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• ORIGINAL RESEARCH •

Clinicopathological and molecular genetic analysis of 4 typical Chinese HNPCC families

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Abstract

AIM: **To study the clinicopathological and mole cular genetic characteristics of typical Chinese hereditary nonpolyposis cotorectal cancer (HNPCC) families.**

METHODS: **Four typical Chinese HNPCC families were analyzed using microdissection, microsatellite instability analysis, immunostaining of hMSH2 and hMLH1 proteins and direct DN A sequencing of hMSH2 and hMLH1 genes.**

RESULTS: **All five tumor tissues of 4 prob ands from the 4 typical Chinese HNPCC families showed microsatellite instability at more than two loci (MSI-H or RER+phenotype). Three out of the 4 cases losthMSH2 protein expression and the other case showed no hMLH1 protein expression. Three pathological germline mutations (2 in hMSH2 and 1 in hMLH1), which had not been reported previously, were identified. The same mutations were also found in other affected members of two HNPCC families,respectively.**

CONCLUSION: **Typical Chinese HNPCC families showed relatively frequent germline mutation of mismatch repair genes. High-level microsatellite instability and loss of expression of mismatch repair genes correlated closely with germline mutation of mismatch repair genes. Microsatellite instability analysis and immunostaining of mismatch repair gene might serve as effective screening methods before direct DNA sequencing. It is necessary to establish clinical criteria and molecular diagnostic strategies more suitable for Chinese HNPCC families.**

Subject headings colorectal neoplasm; hereditary nonpolyposis/ genetic; colorectal neoplasms, hereditary nonpolyposis/ pathology; immunohistochemistry; sequence analysis, DNA

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INTRODUCTION

Hereditary nonpolyposis colorectal cancer (HNPCC), also called Lynch syndrome, is the most common human hereditary cancer predisposition and accounts for 5%-10% of total colorectal carcinomas (CRCs). Tumors in HNPCC families are characterized by an early age (before the age of 50) of onset, an excess of syn-and meta-chronous colore ctal cancers, nigh occurrence in the proximal colon and an increased risk of neoplasms of other organs including endometrium, ovary, stomach, small intestine , pancreas, biliary tract, bladder and ureter[1-5]. The lack of characteristic diagnostic features has prompted the establishment of the so-called Amsterdam criteria for diagnosis: the histologically verified colorectal cancer must occur in at least three relatives (one of whom is a first-degree relative of the other two); the cancer must occur in at least two successive generations; and at least one case must occur at an onset age of less than 50. In addition, familial adenoma touspolyposis (FAP) must be ruled out[6].

Germline mutations of six genes in volved in DNA mismatch repair (MMR), i.e. hMSH2, hMLH1, PMS1, PMS2, MSH6 (also known as GTBP) and MLH3, have been identified in patients with the disease, and t he former two genes account for the large majority of mutations found in families with HNPCC. hMSH2 is localized to chromosome 2p21-22, contains 16 exons, and is predicted to encode a 935 amino acid protein, whereas hMLH1 is localized to chromosome 3p21 and contains 19 exons encoding a 756 amino acid protein[3,7-12]. These genes are required for the correction of DNA mismatches that occur during replication. Defective DNA mismatch repair genes result in microsatellite instability (or called replication errors, RER+). It has been suggested that the presence of replication errors can be a useful marker for HNPCC[13-25]. More recent studies indicate that immunohistochemistry may be a useful alternative strategy for identifying tumors with mismatch repair deficiency. Additionally, immunohisto chemistry provides information on the specific defective gene involved and may, therefore, be cost-effective by limiting the numbers of genes to be sequenced[26-30]. MSI and immunohistochemical analysis may be useful screening methods before MMR gene mutation analysis[22-23,31-34]. Until now there have only be en some case reports of HNPCC in China and no systemic study of molecular genetic aspects of HNPCC had been presented. We have collected 61 Chinese HNPCC families (reported elsewhere) and conducted clinicopathological and molecular genetic analyses of 4 HNPCC families fulfilling the Amsterdam criteria (referred to as typical H NPCC families).

MATERIAL AND METHODS

Patients

Four typical Chinese HNPCC families were taken into the study after informed consent (Figure 1) was made. One proband was female and the other three were male. The onset age was 38, 29, 58 and 67 years respectively, with a mean age of 48. Metachronous and synchronous tumors and their locations are listed in Table 1. Tumor tissues and peripheral white blood cells were collected for the study.

