



## 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.

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## 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, non-truncating 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 colorectal 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  $-20^{\circ}\text{C}$ . 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 endometrioid 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.

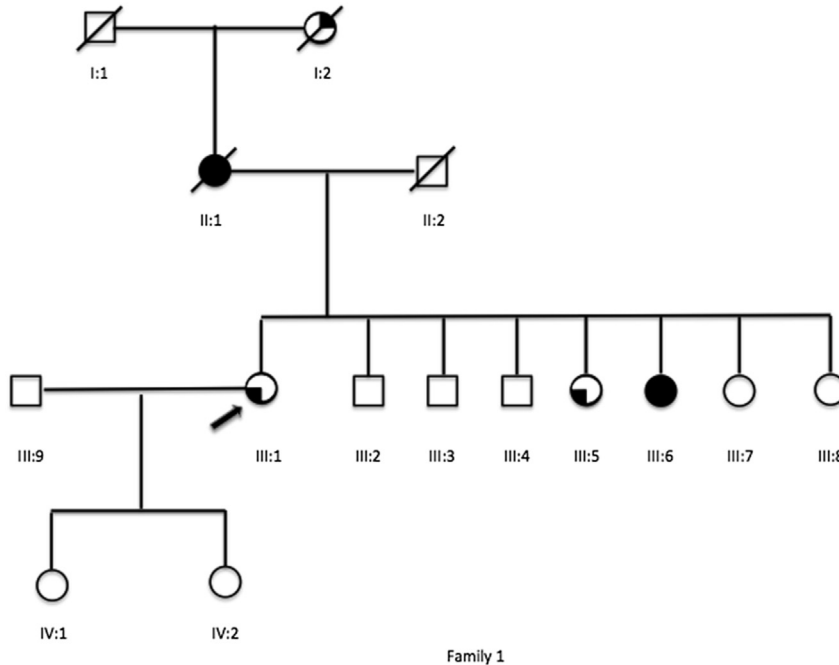


Fig. 1. Family 1 tree.

3.5. MSH2 gene

All coding exons and the intron–exon boundaries of MSH2 gene (16 exons) were analyzed in the proband of family 1 (III:1), by molecular analysis and direct sequencing.

We found a novel mutation consisting in a G deletion belonging to the 914 codon of the exon 16 in the MSH2 gene. This deletion leads to a premature stop codon (position 916), causing a frame-shift and the formation of a truncated protein (Fig. 4).

The combination of the mutation with loss of the wild-type allele compromises MMR genes, thus it promotes genetic instability and tumor formation. The sequencing analysis of the other exons has not revealed any changes.

We extended genetic analysis to the other family members and we found the same mutation in three out of the four proband's sisters (III:5 colon and uterus cancer affected; III:6 uterus cancer affected; III:8 metastatic uterus cancer) and in one proband's daughter (IV:1 healthy). The rest of the family members were unavailable for analysis.

Molecular analysis and direct sequencing of MSH2 gene in the proband of family 2 (II:1) has detected the presence of the mutation previously described by Miyaki et al., 1995 [10]. This mutation consists in an A insertion, in the region surrounding the 227 and the 228 codons of exon 4, which results in frame-shift and creates a stop codon. We extended the analysis to four members of proband's family because the others were unavailable. The mutation was also identified in two proband's sons (III:1 colon ca; III:2 colon ca). In addition, the test didn't show any mutation in proband's sister (II:3) and brother (II:5).

In the proband of family 3 (II:3) we didn't find any mutation in the MSH2 gene. His relatives didn't want to take part in the genetic test.

The genetic analysis of proband's family 4 has shown a G>A substitution, in heterozygous condition, in the codon 322 (GGC → GAC), that led to substitution of a glycine with an asparagine (G322D) described by Olilla S. et al 2008 [11].

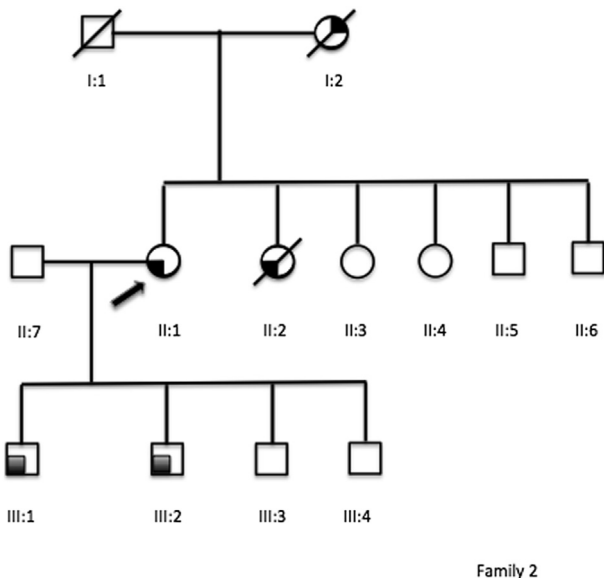


Fig. 2. Family 2 tree.

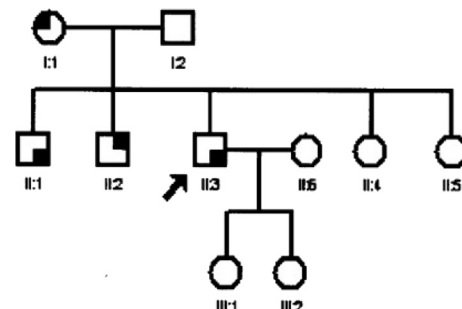


Fig. 3. Family 3 tree.

