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Short report

Atypical HNPCC owing to MSH6 germline mutations: analysis of a large Dutch pedigree


Abstract

Hereditary non-polyposis colorectal cancer (HNPCC) is the most common genetic susceptibility syndrome for colorectal cancer. HNPCC is most frequently caused by germline mutations in the DNA mismatch repair (MMR) genes MSH2 and MLH1. Recently, mutations in another MMR gene, MSH6 (also known as GTBP), have also been shown to result in HNPCC. Preliminary data indicate that the phenotype related to MSH6 mutations may differ from the classical HNPCC caused by defects in MSH2 and MLH1. Here, we describe an extended Dutch HNPCC family not fulfilling the Amsterdam criteria II and resulting from a MSH6 mutation. Overall, the penetrance of colorectal cancer appears to be significantly decreased (p<0.001) among the MSH6 mutation carriers in this family when compared with MSH2 and MLH1 carriers (32% by the age of 80 v >80%). Endometrial cancer is a frequent manifestation among female carriers (six out of 13 malignant tumours). Transitional cell carcinoma of the urinary tract is also relatively common in both male and female carriers (10% of the carriers).

Moreover, the mean age of onset of both colorectal cancer (MSH6 v MSH2/MLH1 = 55 years v 44/41 years) and endometrial carcinomas (MSH6 v MSH2/MLH1 = 55 years v 49/48 years) is delayed. As previously reported, we confirm that the pattern of microsatellite instability, in combination with immunohistochemical analysis, can predict the presence of a MSH6 germline defect.

The detailed characterisation of the clinical phenotype of this kindred contributes to the establishment of genotype-phenotype correlations in HNPCC owing to mutations in specific mismatch repair genes.

Keywords: hereditary non-polyposis colorectal cancer; MSH6
resulting from a frameshift mutation in the MSH6 gene.

Patients
The proband, a 50 year old female, was referred to the Department of Clinical Genetics, Rotterdam by her gynaecologist in 1993. At that time, three of her six sisters were diagnosed with endometrial cancer at 50, 57, and 60 years of age. A fourth sister had developed ovarian cancer (endometroid adenocarcinoma) at the age of 50. The proband's father died at the age of 85 without any symptoms of HNPCC. Her mother underwent a hysterectomy for leiomyomata at 55 and was in good health at 92 years of age.

The differential diagnosis in this family included HNPCC. Mutation analysis of the MLH1 and MSH2 genes by PCR and DGGE in one of the affected sisters of the proband did not show any pathogenic mutation. In the meantime, the proband developed endometrial cancer at the age of 53, the fourth case of endometrial cancer in this sibship.

As soon as MSH6 mutation analysis became available, mutation screening was performed and a frameshift mutation (del T codon 594) in exon 4 was identified. The proband, her affected sisters, and mother were found to carry the same mutation.

At the request of the proband, family members were informed about the findings and were invited to contact the Department of Clinical Genetics for additional information and/or to be tested for the presence of the mutation.

We were also able to link the family to another kindred under investigation at the Department of Clinical Genetics at the Leiden University Medical Centre.

Mutation analysis
In total, DNA testing for the MSH6 mutation was performed in 80 out of 132 living relatives with at least a 25% risk of being carrier of the MSH6 mutation (fig 1), 27 males (out of 63, 43%) and 53 females (out of 69, 77%). Out of these 132 relatives, 11 were previously diagnosed with an HNPCC related tumour and all of them were tested. Two tested unaffected relatives were obligate carriers. Out of 75 carriers at 50% risk and 44 at 25% risk, 46 (61%) and 21 (48%) were tested, respectively (tables 1 and 2).

Ten of the 11 affected subjects were carriers of the familial MSH6 mutation. The patient (IV.6) negative for the mutation was a 55 year old male, diagnosed with numerous tubulovillous adenomas at the age of 53. Since his clinical presentation was suggestive of familial adenomatous polyposis (FAP), though with a delayed age of onset, mutation analysis of the APC gene was performed. This did not show any alteration.

The MSH6 mutation was confirmed in both unaffected obligate carriers.

Out of the 46 tested healthy subjects with a 50% risk for the mutation, 17 (37%) were found to carry the MSH6 mutation; none of those with a 25% risk tested positive (table 2).

All 17 unaffected mutation carriers above the age of 25 years (five males and 12 females) were offered the generally accepted surveillance for HNPCC, namely colonoscopy every one to two years, yearly gynaecological examination with vaginal ultrasound and CA125 blood testing.

The family included five dead obligate mutation carriers, three females and two males. Three of them had been diagnosed with an HNPCC related tumour (table 1). One male reached the age of 83 without having developed any HNPCC related tumour.

We were not able to determine the MSH6 status of five cancer patients in this family: one colorectal cancer (onset >70) in a female patient (III.13) whose 13 descendants tested negative for the mutation; one male with an astrocytoma (III.19) at the age of 75, three of whose four children did not carry the mutation; two breast cancer patients (IV.14, IV.20, onset >50); and one patient with cancer of unknown origin around the age of 40 (III.6).

