



RAPID COMMUNICATION

Clinical and genetic characteristics of Chinese hereditary nonpolyposis colorectal cancer families

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Abstract

AIM: To analyze the clinical characteristics of Chinese hereditary nonpolyposis colorectal cancer (HNPCC) families and to screen the germline mutations of human mismatch repair genes hMLH1 and hMSH2 in the probands.

METHODS: Thirty-one independent Chinese HNPCC families were collected in Zhejiang Province. All of them met Chinese HNPCC criteria. Clinical data about patient gender, site of colorectal cancer, age of onset, history of multiple colorectal cancer, associated extracolonic cancer were recorded. PCR and denaturing high performance liquid chromatography (DHPLC) were employed to screen the mutations. Sequencing analysis was used to find out the exact mutation site and characteristics of the samples showing abnormal DHPLC profiles.

RESULTS: One hundred and thirty-six malignant neoplasms were found in 107 patients including 14 multiple cancers. One hundred and six of the 136 neoplasms (77.9%) were diagnosed as colorectal cancer, with an average age of onset at 48.57 ± 29.00 years. Gastric cancer was the most common extracolonic cancer (10.3%) in these families. Twenty-three different sequence variations in hMLH1 and hMSH2 genes were detected in these 17 families. Fifteen sequence variations were located in the exons, including 5 SNPs, 3 silent mutations, 3 missense mutations, 2 nonsense mutations and 2 frameshift mutations. The latter seven mutations seemed to be pathogenic.

CONCLUSION: Germline mutations of hMLH1 and hMSH2 genes are identified in about one-third HNPCC kindreds fulfilling Chinese HNPCC criteria. Chinese HNPCC families have some particular clinical characteristics, such

as a left-sided predominance, less synchronous or metachronous colorectal cancer, and frequent occurrence of gastric cancer.

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Key words: Colorectal cancer; Hereditary nonpolyposis; DNA mutation analysis; High pressure liquid Chromatography; Oncogenes

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INTRODUCTION

Hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominantly-inherited cancer susceptibility syndrome. It is estimated that HNPCC may account for 5%-10% of the total colorectal cancers (CRC) worldwide^[1]. Clinically, HNPCC families are diagnosed based on the Amsterdam criteria. Although the Amsterdam criteria unify the diagnosis of HNPCC worldwide, they are too rigid to exclude extracolonic cancers associated with HNPCC. New criteria and guidelines have been proposed, such as the Japanese criteria, suspected HNPCC criteria. Based on the suspected HNPCC criteria and the specific characteristics of the tumor spectrum in Chinese population, the Chinese Hereditary Colorectal Cancer Collaboration has established the criteria for Chinese HNPCC families^[2].

Six genes (hMSH2, hMLH1, hPMS1, hPMS2, hMSH6/GTBP and hMLH3) involved in DNA mismatch repair have been proven to be closely related with the development of HNPCC. hMLH1 and hMSH2 genes are thought to be the main genes responsible for HNPCC, because more than 90% detected germline mutations in HNPCC families are located in these two genes^[3]. Denaturing high-performance liquid chromatography (DHPLC) is a mutation pre-screening method^[4]. The major advantage of DHPLC is the low cost and the high speed of analysis. Therefore, in this study, we analyzed the clinical characteristics of our 31 Chinese HNPCC families registered in our cancer institute, and screened the germline mutations of hMLH1 and hMSH2 genes by DHPLC and DNA sequencing.

MATERIALS AND METHODS

Clinical data

Thirty-one families involved in this study fulfilling the Chinese HNPCC criteria were collected in Zhejiang Province. Detailed familial and medical histories were obtained through an interview with the probands, a home visit to extended family members and an extensive review of medical records if available. Peripheral blood samples were collected from all participants after formal written consent was signed. Eligible HNPCC families were registered and family members were followed up intensively. All patients were reviewed by telephone or by outpatient visit at regular intervals. Data concerning sex, site of CRC, age of diagnosis, history of synchronous and/or metachronous CRC, instance of extracolonic cancers, and histopathology of tumors were documented and thoroughly verified.

Genomic DNA preparation and PCR

Genomic DNA was extracted using the QIAamp DNA isolation kit (Qiagen, Valencia, CA) according to the manufacturer's recommendations. PCR was performed using 100 ng of genomic DNA as template. A 25 μ L reaction mixture containing 10–20 pmol of each primer, 1.5 U of Taq DNA polymerase (Transgenomics, UK) with a final concentration of 2 mmol/L Mg^{2+} and 0.2 mmol/L of dNTPs was used. PCR conditions were as follows: an initial denaturing at 95°C for 5 min, followed by 40 cycles at 95°C for 30 s, at 55–60°C for 30 s, at 72°C for 40 s, and a final extension at 72°C for 10 min. A total of 35 sets of primers including 19 sets for hMLH1 gene and 16 sets for hMSH2 gene were used^[15].

