Age at diagnosis of cancer as predictor of mutation occurrence in families suspected of HNPCC

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Abstract. Analysis of significance of age at cancer diagnosis as a factor allowing identification of a subgroup of patients with a high frequency of hMSH2 and hMLH1 mutations among families that fulfil suspected HNPCC criteria was performed. DNA from thirty-one unrelated patients affected by colorectal cancer from families matching the above criteria were studied by direct sequencing for occurrence of hMSH2 and hMLH1 gene mutations. Seven unequivocal constitutional mutations were detected: five in the hMLH1 gene and two in the hMSH2 gene. Additionally, one hMLH1 alteration of unknown significance was found. All seven mutations were found in a subgroup of 19 patients with cancer diagnosed before the age of 50 years. In a subgroup of 12 patients with cancer diagnosed at an older age only one case with hMLH1 alteration of unknown significance was detected. Our results indicate that early age at cancer diagnosis seems to be a crucial pedigree factor in discrimination of patients with hMSH2 or hMLH1 mutations among families suspected of HNPCC and matching criteria I of ICG-HNPCC.

Key words: clinical criteria, germline mutation, mutational analysis, colorectal cancer.

Introduction

Hereditary non-polyposis colorectal cancer [HNPCC, Lynch syndrome] is an autosomal dominant cancer susceptibility syndrome that accounts for up to 8% of all colon cancer cases (LYNCH, De La CHAPELLE, 1999). It is characterised by an early onset of colorectal cancer (CRC) and increased frequency of other cancers, including adenocarcinomas of the endometrium, ovary, stomach, small bowel, hepatobilary system, pancreas, and urological tract (AARNIO et al. 1999). Families with an aggregation of CRCs or other HNPCC-associated cancers are

identified as HNPCC families if they fulfill the following so-called Amsterdam II criteria defined by the International Collaborative Group on HNPCC: (1) the presence of histologically verified HNPCC-associated cancers in at least three relatives (one of whom is a first degree relative of the other two); (2) the presence of the disease in at least two successive generations; and (3) age at onset of cancers of less than 50 years in at least one of the relatives (VASEN et al. 1999). The Amsterdam criteria are very restrictive: in many families being actually affected by HNPCC they cannot be matched due to a small family size, low penetrance or just lack of medical information about relatives. Therefore, Park and other ICG-HNPCC members defined criteria for diagnosis of suspected HNPCC cases. Those criteria are summarised in Table 1 (PARK et al. 1999).

Table 1. Suspected HNPCC criteria according to PARK et al. (1999)
At least one item from both category 1 and category 2 should be fulfilled.

Suspected HNPCC criteria I
– Category 1
A-Vertical transmission of colorectal cancer
or

B-At least 2 siblings affected with colorectal cancer in family
and
-Category 2:
C-Multiple colorectal tumours (including polyps)
or
D-At least one CRC diagnosed before 50
or
E-Development of extracolonic cancer in family members
(endometrium, urinary tract, small intestine, stomach, hepatobiliary system, or ovary)
Suspected HNPCC criteria II
One colorectal cancer with: Early age of onset (<40 years)
or
Endometrial, urinary tract or small intestine cancer in the index patient or sibling
(one aged <50 years)
or
Two siblings with other integral HNPCC extracolonic cancer (one aged <50 years).

Genetically, HNPCC has been linked to a deficiency in DNA mismatch repair (MMR), which can be characterised by the presence of DNA microsatellite instability (MSI) (THIBODEAU et al. 1996). Currently, at least five genes have been known to be associated with HNPCC, including hMSH2 (LEACH et al. 1993), hMLH1 (PAPADOPOULOS et al. 1994), hPMS2, hPMS1 (NICOLAIDES et al.1994) and hMSH6 (AKIYAMA et al. 1997). The genes hMSH2 and hMLH1 appear to account for approximately 60% of all HNPCC families (WANG et al. 1999, HEINIMANN et al. 1999), whereas the relative contribution of hPMS1, hPMS2

