Journal of Sciences, Islamic Republic of Iran 20(1): 7-12 (2009) http://jsciences.ut.ac.ir
University of Tehran, ISSN 1016-1104

Evaluation of MLH1 and MSH2 Gene Mutations in a Subset of Iranian Families with Hereditary Nonpolyposis Colorectal Cancer (HNPCC)

M. Salehi,[1] S. Amani,[1,2] S. Javan,[3] M.H. Emami,[2,4]
M.R. Salamat,[5] and M.R. Noori Daloii[6,*]

[1] *Department of Genetics, Medical School, Isfahan University of Medical Sciences, Isfahan, Islamic Republic of Iran*
[2] *Poursina Hakim Research Center, Bozorgmehr Avenue, Isfahan, Islamic Republic of Iran*
[3] *Medical Immunology Section, Central Diagnosis Lab. Iran University of Medical Sciences, Tehran, Islamic Republic of Iran*
[4] *Department of Internal Medicine, Medical School, Isfahan University of Medical Sciences, Isfahan, Islamic Republic of Iran*
[5] *Department of Medical Engineering, Medical School, Isfahan University of Medical Sciences, Isfahan, Islamic Republic of Iran*
[6] *Department of Medical Genetic, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran*

Received: 9 August 2008 / Revised: 5 November 2008 / Accepted: 24 December 2008

Abstract

Hereditary nonpolyposis colorectal cancer is the most common form of hereditary colorectal cancers accounting for 5 to 10% of all colon carcinoma. It is inherited in an autosomal dominant mode and caused by hereditary mutations in mismatch repair genes (MMR) chiefly MLH1 and MSH2. The lifetime risk of colon cancer in affected persons is 80%. Screening, prevention strategies and consequently treatment options will be improved by understanding of the genetic basis of this disorder. The aim of this study was to assess mutations in MLH1 and MSH2 genes in a subset of Iranian HNPCC patients. The families that fulfill Amsterdam criteria were selected as HNPCC families. Genomic DNA was extracted from the peripheral blood of the samples and mutations of MLH1 and MSH2 were detected by PCR-single strand conformation polymorphism (PCR-SSCP) and DNA sequencing techniques. Hereditary mutations were found in 20 cases. Of these mutations, 14 were found in MLH1 and 6 in MSH2 genes thus MLH1 gene had higher mutation rate than MSH2. Eighteen out of 20 detected mutations in our population were previously reported and two were novel. Our results demonstrated that mutation range as well as genes involved in HNPCC is different from one region to other and characterizing mutations could be very helpful in diagnosis of the at risk individuals.

Keywords: HNPCC; Lynch syndrome; MSH2; MLH1; Iran

Introduction

Colorectal cancer (CRC) is the second leading cause of cancer death in the United States and the sixth or seventh cause of cancer death in the developing countries (1, 2). CRC is shown in three specific figures including, sporadic, familial and inherited. Nearly 10 % of the patients have an inherited predisposition to colon

[*] Corresponding author, Tel.: +98(21)66491090, Fax: +98(21)88953003, E-mail: nooridaloii@sina.tums.ac.ir

cancer (3). Hereditary nonpolyposis colorectal cancer (HNPCC) and Familial Adenomatous Polyposis (FAP) are two forms of the hereditary colorectal cancers (4-6).

HNPCC or Lynch syndrome is the most common form of hereditary colorectal cancers accounts for 5 to 10% of entire colorectal cancer population and inherited in an autosomal dominant manner (7-13). Based on previous studies in our country clinical diagnosis of HNPCC was observed in 4.7% of probands in Tehran Province (14)

This syndrome is characterized by an early age of onset of CRC (average age 45 years) which is earlier than the age of CRC in general population, proximal predominance of colorectal cancer, multiple synchronous or metachronous primary tumors and tendency to have multiple extra colonic cancers. Carcinomas of some part of the body such as endometrium, gastric, small bowel, pancreas, hepatobiliary tract, brain as well as transitional carcinoma of the ureter, renal pelvis and skin lesions are the most common of these extra colonic cancers (15-20). Affected women may have up to 60% lifetime risk of endometrial cancer, although their risk of CRC tends to be lower than the affected males (21).

Unlike FAP, which is characterized by hundreds of polyps in the colon there is no dependable clinical sign for HNPCC diagnosis, so Amsterdam criteria was proposed by the international collaborative group on HNPCC in 1990. In fact these criteria were developed to facilitate genetic analysis in high-risk families and to allow identical worldwide studies. According to these criteria there should be at least 3 relatives with colorectal cancer. All of the following criteria should also be present: 1- One should be a first degree relative of the other two, 2- At least two successive generations should be affected, 3- At least one colorectal cancer should be diagnosed before the age of 50 (22-23). Since these criteria are strict and exclude small families, alternatives such as the modified Amsterdam, Amsterdam II and Bethesda criteria have been developed (24).

