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Short report

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

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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.

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Keywords: hereditary non-polyposis colorectal cancer; *MSH6*

Hereditary non-polyposis colorectal cancer (HNPCC) is one of the most common autosomal dominant conditions predisposing to cancer. HNPCC carriers are at risk for the development of a broad spectrum of malignancies including tumours of the colon, endometrium, stomach, small intestine, hepatobiliary system, ureter, renal pelvis, and ovary.[1] HNPCC is caused by germline mutations in DNA mismatch repair (MMR) genes, predominantly *MSH2* on chromosome 2p16[2] and *MLH1* on chromosome 3p21.[3] In a minority of cases, mutations have been described in other MMR genes like *PMS1* and *PMS2*.[4] Recently, germline mutations in the *MSH6* gene on chromosome 2p15 were recognised as a frequent cause of atypical HNPCC, that is, not complying with the clinical "Amsterdam criteria".[5–9]

The involvement of MMR genes in HNPCC is reflected by the occurrence of instability of simple repetitive DNA sequences (microsatellite instability or MSI) in tumours from HNPCC patients. Accordingly, more than 90% of the colorectal tumours and at least 75% of the endometrial tumours from HNPCC patients display MSI.[10–12] In sporadic colorectal and endometrial cancer, MSI is found in about 15-30% of tumours.[13 14]

The first reports on *MSH6* germline mutations have indicated that the clinical phenotype of the affected families may differ from the "classical" HNPCC caused by *MSH2* and *MLH1* mutations.[5–9] The penetrance of colorectal cancer seemed to be reduced. However, endometrial cancer was likely to represent a more important clinical manifestation among female *MSH6* carriers. The reported mean age of onset of colorectal and endometrial cancer appeared to be delayed in families with *MSH6* germline mutations, 50 years and older compared to 44 (*MSH2*) and 41 (*MLH1*) years of age[15] and 53 years and older compared to 49 (*MSH2*) and 48 (*MLH1*) years, respectively.[7] Notably, MSI analysis of tumours from *MSH6* mutation carriers suggested a reduced penetrance of the MSI-H phenotype and preferential instability at mononucleotide repeats.[7–9 12]

Here, we report the clinical and molecular characterisation of a large HNPCC kindred