DHPLC analysis

DHPLC analysis was carried out on an automated HPLC device equipped with a DNA separation column (WAVE: Transgenomic, San Jose, CA, USA) as previously described^[6].

DNA sequencing

PCR products displaying abnormal DHPLC peak were purified with microconcentrator filters (Amicon, Beverly, MA) and sequenced with a Bioasia-1524-030/3730 DNA sequencer. All mutations were sequenced in both directions.

RESULTS

Clinical characteristics of HNPCC families and probands

A total of 31 kindreds meeting the Chinese HNPCC criteria or the Amsterdam criteria were studied. One hundred and thirty six malignant neoplasms were found in 107 patients (14 multiple cancers), including 106 CRCs, 14 gastric cancers, 3 esophageal cancers, 2 lung cancers, 2 cervical cancers, 2 leukemia, 1 breast cancer, 1 ovarian cancer, 1 oral cancer, 1 thyroid cancer, 1 hepatic cancer, 1 urinary cancer, 1 malignant histiocytosis. CRC accounted for 77.9% (106/136) of the cancers. Nine multiple colorectal tumors and 5 colorectal tumors were associated with extracolonic cancer, accounting for 8.5% and 4.5% of the total CRCs, respectively. Of the 106 CRCs, 18 were located in the

right-sided colon, 8 in the transverse colon, 15 in the left-sided colon, 25 in the rectum and 40 in unidentified site. Left-sided colon cancers constituted of 60.6% (40/66) of all CRCs. Individuals suffering from gastric cancer accounted for 10.3% (14/136) of total patients. The average age of malignant neoplasm onset in all the patients was 48.82 ± 13.32 years and the ratio of males to females was 1.7:1. The average age of CRC patients was 48.57 ± 29.00 years. All predispositions in kindreds were followed up.

Mutations of hMLH1 and hMSH2 genes

A total of 23 abnormal peak profiles were found in 31 probands from 31 families. Finally, 17 probands were identified with 23 base variations by sequencing analysis, of which 7 base substitutions were located in the hMLH1 gene (6 in the exons, 1 in the intron) while the other 16 base substitutions were located in the hMSH2 gene (9 in exons, 7 in introns). Of the fifteen base substitutions in coding sequences, five were proven to be polymorphisms (SNPs), while the other ten were proven to be mutations, including 3 missense mutations (samples H4, H11 and H12), 2 nonsense mutations (samples H20 and H27), 2 frameshift mutations (samples H24 and H29), and 3 silent mutations (samples H5, H16 and H28). The detailed information is shown in Table 1 and Figure 1.

DISCUSSION

HNPCC is characterized by dominant right colon localization, early age of onset, high prevalence of synchronous and metachronous tumors, and certain extracolonic cancers^[14]. In our study, patients with Chinese HNPCC developed CRC at an average age of 48.57 ± 29.00 years. As compared to the onset of sporadic colorectal malignancies at the age approximately 60 years, the onset of HNPCC was much earlier, similar to that in Western countries. In the present study, synchronous and metachronous colorectal cancers only accounted for 8.5% of all CRCs, lower than those in Western countries. The reason is still unclear. Left-sided colorectal cancers accounted for 60.6% (40/66) of all CRCs. This result might be due to the high incidence of left-sided colon cancer (including rectal cancer) in Chinese population. Gastric cancer was the most frequent extracolonic cancer in our 31 families, while endometrial cancer was the most common in Western countries. The reason might be due to the high incidence of gastric cancer in Chinese population. Liu *et al.*^[15] have reported similar results found in their cohort of Chinese HNPCC families. These characteristics might truly reflect the specific clinical phenotype of Chinese HNPCC.

In this study, we screened mutations of the hMLH1 and hMSH2 genes in 31 Chinese HNPCC families by DHPLC and PCR as well as DNA sequencing. Twenty-three sequence abnormalities were detected in 17 probands, 15 in exons and 8 in introns. Base variants identified were compared with those described in the human gene mutation database (HGMD) (<http://www.uwcm.ac.uk/uwcm/mg/hgmd0.html>). Seven of the 15 sequence abnormalities including 3 missense mutations (H4, H11, H12), 2 nonsense mutations (H20, H27), and 2 frameshift mutations (H24, H29) in coding regions of the hMLH1

Table 1 hMSH2/hMLH1 gene sequence variations identified by sequencing in Chinese HNPCC