HNPCC is associated with germline mutations in genes coding for DNA mismatch repair (MMR) proteins. MSH2 on chromosome 2p16 and MLH1 at 3p21 are frequently affected MMR proteins. Rare mutations are also found in MSH6 on chromosome 2p16, PMS1 and PMS2 at 2p3 and 7p11 respectively (25-28).

Predictive genetic testing for HNPCC offers the occasion to identify whether an asymptomatic person inherited the genetic predilection and therefore is useful for removing the doubt about the carries status. Furthermore Identification of mutations in MMR genes facilitates genetic testing of at risk relatives and thus an objective evaluation of their lifetime cancer risk and participation in severe surveillance programs. Lifetime risk for patients with MMR gene mutations is over 80% therefore, intensive medical follow-up is recommended and regular surveillance reduces morbidity and mortality from colon cancer. For individuals who did not inherit mutation intensive medical follow-up is not necessary (29-31).

This study was performed to evaluate the germline mutations of MLH1 and MSH2 in a group of Iranian HNPCC families.

Materials and Methods

Patient Samples

Referred patients and families to CRC clinic were classified as HNPCC group according to the Amsterdam criteria. After genetic counseling written informed consent was obtained from each patient. Five ml blood was obtained from each sample and DNA extracted using salting out method as described previously (32).

PCR Amplification

Extracted DNA was PCR amplified using specific primers for each exon of MSH2 and MLH1 genes (33). All PCR amplification was performed in 25 µl volume containing 1.2 µl (50-100 ng) template DNA, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.2 µM R-primer, 0.2 µM F-primer, and 0.2 µl of 5U/µl Taq DNA polymerase. PCR cycles included initial denaturation at 95°C for 5 minutes followed by 32 cycles of 95°C for 1 minute, 58°C for 1 minute, 72°C for 1 minute and final extension at 72°C for 10 minutes. Annealing temperature was different in each PCR amplification depending on primer set.

SSCP Analysis and Sequencing

Prior to loading on Non-denaturating polyacrylamide gel, PCR products were heated to 95°C for 4 minutes to denature the amplicons and then quenched on ice. Electrophoresis was performed at 20 mA for 18 h at room temperature. The ratio of acrylamide: bisacrylamide in Non-denaturing polyacrylamide gel containing 10% glycerol was 30:0.8. After electrophoresis the DNAs on the gel were detected by silver staining using standard methods. The nucleotide sequences of the PCR products that showed an abnormal electrophoretic mobility on SSCP gel were determined using direct sequencing, (Gene Fanavaran Company; Tehran, Iran).

Results

In this study we searched for mutations in the MLH1 and MSH2 genes in 32 HNPCC Iranian families by PCR-SSCP method.

Germline mutations of MLH1 and MSH2 genes were identified in 20 samples (62.5%). Of these, 14 mutations were found in MLH1 (70%) and 6 in MSH2 (30%) genes. Nonsense mutation in exon 8 of MLH1 gene at position 694 was detected in two patients. In addition 7 nonsense and 5 missense mutations were also detected in MLH1 gene. Four of the detected MSH2 gene mutations were reported previously, including 3 missense and 1 nonsense mutations, and 2 were novel.

The novel mutations were T deletion at 2586 and G deletion at 2556 in exon 15 of MSH2 gene.

Mutations were detected on a non-denaturating gel by analyzing migration patterns due to the conformational variability of single-stranded DNA and sequencing. Figure 1 represents an example of the SSCP gels that was detected a potential mutation in exon 15 of the MLH1 gene.

Figure 2 represents sequencing results of the differentially migrated bands, in which one of the novel mutations in MSH2 gene is demonstrated. The results of the detected mutations are summarized in Table 1.

Discussion

In a group of distinct inherited syndromes, germline mutations result in an autosomal dominant disorder with a remarkable tendency for colorectal cancer named hereditary nonpolyposis colorectal cancer (HNPCC) (9). HNPCC is caused by germline mutations in the mismatch repair (MMR) genes including, MSH1, MSH2, MSH6, PMS1 and PMS2. Of these, MSH2 and MLH1 have been related with the enormous majority of HNPCC families (34, 21).

A better understanding of the molecular basis of hereditary nonpolyposis colorectal cancer syndrome would have deep consequences for both the diagnosis and treatment of (pre)malignant neoplastic lesions (35).

