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.

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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

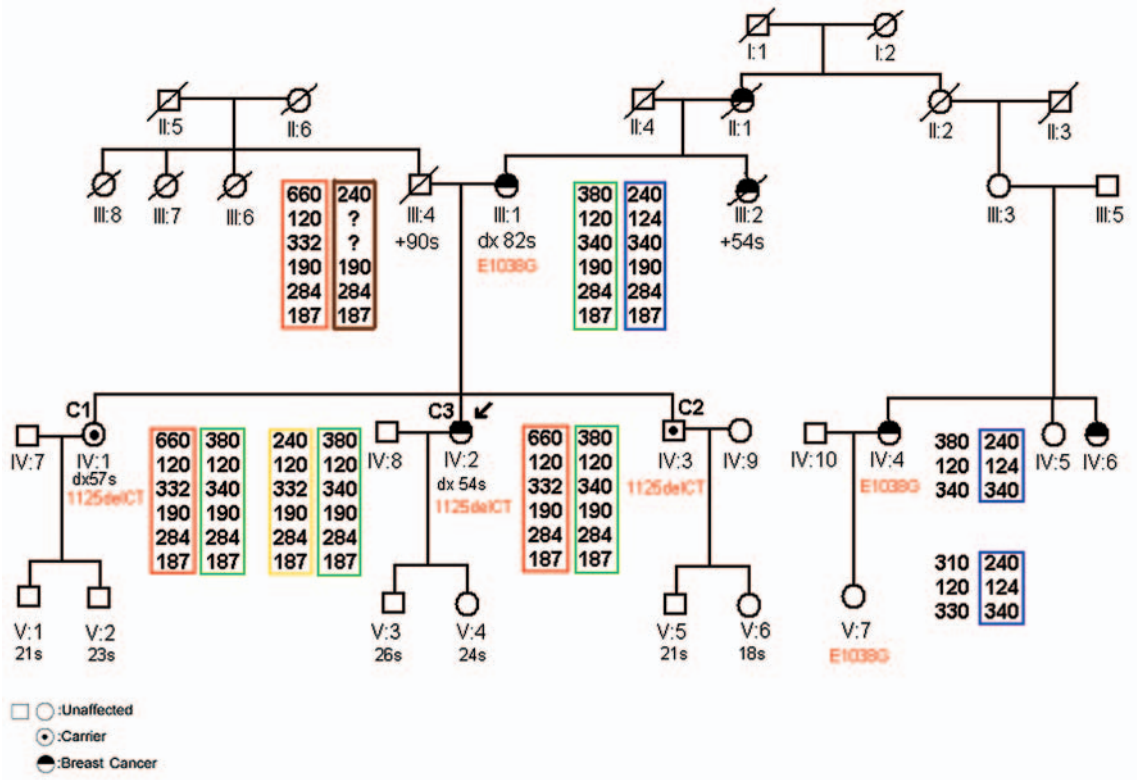

Fig A. Pedigree of family. Affected males and females are indicated by a semi-blackened square or circle. The proband is identified by an arrow. Numbers under the symbols refer to age at diagnosis if preceded by a "dx", age at death if preceded by a "+", and current age in years if the number has no preceding symbol. The numbers vertically below the individual indicate the marker alleles.

(www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/). 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.

Our data document in an unquestionable way where the mutated haplotype was inherited from.

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References

1 Belogianni I, Apessos A, Mihalatos M, Razi E, Labropoulos S, Petounis A, Gaki V, Keramopoulos A, Pandis N, Kyriacou K, Hadjisavvas A, Kosmidis P, Yannoukakos D and Nasioulas G: Characterization of a novel large deletion and single point mutations in the BRCA1 gene in a Greek cohort of families with suspected hereditary breast cancer. BMC Cancer *7;4(1)*: 61, 2004.

2 Nathansos KN, Wouster R and Webber BL: Breast cancer genetics: What we know and what we need. Nature Medicine *7*: 552-556, 2001.

3 Miolo G, Puppa LD, Santarosa M, Giacomi C, Veronesi A, Bidoli E, Tibiletti MG, Viel A and Dolcetti R: Phenotypic features and genetic characterization of male breast cancer families: identification of two recurrent BRCA2 mutations in north-east of Italy. BMC Cancer *6*: 156 DOI 10.1186/1471-2407-6-156, 2006.

4 Friedrichsen D, Malone K, Doody D, Daling J and Ostrander E: Frequency of CHEK2 mutations in a population based, case-control study of breast cancer in young women. Breast Cancer Res *6*: R629-R635, 2004.

5 Richards WE, Gallion HH, Schmittschmitt JP, Holladay DV and Smith SA: BRCA1-related and sporadic ovarian cancer in the same family: implications for genetic testing. Gynecology Oncology *75*: 468-472, 1999.

Internet Resources: Open Access On-Line Breast Cancer Mutation Data Base hosted by NHGRI: URL www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/ (Date last visited 04-05-2009).

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