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World J Gastroenterol 2008 January 14; 14(2): 298-302
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Detection of hMSH2 and hMLH1 mutations in Chinese hereditary non-polyposis colorectal cancer kindreds

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Supported by The Special Funds of China Education Ministry for Returnees, No. 2003-14
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Received: June 20, 2007 Revised: September 9, 2007

Key words: Screening; Human mutS homology 2 gene; Human mutL homology 1 gene; Colorectal cancer; Heredity

http://dx.doi.org/10.3748/wjg.14.298

Zhang CH, He YL, Wang FJ, Song W, Yuan XY, Yang DJ, Chen CQ, Cai SR, Zhan WH. Detection of hMSH2 and hMLH1 mutations in Chinese hereditary non-polyposis colorectal cancer kindreds. *World J Gastroenterol* 2008; 14(2): 298-302

http://www.wjgnet.com/1007-9327/14/298.asp

Abstract

AIM: To establish and validate the mutation testing for identification and characterization of hereditary non-polyposis colorectal cancer (HNPCC) in suspected Chinese patients.

METHODS: Five independent Chinese kindreds with HNPCC fulfilling the classical Amsterdam criteria were collected. Genomic DNA was extracted after informed consent was obtained. The coding region of hMSH2 and hMLH1 genes was detected by polymerase chain reaction (PCR) and denaturing high-performance liquid chromatography (DHPLC). Mutations identified in the proband by DHPLC were directly sequenced using a 377 DNA sequencer, analyzed with a basic local alignment tool (BLAST), and tested in the corresponding family members by direct DNA sequencing.

RESULTS: Mutations were identified in two Chinese HNPCC kindreds. One was the missense mutation of hMSH2 c.1808A→G resulting in Asp 603 Gly identified in the proband of the fifth HNPCC (HNPCC5) kindred. In the HNP5 kindred, three family members were found to have this mutation and two of them had colorectal cancer. The other mutation of hMLH1 c.1882A→G was identified in the HNP2 kindred's proband, which might be the nonsense mutation analyzed by BLAST.

CONCLUSION: Pedigree investigation and mutation testing of hMSH2 and hMLH1 are the practical methods to identify high-risk HNPCC patients in China.

INTRODUCTION

Hereditary non-polyposis colorectal cancer (HNPCC) syndrome is characterized by autosome-dominantly inherited predisposition to early colorectal carcinoma and extracolonic epithelia-derived tumors most often located in the gastrointestinal and urogenital tracts[1]. The mean age of HNPCC and sporadic colorectal cancer (CRC) patients at diagnosis is 42 years and 65 years, respectively. HNPCC, accounting for approximately 5%-15% of all CRCs, is categorized as Lynch I or Lynch Ⅱ syndrome (according to revised Amsterdam criteria)[2]. Germline mutations of the mismatch repair (MMR) genes identified in HNPCC kindreds, including *MSH2*, *MLH1*, *MSH6*, *PMS1* (promotion of mutS homology 1), *PMS2*, and *MLH3*, have been proved as the major cause for HNPCC by linkage analysis. Mutations in two of these MMR genes, *MSH2* and *MLH1*, account for the majority of the kindreds with HNPCC[3]. Thus, pedigree investigation and MMR gene testing, as the basis for efficient prevention and treatment of HNPCC, are most often used in early diagnosis of at-risk family members and in confirmation of the diagnosis of HNPCC.

CRC is the third life-threatening cancer in China. However, HNPCC pedigree and its predisposition gene have not been extensively studied. We have established a CRC database since 1994 and the follow-up rate is above 90%. We have recently paid attention to hereditary colorectal cancer in South China and found that about 3% of CRC patients have multiple CRCs to which the young are vulnerable[4,5]. Thus, we collected those CRC families and finally obtained eleven independent Chinese kindreds with HNPCC by deep pedigree investigation until January,

2004. Five of them fulfilled the classical Amsterdam criteria. To identify high-risk populations with HNPCC, we tested hMSH2 and hMLH1 mutations in these classical kindreds.

MATERIALS AND METHODS

Patients

The clinical diagnosis of classical HNPCC was established and verified at the Department of Gastrointestinopan creatic surgery, the First Affiliated Hospital of the Sun Yat-Sen University[2]. Five independent Chinese kindreds with HNPCC fulfilling the classical Amsterdam criteria were collected. The study was approved by the Ethical Committee of Sun Yat-Sen University.