Identification of mutations in MMR genes enables at risk relatives to be informed about their cancer risks and to benefit from intensive surveillance programs that have been proven to reduce their overall mortality by 65% (36).

In this study we examined MLH1 and MSH2 gene mutations among a group of Iranian families affected by HNPCC.

We detected 20 mutations, 6 of them in MSH2 and 14 in MLH1 genes. The mutation rate in our study population (62.5%) was higher in comparison with some other studies. For example the mutation rate in a survey on Taiwanese hereditary non-polyposis colorectal population was lower than our study. The mutation rate of MSH2 and MLH1 genes in that study was 0% and 20% respectively. In that study 72 Taiwanese affected individual that fulfilled Amsterdam criteria had been analyzed using RNA and DNA based methods (37). It seems that the observed differences in mutation frequencies in these studies are probably because of geographical or genetic differences between two populations.

The ratio of mutations is different between MLH1 and MSH2 genes in various countries. In our population the MLH1 gene had higher mutation rate than MSH2 gene (70% in MLH vs. 30% in MSH2 gene). Same as our study, mutation rate in MLH1 (32%) gene has been reported to be higher than MSH2 (8%) in HNPCC families in Brazil (16). The rate of mutations in MLH1

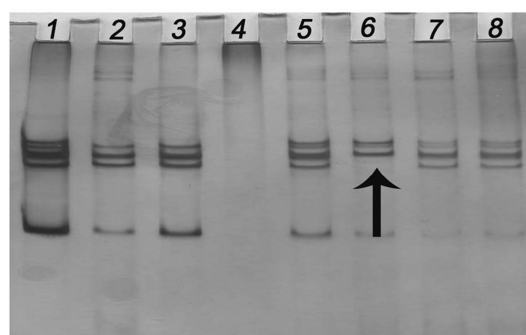

Figure 1. Representative of an acrylamide gel electrophoresis of PCR products of exon15 of MLH1 gene. Arrow shows the abnormal mobility band. The PCR products were run on polyacrilamide gel and then silver stained. The lanes 1 is from healthy control and lanes 2-8 are from patient samples. The lane 6 probably shows mutation because of appearance different patterns on the gel.

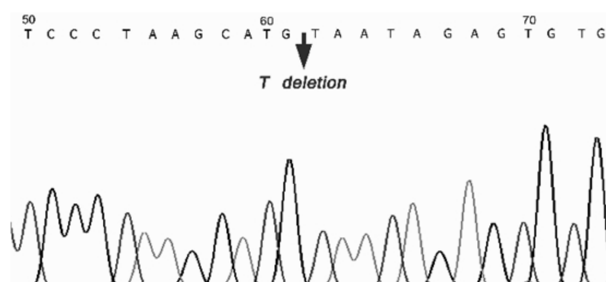

Figure 2. Representing one of the sequencing results. The base number2587 is deleted in exon 15 of MSH2 gene in patient compared to normal gene.

Table 1. The detected MLH1 and MSH2 genes mutations in our subset of HNPCC families

Gene Name	Exon	Position	Nucleotide change	Protein change
MLH1	15	1693	A>T	p.Ile565Phe
MLH1	15	1721	T>C	p.Leu547Pro
MLH1	19	2135	G>A	p.Trp712X
MLH1	19	2142	G>A	p.Trp714X
MLH1	19	2146	G>A	p.Val716Met
MLH1	3	230	G>A	p.Cys77Tyr
MLH1	3	265	G>T	p.Glu89X
MLH1	2	184	C>T	p.Gln62X
MLH1	17	1988	A>G	p.Glu663Gly
MLH1	8	649	C>T	p.Arg217Cys
MLH1	8	649	C>T	p.Arg217Cys
MLH1	6	506	C>T	p.Pro169Leu
MLH1	3	265	G>T	p.Glu89X
MLH1	12	1151	T>A	p.Val384Asp
MSH2	13	2011	A>T	p.Asn671Tyr
MSH2	13	2038	C>T	p.Arg680X
MSH2	13	2071	dupA	p.Ile691AsnfsX8
MSH2	12	1939	G>T	p.Glu647X
MSH2	15	2586	Tdel	Frame shift
MSH2	15	2556	Gdel	Frame shift

gene in a study on Korean HNPCC families by Young-Kyoung Shin in 2004 is similar to our study. In that study 9 of 12 detected mutations was in MLH1 gene (70%) (39). In contrast Long Cui in 2004 reported that in Chinese HNPCC families mutation frequency in MSH2 and MLH1 genes have been 66.67% and16.67% respectively (35). These results emphasize the importance of ethnic group in type and frequency of HNPCC causing mutations.