Table 1 Clinicopathological charcateristics, MSI status, immun ostaining and the affected MMR genes

Family	Onsetage	Number of CRC	Tumor site	MSI status	Immunostaining		Gene
					hMSH2	hMLH1	
H2	29	4	RightColonX2; rectum X2	4/5	—	+	hMSH2
H9	67	2	Hepatic flexure; transverse colon	4/5; 5/5	+	—	hMLH1
H11	58	2	Rectum X2	4/5	—	+	hMSH2?
H27	38	2	Cecum; transversecolon	3/5	—	+	hMSH2

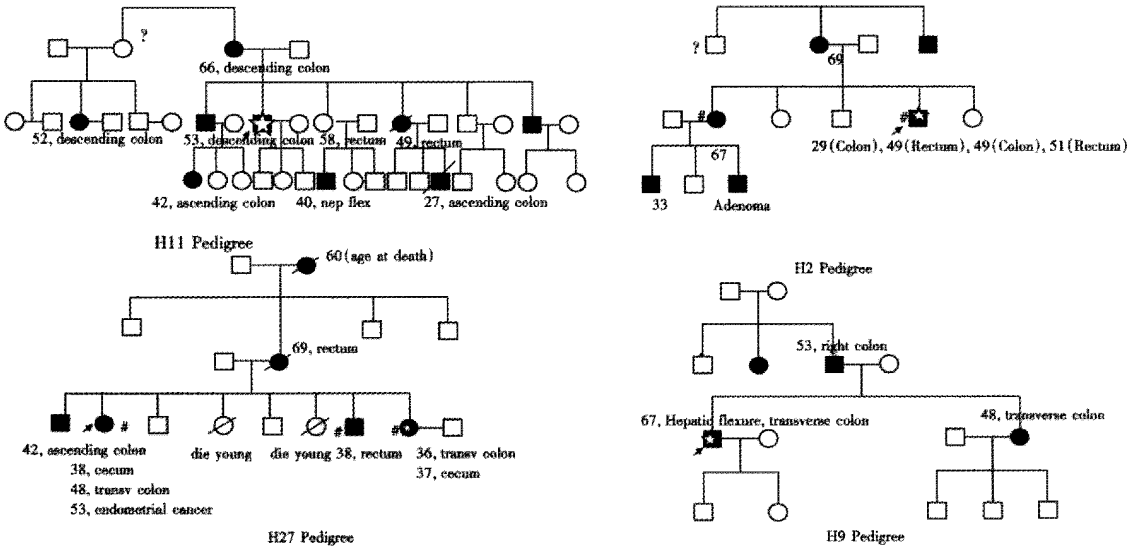

Figure 1 Four typical Chinese HNPCC pedigrees.

Microdissection and minimal amount of DNA extraction

One 5 μm and four 7 μm paraffin-embedded sections were deparaf finized. The 5 μm slide stained with HE served as control. The 7 μm ones were lightly stained with hematoxylin for microdissection. The microdissection was performed under the dissection microscope with a scalpel. Tumor cells should account for at least 80% of the total cells isolated. The microdissected tissues were transferred directly into a centrifugation tube with 150 μL cell lysis buffer (0.5 mol·L^{-1} Tris, 20 mmol·L^{-1} EDTA, 10 mmol·L^{-1} NaCl, 10 g·L^{-1} SDS, 0.5 g·L^{-1} Proteinase K). The subsequent DNA extraction was performed according to the protocol of the DNA extraction kit (Daxia Biotech Ltd, Shanghai). Genomic DNA from per ipheral white blood cells was also extracted with a large volume.

Microsatellite instability analysis

Matched normal and tumor DNA were investigated with a panel of 5 microsatellite markers (mononucleotide repeats BAT26 and BAT25, dinucleotide repeats D5S346, D2S123 and mfd15)[31]. The primer sequences have been published elsewhere[35]. The primer pairs were synthesized by Shenyou Biot ech Ltd. Each forward primer was labeled with a fluorescent dye at 5' end (Fam, Tamara or Joe) to enable the PCR products detectable by an ABI automated DNA sequencer. After successful amplification, the 2 μL PCR product was mixed with 12.5 μL dei one d formamide and 2 μL 350 Rox Sizer. The mixture was denatured, snap cooled and electropheresed on ABI 310 automated DNA sequencer according to the manufacturer' s recommendation. The electrophoresis results were analyzed by GeneScan Software (Applied Biosystems, Incorporated, Foster City, CA). MSI was determined according to Gebert *et al* [36]. Additional peaks (bands) at a microsatellite locus in the tumor compared with the normal tissue from the same patient were interpreted as microsatellite instability (MSI). Cases with MSI in more than 2 of the 5 loci were interpreted as exhibiting high microsatellite instability (MSI-H).