DNA isolation

Peripheral blood samples were collected from both patients and their family members in each Chinese kindred after informed consent was obtained for genetic analysis. DNA was extracted directly from leukocytes following the standard procedures.

All exons of *MLH1* and *MSH2* were amplified for mutation testing. Samples used were amplified in 20 mL reaction volume containing approximately 100 ng DNA in 50 mmol/L KCl, 10 mmol/L Tris-HCl, 1.5 mmol/L $MgCl_2$, 200 mmol/L each dNTP, 0.01% gelatin, 1 U Taq-polymerase, and 20-40 pmol each primer. The PCR amplification conditions were as follows: denaturation at 95℃ for 5 min, followed by 30 cycles of denaturation at 95℃ for 30 s, annealing at fragment-specific annealing temperature for 1 min, extension at 72℃ for 1 min, and a final extension at 72℃ for 7 min. The primers, annealing temperature, and size of the PCR products for each of the investigated hMLH1 and hMSH2 exons are described elsewhere[6-8]. The primers were synthesized by Shanghai Bio-Chemical Corporation.

DHPLC analysis

The amplified PCR fragments were screened for sequence variants by denaturing high pressure liquid chromatography (DHPLC) on a WAVE DNA fragment analysis system (Transgenomic, San Jose, CA, USA). The running conditions for each amplicon (available upon request) were determined by the Wavemaker 3.4.4 software (Transgenomic, San Jose, CA, USA) based on the DNA sequence. Five mL of each PCR product (containing 50-100 ng of DNA) was denaturated at 95℃ for 3 min and then gradually reannealed by decreasing the sample temperature from 95℃ to 65℃ over 30 min. PCR products were then separated at a flow rate of 0.9 mL/min with a linear acetonitrile gradient. Generally, analysis took approximately 10 min including column regeneration and re-equilibration to starting conditions. The column mobile phase consisted of a mixture of 0.1 mol/L triethylamine acetate (pH 7.0) with (buffer B) or without (buffer A) 25% acetonitrile. Samples displaying variant elution peaks in each run were chosen for sequence analysis. For some amplicons displaying variations in elution profiles of control samples, all samples were sequenced.

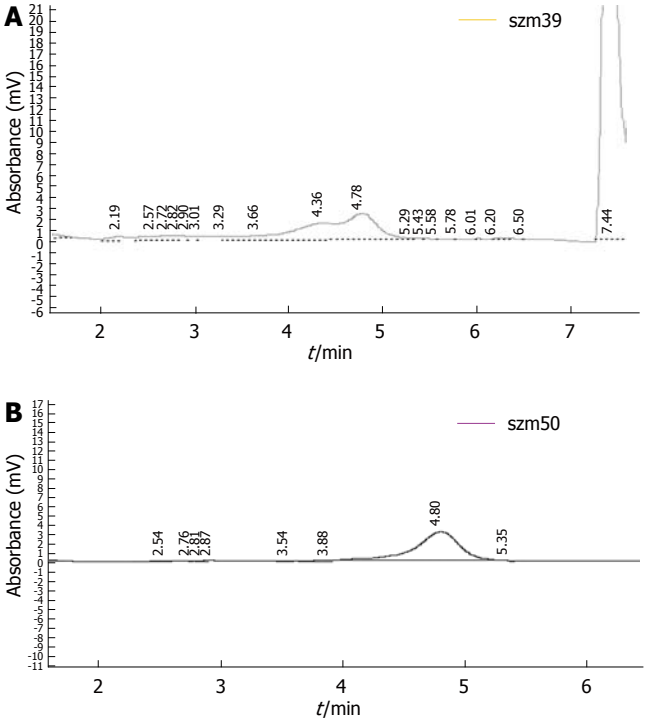

Figure 1 DHPLC showing variant elution peaks (**A**) and normal elution peaks (**B**) in exon 12 of hMSH2 gene.

DNA sequencing

DHPLC variants were confirmed by direct sequencing of independently amplified PCR products of the amplicons, in both sense and antisense direction, using the same primers. Sequencing was performed with Big Dye™ terminator cycle sequencing ready reaction kit (Applied Biosystems, Inc., Foster City, CA) on an ABI Prism™ 377 DNA sequencer following the standard conditions recommended by the manufacturer. Sequences obtained were aligned and compared to published wild-type sequences by Sequencher 3.1.1 analysis software.