Upon checking the variants registered in Human Genome Mutation Database we found that 18 of the mutations were reported previously. Nine nonsense and 5 missense mutations were detected in MLH1 gene. The nonsense mutations in exon 8 at 649 were observed in two patients. The mutations in MSH2 gene including 2 nonsense, 2 missense and two frameshift mutations. The frameshift mutations in MSH2 gene have not been reported previously. These mutations were T and G deletion at position 2586 and 2556 in exon 15 MSH2 genes respectively.

The mutations frequency in MLH1 and MSH2 genes was different in various studies. For example mutations in exons 15 (1693, A>T) and 17 (1988, A>G) of MLH1 were reported only once previously (38, 39). Other detected mutations in this study have been recorded frequently from other places (42).

These differences in frequency of mutations also have been observed in MSH2 gene (43, 44).

From our results as well as others it can be concluded that mutation range and genes involved in HNPCC is unique in each region and knowing this spectrum is very important in diagnosis of at risk individuals in HNPCC families.

Acknowledgment

This work was funded by the Deputy for Research, Isfahan University of Medical Sciences. We also would like to thank Poursina Hakim Research Center for their help and support.

References

1. Annie Yu H-J., Lin K.M., Ota D.M., Lynch H.T. Hereditary non-polyposis colorectal cancer: preventive management. *Cancer Treat Rev,* **29**: 461-470 (2003).
2. Giraldo A., Gomez A., Salguero G., Garcia H., Aristizabal F., Gutierrez O., Angel L.A., Padron J., Martınez C., Martınez H., Malaver O., Florez L., Barvo R. MLH1 and MSH2 mutations in Colombian families with hereditary non-polyposis colorectal cancer (Lynch syndrome) description of four novel mutations. *Fam Cancer,* **4**: 285-290 (2005).
3. Calvert M.D., Frucht H. The genetics of colorectal cancer. *Ann Intern Med,* **137**: 603-612 (2002).
4. Hedge M., Blazo M., Chong B., Prior T., Richards C. Assay validation for identification of hereditary non-polyposis colon cancer-causing mutations in mismatch repair genes MLH1, MSH2, and MSH6. *J Mol Diagn,* **7**: 525-34 (2005).
5. Woods M.O., Hyde A.J., Curtis F.K., Stuckless S., Green J.S., Pollett A.F., Robb J.D., Green R.C., Croitoru M.E., Careen A., Chaulk J.A., Jegathesan J., McLaughlin J.R., Gallinger S.S., Younghusband H.B., Bapat B.V., Parfrey P.S. High frequency of hereditary colorectal cancer in Newfoundland likely involves novel susceptibility genes. *Clin Cancer Res*, **11**: 6853-6861 (2005).
6. Cunningham J.M., Kim C.Y., Christensen E.R., Tester D.J., Parc Y., Burgart L.J., Halling K.C., McDonnell S.K., Schaid D.J., Walsh Vockley C., Kubly V., Nelson H., Michels V.V., Thibodeau S.N. The frequency of hereditary defective mismatch repair in a prospective series of unselected colorectal carcinoma. *Am J Hum Genet*, **69**: 780-790 (2001).
7. Mecklin J.P., Järvinen H.J. Surveillance in Lynch syndrome. *Fam Cancer*, **4**: 267-271 (2005).
8. Wehner M., Mangold E., Sengteller M., Friedrichs N., Aretz S., Friedl W., Propping P., Pagenstecher C. Hereditary non-polyposis colorectal cancer: pitfalls in deletion screening in MSH2 and MLH1 genes. *Eur j Hum Genet,* **13**: 983-986 (2005).