and hMSH6 together seems to be very small, representing probably less than 10% of all families (see: www.nfdht.nl in internet). Finding of hMSH2 and hMLH1 abnormalities is of significant practical value since it allows establishment of unequivocal diagnosis in families suspected of HNPCC, and in families with identified constitutional mutations it allows exclusion of about 50% of relatives from intensive and costly surveillance and aggressive treatment programmes. The search for hMSH2 and hMLH1 mutations is still complex, time-consuming and thus expensive. There are no doubts about the need for extended molecular analyses in all families that fulfil Amsterdam criteria. The question when to apply hMSH2 and hMLH1 gene testing in other groups of CRC patients is still open. Several research groups have been working on logistical models of preselection of patients for sequencing. Preselection models include assessment of pedigree/clinical data, analyses of MSI, and gene expression at protein level by immunohistochemistry (THIBODEAU et al. 1996, AALTONEN et al. 1998, HEINIMANN et al. 1999, LAMBERTI et al. 1999, LOUKOLA et al. 1999, PARK et al. 1999, WIJNEN et al. 1999, DEBNIAK et al. 2000, LIU et al. 2000, SYNGAL et al. 2000). Analyses of literature data on correlation between occurrence of hMSH2/hMLH1 constitutional mutations and pedigree/clinical features allowed us to hypothesise that in all Amsterdam-negative families with CRC aggregation, the prevalence of mutations within hMSH2 and hMLH1 is very high and sequencing without any preselection can be applied if cancers are diagnosed at an early age (HEINIMAN et al.1999, LAMBERTI et al. 1999, WANG et al. 1999, WIJNEN et al.1999, LIU et al. 2000, SYNGAL et al. 2000). In literature there are no direct data showing such a correlation. Therefore, we decided to compare the frequencies of hMSH2/hMLH1 mutations between two subgroups of our patients that fulfil suspected HNPCC criteria discriminated by presence or absence of CRCs diagnosed under the age of 50 years.

Material and methods

Patients

Thirty-one unrelated patients affected by colorectal cancer from families suspected of HNPCC according to criteria I of ICG-HNPCC (PARK et al. 1999) were diagnosed at the Hereditary Cancer Center, Pomeranian University of Medicine, Szczecin, Poland. Our study concerned only cases matching criteria I of suspected HNPCC because patients that fulfil criteria II are identified in our centre extremely rarely. The criterium of cancer diagnosis under the age of 50 was matched in 19 cases. Patients from families that unequivocally fulfil Amsterdam criteria II (VASEN et al. 1999) were excluded.

DNA isolation and sequencing

Peripheral blood samples were collected from the patients after obtaining informed consent. DNA was extracted directly from leukocytes by the classic phenol purification method. Genomic exons and exon-intron junctions of hMLH1 and hMSH2 were amplified with primers under the same conditions as previously described (KOLODNER et al. 1995, WIJNEN et al.1995). Dye-terminator cycle sequencing reactions were performed with the use of the ABI PRISM Dye-terminator Cycle Sequencing Ready Reaction kit (Perkin Elmer) according to the recommended protocol. Automated fluorescence analysis was performed on the 373 A DNA Sequencer (ABI: Perkin-Elmer).

Results

Occurrence of germline mutations and fulfillment of particular items of suspected HNPCC criteria I in studied cases are summarised in Table 2. In the studied series we found seven unequivocal constitutional mutations: five in the hMLH1 gene and two in the hMSH2 gene. Additionally, one hMLH1 alteration of unknown significance was detected. Germline alterations identified in studied families are presented in Table 3.

All seven mutations were detected in the subgroup of patients with at least one CRC diagnosed under the age of 50 years. The mutation ratio in this subgroup was

Table 2. Fulfillment of suspected HNPCC criteria and occurrence of germline mutations in the studied patients

Patient no.	Suspected HNPCC criteria I					
	A	or B	and C	or D	or E	Mutation
1	2	3	4	5	6	7
1	+			+		
2	+			+		
3	+			+		
4	+		+	+		+
5	+			+		+
6		+		+		
7	+			+		+
8	+			+		+
9	+			+		
10	+			+		
11	+			+		
12	+			+		+

1	2	3	4	5	6	7
13	+			+		
14	+			+		
15		+				
16	+		+			
17	+			+		
18		+		+		+
19		+		+		+
20	+		+			
21	+		+			
22	+		+			
23	+		+			
24		+	+			
25	+				+	
26	+				+	
27	+		+			
28		+			+	
29	+				+	
30	+		+			
31		+			?	

