

Introduction:

Human Non-Polyposis Colorectal Cancer (HNPCC) is a genetic disorder due to a mismatch repair (MMR) gene defect, transmitted in an autosomal dominant manner. It predisposes to the development of a wide spectrum of tumors which usually display microsatellite instability (MSI) as a result of frequent errors during the replication of short nucleotide repeats. The identification of the germline mutation allows mutation carriers to be included in lifesaving cancer surveillance programs (1). It has been established that mutation carriers have higher risk of developing colorectal, endometrial, stomach, small bowel, biliary tract, urinary tract, brain, and ovarian cancers (2) but it is not uncommon to see also 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 do not evoke any strong relationship with the MMR gene defect. In an extensive screening study of 59 multiple-case BC families; Wong et al (5) did not identify any genetic abnormality that might implicate MSH2 as a BC susceptibility gene. In an immunohistochemical staining of 211 BC specimens, Khilko et al (6) did not show any loss of MMR protein expression. On the other hand, population-based studies of HNPCC kindreds did not show an increased relative risk of BC (2,3). Moreover when BC does occur, MSI is usually absent. The purpose of this communication is to describe a case of breast cancer suggesting strongly a relationship with the MSH2 gene defect.

Material and methods:

Starting from a 56 years old proband with relapsed ovarian carcinoma, a detailed family history was taken from 59 members, 35 of them (18♀ and 17 ♂) consented to participate in the DNA screening test which revealed 17 carriers of a point mutation of the intron 3 (G→A) at the splice donor site of MSH2 gene. These carriers were included in a regular follow-up program that included a colonoscopy starting from the age of 20 years and a gastroduodenoscopy starting from the age of 30 years every 1-2 years, a complete clinical examination, a chest X-ray, a pelvic and abdominal ultrasound, serum CEA, CA 19-9, and CA 125 levels every year. An eight-year follow-up results of this family was published in a previous report (7). The surveillance program was then carried on according to the initial plan with planning to perform MSI analysis and immunohistochemistry in all new tumor specimens. 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, RIITGFβ, NR21, NR22, and NR24. 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 immuno-staining was also performed in paraffin embedded tumor and normal tissues for MSH2, MLH1, MSH6, PMS2, CK7 and CK20.

Results and discussion:

Our HNPCC family was revealed by gynecological tumors. The pedigree analysis shows two ovarian, two endometrial and two small intestinal carcinomas in addition to the thirteen colon cancers. It also includes a skin cancer, a breast cancer and a brain tumor but no gastric, urinary or biliary-pancreatic cancer. As shown in figure 1, extracolonic cancer alone was noted in two patients (13%), extracolonic first manifestation, was observed in three patients (20%), Only colonic cancers in six patients (40%) and extracolonic in conjunction with colorectal cancer in six patients (40%).

Since the previous update on this pedigree (7), no new colorectal or gynecological tumor was screen-detected. Only an unforeseen breast cancer in a 26 years old pregnant female has occurred. No family history of breast cancer was noted in any branch of the family. 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 (III 34) 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 on immunostaining. This metastatic breast cancer did not respond to standard chemotherapy regimens including Trastuzumab, Taxanes and Doxorubicin. Micro satellite Instability (MSI) was present in 4 out of 8 loci in the tumor specimen. The immuno-histo-chemical (IHC) analysis revealed a negative expression for CK20 and MSH2 and a positive expression for CK7, MSH6 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).

Family history is best assessed via construction of a comprehensive pedigree that includes at least 3 generations. However, uncertainties may remain. Cancer in HNPCC kindred may be due to mutations in other genes. With the typical manifestations of HNPCC syndrome in branches D and F of our pedigree as shown in figure 1, branch F exhibits two striking events in the mutation carriers; an early onset brain astrocytoma at 30 years and an early-onset breast cancer at 26 years which may suggest that more than one gene may contribute to the genetic risk. The co-segregation of the germline mutation with the cancer phenotype is a relevant criterion of pathogenicity. Furthermore, the absence of MSH2 enzyme expression and the high MSI pattern of the breast tumor indicate that this tumor is a consequence of the MSH2 gene defect.

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) and should be monitored on an equal footing with other established tumor sites.