Family	Gene/exon	point of mutation	Peptide change	Mutation result	Significance	Reported by
H3	hMLH1/12	g.1151T > A,GTT-GAT	Val-Asp, V384D		SNP	Wang Y <i>et al</i> ^[7]
H4	hMLH1/2	g.199G > A,GGG-AGG	Gly-Arg, G67R	missense mutation	pathological	Sasaki S <i>et al</i> ^[8]
H5	hMSH2/1	g.54C > G,GGC-GGG	Gly-Gly, G18G	silent mutation	synonymous	
H6	hMSH2/7	g.1276 + 47T>A		intronic	uncertain	
H7	hMLH1/8	g.637G > T,GTG-TTG	Val-Leu, V213L		SNP	
H11	hMLH1/15	g.1668-20A > G		intronic	uncertain	
	hMSH2/12	g.1886, A > G,CAA-CGA	Gln-Arg, Q629R	missense mutation	pathological	Kim JC <i>et al</i> ^[9]
H12	hMSH2/15	g.2516, A > G,CAT-CGT	His-Arg, H839R	missense mutation	pathological	
H13	hMSH2/10	g.1661 + 12G > A		intronic	SNP	Scott RJ <i>et al</i> ^[10]
H15	hMSH2/1	g.211 + 9C > G,		intronic	SNP	
H16	hMLH1/8	g.649, C > G,CGC-TGC	Arg-Cys, R217C		SNP	Miyaki M <i>et al</i> ^[11]
	hMSH2/7	g.1221, C > G,CTC-CTG	Leu-Leu, L407L	silent mutation	synonymous	
	hMSH2/13	g.2006-6T > C		intronic	SNP	
H20	hMLH1/19	g.2250, C > G,TAC-TAG	Tyr-X, Y750X	nonsense mutation	truncated peotein	Syngal S <i>et al</i> ^[12]
H21	hMSH2/1	g.23C > T, ACG-ATG	Thr-Met, T8M,		SNP	Nomura S <i>et al</i> ^[13]
	hMSH2/1	g.211 + 9C > G,		intronic	SNP	
H22	hMSH2/1	g.23C > G, ACG-ATG	Thr-Met, T8M		SNP	Nomura S <i>et al</i> ^[13]
	hMSH2/1	g.211 + 9C > G,		intronic	SNP	
H24	hMSH2/11	g.1664, delA,	Stopat odon556	frameshift mutation	truncated protein	
	hMSH2/11	g.1662-2A > G		intronic	uncertain	
H27	hMSH2/11	g.2292G > A, TGG-TGA	Trp-Stop, W764X	nonsense mutation	truncated protein	
H28	hMSH2/5	g.795T > C,GTT-GTC	Val-Val, V265V	silent mutation	synonymous	
H29	hMLH1/14	g.1591delGT	Stop at codon555	frameshift mutation	truncated protein	

