The *APOBEC3B* deletion - determination of its consequences on mRNA level and analysis of its role in familial breast cancer predisposition

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Recently, a group of candidate breast cancer genes has expanded by the intensively studied *APOBEC3B* gene. The APOBEC3B protein is reported to be a possible mutagenic enzyme responsible for induction of specific localized somatic hypermutation patterns, so called "*kataegis*" (from the Greek for thunderstorm) in several cancer types (primarily breast cancer). It was presumed that investigation of CNVs may uncover a substantial part of still unidentified genetic loci related to the susceptibility to various complex diseases. One of common CNVs is deletion of *APOBEC3B* which occurs with a high allelic frequency in East Asian, Amerindian and Oceanic populations (range between 37% to 93%), and with a moderate allelic frequency in African and European populations (1% and 6%, respectively). Association of the *APOBEC3B* deletion with breast cancer predisposition has been revealed in Chinese population and among American women of European ancestry (OR=1.31/1.76 associated with one/two copies deletion and OR=1.21/2.29, respectively). In the framework of preliminary research, we confirmed that the germline *APOBEC3B* deletion occur with an allelic frequency of ~5% in Polish/European population and with somewhat higher frequency in a group of breast cancer patients. Therefore, the objective of our study was to define consequences of the *APOBEC3B* deletion on expression level and to confirm its role in breast cancer susceptibility in larger case-control group from Polish population.

Our APOBEC3B analysis encompassed two major research aims. In the framework of the first aim, we performed analysis of the relation between *APOBEC3B* genotype and alteration of the expression level of APOBEC3B and functionally related APOBEC3A. In this analysis, we exploited publically available expression data as well as our own qPCR/ddPCR expression results obtained analyzing the selected cell lines with different *APOBEC3B* genotypes. Additionally, we defined structure of fusion mRNA (1 nucleotide resolution) that is transcribed from the allele with the *APOBEC3B* deletion, which presence determines generation of hybrid APOBEC3A/APOBEC3B transcript (APOBEC3A with 3'UTR of APOBEC3B). Determination of the fusion transcript structure allowed to distinguish it from canonical APOBEC3A and APOBEC3B transcripts at the expression level.
In the framework of the second aim, an analysis of the *APOBEC3B* deletion association with breast cancer risk was conducted with the use of 2000 DNA samples from women with breast cancer and 2000 adjusted controls derived from International Hereditary Cancer Center (IHCC) in Szczecin. The association analysis was performed with the use of A3B PCR test. The obtained results were validated with the use of independent, specially designed assay based on the multiplex ligation-dependent probe amplification (MLPA) method. The performed analysis revealed lack of the association of the *APOBEC3B* deletion with breast cancer in Polish population.

Both PCR-based test and the designed MLPA assay will be freely available to research community and can be used to study the *APOBEC3B* deletion in other populations or other cancer types.

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