Immunostaining for hMSH2 and hMLH1

Sections of 4 μm were prepared from 100 mL·L^{-1} neutral buffered formalin-fixed and paraffin-embedded tumor tissue. After deparaff inization and rehydration, the sections were pretreated with microwave (4 min×4 at 900 W) in 0.1 mol·L^{-1} citrate buffer and were then incubated overnight at 4℃ with a monoclonal antibody against the hMSH2 prepared with the carboxy-terminal fragment (FE11, Oncogene Research Products, Cambridge, MA) and a monoclonal antibody against the hMLH1 prepared with full-length protein (G168-728, PharMingen, S an Diego, CA) at 1:40 dilutions. The antibodies were detected by the Envison two-step method (Dako, Denmark) using diaminobenzidine as the chromagen. The slides were counterstained with hematoxylin. Diminished expression of hMSH2 or hMLH1 in cancer tissues were demonstrated when there was complete absence of detectable nuclear staining of neoplastic cells. Infiltrating lymphocytes as well as normal colonic crypt epithelium next to the tumor area served as internal positive controls. Two pathologists assessed all cases without any knowledge of microsatellite instability or germline mutation status.

Sequencing analysis

All 19 exons of hMLH1 gene and all 16 exons of hMSH2 gene (including all intron - exon borders) from proband's genomic DNA were individually amplified in a thermocycler (Perkin-Elmer 9700, Applied Biosystems.). All the primers were kindly provided by Prof. von Knebel in the Division of Molecular Diagnostics and Therapy, Department of Surg ery, University of Heidelberg. Either sense or antisense was anchored with a M13 primer that benefits the subsequent

sequencing. PCR reaction was set in 25 μL volume containing 100 ng genomic DNA. The PCR products were purified using the Q IAquick-spin PCR purification kits (Qiagen Inc., Chatsworth, CA) and were subjected to direct sequencing with M13 forward primers using the ABI PRISM dye terminator cycle sequencing kit (Applied Biosystems) according to the manufacturer's instructions. The electrophoresis was performed on an ABI 310 automated sequencer. Search of the same mutation in additional family members was performed in the family with a detected mutation.

RESULTS

Clinicopathological characteristics are shown in Table 2.

Microsatellite instability

All 5 tumors of the 4 HN PCC probands showed microsatellite instability at more than two loci (MSI-H, or called RER+ phenotype) (Table 1, Figure 2). One tumor displayed MSI in 5/5 loci, three tumors showed MSI in 4/5 loci and the others had MSI in 3/5 loci.

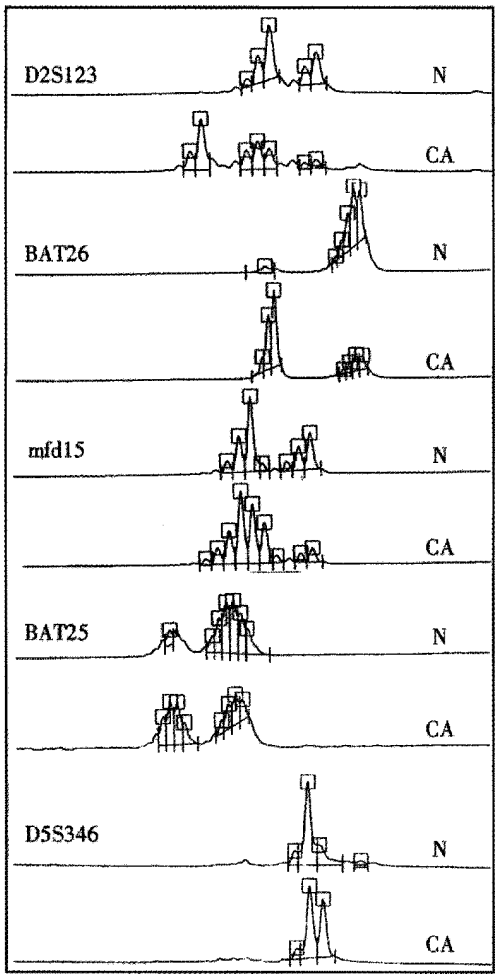

Figure 2 MSI status of H2 proband. Microsatellite analysis of H2 proband with five microsatellite markers, MSI in 4/5 loci

Loss of expression of hMSH2 and hMLH1 protein

Lack of hMLH1 immunostaining was observe d in tumors from H9 proband. Tumors from probands of H2, H11 and H27 were negative for hMSH2 immunostaining (Table 1, Figures 3 and 4).

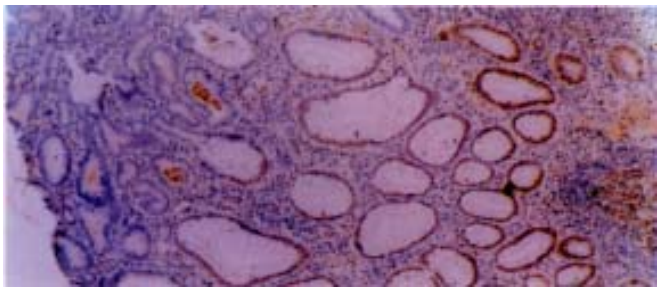

Figure 3 Immunohistochemical staining. No hMSH2 protein expression in adenoma and carcinoma areas of H11 proband tumor section, infiltrating lymphocytes as well as normal colonic crypt epithelium next to the tumor showed nuclear staining of the hMSH2 protein. ×100