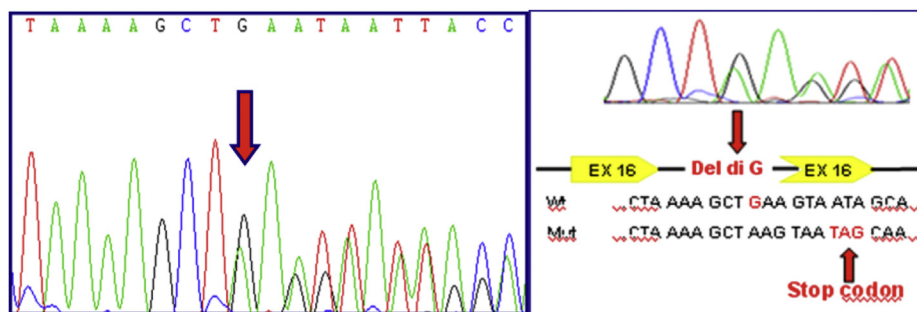


Fig. 4. Deletion G at the 914 codon of the exon 16 in the MSH2 gene. Deletion G leads to a stop codon (position 916) resulting in a truncated protein.

### 3.6. MLH1 gene

Molecular analysis by direct sequencing of the 19 coding exons and the intron–exon boundaries of the MLH1 gene didn't show any mutation in the proband (III:1) of the family 1 and in the proband (II:1) of the family 2. These results did not lead to extend the genetic test to the others family members.

In the proband (II:3) of the family 3 the molecular analysis has shown an A>G substitution (ATC→GTC), in heterozygous condition, that led to a substitution of an isoleucine with a valine at codon 219 (I219V), reported by Tomlinson et al., 1997 [12] as a polymorphism with a frequency of 13%.

The same polymorphism in homozygous condition was found in the proband of family 4.

## 4. Discussion and conclusion

We carried out molecular analysis of the hMSH2 and hMLH1 genes implicated in the HNPCC syndrome in a cohort of four Caucasian families with suspected HNPCC.

The most of mutations identified in the MSH2 and MLH1 genes are small insertions/deletions leading to frame-shifts and truncated protein products [13].

In MLH1 and MSH2 genes we identified four mutations, one of which is in the MLH1 gene and three are in the MSH2 gene. In the MLH1 gene are present mainly missense mutations, while in the MSH2 gene are the most frequent alterations that lead to truncated proteins [14].

About the variant of MLH1 gene present in the proband of families 3 and 4 it is not possible to determine whether it is a novel mutation or a mutation inherited from a parent, because the family did not take part in the screening.

In this study we identified a novel mutation that consists of a G deletion at 914 codon in the MSH2 gene, this deletion lead to a stop codon resulting in a truncated protein. To define the pathogenicity of the novel mutation, we followed its segregation with the disease phenotype in the majority of relatives confirming the pathogenic nature of the mutation.

Mutation G322D found in the MSH2 gene leads to variable and contradictory interpretations of its pathogenicity, in fact it has been described both as pathogenic and non-pathogenic in literature and databases. The majority of the published data discusses MSH2 G322D as a neutral polymorphism, it has also been hypothesized to be a low penetrance allele [11].

In contrast with these interpretations, a recently published population-based study raises a possibility of G322D being instead of pathogenic a protective allele further complicating its classification [15].

Many mutations have been identified in MMR genes, this genotypic diversity is reflected in the phenotypic characteristic

variety of the syndrome. The phenotypic diversity may indicate that other factors can influence the penetrance of the molecular defect.

Clinicians make use of the Amsterdam criteria that were initially proposed to select kindreds suitable for the localization and the identification of genes involved in the syndrome [16].

However, several reports have shown that the clinical of HNPCC based on the Amsterdam criteria is more stringent than a definition based on the involvement of MMR genes. The Amsterdam criteria include only the age of onset and the number of CRCs within a family. Considering the cost of screening methodologies, it appears essential to identify pathological and clinical factors that predict the outcome of genetic analysis. Based upon our results and in accordance with a report by Wijnen et al., 1998 [17], the presence of multiple tumors in a single member of a family and the presence of endometrial cancer in a family should be included among the guidelines for referring patients for genetic testing.

In conclusion, genetic analysis allows to identify carriers of mutation in pre-symptomatic phase and this is of crucial importance for subjects belonging to families at risk of colon cancer. Asymptomatic subjects in which the molecular diagnosis indicates the presence of mutations are directed to a closer clinical monitoring and they are inserted into a screening program in order to detect early the presence of polyps before they transform into cancer.

Increased knowledge about inherited susceptibility for cancer and the identification of genes associated with cancer risk has increased the need for individuals with training in genetics to work closely with oncology professionals in the familial cancer arena [18–24]. Genetic counselors can provide a variety of useful services: They may function as clinical coordinators of a family cancer risk counseling (FCRC) program and serve as study coordinators on research teams. In the oncology practice setting, genetic counselors who are trained to do cancer risk counseling can help ascertain and evaluate familial clusters of cancers. In the context of FCRC, the genetic counselor can educate family members about risk factors for cancer and the significance of a positive family history, assess psychosocial functioning and provide psychosocial support and referrals. Genetic susceptibility testing should be offered only with appropriate genetic counseling.

### Ethical approval

This is a retrospective study based only on the analyses of recorded data and then no Ethical Approval was necessary.

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All Authors have no source of funding.



## Author contribution

**Andrea Cavallaro:** Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

**Vito Emanuele Catania:** Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

**Angela Russo:** Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

**Bartolomea Ficili:** Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

**Fabrizio Romano:** Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

**Andrea Valentino Failla:** Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

**Alessandro Cappellani:** Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

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**Vincenzo Trichilo:** Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

**Massimo Libra:** Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

**Salvatore Travali:** Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

## Conflicts of interest

All Authors have no conflict of interests.

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