

Short reports

A proven de novo germline mutation in HNPCC

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Abstract

Hereditary non-polyposis colon cancer (HNPCC) is a heterogeneous group of tumour predisposition syndromes caused by germline mutations in at least four different mismatch repair genes. HNPCC patients are prone to the development of carcinomas of the intestinal tract and other specific sites. Identification of presumptive HNPCC patients is primarily based on a positive family history of colorectal cancer in at least two generations. In the course of mutation screening of the *MLH1* and *MSH2* genes in patients manifesting a carcinoma of the HNPCC tumour spectrum before the age of 45 years, we identified a germline *MSH2* 344delA frameshift mutation in a male proband. This index patient, at the age of 25 years, initially developed a large rectal adenoma that was removed by polypectomy. Ten years later he was operated on for an invasive right sided colon carcinoma in the caecum (International Union Against Cancer (UICC) stage III). The mother and father, aged 61 and 66 years, respectively, were healthy and had no family history of colorectal cancer. Subsequent molecular analyses excluded the germinal *MSH2* 344delA alteration identified in their son and at the same time paternity was confirmed with a set of informative polymorphic markers. Thus, the genetic alteration identified in our patient definitely represented a de novo germline mutation in one of the major HNPCC genes. This case report of a patient with colorectal cancer at a relatively young age with no family history is intended to encourage mutation screening of the *MSH2* and *MLH1* genes in similar cases to find out whether this group of patients contains an increased proportion of de novo mutations in mismatch repair genes.

(J Med Genet 1999;36:919-921)

Keywords: HNPCC; *MSH2*; mutation

Presymptomatic identification of germinal mutations in tumour predisposition syndromes represents an enormous challenge to human molecular genetics. In the case of hereditary non-polyposis colorectal cancer (HNPCC),

identification of disease causing mutations is hampered by several complications. First, mutations scattered all over at least four different mismatch repair genes have been shown to cause HNPCC.¹ Moreover, missense mutations in these mismatch repair genes may represent rare polymorphisms or disease causing mutations.^{2,3} Second, it can be difficult to diagnose HNPCC solely on the basis of intestinal manifestations, for instance, if attenuated familial adenomatous polyposis (AFAP) with a reduced number of colorectal polyps has to be considered as an alternative diagnosis.⁴ Third, application of the strict "Amsterdam criteria" for the selection of probands for mutation screening in mismatch repair genes definitely excludes cases of HNPCC, as discussed previously⁵ and confirmed in the present case.

We analysed a panel of 20 probands, who had a carcinoma of the HNPCC tumour spectrum before the age of 45 years. However, these patients were not preselected for microsatellite instability (MSI) or loss of *MSH2* and *MLH1* protein expression in their tumours.⁶ Nevertheless, we expected that early onset of malignancy, at least in a proportion of these presumptive HNPCC patients, could be the result of germline mutations in one of the known mismatch repair genes.

A combined approach of reverse transcription-polymerase chain reaction (RT-PCR) based protein truncation test and single strand conformation polymorphism (SSCP) analysis as screening procedures was used for the detection of genetic alterations in both the *MSH2* and *MLH1* genes. In four out of 20 patients selected only by the criterion of early manifestation of carcinoma, we were able to define germline mutations located in one of the two major mismatch repair genes.

It turned out that one of the four proven HNPCC patients was a male index patient, who did not fulfil the hereditary aspects of the Amsterdam criteria, that is, three affected family members in two generations. However, he underwent polypectomy at the age of 25 years, when a large rectal polyp (at 10 cm ab ano) had to be resected. Ten years later, in 1997, significant anaemia (haemoglobin 7.2 g/dl) was observed. Subsequently, examination by CT scan showed a tumour with a diameter of 3 cm located in the caecum. A right sided hemicolectomy was performed and histopathological evaluation of the resected tumour defined an

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Revised version received 17 May 1999
Accepted for publication 8 July 1999

ulcerated, moderately differentiated adenocarcinoma on the grounds of a tubulovillous adenoma. Infiltration of the submucosa, invasion of multiple lymphatic vessels, and metastases in stem and diverging lymph nodes were observed (UICC stage III). A recent microsatellite analysis of tumour DNA using the markers BAT26, D2S123, D5S346, and D17S250⁶ showed microsatellite instability (MSI) at all four loci tested (not shown), which is in accord with mismatch repair deficiency in the carcinoma.