g: genomic DNA

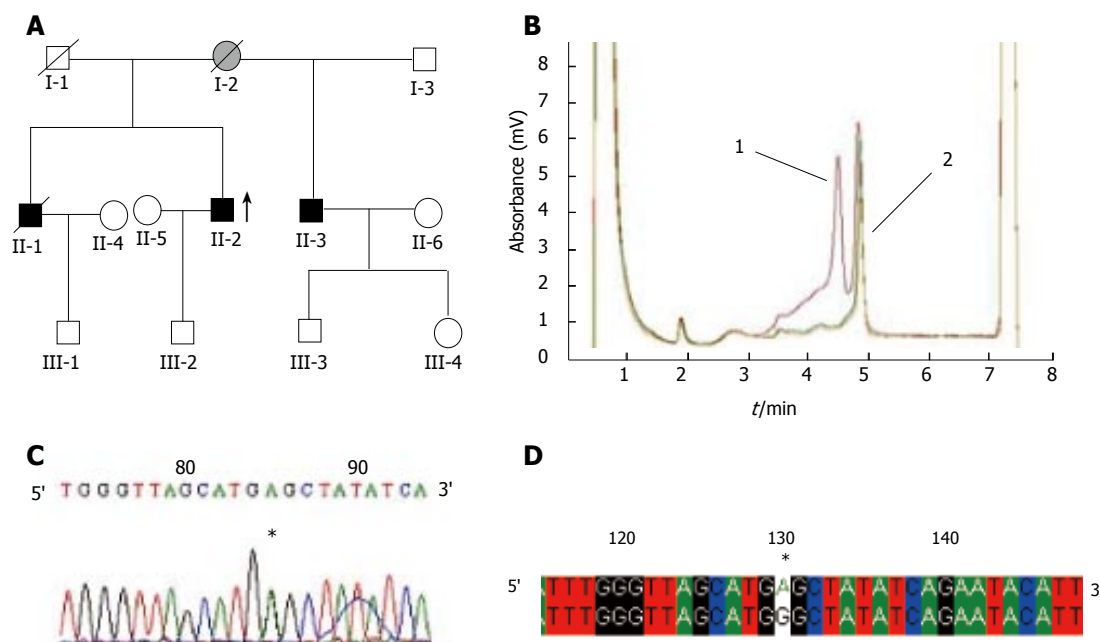


Figure 1 Mutations of hMLH1 and hMSH2 genes. **A:** pedigree map. I-2: cervical cancer at age of 42 years; II-1: rectum cancer at age of 38 years; II-2: proband, transverse colon cancer at age of 51 years; II-3: transverse colon cancer at age of 49 years; **B:** DHPLC analysis. 1: proband's abnormal profile; 2: normal control; **C:** sequencing graphs of mutation. *: the position of mutation G > A; **D:** mutation analysis. upper lane: result of proband's sequencing; lower lane: normal control.

and hMSH2 genes might probably affect the protein functions. Detailed information of these mutations is listed in Table 1. Among them, mutations of H4, H11 and H20 have been reported before^[8,9,12], while the other four mutations of H12, H27, H24 and H29 are novel. The mutation rate was 22.6% (7/31). Nearly half of the mutations were missense mutations. Three (H5, H16, H28) of the fifteen sequence abnormalities in coding sequences are novel silent mutations in exons 1, 7, 5 of the hMSH2 gene, respectively. The last five (H3, H7, H16, H21 and H22) of

the fifteen sequence abnormalities in coding sequences are known as SNPs^[7,10,11,13].

The missense mutation detected in sample H4 located at codon 67 in exon 2 of the hMLH1 gene, has been reported as a pathological mutation^[8]. The missense mutation detected in sample H11 located at codon 629 in exon 12 of the hMSH2 gene has also been reported^[9]. In sample H12, a novel missense mutation at codon 839 in exon 15 of the hMSH2 gene was first discovered in our study, which is located in the Muts V structural motif

(peptides 619-854) of the hMSH2 gene and leads to amino acid change from histidine to arginine. In order to determine the significance of this mutation, 100 blood samples from cancer-free donors were tested under the same conditions and none of the 100 normal samples harbored the same base change as sample H12, suggesting that this base change is pathologic.

The nonsense mutation detected in sample H20 altered nucleotide 2250 (genomic DNA) from C to G at codon 750 in exon 19 of the hMLH1 gene, resulting in a stop codon instead of tyrosine, forming a truncated protein of MutL losing 6 peptides from 751 residue to 756 residue. This nonsense mutation has been reported by Syngal *et al.*^[12]. The other nonsense mutation in sample H27 altered nucleotide 2292 (genomic DNA) from G to A at codon 764 in exon 11 of the hMSH2 gene, leading to the substitution of a stop codon TGA for tryptophan condon TGG, forming a truncated protein of MutC structural motif (peptides 619-854) losing 170 peptides from 765 residue to 934 residue, suggesting that these nonsense mutations may have a causative effect on cancer predisposition and further studies are needed to examine their co-segregation with the phenotype.

Two frameshift mutations detected in samples H24 and H29 have not been reported previously. In sample H24, the base A deleted at codon 555 in exon 11 of the hMSH2 gene led to a stop codon at codon 556. While in sample H29, the GT deleted at codon 531 in exon 14 of the hMLH1 gene led to a stop codon at codon 555. Both mutations produced truncated proteins with insufficient function.

Of the 23 base variants, 8 were located in intronic regions. Five of these 8 variants have been previously reported and known as SNPs (<http://www.ncbi.nlm.nih.gov/>), the significance of the other 3 remains uncertain.

DHPLC is an elegant, new method for mutation detection. It detects heteroduplex formation in renatured PCR fragments possessing heterozygous sequence variations, and has been applied successfully to mutation screening of various genes^[3]. In the past few years, we have searched successfully for mutations in hMLH1 and hMSH2 genes in 29 HNPCC families and 34 suspected HNPCC families with PCR-SSCP and DNA sequencing methods^[16]. However, SSCP is inefficient in detecting mutations and misses 20% of point and frameshift mutations. Though DNA sequencing is supposed to be a screening method with a high sensitivity, it is time consuming and expensive. It was reported that the sensitivity of DHPLC for screening mutations of the hMLH1 and hMSH2 genes can reach 97% without false negatives^[6]. Furthermore, the system is highly automatic, the running time per sample averages only 7 min. Because of these advantages of DHPLC, it enables a broad screening of HNPCC families for human mismatch repair gene alterations in routine analysis.

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