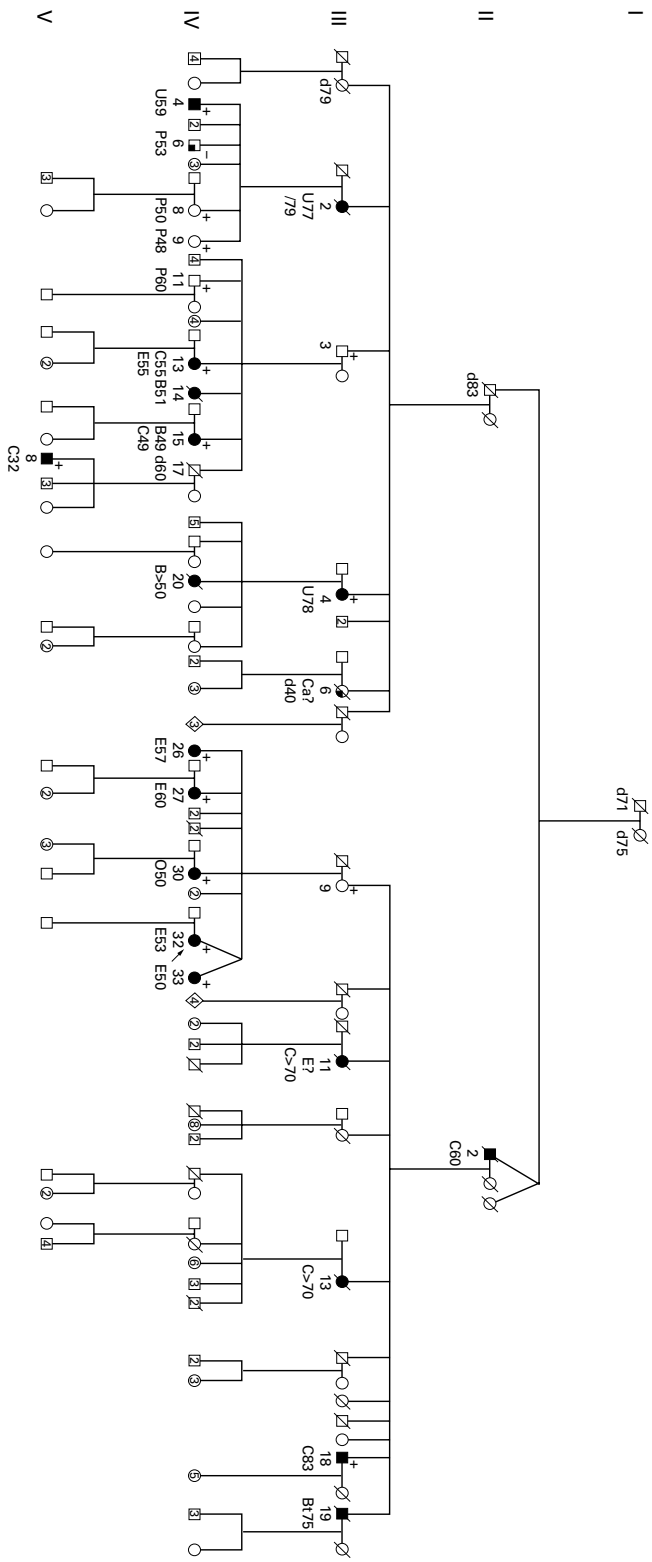

Figure 1 Pedigree of MSH6 family. C = colorectal cancer. E = endometrial cancer. U = urinary tract cancer. P = polyp. O = ovarian cancer. B = breast cancer. Bt = brain tumour. Ca? = cancer of unknown origin. + = MSH6 mutation positive. – = MSH6 mutation negative.

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.[7] 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

Table 1 (Pre)malignant tumours in the extended Dutch MSH6 family

Patient	*Tumour*	*Age at diagnosis*	*MSH6 mutation*
II.2	Colorectal cancer	60	Obligate carrier
III.2	Transitional cell carcinoma right pyelum & transitional cell carcinoma left ureter	77 79	Obligate carrier
III.4	Transitional cell carcinoma	78	+
III.6	Unknown	40	Not tested
III.11	Endometrial carcinoma Colon carcinoma	? >70	Obligate carrier
III.13	Colon carcinoma	>70	Not tested
III.18	Colon carcinoma	83	+
III.19	Astrocytoma	75	Not tested
IV.4	Transitional cell carcinoma ureter	59	+
IV.6	Multiple adenomatous polyps of the colon	53	−
IV.8	Adenoma	50	+
IV.9	Adenoma	48	+
IV.11	Tubulovillous adenoma	60	+
IV.13	Endometrial & rectal carcinoma	55	+
IV.14	Breast cancer	51	Not tested
IV.15	Breast & colon carcinoma	49	+
IV.20	Breast cancer	>50	Not tested
IV.26	Endometrial carcinoma	57	+
IV.27	Endometrial carcinoma	60	+
IV.30	Endometroid adenocarcinoma ovary	50	+
IV.32	Endometrial carcinoma	53	+
IV.33	Endometrial carcinoma	50	+
V.8	Colorectal cancer	32	+

+ indicates the presence of the *MSH6* mutation.
– indicates the absence of the *MSH6* mutation.

Table 2 Results of mutation analysis

	Male			*Female*			*Total*
Pretest risk	100%*	50%	25%	100%*	50%	25%	
Tested	5	15	7	8	31	14	80
MSH6+	4	4	0	8	13	0	29
MSH6−	1	11	7	0	18	14	51
Not tested	0	19	17	0	10	6	52
Total	5	34	24	8	41	20	132

*Relatives with an HNPCC related tumour and obligate carriers.

(for female carriers), and yearly urine sediment testing and cytology. During the first screening the following diagnoses were made: a tubulovillous adenoma with dysplasia in the proximal rectum of a 60 year old male; an invasive ductal adenocarcinoma of the breast and an adenocarcinoma of the colon in a 49 year old female; a small tubular adenoma and endometrial hyperplasia in a 50 year old female; and an adenoma in a female aged 48. Of the remaining 13 carriers, four were under the age of 45 and four were above 70 years without any symptoms of the genetic predisposition. However, a 92 year old female underwent a hysterectomy because of leiomyomata of the uterus at the age of 55.

The family included five dead obligate mutation carriers, three females and two males. Three of them had been diagnosed with a 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-Meyer 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 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).

