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Original research

Molecular screening in Sicilian families with hereditary non-poliposis colorectal cancer (H.N.P.C.C.) syndrome: Identification of a novel mutation in MSH2 gene

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ABSTRACT

HNPCC is an autosomal inherited cancer syndrome characterized by germinal and somatic mutations of DNA mismatch repair (MMR) genes. The inherited mutation in one allele together with an acquired defect in the other allele of an MMR gene leads to accelerate tumor progression.

In this study we analyzed a cohort of 11 subjects belonging to four Sicilian families with HNPCC suspected by molecular analysis of coding regions of hMSH2 (NC_000002) and hMLH1 (NC_000003) genes.

Molecular analysis has detected the presence of two mutations in gene MSH2 and one mutation in MHL1 gene. In addition, we found a novel mutation consisting in a G deletion at 914 codon of the exon 16 in the MSH2 gene. This deletion leads to a stop codon due to a frame-shift, resulting in a truncated protein.

We extended genetic analysis to the other family members and the same mutation was detected in three sisters and in one of the two healthy daughters.

This mutation is correlated with clinical findings revealed in genealogic tree and it represents a novel mutation responsible of HNPCC.

1. Introduction

Colorectal cancer (CRC) is one of the most common and preventable forms of cancers worldwide. Its incidence varies among different populations with the highest incidence reported from Western and industrialized countries.

Several genetic and environmental factors contribute to the development of cancer and it is estimated that up to 35% of all colorectal cancers are caused by a genetic predisposition [1–4].

Hereditary non-polyposis colorectal cancer (HNPCC) is the most common inherited syndrome predisposing to colorectal cancer (CRC), accounting for approximately 5–10% of all cases CRC [5,6].

Approximately 10%–15% of patients with colorectal cancer have a family history of colorectal cancer, and 5% of patients have early-onset (45 years) colorectal cancer. In the etiology of colorectal cancer in these cases, several genetic factors are likely to play a partial role, as do dietary and other environmental influences.

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Lynch syndrome patients are also at risk of developing extracolonic malignancies in a variety of organs such as uterus, small bowel, stomach, ovary, bladder, pancreas, urinary tract and the brain cancer. The syndrome is characterized by early onset epithelial cancers, proximal predominance of colorectal cancer, excess of synchronous and metachronous tumors.

HNPCC is an autosomal dominant condition caused by a defect in one of the mismatch repair (MMR) genes [5]. Germline mutations of genes involved in post-replicative DNA mismatch repair (MMR), in particular hMLH1, hMSH2, hPMS1, hPMS2, hMSH3, and hMSH6, are thought to be responsible for HNPCC as they cosegregate with the HNPCC phenotype [7,8].

However, the majority of mutation (90%) have been identified in hMLH1 and hMSH2 genes and only 10% in hMSH6 gene [9].

Most of them are small insertions/deletions leading to a frameshift, resulting truncated protein with loss of function. About 10–30% of mutations responsible for HNPCC are large genomic rearrangements scattered throughout the coding regions of the two genes.

Due to the genetic heterogeneity and clinical variability among HNPCC families, the identification of germline mutations becomes laborious.

An inherited defect in one of these genes, combined with an acquired defect in the wild-type allele, compromise MMR and thus promote genetic instability and tumorigenesis. Conversely, nontruncating mutations can either be neutral variations or lead to a highly increased cancer risk and LS.

Usually, clinical characteristics described in Amsterdam I, II criteria are used to discriminate an HNPCC from a sporadic colonrectal cancer. The use of these criteria does not offer an optimal screening strategy to predict the subsequent detection of a pathogenic germline mutation, because a largely variability (i.e. age onset, tumor spectrum) is usually present within family members and subjects carrying the same mutation. Part of the phenotypic variation between carriers of similar mutations may be explained by different life styles or the existence of modifying genes.

In addition, the Amsterdam criteria tend to exclude HNPCC families with only extracolonic tumors and patients with a limited family history.