Mutations identified in the kindred's proband were tested among their family members by direct DNA sequencing.

RESULTS

Among the 5 probands of the five Chinese HNPCC kindreds, 180 PCR products were screened by DHPLC. Two PCR products found with variant elution peaks by DHPLC were identified to have mutations. One was hMSH2 c.1808A→G resulting in Asp 603 Gly identified in the HNP5 kindred's proband and tested among the family members (Figures 1-3). The other was hMLH1 c.1882A →G identified in the HNP2 kindred's proband (Figures 4 and 5), which was not tested among the family members, because it might be the nonsense mutation analyzed by BLAST.

As shown in Figure 3, in the HNPCC5 kindred, there were four colorectal carcinoma patients in two successive generations, and three of these were diagnosed before the age of 45 years. The proband developed endometrial carcinoma at the age of 61 years, bladder carcinoma at the age of 66 years and colorectal carcinoma at the age of 72

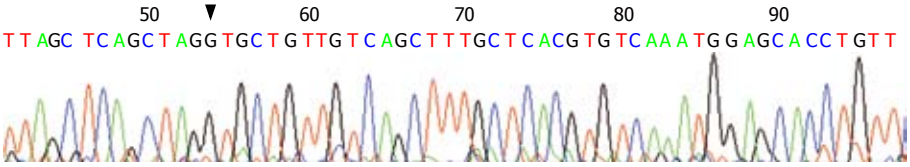

Figure 2 HPLC variants confirmed by direct sequencing displaying a single nucleotide substitution of c.1808A→G in exon 12 of hMSH2 gene.

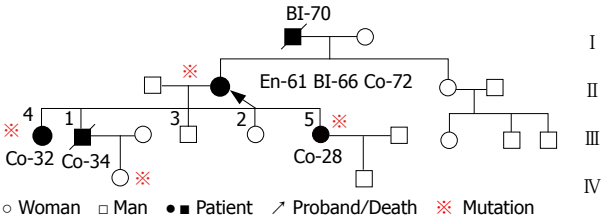

Figure 3 Pedigree tree of HNPCC 5 kindred. Co: Colon; Bl: Bladder; En: Endometrium.

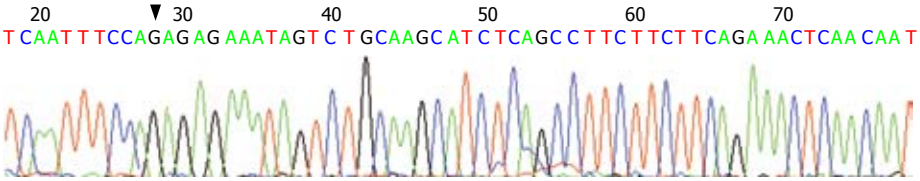

Figure 4 DHPLC variants confirmed by direct sequencing revealing a single nucleotide substitution of c.1882A→G in exon 16 of hMLH1 gene.

years, while his father developed bladder carcinoma at the age of 70 years. In addition, patient Ⅲ-1 had colorectal carcinoma at the age of 34 years and died of synchronous hepatic metastasis.

In the HNP5 kindred, each PCR product from nine proband's family members was tested by direct DNA sequencing. Only three of them were found to have the missense mutation in hMSH2 at position A1808G. The missense mutation sequence variant was found in exon 12 of hMSH2 gene. It was a single nucleotide substitution of c.1808A→G (Figure 2), which resulted in Asp 603 Gly of hMSH2 (NCBI Ref. Seq. NM 000251 and NP 000242 for mRNA and protein, respectively). Proband and patient Ⅲ-4 and -5 had this mutation and developed colorectal carcinoma. Patient Ⅲ-1's daughter had no colorectal disease even though she had this mutation, and was still under follow-up. No mutation was found in the others.

DISCUSSION

CRC is one of the most common cancers and its clinical selection criteria for cancer families were first established in Amsterdam in 1990 by the International Collaborative Group on HNPCC and modified in 1999[2]. Detection of constitutional mutations in genes associated with predisposition to cancer is practical in molecular diagnosis. Data suggest that molecular testing is much more efficient, if analyses are focused on a limited number of alterations[9-13]. Detection of germline mutation carriers is an efficient method to define high-risk CRC patients. Identification of germline mutations of either hMSH2 or hMLH1 could be performed in 50%-70% of families meeting the Amsterdam criteria for HNPCC, whereas the families not complying with these criteria show a much lower frequency of the MMR gene mutations[14-17]. Thus, hMSH2 or hMLH1 gene testing is most often used in early diagnosis of at-risk family members with HNPCC.