Using the markers recommended by Boland *et al*,[16] MSI analysis showed variable patterns (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

	Subject															
	III.2		*III.4*	*III.13*	*III.18*	*IV.4*		*IV.6*	*IV.13*		*IV.15*		*IV.26*	*IV.27*	*IV.30*	*IV.32*
Tumour type	U	U	U	C	C	U	U	P	R	E	C	B	E	E	O	E
Microsatellite status	H	H	L	NT	H	H	L	S	H	L	S	H	H	L	H	S
Mononucleotide markers																
BAT25*	+	+	+	NT	+	+	−	−	+	−	−	+	+	−	+	−
BAT26*	NT	+	−	NT	+	+	+	−	+	+	−	+	+	+	+	−
BAT40	+	+	+	NT	+	+	+/−	−	+	+	+	+	NT	+	+	+
Dinucleotide markers																
D2S123*	+	NT	−	NT	−	−	NT	−	−	−	−	−	−	−	−	−
D5S346*	−	+	−	NT	+	−	−	−	+	−	−	−	−	−	−	−
D17S250*	+	NT	−	NT	+	−	−	−	+	−	−	−	−	−	−	−
Immunohistochemistry																
MLH1	NT	NT	+	++	+	+	NT	+	+	++	+	+	+	+	++	+++
MSH2	NT	NT	+	++	++	+	NT	+	−	+	+	+	++	+	+	++
MSH6	NT	NT	−	+	−	−	NT	+	−	−	−	−	−	−	NT	−

* = markers recommended by NCI for MSI analysis.[16] + = Unstable. − = Stable. NT = not tested/not conclusive. H = MSI high according to NCI recommendations.[16] L = MSI low according to NCI recommendations.[16] S = MSI stable according to NCI recommendations.[16] C = colon tumour. U = urinary tract tumour. E = endometrial tumour. O = ovarian tumour. R = rectal tumour. P = polyp. B = breast tumour.
Immunohistochemistry: +++ = strong staining, ++ = moderate staining, + = weak staining, − = no staining.

the exception of III.13 and IV.6. Patient III.13 could not be tested for either the presence of the germline mutation or for MSI, and is likely to represent a phenocopy, in view of the late age of onset (colon carcinoma at >70 years) and the failure to detect the *MSH6* mutation in her 13 descendants. We were able to perform MSI analysis in a single adenoma from patient IV.6 that did not display instability for any of the tested markers. MLH1 and MSH2 IHC analysis was positive in all samples with the exception of the rectal carcinoma from IV.13, negative for MSH2.

Discussion

Genetic heterogeneity and variable phenotypic expression represent major complications in the diagnosis and clinical management of HNPCC, one of the most common inherited predispositions to multiorgan tumorigenesis. Although the hallmark of this condition is represented by colorectal lesions, a broad range of cancer types, including tumours of the endometrium, stomach, small intestine, hepatobiliary system, and ureter, are established clinical expressions of HNPCC. The vast majority of HNPCC cases are known to result from germline mutations in the DNA mismatch repair genes *MSH2* and *MLH1*.[17] In these kindreds, high penetrance and early onset of colorectal and endometrial cancer are the major clinical features. The set of diagnostic criteria, Amsterdam criteria I and II, established by the International Collaborative Group on HNPCC[18 19] well serve the purpose of selecting families with a high likelihood of carrying *MSH2* and *MLH1* mutations.[17] More recently, atypical HNPCC families, that is, not complying with the Amsterdam criteria (ACI and II) though clearly suggestive of a HNPCC-like inherited condition, have been reported to be caused by germline mutations in a third MMR gene, namely *MSH6*.[7–9] Preliminary phenotypic analysis of these families showed a reduced occurrence of colorectal cancer and a prevalence of endometrial tumours.

Here, we have presented a detailed analysis of an extended Dutch HNPCC pedigree resulting from an inactivating germline mutation of the *MSH6* gene.

The expression of the *MSH6* mutation in this family suggests a reduced penetrance and a delayed age of onset for colorectal cancer. Using the Kaplan-Meyer method, 7% of the carriers developed colorectal cancer by the age of 50 years and 32% by the age of 80 years. This is significantly reduced ($p<0.001$) compared to "classical" HNPCC caused by *MSH2* and *MLH1* (~55% by age 50 and >80% by age 80).[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.[15] 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 analysis[16] 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.13E, 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.[12]

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

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 polyposis[24] rather than HNPCC. Accordingly, MSI analysis of the colonic polyps was negative (MSS) and normal MSH6 expression was found by IHC. Failure to detect the *MSH6* mutation in this patient confirmed that the attenuated polyposis is likely to result from an unlinked genetic predisposition.

In conclusion, the clinical features associated with *MSH6* germline mutations strongly support the inclusion of endometrial cancer and transitional cell cancer of the ureter and renal pelvis in the diagnostic criteria for HNPCC in the new Amsterdam Criteria (ACII) as formulated by the ICG-HNPCC.[19] In addition, we propose to perform MSI in combination with IHC in all Amsterdam negative families with a clustering of endometrial or urinary tract cancers. The combined MSI and IHC analysis will direct subsequent mutation analysis for *MSH6*.

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