Recently, molecular investigations of mutations that lead to loss of function in the mismatch repair genes have been used to correctly identify patients with HNPCC.

The identification of a germline mutation in the proband is crucial to extend the molecular analysis of family members and offered a surveillance program that will hopefully reduce cancer mortality.

Mutations in hMSH2 (NC_000002) and hMLH1 (NC_000003) genes were analyzed in patients from Caucasian families suspected HNPCC by clinical examination.

2. Materials and methods

All patients recruited are HNPCC suspected. We enrolled four subjects. The purpose of the study was explained, and informed consent was obtained from all participating patients. Personal and familial cancer history was collected, including site and type of cancer. Peripheral blood was obtained from the probands and family members.

Screening was completed in 4 families. Testing was initially carried out on DNA from an affected family member and upon detection of described mutations and a novel inactivating mutation, the rest of the family members were directly tested for these mutations.

Total genomic DNA was extracted from peripheral blood lymphocytes using standard method phenol-chloroform, and stored at −20 °C. All coding exons and the intron–exon boundaries of both MSH2 (16 exon) and MLH1 (19 exon) genes were amplified from genomic DNA by polymerase chain reaction (PCR) method, using specific primers for each region. Primers were selected using the PRIMER Program. Amplification products were run by 1% agarose gel electrophoresis stained with SYBR Safe DNA gel stain. Genomic DNA was used to amplify via polymerase chain reaction (PCR) the regions of both MLH1 and MSH2 genes. PCR products were purified with Microcon Centrifugal filter devices (Millipore) and sequenced by an automated ABI PRISM 310 sequencing apparatus (Applied Biosystem, Foster City, CA). Sequencing reactions were carried out by the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystem) according to the protocol suggested by the manufacturer.

3. Results

3.1. Family 1

The proband (III:1) is a 44 years-old woman with a right colon cancer pT3N0 G2. Her mother (II:1) was suffering from colon cancer and uterus cancer, but she died of colon cancer. Her father (II:2) died due to emphysema. The grandmother (I:2) had a breast cancer; one of the four sisters (III:5) had both uterus (45 y) and colorectal cancer (53 y), two of the others three sisters had only an endometrial cancer (III:6 53 y) (III:8 44 ys, with metastases at onset), while all the three brothers exhibit no malignancy. The proband's two daughters (IV:1; IV:2), 22 and 24 years old, were in good health, the older with the mutation, the younger no. Four years later, 48 years old, the proband developed an endometrial adenocarcinoma pT1b (IB FIGO)Nx (Fig. 1).

3.2. Family 2

The proband (II:1) is a 51 years-old woman with a right colon cancer pT3pN2 (4/24 lgh) G2. The father (I:1) died of a stroke. The mother (I:2) died of a malignant ovarian tumor. The proband has three sisters, one of which had died because of colon cancer at the age of 34 years (II:2), one had no mutations, the about the third we do not have news. One of the two brothers had no mutation, the other was not studied.

The four proband's sons were submitted to molecular analysis; two sons developed colon cancer (III:1 with a right colon cancer T3N0 G2 at the age of 35 y; III:2 Left colon cancer in situ pT1s G2 at the age of 38 y). The other two sons were in good health. Two years after the colorectal neoplasia, the proband had a low differentiated endometroid adenocarcinoma with a wide squamous metaplasia PT2N0G3, FIGO II. Six months after she had a low differentiated endometrioid lung metastases (Fig. 2).

3.3. Family 3

The proband (II:3) is a 47 years-old man dx colon cancer affected. His mother (I:1) stomach adenocarcinoma affected. His father (I:2) affected by HHC. The proband has two brothers and two sisters. The sisters were in good health. The brothers are affected by colon adenocarcinoma (II:1) and breast cancer (II:2), respectively. The proband has two daughters, 20 and 24 years old (Fig. 3).

3.4. Family 4

The proband is a 28 years-old woman sx colon cancer affected. The grandfather is dead because of the colon cancer. Her father and her mother are 58 and 60 years-old respectively. The proband has one brother of 19 years-old and he is in good health.