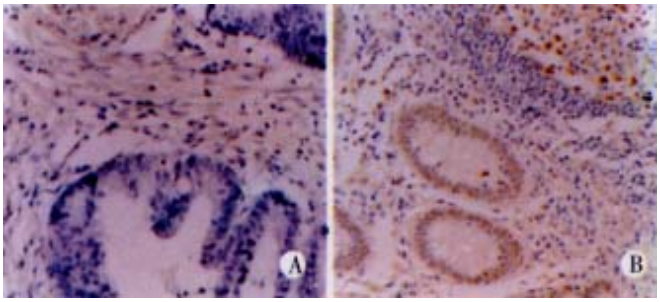

Figure 4 Immunohistochemical staining. A: No hMLH1 protein expression in carcinoma area of H9 proband tumor section. ×400
B: Infiltrating lymphocytes as well as normal colonic crypt epithelium next to the tumor showed nuclear staining of the hMLH1 protein.×200

Germline mutation of hMSH2 and hMLH1 gene

Germline mutations were found in all four probands. Three of four were definitely pathological mutations that had not been reported previously (family H2, H9, H27). The first pathological mutation was a transition of C to T in exon 13 (codon 680) of hMSH2, which leads to a stop codon (CGA-TGA) (family H2)(Figure 5). The second mutation was a 24 bp deletion in exon 11 (codon 305) of hMLH1 (family H9) (Figure 6). The third mutation was one "A" insertion at codon 206 of exon 3 of hMSH2 leading to a stop codon 73 bp downstream (family H27) (Figure 7). All 3 mutations give rise to protein truncation or protein structure alteration. In addition, the affected sister of H2 proband also carried t he same mutation in exon 13 of hMSH2. One sister and one brother of H27 porband also suffered from colorectal cancer at young age (her sister at 36 in transvers al colon and at 47 in cecum; her brother at 38 in rectum), both carrying the sam e germline mutation as their proband does. In H11 proband, a missense mutation in exon 1 of hMSH2 was identified. In order to determine whether this alteration represents a neutral polymorphism or a disease causing mutation, we assessed all possible family members and found no definite relationship between the base change and the disease. So it could not be demonstrated to be pathological and might be a single nucleotide polymorphism (SNP).

Table 2 Pathological germ-line mutations in the hMLH1and hMSH 2 genes

Family	Gene	Exon	Codon	Mutation	Nucleotide Change
H2	hMSH2	13	680	Nonsense	CGA-TGA(stop codon)
H9	hMLH1	11	305	In-frame deletion	24 bp deletion
H27	hMSH2	3	206	Frame shift	1 bp(A) insertion; stop at 73 bp downstream of the mutation

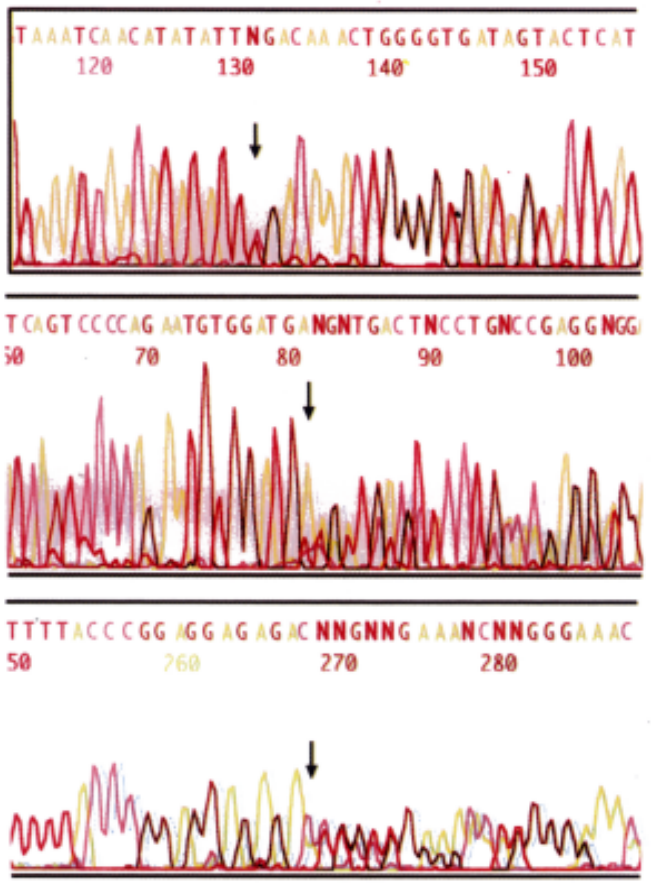

Figure 5 C-T transition (CGA-TGA) at codon 680 in exon 13 of hMSH2 gene in H2 proband, resulting in a stop codon.
Figure 6 A 24 bp deletion at codon 305 in exon 11 of hMLH1 gene in H9 proband.
Figure 7 A 1 bp insertion at codon 206 in exon 3 of hMSH2 gene in H27 proband, resulting in a stop codon 73 bp downstream of the mutation.