Owing to the fact that both metachronous tumours appeared at a very early age, genetic susceptibility was considered. Pedigree analysis, however, did not support a hereditary colorectal cancer predisposition syndrome. The mother and father, aged 61 and 66 years respectively, had no personal or family history of colorectal cancer. Nevertheless, we included leucocytic RNA and DNA of this patient in our *MSH2* and *MLH1* mutation screening. Based on the findings of a truncated *MSH2* specific in vitro translation product of some 20 kDa and an aberrant single strand conformation polymorphism in exon 2, preliminary evidence was obtained for a germinal mutation in the *MSH2* mismatch repair gene (data not shown). Genomic DNA obtained from leucocytes of the index patient was amplified with *MSH2* exon 2 specific primers⁷ and the purified PCR product pool was sequenced on both strands as

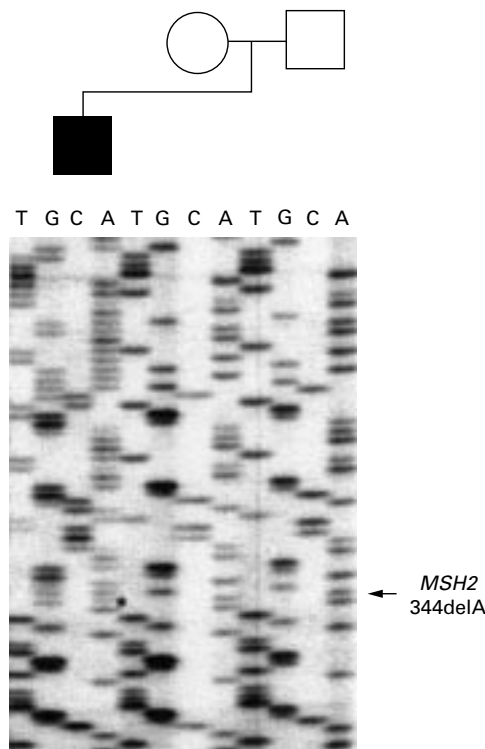


Figure 1 DNA sequence analysis of *MSH2* exon 2 in colorectal cancer patient and in his healthy parents. Genomic DNA was isolated from leucocytes and PCR amplified with the help of *MSH2* exon 2 flanking primers extended by M13 derived sequences at the 5' ends. Dye modified complementary M13 primers were used in conjunction with a commercial dideoxy sequencing kit (Biozym, Hameln, Germany) to obtain sequence reaction products which were analysed on an automated LiCor sequencing apparatus (MWG, Ebersberg, Germany).⁸ The identified mutation is marked by an asterisk.

Table 1 Paternity testing*

Marker	Father	Son (proband)	Mother
FGA	1 - 3	3 - 4	2 - 4
D1S80	2 - 3	2 - 1	1 - 3
D3S1359	3 - 3	3 - 2	1 - 2
D4S413	2 - 3	2 - 4	1 - 4
D6S311	1 - 2	2 - 1	1 - 1
D7S507	1 - 2	2 - 3	3 - 3
D9S168	1 - 4	1 - 2	2 - 3
D9S288	3 - 4	3 - 1	1 - 2
D17S855	2 - 2	2 - 1	1 - 1
D22S264	2 - 3	2 - 3	1 - 3
D22S941	2 - 3	3 - 1	1 - 2

*Alleles identified as length variants have been numbered according to their increasing sizes.

