

Introduction:

The identification of the germline mutation in a lynch syndrome family allows mutation carriers to be included in lifesaving cancer surveillance programs (1). Sporadic form in a cancer family makes the interpretation of the pedigree difficult. It is not uncommon to see lynch syndrome pedigrees with breast cancer (BC); however, there is no agreement as to whether breast cancer is part of the disease spectrum (2,3,4). Most genetic and immunohistochemical studies on familial and sporadic breast cancers did not evoke any strong relationship with the mismatch repair (MMR) gene defect. Muller et al (3) found that synchronous and metachronous breast cancers from Lynch syndrome families arise sporadically because they display a stable micro satellite pattern and normal MMR protein expression. Wong et al (5) in an extensive screening study of 59 multiple-case BC families; did not identify any genetic abnormality that might implicate MSH2 as a BC susceptibility gene. Khilko et al (6) in an immunohistochemical staining of 211 BC specimens did not show any loss of MMR protein expression. We describe in the present letter a case of breast cancer, in which the microsatellite instability (MSI) and the immunohistochemical (IHC) studies suggest strongly a relationship with the MSH2 gene defect.

Methods:

Seventeen carriers of MSH2 gene point mutation, at the splice donor site of intron 3, were included in a regular surveillance program. An eight-year follow-up result of this family was published in a previous report (7). The MSI analysis was performed after PCR amplification of tumor and normal surrounding borders DNA at eight loci containing mononucleotide and dinucleotide repeated sequences using fluorescent specific primers for each locus: BAT-25, BAT-26, D2S123, D17S250, RIITGF β , NR21, NR22, and NR24. The PCR products were electrophoresed for three hours in an Applied Biosystems (ABI) Prism sequencer. Data were collected using the GeneScan program for fragment analysis and alterations in the micro satellites were detected by comparing normal tissue and tumor tissue DNA strands in neighboring lanes. The IHC staining was also performed in paraffin embedded tumor and normal tissues for MSH2, MLH1, MSH6, PMS2, CK7 and CK20.

Results and discussion:

Although no new colorectal or gynecological tumor was screen-detected since the previous update on this pedigree (7). Only an unforeseen breast cancer in a 26 years old pregnant female (III 34) has occurred. No family history of breast cancer was noted in any branch of the family. No breast or ovarian cancer was known in any paternal family member of this patient. Screening for breast cancer was not part of our surveillance program, since there was no reason to suspect such a tumor as early as 26 years and during pregnancy. This patient who carries the causative germline mutation developed an infiltrating ductal carcinoma of the left breast with distant metastases at the first

presentation. The tumor was of high histological grade with negative estrogen receptors and positive c-erb B2 oncoprotein expression. This metastatic breast cancer did not respond to standard chemotherapy regimens including Trastuzumab, Taxanes and Doxorubicin.

MSI was present in 4 out of 8 loci in the tumor specimen. The IHC analysis revealed a negative expression for CK20, MSH2 and MSH6 and a positive expression for CK7 and MLH1 proteins, which confirm the mammary origin of the tumor and the role of the defective MSH2 gene in the pathogenesis (Tables 1 and 2).

The sensitivity of MSI analysis in tumors from known MMR gene mutation carriers is estimated to be 96% and IHC analysis has a sensitivity of 100% in detecting MMR deficiency in carriers of a pathogenic mutation and in predicting which gene of MLH1, MSH2 or MSH6 is involved (8).

The MMR gene mutation carriers are at high risk of developing Lynch Syndrome-related cancers. Some of them develop sporadic cancers due to polymorphisms in other genes. These sporadic tumors usually exhibit stable microsatellite patterns and normal MMR protein expressions. The absence of high-MSI pattern in a breast cancer specimen from MSH2 mutation carrier indicates that the development of such a tumor is unrelated to MMR gene impairment, despite the presence of the constitutional mutation. However, an MSI-positive phenocopy in an early onset breast cancer, that did not express the MSH2 protein in IHC is highly suggestive of its belonging to the tumor spectrum of the disease (9,10). Furthermore, the co-segregation of the germline mutation with the cancer phenotype is an additional relevant criterion of pathogenicity.

The efficacy of the surveillance program in reducing mortality from recognized Lynch Syndrome-related cancers in mutation carriers has been proven. All other cancers occurring in Lynch syndrome families should undergo MSI analysis and immunostaining for the MMR gene protein expression in order to better assess the phenotype-genotype relationship and change the surveillance guidelines accordingly.

