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Accurate detection and quantification of epigenetic and genetic second hits in *BRCA1 and BRCA2*-associated hereditary breast and ovarian cancer reveals multiple co-acting second hits

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Background. This study characterizes the **second hit spectrum in *BRCA1* and *BRCA2*-associated breast and ovarian cancers** at both gene loci to investigate if second hit mechanisms are mutually exclusive or able to coincide within the same tumor.

Methods. Loss of heterozygosity, somatic point mutations and copy number alterations along with promoter methylation were studied in **56 breast and 15 ovarian** cancers from *BRCA1* and *BRCA2* germline mutation carriers. A mathematical methodology was introduced to quantify the tumor cell population carrying a second hit.

Results. **Copy neutral LOH** was the most prevalent LOH mechanism in this cohort (BC 69%, OC 67%). However, only 36% of BC and 47% of OC showed LOH in all cancerous cells. Somatic **intragenic deletions** and **methylated subclones** were also found in combination with (partial) loss of heterozygosity. Unequivocal deleterious somatic point mutations were not identified in this cohort.

Conclusion. **Different mechanisms** inactivating the wild type allele are **present within the same tumor** sample at various extents. Results indicate that *BRCA1/2*-linked breast and ovarian cancer cells are predominantly characterized by LOH, but harbor a complex combination of second hits at various frequencies.