Legend : + present
? germline alteration of unknown significance

Table 3. Germline alterations in studied families

DNA changes	Consequence	Patient no.
hMSH2/SD 5 IVS5+3A>T	in frame del exon 5	5
hMSH2/7 1216C>T	R406X	8
hMLH1/1 37delG	frameshift	19
hMLH1/4 350C>T	T117M	7
hMLH1/4 355insAA	frameshift	12
hMLH1/10 883delAGgt	out of frame del exon 10	18
hMLH1/18 2041G>A	A681T	4
hMLH1/18 2059C>T	R687W	31

36.8% (7/19). In the subgroup of cases with cancer diagnosed at the age of over 50 years only one case of hMLH1 alteration of unknown significance was detected.

Discussion

Our results suggest that age at diagnosis is a powerful criterium in identification of subgroups of suspected HNPCC cases with a very high incidence of hMSH2 or hMLH1 germline mutations. Among cases that fulfil suspected HNPCC criteria I we were able to detect mutations in 36.8% (7/19) of cases, provided that one case of CRC was diagnosed at the age under 50 years. Recently Wijnen has developed a logistic model based on analysis of pedigree and clinical data, aimed to select those patients for whom the probability of finding a hMSH2 and hMLH1 mutation is higher than 20% (WIJNEN et al. 1999). Mutation ratio in the subgroup selected in such a way is so high that according to that author it is not necessary to use preselection of cases by assessment of cancers for microsatellite instability before mutational hMSH2 and hMLH1 analysis. The value of 20% in Wijnen's model can be exceeded practically only for families that fulfil Amsterdam II criteria. Thus, all other cases matching suspected HNPCC criteria I should be studied for MSI before mutation analysis. Additionally it is important to note that in his model the sensitivity of finding mutations cannot exceed 90%, because some authors reported that more than 10% of cancer patients with hMLH1/hMSH2 mutations were MSI-negative (FARRINGTON et al. 1998). Also in our series MSI was not shown by tumour analysis in patient 4 with germline hMLH1 mutation in exon 18 (DEBNIAK et al. 2000). According to our results the subgroup of patients for whom MSI analyses are not justified can be further extended by cases matching items A or B and obligatory item D from Park's criteria I. In our families with mutations, HNPCC was not diagnosed definitively on the basis of pedigree/clinical criteria because of small family size (5 families) or lack of verified medical information (2 families). Many genetic counsellors, working routinely with patients from families with cancer family syndromes, experienced that collection of pedigree and medical data from/about relatives is frequently very laborious, time-consuming and sometimes related to social, ethical and psychological difficulties. Park's criteria can be matched on the basis of just nuclear pedigree analysis. Our results suggest that in order to effectively find hMSH2 and hMLH1 mutations in families that fulfil suspected HNPCC criteria I it is actually not necessary to work intensively on cancer family histories, provided that CRC is diagnosed under the age of 50. It may be that occurrence of multiple (item C) or extracolonic HNPCC-associated tumours (item E) are not independent factors useful in efficient discrimination of hMSH2 and hMLH1 mutation carriers. Our material is relatively small but the results obtained indicate that the probability of finding hMSH2 and hMLH1 constitutional abnormalities in families matching only items A or B and C or E will be very low. According to literature data, differ-

ent factors may be important in identification of groups of patients with a high frequency of constitutional mutations in HNPCC genes other than hMLH1 and hMSH2. For example, occurrence of CRC under 50 years of age seems to be less important in the search for hMSH6 mutations (KOLODNER et al. 1999, WIJNEN et al.1999). Early age at cancer diagnosis seems to be a crucial pedigree factor in discrimination of patients with hMSH2 or hMLH1 mutations. After verification of our results by examination of a larger series of patients from different centres, the findings reported in this paper may allow to define a more precise and more useful model of HNPCC diagnosis.

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