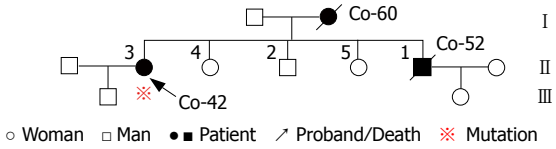

Figure 5 Pedigree tree of HNPCC 2 kindred. Co: Colon.

To identify a population-specific panel of mutations, it is crucial to describe them in all ethnic groups.

Even though CRC is common in China, few studies on HNPCC pedigree and its predisposition gene are available[4,17]. In our study, HNPCC kindreds were collected and hMSH2 and hMLH1 gene mutations were tested in order to find high-risk CRC populations. Two mutations were found among the five Chinese HNPCC kindreds. In the 5HNPCC kindreds, missense mutation was found in four members, three of them developed colorectal carcinoma. Although it has not been confirmed as a germline mutation yet, it may be an important factor for CRC development. Thus, the patient Ⅲ-1's daughter with this mutation should be closely followed up.

Approximately 20% of patients with colorectal cancer have a genetic component and HNPCC is the most common autosome dominant hereditary syndrome predisposing to colorectal cancer[18,19]. Various methods have been described to screen for HNPCC and directly test for mismatch repair gene mutations[20-27]. For patients with available tumor specimens, MSI and IHC are widely performed. Ruszkiewicz *et al*[28] reported that immunohistochemistry is an alternative method for assessment of MSI status, which is fast and relatively inexpensive compared with MSI testing. Some reports[26,29,30] suggest that detection of MSI and IHC for hMSH2/hMLH1 proteins is a reliable pre-screening test for hMLH1/hMSH2 germline mutations in families suspected of having HNPCC. Because China is a developing country with a large population and the incidence rate of CRC

increases, a screening protocol specific for the Chinese population is necessary to detect the high-risk populations with HNPCC. hMSH2 and hMLH1 gene mutation testing is a practical method for detecting HNPCC in Chinese population. Meanwhile, further research should be performed in Chinese HNPCC kindreds with germline mutations.

COMMENTS

Background

Approximately 20% of colorectal cancer patients have a genetic component and hereditary non-polyposis colorectal cancer (HNPCC) is the most common autosome dominant hereditary syndrome predisposing to colorectal cancer. Various methods have been described to screen for HNPCC and directly test for mismatch repair gene mutations. Even though CRC is common in China, few studies on HNPCC pedigree and its predisposition gene are available.

Research frontiers

Data suggest that molecular testing is much more efficient when analyses are focused on a limited number of alterations. Detection of germline mutation carriers is an efficient method to define high-risk CRC patients. Germline mutations of either hMSH2 or hMLH1 can be identified in 50%-70% of families meeting the Amsterdam criteria for HNPCC. Thus, hMSH2 or hMLH1 gene testing is most often used in early diagnosis of at-risk family members with HNPCC.

Innovations and breakthroughs

Five independent Chinese kindreds with HNPCC fulfilling the classical Amsterdam criteria were collected. Mutations were identified in two Chinese HNPCC kindreds. One was the missense mutation of hMSH2 c.1808A→G resulting in Asp 603 Gly, the other was the mutation of hMLH1 c.1882A→G .

Applications

Molecular pathological tests should be performed in order to identify individuals and at-risk family members with HNPCC. Close follow-up and intensive surveillance should be performed.

Terminology

Denaturing high-performance liquid chromatography (DHPLC) is a method for separating DNA duplexes that differ in the identity of one or more base pairs. The method is believed to be most efficient at the site of mutation. Basic local alignment search tool (BLAST) is powerful to compare novel sequences with previously characterized genes. Both functional and evolutionary information can be obtained from well designed queries and alignments. BLAST 2.0 can rapidly search for nucleotide and protein in their databases.

Peer review

The results of this clinical study indicate that mutation testing for hMSH2 and hMLH1 by PCR and direct sequencing is a preferable method to identify high-risk HNPCC patients.

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