DISCUSSION

In China, the first clinical report of HNPCC cases was published by Mo *et al*[37] in 1996. Attention has been paid to this kind of hereditary tumor syndrome thereafter by Chinese sc holars[38-39]. Our study is a postlude of Mo's report. Three male prob ands and one female proband out of the 4 typical Chinese families had a mean onset age of 48 years. Three displayed proximal CRCs and one had a rectum cancer, all showed synch ronous and/or metachronous tumors, with one having metachronous endometrial cancer. These families fulfilled the strict HNPCC criteria. The four probands mentioned above had altogether 10 tumors. The current study is the first report with comprehensive microsatellite analysis, immunohistochemistry and direct mutation a nalysis of mismatch repair genes in Chinese HNPCC study.

Microsatellite is highly polymorphical, thus it has been widely considered as an ideal genetic marker. Microsatellite instability reveals loss of the function of mismatch repair gene s. It can serve as a reliable preliminary screening strategy of HNPCC family as several studies have shown that microsatellite instability occurs in about 80%-90% of HNPCC tumors[13-25]. Microsatellite instability was also found in 15%-20% of sporadic colorectal carcinomas[36,40]. In the current study, we adopted a panel of five sensitive microsatellite markers accepted by the International Collaborative Group for HNPCC and the National Cancer Institute to detect the MSI status[35]. One hundred percent (5/5) of the five tumors displayed high-level microsatellite instability, which suggests that Typical Chinese HNPCC families show high level defection of mismatch repair function in the affected patients.

The majority of HNPCC cases are associated with the mutation of hMSH2 and hMLH1 genes. Recent studies showed that the immunostaining of proteins produced by these two genes could serve as a convenient, rapid and cheap approach in screening HNPCC families[26-30]. In our study, the tumors from H2, H11 and H27 probands lost the expression of hMSH2 protein, while germline mutation of hMSH2 gene was only detect ed in H2 and H27 probands. The tumor of H9 proband showed no hMLH1 protein expression and a germline mutation of hMLH1 gene was identified. Although H11 proband had a tumor displaying no expression of hMSH2 protein, no pathological germline mutation had been detected. In general, microsatellite instability status and immunohistochemical alteration of hMSH2 and hMLH1 proteins correlated closely with each other. Immunohistochemistry is also very useful in screening HNPCC families.

Direct gene sequencing remains the most reliable method for HNPCC diagnosis. Mutations of hMSH2 and hMLH1 accounted for 25%-86% of the total cases[41-46]. Two main reasons were suggested for the discrepancies of mutation detection rate: firstly, different clinical criteria for selecting HNPCC families were adopted in various studies; secondly, the methods used by individual investigators varied. Till now more than three hundred different predisposing mutations have been reported, mainly affecting the MMR genes hMLH1 (about half), hMSH2 (about 40%) and MSH6 (about 10%)[7]. There appeared no hot spot mutations among those found in these mutations. The three pathological mutations (two in hMSH2 and one in hMLH1) found in our collectives are all novel mutations that had not been reported before (http:/ www.nfdht.nl/database/mdbchoice). The rate of mutation is 75% (3/4). The one A-insertion frame shift mutation in H27 proband gave rise to stop codon 73 bp downstream. H9 proband showed 24 bp deletion mutation in exon 11 of hMLH1 gene. The third mutation was a base substitution resulting in a stop codon. All of the detected mutations resulted in the truncation or structure alteration of the proteins. The mutations existed also in genomic DNA from other affected family members. That all mutations in our study appear new demonstrates the wide spectrum of the mutation responsible for HNPCC. The mutation may be different in a variety of races and geographical regions. It is therefore very important to develop HNPCC screening and genetic analysis strategies in China. The remaining family proband (H11) possessed a missense mutation (CCG-CAG, Pro-Glu). Its pathological meaning could not be demonstrated, because the presence and absence of cancer in this family showed no relation with the base change, suggesting that this may be only a polymorphism, i.e., SNP. This result could not explain the phenomenon of the lost of hMSH2 protein in the tumor of H11 proband. Curia et al con sidered that the possible germline mutation of such cases was located outside the coding region and intron-exon borders. Such mutations have the potential to affect the transcription, processing, and/or stability of mRNA encoded by the corresponding allele, resulting in germ-line transcript imbalance that should be detectable in normal tissues or PBLs. Such imbalance could be investigated by primer extension assays[47]. This kind of screening is necessary for the family like this, so as to discover the abnormality not detectable by sequencing.

