Elucidation of the Transmission of a Novel Mutation in BRCA1 (1125delCT) in a Family with Multiple Cases of Breast Cancer

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Abstract. In a family with multiple members affected by breast cancer we identified the novel mutation 1125delCT (exon 11) in BRCA1. Three out of three offsprings have the novel mutation while the mother affected by breast cancer does not carry the mutation. Linkage analysis revealed the transmission of the healthy haplotype from the mother to the three offsprings while the children inherited the mutated haplotype from the father. Our data document in an unquestionable way where the mutated haplotype was inherited from. In some families, although the transmission pathway seems obvious, the molecular analysis yields surprising results.

Germline mutations in the BRCA1 and BRCA2 genes are highly associated with predisposition to breast and ovarian cancer. It has been estimated that female mutation carriers have a lifetime risk of developing breast cancer of 60-80% (1, 2). A large number of mutations in the BRCA1 gene have been identified most of which are point mutations, small deletions and insertions leading to premature termination of protein synthesis and therefore inactivation of the protein (www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/). In a study by Miolo et al. (3) a family with the 9106C>T mutation was analysed and it was shown that common haplotypes for both microsatellite and intragenic polymorphisms segregated with the mutation. In the current study a family with several members affected by breast cancer was screened for BRCA1 mutations. The aim of the study was to elucidate the transmission of a novel mutation in the BRCA1 gene (1125delCT) in the family, using linkage analysis.

Case Report

In the family under study there are six subjects that developed breast cancer in three generations (Figure 1). Genetic material was obtained from four affected subjects only. The proband (IV:2) was diagnosed with breast cancer at the age of 54. The proband’s mother (III:1) developed breast cancer at the age of 82 while the proband’s father (III:4) died of natural causes at the age of 90.

The maternal aunt (III:2) also developed breast cancer and died at the age of 54 and the proband’s maternal grandmother was also diagnosed with breast cancer. Two of the proband’s second cousins from the maternal side (IV:4 and IV:6) were also diagnosed with breast cancer.

Materials and Methods

The methodology used has been previously described (1). Briefly, genomic DNA was purified from peripheral blood leukocytes using standard extraction protocols. The complete coding sequence of BRCA1 and BRCA2 including splice junctions were amplified by PCR followed by Automated Cycle Sequencing for both strands with the ABI Prism® 310 Genetic Analyzer. Germline rearrangements of BRCA1 and BRCA2 gene were also analysed by multiplex ligation dependent probe amplification (MLPA). Linkage analysis was performed using the following variable number tandem repeats (VNTRs): D17S2220, D17S2226, D17S2224, and D17S5.

Results and Discussion

DNA sequencing of the proband (IV:2) revealed the pathogenic mutation 1125delCT (exon 11). This 2bp deletion is a frame shift mutation leading to the creation of a premature stop codon at position 1131 of the BRCA1 protein.
The same mutation was identified in the proband’s brother (IV:3) and sister (IV:1) who have not been affected by cancer so far. In order to understand the transmission pathway we sequenced the proband’s mother (III:1) who surprisingly did not carry the pathogenic mutation. We hypothesize that the mutated haplotype has been transmitted by the father’s side (III:4) who is deceased and whose genetic material was not available. In order to substantiate this hypothesis linkage analysis was performed. The haplotype of the father (III:4) was determined based on the information extracted from genotyping the children and the mother. Nevertheless one VNTR was informative.

The data summarized in Figure 1, show that there is indeed a healthy haplotype (green) transmitted from the mother to the three offsprings. The data show that two of the children inherited the mutant haplotype (red) from the father (III:4). In the proband (IV:2), the VNTR D17S5 matched with the healthy haplotype from the father (III:4). This can be explained by an event of homologous recombination since the chromosomal distance of the locus of the VNTR D17S5 and the BRCA1 allows this to occur (yellow).

The proband’s mother (III:1) also carries a known polymorphism (E1038G), which has been identified in members of her side of the family. This polymorphism is located in the same exon as the mutation and is in the block of the blue haplotype. Two of the proband’s second cousins from the maternal side (IV:4 and IV:6) were also diagnosed with breast cancer and carry the polymorphism as well. However, it could be argued that the proband’s mother (III:1) carries another mutation in BRCA1 or BRCA2 or genomic rearrangements not transmitted to the children. In order to complete the transmission picture of this family, since decisions had to be made regarding the clinical management, we screened the proband’s mother for the whole BRCA1, for genomic rearrangements in both genes as well as point mutations in BRCA2.

The proband’s mother (III:1) was also screened for the CHEK2delC which has been associated with increased risk of developing breast cancer and has been detected in a high frequency in BRCA1/BRCA2 negative families (4). The screening did not reveal any pathogenic mutations. The immunohistochemical staining for estrogen (ER) and progesterone receptors (PgR) were negative in the proband’s
tumor (IV:2) while in the proband’s mother’s tumor (III:1) the estrogen receptors were negative and the progesterone receptors were positive. These data are in agreement with the published data regarding BRCA1 carriers (5). The mutation that is responsible for the breast cancer in the probands maternal family is unclear from these investigations, even though, because of the number of patients (4) it is almost definite that genetic predisposition is also at play in those cases.

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References


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