detailed previously.⁸ Fig 1 shows the identification of a single base pair deletion at nucleotide position 344 (Acc No U04045), designated *MSH2* nt344delA. As a consequence of this deletion, a frameshift is generated leading to an elongation of the *MSH2* polypeptide chain by 58 aberrant amino acids beyond codon 114. Thus, a premature stop codon finally terminates translation after 172 amino acids. Mutation screening of the entire *MLH1* gene by protein truncation test and SSCP excluded a genetic alteration of this mismatch repair gene. Since incomplete penetrance of HNPCC alleles could result in lack of clinical symptoms in gene carriers of the preceding generation, we looked for the same mutation in leucocyte DNA of the patient's parents. Neither the mother nor the father carried the genetic alteration identified in their son (fig 1). To confirm paternity a panel of 19 highly polymorphic markers was analysed. All of them, including the 11 informative markers listed in table 1, were in concordance with paternity. Thus, the identified germline mutation of the index patient definitely represented a de novo mutation in the *MSH2* gene; this information was given to the patient in the context of a genetic counselling session.

The conclusion can be drawn from this case report that patients with de novo mutations in HNPCC genes do indeed exist. However, they escape molecular detection if a positive family history of colorectal cancer is required as a selection criterion for mutation screening in mismatch repair genes. Therefore, it is a matter of definition of appropriate clinical criteria to identify such potential new mutations in the first generation. We feel that in the complete absence of a family history of colorectal cancer, a major indicator for a germline mutation in one of the HNPCC genes is the early age of onset of a carcinoma belonging to the HNPCC tumour spectrum. Whether a manifestation of metachronous tumours, as happened in our case, does increase the probability of a de novo mutation in *MSH2* or *MLH1* has to be determined on the basis of additional cases. A systematic analysis of *hMSH2* and *hMLH1* in 50 young colon cancer patients (<30 years) showed a pathogenic mutation in 28% of the probands analysed.⁹ Of course one could raise the question of whether new mutations in mismatch repair genes would also be identified in those patients developing a first colorectal carcinoma at a later age. It would require much work to screen molecularly those colorectal

cancer cases that appear to be sporadic rather than based on a germline mutation. On the other hand, analysis of the microsatellite instability status in malignant tumours of potential HNPCC patients should prove a useful pre-screening parameter.

Since de novo mutations in dominantly inherited disorders are predominantly paternal, we tried to find out the parental derivation of the mutated allele. To this end we sequenced cDNA clones of the patient's normal and mutated *MSH2* transcript. The sequence information obtained was then compared with data derived from the analysis of genomic DNA of the patient. We did not detect heterozygosity of any of the known exonic polymorphisms (<http://www.nfdht.nl/database/msh2-poly.htm>). Only one polymorphic site located in intron 10 was informative; however, the physical distance between it and the genomic alteration in exon 2 was too large to connect both sequence alterations by genomic PCR. Therefore, we were unable to distinguish between maternal and paternal origin of the spontaneously mutated chromosome.

Finally, it should be mentioned that we would have performed a colectomy instead of a hemicolectomy if the *MSH2* germline mutation of our HNPCC patient had been identified before the operation. Most authors recommend subtotal colectomy in patients with colorectal cancer and genetically proven HNPCC.^{10 11} Others, however, recommend resection of the tumour and colonoscopy every one to two years for life. However, to our knowledge there are no randomised studies and therefore no data showing a better outcome for one of these two groups.

The case presented should encourage mutation screening preceded by MSI analysis in

patients with early onset of HNPCC related carcinomas (before the age of 45 years) even in the absence of a family history, to find out whether this group harbours an increased proportion of de novo germline mutations in the *MSH2* or *MLH1* genes.

This paper is dedicated to Professor Dr R A Pfeiffer on the occasion of his retirement with thanks for his continuous support of molecular diagnostic developments in the field of inherited tumour predisposition syndromes. The expert technical assistance of Mrs B Schenker and A Vogler is gratefully acknowledged.

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