Since HNPCC has many characteristics different from those of sporadic colorectal cancer, it is necessary to distinguish between them. HNPCC has better prognosis and shows more resistance to the chemotherapeutic drugs (for example, 5-FU, cisplatinum, etc). MMR gene mutation analysis will give both HNPCC proband and his family members better management and surveillance, and it will also support genetic counseling as well as gene therapy in the future. To the proband himself, it is helpful for us to conduct positive and

effective therapy to reduce the occurrence of possible me tachronous multiple colorectal cancer. To the mutation carriers in his family who have not yet suffered from colorectal cancer, close follow-up and early diagnosis are more likely to be performed. Colonoscopy every 1 to 3 years starting at age of 25 is recommended. To the non-mutation carriers, we should free them from unnecessary psychological and economical burden[48-50].

The current report is only a first description of our study at the initial stage. We hope that it could provide a guidance to the surgeons and pathologists in China who are closest to the patients. A wider survey with more kindreds in detail and a deeper analys is of the tumor spectrum of Chinese HNPCC kindreds remain a heavy assignment for us.

REFERENCES

1 Anwar S, Hall C, White J, Deakin M, Farrell W, Elder JB. Hereditary non-polyposis colorectalcancer: an updated review. *Eur J Surg Oncol*, 2000; 26:635-645

2 Lynch HT, Lynch JF. Hereditary nonpolyposis colorectal cancer. *Semin Surg Oncol*, 2000;18:305-313

3 Marra G Boland CR. Hereditary nonpolyposis colorectal cancer: the syndrome, the genes, and the historical perspectives. *J Natl Cancer Inst*, 1995; 87: 1114-1125

4 Lynch HT, Smyrk T, Lynch JF. Molecular genetics and clinical-pathology features of hereditary nonpolyposis colorectal carcinoma (Lynch syndrome). *Oncology*,1998;55:103-108

5 Lynch HT. Hereditary nonpolyposis colorectal cancer(HNPCC). *Cytogenet Cell Genet*,1999;86:130-135

6 Vasen HFA, Watson P, Mecklin J-P, Lynch HT. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC Lynch syndrome) proposed by the international collaborative group on HNPCC. *Gastroenterology*, 1999; 116: 1453-1456

7 Peltomaki P. Deficient DNA mismatch repair: a common etiologic factor for colon cancer. *Hum Mol Genet*,2001; 10:735-740

8 Peltomake P. DNA mismatch repair and cancer. *Mutation Res*,2001; 488:77-85

9 Plaschke J, Kruppa C, Tischler R, Bocker T, Pistorius S, Dralle H, Ruschoff J, Saeger HD, Fishel R, Schackert HK. Sequence analysis of the mismatch repair gene hMSH6 in the germlineof patients with familial and sporadic colorectal cancer. *In J Cancer*,2000;85:606-613

10 Wagner A, Hendriks Y, Meijers-Heijboer EJ, de Leeuw WJF, Mo rreau H, Hofstra R, Tops C, Bik E, Brocker-Vriends AHJT, vander Meer C, Lindhou t D, Vasen HFA, Breuning MH, Cornelisse CJ, van Krimpen C, Niermeijer MF, Zwinde rman AH, Wijnen J, Fodde R. Atypical HNPCC owing to MSH6 germline mutations: analysis of a large Dutch pedigree. *J Med Genet*, 2001;38:318-322

11 Lipkin SM, Wang V, Russell J, Banerjee-Basu S, Baxevanis AD, Lynch HT, Elliott RM, Collins FS. MLH3: a DNA mismatch repair gene associated with mammalian microsatellite instability. *Nature Genet*, 2000;24:27-35

12 Potter JD. Colorectal cancer: molecules and populations. *J Natl Cancer Inst*, 1999;91:916-932

13 Aaltonen LA, Peltomaki P, Leach FS, Sistonen P, Pylkkanen L, Mecklin JP, Jarvinen H, Powell SM, Jen J,Hamilton SR, Petersen GM, Kinzler KW, Vogelstein B, de la Chapelle A. Clues to the pathogenesis of familial colorectal cancer. *Science*, 1993; 260:812-816

14 Ji XL. Instability of microsatellites: new focus on gene studies. *Shijie Huaren Xiaohua Zazhi*, 1999;7:372-374

15 Zhang LL, Zhang ZS, Zhang YL, Wu BP, Guo W, Liu XX, Zhou DY. Microsatellite instability inmultiple primary colorectal cancers. *Shijie Huaren Xiaohua Zazhi*,1999;7 :397-399

16 Aaltonen La, Salovaara R, Kristo P, Canzian F, Hemminki A, Peltomaki P, Chadw ick RB, Kaariainen H, Eskelinen M, Jarvinen H, Mecklin JP, de la Chapelle A. Inc idence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. *N Engl J Med*, 1998;338:1481-1487

