

Genomes of *BRCA1* mutation and epimutation carriers appear to acquire specific epigenetic signature

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The contribution of genome wide methylation changes observed in peripheral blood of *BRCA1* epimutation and germline mutation carriers, as well as women before diagnosis of breast cancer is poorly understood. We used InfiniumMethylationEPIC BeadChip microarray technology (Illumina) and analyzed genome wide methylation patterns (over 850 000 CpG sites) in blood cells of 43 women with one of three *BRCA1* founder mutations (mean age=52.91), which were cancer free at the time of sampling and did not develop cancer within 12.9 years of follow up, 29 women with detectable in blood *BRCA1* epimutation (mean age=62.69) with 7.22 years follow up, and 19 women with neither mutation nor epimutation, which developed cancer on average 4.62 years from sampling. The controls in our experiment were 21 healthy women with neither mutation nor epimutation and cancer free follow up of 8.17 years. All samples were acquired from The International Hereditary Cancer Center (IHCC) Biobank.

In the analysis we first identified CpG sites with statistically significantly different methylation levels between women in each of the analyzed groups and controls (FDR corrected $p \leq 0.05$ methylation difference of more than 5%). We then, assessed the differences in the identified methylation signatures between groups of studied women. Each of the groups displayed different number of methylation changes, with *BRCA1* epimutation carriers harboring the highest number of 5163 differently methylated CpG sites, germline mutation carriers 2473 CpG sites, and cancer free women, who developed cancer after follow up, 12 CpG sites. This indicates that only epimutation and germline mutation carriers display increased number of genome wide methylation changes, but only a minor number of identified DMPs overlap between analyzed groups of women. Nevertheless, the biological processes identified with Gene Set Enrichment Analyses (GSEA) for epimutation and germline mutation carriers, based on genes annotated to identified methylation changes carriers were remarkably coherent. These terms included various processes occurring within mammary tissue, such as mammary gland bud morphogenesis, mammary gland specification, mammary gland bud formation, and fibroblast growth factor receptor signaling pathway, involved in mammary gland specification. Overall, our results suggest that both *BRCA1* epimutation, as well as germline mutation carriers acquire methylation changes that are not present in cancer free women and healthy women five years before cancer diagnosis, and those changes affect processes, which disruption has been shown to occur during breast cancer pathology.

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