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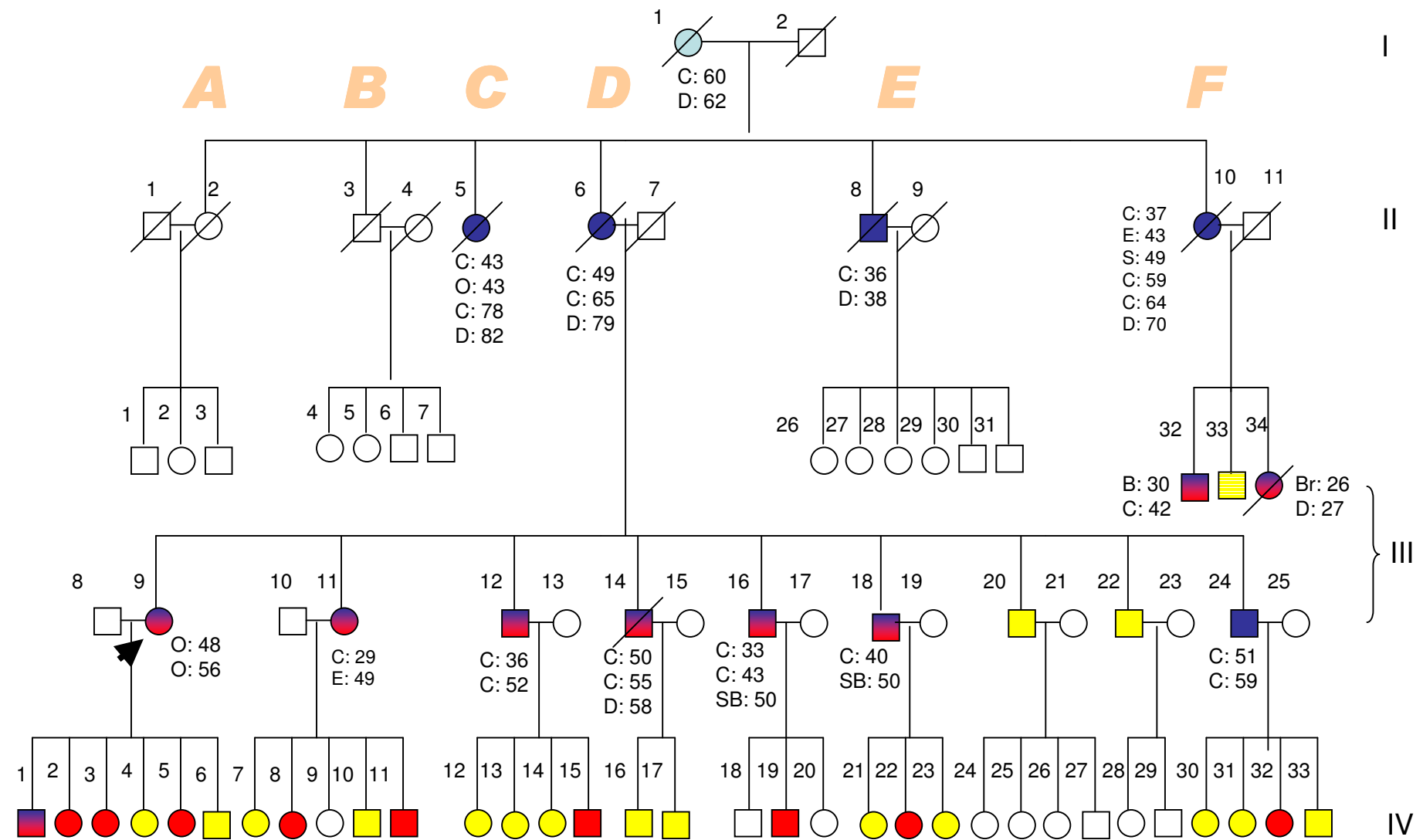
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Table 1: Microsatellite Instability (MSI) test results:

Locus	Stable	Unstable
BAT-25		×
BAT-26		×
D2S123/AFM093X43	×	
D17S250/MFd	×	
RIITGFβ	×	
NR21	×	
NR22		×
NR24		×

Table 2: Immunohistochemical (IHC) test results

	Tumor tissue	Normal tissue
hMLH1	Positive	Positive
hMSH2	Negative	Positive
hMSH6	Negative	Positive
PMS2	Positive	Positive
CK7	Positive	
CK20	Negative	



C: 21

- Familial history
- Histological confirmation
- MSH2 mutation carrier
- DNA test negative
- DNA test positive Histology confirmation

- C: Colon cancer
- O: Ovarian cancer
- SB: Small bowel cancer
- B: Brain tumor
- S: Skin cancer

- Br: Breast cancer
- D: Death

Figure 1