17 Lamberti C, Kruse R, Ruelfs C, Caspari R, Wang Y, Jungck M, Mathiak M, Malayeri HRH,Friedl W, Souerbruch T, Propping P. Microsatellite instability: a useful tool to select patientsat high risk for hereditary non-polyposis colorectal cancer-a study in different groups of patients with colorectal cancer. *Gut*, 1999; 44:839-843

18 Liu T, Wahlberg S, Burek E, Lindblom P, Rubio,Lindblom A. Microsatellite instability as a predictor of amutation in a DNA mismatch repair gene in familial colorectal cancer. *Genes Chromosomes Cancer*, 2000; 27:17-25

19 Pedroni M, Tamassia MG, Perc esepe A, Roncucci L, Benatti P, Lanza G, Gafa R, Di Gregorio C, Fante R, Losi L, Gallinari L, Scorcioni F, Vaccina F, Rossi G, Cesinaro AM, Pona De Leon M. Microsatellite instability in multiple colorectal tumors. *In J Cancer*, 1999;81:1-5

20 Calistri D, Presciuttini S, Buonsanti G, Radice P, Gazzoli I, Pensotti V, Sala P, Eboli M, Andreola S, Russo A, Pierotti M, Bertario L, Ranzani GN. Microsatellite instability in colorectal-cancer patients with suspected genetic predisposition. *In J Cancer*,2000;89:87-91

21 Frazier ML, Su LK, Amos CI, Lynch PM. Current applications of genetic technology in predispositon testing and microsatellite instability assays. *J Clin Oncol*, 2000;18(21s):70s-74s

22 Loukola A, Eklin K, Lai ho Paivi, Salovaara R, Kristo P, Jarvinen H, Mecklin JP, Launonen V, Aaltonen LA . Microsatellite markers analysis in screening for hereditary nonpolyposis colorectal cancer (HNPCC). *Cancer Res*, 2001;61:4545-4549

23 Terdiman JP, Gum JP, Conrad PG, Miller GA, Weinberg V, Crawley SC, Levin TR, Reeves C, Schmitt A, Hepburn M , Sleisenger MH, Kim YS. Efficient detection of hereditary nonpolyposis colorectal cancer gene carriers by screening for tumor microsatellite instability before germline genetic testing. *Gastroenterology*, 2001;120:21-30

24 Dietmaier W, Wallinger S, Bocker T, Kullmann F, Fishel R, Ruschlff J. Diagnosis microsatellite ins tability: definition and correlation with mismatch repair protein expression. *Cancer Res*, 1997:57:4749-4756

25 Wu BP, Zhang YL, Zhou DY, Gao CF, Lai ZS. M icrosatellite instability, MMR gene expression and proliferation kinetics in colorectal cancer with famillial predisposition. *World J Gastroenterol*, 2000;6: 902-905

26 Marcus VA, Madlensky L, Gryfe R, Kim H, So K, Millar A, Temple L K, Hsieh E, Hiruki T, Narod S, Bapat BV, Gallinger S, Redston M. Immunohistoche mistry for hMLH1 and hMSH2:a practical test for DNA mismatch repair-deficient t umors. *Am J Surg Pathol*, 1999; 23: 1248-1255

27 Cawkwell L, Gray S, Murgatroyd H, Sutherland F, Haine L, Longfellow M, O'Loughlin S, Cross D, Kronborg O, Fenger C, Mapstone N, Dixon M, Quirke P. Choice of management strategy for colorectal cancer based on a diagnostic immunohistochemical test for defectivemismatch repair. *Gut*, 1999; 45:409-415

28 Bocker T, Cuesta KH, Burkholder S, Barus evicius A, Rose D, Kovatich AJ, Boman B, Fry R, Fishel R, Palazzo JP. Colorectal carcinomas with high microsatellite instability: defining a distinct immunologic and molecular entity with respect to prognostic markers. *Hum Pathol*, 2000;31:1506-1514

29 Chaves P, Cruz C, Lage P, Claro I, Cravo M, Leitao CN, Soares J. Immunohistochemical detection of mismatch repair gene proteins as a useful tool for the identification of colorectal carcinoma with the mutator phenotype. *J Pathol*, 2000;191:355-360

30 Chiaravalli AM, Furlan D, Facco C, Tibiletti MG, Dionigi A, Casati B, Albarello L, Riva C,Capella C. Immunohistochemical pattern of hMSH2/hMLH1 in familial and sporadic colorectal, gastric,endometrial and ovarian carcinomas with instability in microsatellite sequences. *Virchows Arch*, 2001; 438:39-48

31 Bocker T, Ruschoff J, Fishel R. Molecular diagnost ics of cancer predisposition: hereditarynon-polyposis colorectal carcinoma and mismatch repair defects. *BiochimicaetBiophysicaActa*, 1999;1423:O1-O10