The efficacy of the surveillance program in reducing mortality from recognized HNPCC-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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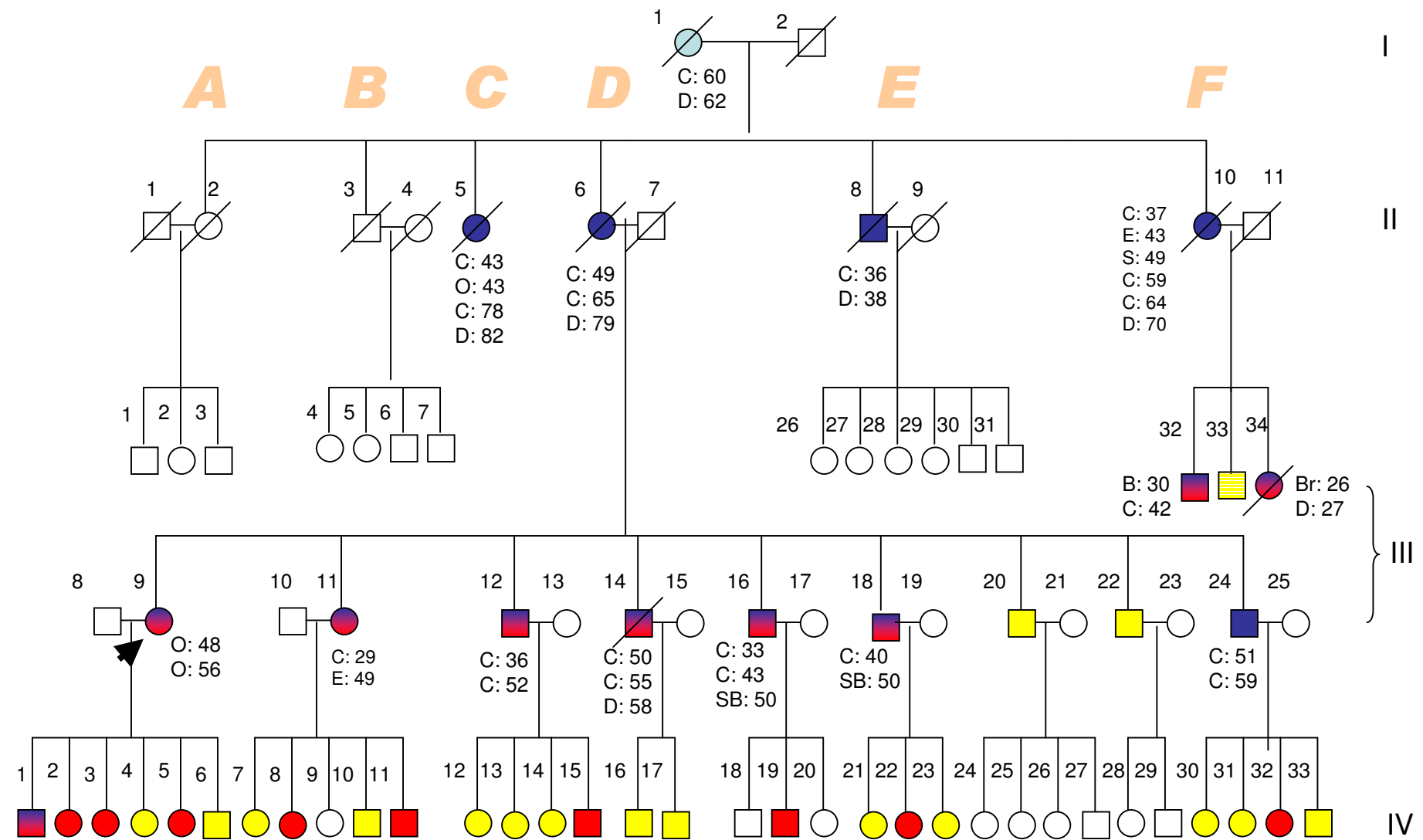
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Table 1: Microsatellite Instability (MSI) test results:

| 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 | Positive | 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
- Br: Breast cancer
- E: Endometrial cancer
- SB: Small bowel cancer
- B: Brain tumor
- D: Death

Figure 1