Abstract

BACKGROUND Multigene panels are routinely used to search for predisposing mutations in families considered at high risk of breast cancer. The number of variants of unknown significance (VUS) thereby identified increases with the number of sequenced genes. We aimed to determine whether tumor sequencing can help reclassify germline VUS based on second events hitting the same gene. METHODS Whole-exome sequencing (WES) was performed on whole-blood DNA from 70 unrelated breast cancer patients referred for genetic testing and without *BRCA1*, *BRCA2*, *TP53* or *CHEK2* mutations. Rare variants considered as a VUS based on ACMG guidelines were retained in a list of 735 genes. WES was performed on matched tumor DNA to look for somatic second hits (copy number alteration or second mutation) in the same genes. RESULTS Sixty-eight patients (97%) carried at least one germline VUS (4.7 ± 2.0 variants per patient). Of the 329 VUS, 55 (17%) presented a somatic second hit in the paired tumor tissue. Two were a second somatic mutation, whereas 53 were copy number alterations. This resulted in relative tumor enrichment or depletion of the germline variant for 28 and 25 VUS, respectively. Estimation of tumor mutation burden and homologous recombination deficiency through mutational signatures and allelic imbalance analysis could highlight bi-allelic gene inactivation in relevant cases. Clinical measures were proposed for six patients carrying a germline variant on *PALB2*, *PMS2*, *PMS1*, *MUTYH* or *NTHL1*. Variant disclosure without clinical measures was proposed for five patients carrying a variant on *MRE11A*, *ATM*, *WRN* or *TP53* (functional testing ongoing for the latter). Germline and tumor DNA analysis of an affected relative of the patient carrying the germline *MRE11A* variant demonstrated the co-segregation of the variant as well as the presence of the same somatic second hit. Seven VUS on clinically actionable breast cancer predisposing genes (*PALB2*, *BRCA2*, *CDH1*, *ATM* and *BARD1*) were downgraded to probably benign variants as the germline variant was depleted in the tumor. One patient presented a significant tumor enrichment of a germline VUS in the C-terminal domain of the oncogene *ERBB2*. In vitro expression of this variant caused downstream signaling pathway activation. CONCLUSION Tumor sequencing is a powerful approach to refine VUS in cancer-predisposing genes in high-risk breast cancer patients. In this series, the strategy provided clinically relevant information for 11 out of 70 patients (16%).

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