32 Terdiman JP, Conrad PG, Sleisenger MH. Genetic testing in hereditary colorectal cancer: indications and procedures. *Am J Gestroenterol*, 1999;94:2344-2356

33 Debniak T, Kurzawski G, Gorski B, Kladny J, Domagala W, Ludinski J. Value of pedigree/clinical data, immunohistochemistry and microsatellite instability analyses in reducing the cost of determining hMLH1 and hMSH2 gene mutations in patients with colorectal cancer. *Eur J Cancer*, 2000; 36:49-54

34 Dieumegard B, Gr andjouan S, Sabourin J-C, Bihan ML, Lefrere I, Bellefqih, Pignon J-P, Roougier P, Lasser P, Benard J, Couturier D, Paillerets BB. Extensive molecular screening for hereditary nonpolyposis colorectal cancer. *Br J Cancer*, 2000; 82:871-880

35 Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt R W, Meltzer SJ,Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S. A national cancer institute workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res*, 1998; 58:5248-5257

36 Gebert J, Sun MH, Ridder R, Hinz ULF, Lehnert T, Moller P, Schackert HK, Herfarth C, von knebel Doeberitz M. Molecular profiling of sporadic colorectal tumors by microsatellite analysis. *Int. J. Oncology*, 2000; 16:169-179

37 Mo SJ, Cai H, Cai SJ. Hereditary non-polyposis colorectal cancer: a report of 10 Chinese families. *Chin J Dig*, 1996; 16:326-328

38 Sun MH, Sun LX. Her editary nonpolyposis colorectal cancer and mismatch repair genes. *J Clin Exp Pathol*, 1999; 15(Suppl): 58-60

39 Gao SK, Xu WH. Hereditary nonpolyposis colorectal cancer. *Huaren*

Xiaohua Zazhi, 1998; 6:70-71

40 Chen J, Gu HG, Lin WH, Luo YH. Microsatellite instability analysis in 46 sporadiccolorectal carcinome. *Shijie Huaren Xiaohua Zazhi*,2000; 8:350-352

41 Peltomaki P, Vasen HFA, and the International Collaborative Group on Hereditary Nonpolyposis colorectal cancer . Mutations predisposing to hereditary nonpolyposis colorectal cancer: database and results of a collaborative study. *Gastroenterology*, 1997; 113:1146-1158

42 Bai YQ, Akiyama Y, Nagasaki H, Lu SL, Arai T, Morisaki T, Kitamura M, Muto A, Nagashima M, Nomizu T, Iwama T, Itoh H, Baba S, Iwai T. Predominant germline mutation of the hMSH2 gene in Japanese hereditary nonpolyposis colorectal cancer kindreds. *In J Cancer*, 1999; 82:512-515

43 Planck M, Koul A, Fernebro E, Borg A, Kristoffersson U, Olsson H, Wenngren E, Mangell P, Nilbert M. hMLH1, hMSH2 and hMSH6 mutations in hereditary nonpolyposis colorectal cancer families from southern Sweden. *In J Caner*, 1999;83:197-202

44 Ponz de Leon M, Pedroni M, Benatti P, Percesepe A, Di Gregorio C, Foroni M, Rossi G, Genuardi M, Neri G, Leonardi F , Viel A, Capozzi E, Boiocchi M, Roncucci L.Hereditary colorectal cancer in the general population: from cancer registration to molecular diagnosis. *Gut*, 1999; 45:32-38

45 Yuan Y, Huang J, Zheng S, Mutation of human mismatch repair genes in hereditary nonpolyposis colorectal cancer (HNPCC) families. *Chin J Oncol*, 1999;21:105-107

46 Yuan Y, Han HJ, Zheng S, Park JG. Germline mutations of hM LH1 and hMSH2 genes in patients with suspected hereditary nonpolyposis colorectal cancer and sporadic early-onset colotectal cancer. *Dis Colon Rectum*, 1998;41:434-440

47 Curia MC, Palmirotta R, Aceto G, Messerini L, Veri MC, Crognale S, Valanzano R, Ficari F, Fracasso P, Stigliano V, Tonelli F, Casale V, Guadagni F, Battista P, Mariani-Costantini R,Cama A. Unbalanced Germ-line expression of h ML H1 and hMSH2 alleles in hereditary nonpolyposis colorectal cancer. *Cancer Res*, 1999; 59:3570-3575

48 Vasen HFA, Wijnen J. Clinical implications of genetic testing of hereditary nonpolyposis colorectal cancer. *Cytogenet Cell Genet*, 1999 ; 86:136-139

49 Vasen HFA. Clinical diagnosis and management of hereditary colorectal cancer syndromes. *J Clin Oncol*, 2000;18(21s):81s-92s

50 Park JG. Genetic diagnosis and management of hereditary nonpolyposis colorectal cancer. *World J Gastroenterol*, 1998;4(Suppl 2):55

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