gBRCA1/2* cases. ASJ ancestry was independently associated with higher TILs beyond other relevant covariates. These findings suggest that inherited DNA repair defects and their downstream mutational processes, together with age and ancestry, shape the tumor microenvironment and may inform therapeutic stratification.