In total, 34 mutation carriers (29 alive and five dead) were identified in this pedigree, 17 of whom developed a (pre)malignant tumour (table 1). Of the carriers with a malignancy, four had two primary HNPCC related tumours. Using the Kaplan-Meier method, 7% of the carriers developed colorectal cancer by the age of 50 years and 32% by the age of 80 years (mean age of onset 55 years, ranging from 32 to 83 years). Of the female carriers, 52% developed endometrial cancer by the age of 80 years and all of them were diagnosed above the age of 50 years. Three carriers had a papillary transitional cell carcinoma of the urinary tract (10% of the carriers).

Microsatellite instability (MSI) and immunohistochemical (IHC) analyses

We performed MSI analysis and/or immunohistochemistry with antibodies against MLH1, MSH2, and MSH6$^{12}$ on tumour samples derived from 12 subjects; in four cases, two independent tumour samples were tested (table 3). However, all tumour samples derived from the MSH6 carriers displayed instability of at least one mononucleotide marker when an extended set of markers, including BAT40, was used.

Immunohistochemical (IHC) analysis of the MSH6 protein in tumour sections showed no expression in any of the samples tested, with

Table 3 Microsatellite and immunohistochemical analysis in tumours from 12 affected relatives

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resulting from an inactivating germline mutation.15 Admittedly, these calculations are based on a limited number of cases and may represent an overestimate because not all healthy eligible relatives were tested for the MSH6 mutation. Moreover, the selection bias introduced when studying a family with such a striking clinical history may also lead to an overestimation of the penetrance. The mean age of onset of the colorectal carcinomas in this family is 55 years. This is delayed when compared with HNPCC families caused by MSH2 (44 years) and MLH1 (41 years) mutations.17 This delayed age of onset may also bias the estimated penetrance. Notably, the youngest diagnosed case was at 32 years, implying that periodic screening recommendations should not differ from those established for classical HNPCC until more data are available.

In this family, endometrial cancer is the most common tumour type (six out of 13 malignant tumours) among female carriers. All the endometrial cancers were diagnosed above the age of 50 and their mean age of onset was 55 years, that is, five to 10 years later when compared with “classical” HNPCC caused by mutations in MLH1 and MSH2.7 20

Another striking clinical phenotype in this family is the papillary transitional cell carcinoma of the ureter and renal pelvis observed in three relatives (10% of the carriers). Notably, the lifetime cumulative risk of this tumour type in MLH1 or MSH2 mutation carriers is only 2.6%.21

As previously reported by us and others,7  9 the MSI phenotype caused by loss of MSH6 function is reduced when compared with MLH1 and MSH2, and differs in its predominance at mononucleotide runs. This is in agreement with previous studies on yeast and mouse model systems.22 23 From this point of view, the set of markers previously recommended by NCI for MSI analysis16 may not be suitable for MSH6 mutation carriers and should be complemented with a set of mononucleotide markers. In six of the cases reported here (III.4, IV.4U, IV.1E, IV.15C, IV.27, and IV.32) (table 3), instability was observed at only one or two mononucleotide repeats (one of which was not included in the NCI panel) leading to an MSI-low or stable classification. In five of these cases, IHC was performed and indicated the loss of MSH6 in the tumour. Therefore, we recommend IHC analysis in MSI-L and MSS tumours from cases with a family history suggestive of HNPCC.11

We also show the feasibility of the IHC approach not only on colorectal tumours but also in carcinoma of the endometrium, urinary tract, and breast. The latter finding is relevant for the inclusion of breast cancer in the HNPCC tumour spectrum: breast tumour samples from IV.15 showed both MSI-high and a negative IHC staining pattern in accordance with the presence of the MSH6 germline mutation in this person. Two additional perimenopausal breast cancer cases were found in the present study (IV.14 and IV.20). However, as no material was available from these patients, we could not establish their MSH6 mutation carrier status.

IHC analysis was also helpful in the assessment of the likely phenocopy status relative to III.13. As a limited amount of a colorectal carcinoma was available from this dead patient, we limited our analysis to IHC and found normal MSH6 protein expression. Moreover, all her descendants tested negative for the mutation. Another dead patient from

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this family, III.19, was diagnosed with astrocytoma at the age of 75. Again, no archival material was available from this patient. However, the delayed age of onset and the fact that three of his children tested negative for the mutation, do not allow us to draw any conclusion on the relationship between the presence of the brain tumour and the MSH6 defect.

Patient IV.6 presented with a clinical phenotype more suggestive of attenuated polyposis24 rather than HNPCC. Accordingly, MSI analysis of the colonic polyps was negative (MSS) rather than HNPCC. MSI analysis of the tumour and the relationship between the presence of the brain tumours from HNPCC patients. J Pathol 2000;192:328-35.