9. Strate L.L., Syngal S. Hereditary colorectal cancer syndromes. *Cancer Causes Control,* **16**: 201-213 (2005).
10. Abdel-Rahman W.M., Mecklin J.P., Peltomäki P. The genetics of HNPCC: Application to diagnosis and screening. *Cril Rev Oncol Hematol,* **58**: 208-220 (2006).
11. Lynch H.T., Riley B.D., Weissman S.M., Coronel S.M., Kinarsky Y., Lynch J.F., Shaw T.G., Rubinstein W.S. Hereditary non-polyposis colorectal carcinoma (HNPCC) and HNPCC-like families: Problems in diagnosis, surveillance, and management. *Cancer,* **100**: 53-64 (2004).
12. Kouraklis G., Misiakos E.P. Hereditary non-polyposis colorectal cancer (Lynch Syndrome): criteria for identification and management. *Dig Dis Sci,* **50**: 336-344 (2005).
13. Silva R.V., Garicochea B., Cotti G., Maranho I.C., Cutait R. Hereditary non-polyposis colorectal cancer identification and surveillance of high risk families. *Clinics*, **60**: 251-6 (2005).
14. Mahdavinia M., Bishehsari F., Ansari R., Norouzbeigi N., Khaleghinejad A., Hormazdi M., Rakhshani N., Malekzadeh R. Family history of colorectal cancer in Iran. *BMC Cancer,* **5**: 112 (2005).
15. Kámory E., Tanyi M., Kolacsek O., Olasz L., Tóth L., Damjanovich L., Csuka O. Two germline alterations in mismatch repair genes found in a HNPCC patient with poor family history. *Pathol Oncol Res,* **12**: 228-233(2006).
16. Rossi B.M., Lopes A., Oliveira Ferreira F., Nakagawa W.T., Napoli Ferreira C.C., Casali Da Rocha J.C., Simpson C.C., Simpson A.J. hMLH1 and hMSH2 gene mutation in Brazilian families with suspected hereditary non-polyposis colorectal cancer. *Ann Surg Oncol*, **9**: 555-561 (2002).
17. Akrami S.M. Genetics of hereditary non-polyposis colorectal cancer. *Arch Iranian Med*, **9**: 381-389 (2006).
18. Halbert C.H., Lynch H., Lynch J., Main D., Kucharski S., Rustgi A.K., Lerman C. Colon cancer screening practices following genetic testing for hereditary non-polyposis colon cancer (HNPCC) mutations. *Arch Intern Med,* **164**: 1881-1887 (2004).
19. Heminki K., Chen B. Familial association of colorectal adenocarcinoma with cancers at other sites. *Eur j cancer,* **40**: 2480-2487 (2004).
20. Geary J., Thomas H.J., Mackay J., Dorkins H., Barwell J., Hodgson S.V. The management of families affected by hereditary non-polyposis colorectal cancer (HNPCC). *Fam Cancer,* **6**: 13-19 (2007).
21. Fidalgo P., Almeida M.R., West S., Gaspar C., Maia L., Wijnen J., Albuquerque C., Curtis A., Cravo M., Fodde R., Leitao C.N., Burn J. Detection of mutations in mismatch repair genes in Portuguese families with hereditary non-polyposis colorectal cancer (HNPCC) by a multi-method approach. *Eur J Hum Genet,* **8**: 49-53 (2000).
22. Vasen H.F.A. Clinical description of the Lynch syndrome [hereditary non-polyposis colorectal cancer (HNPCC)]. *Fam Cancer,* **4**: 219-225 (2005).
23. Tejpar S., Van Cutsem E. Molecular and genetic defects in colorectal tumorigenesis. *Best Prac Res Clin Gastroenterol*, **16**: 171-185 (2002).
24. Chung D.C., Rustgi A.K. The hereditary non-polyposis colorectal cancer syndrome: genetics and clinical implications. *Ann Intern Med*, **138**: 560-570 (2003).
25. Bergman M., Wolf B., Karner-Hanusch J. Hereditary colorectal cancer- guidelines for clinical routine. *Eur Surg*, **38**: 59-62 (2006).
26. Plotz G., Zeuzem S., Raedle J. DNA mismatch repair and Lynch syndrome. *J Mol Histol,* **37**: 271-283 (2006).
27. Peltomaki P. Lynch syndrome genes. *Fam Cancer*, **4**: 227-232 (2005).
28. Ponz de leon M,. Roncucci L. The cause of colorectal cancer. *Dig Liver Dis,* **32**: 426-39 (2000).
29. Claes E., Denayer L., Evers-Kiebooms G., Boogaerta A., Legius E. Predictive testing for hereditary non-polyposis colorectal cancer: motivation, illness representations and short-term psychological impact. *Patient Educ and Couns*, **55**: 265-274 (2004).
30. Dovrat S., Figer A., Fidder H.H., Neophytou P., Fireman Z., Geva R., Zidan J., Flex D., Meir S.B., Friedman E. Mutational analysis of hMsh6 in Israeli HNPCC and HNPCC-like families. *Fam Cancer,* **4**: 291-294 (2005).
31. Jacob S., Praz F. DNA mismatch repair defects: role in colorectal carcinogenesis. *Biochimie,* **84**: 27-47 (2002).
32. Miller S.A., Dykes D.D., Polesky H.F. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acid Res,* **16**: 1215 (1988).
33. Beck N.E., Tomlinson I.P., Homfray T., Frayling I., Hodgson S.V., Harocopos C., Bodmer W.F. Use of SSCP analysis to identify germline mutations in HNPCC families fulfilling the Amsterdam criteria. *Hum Genet*, **99**: 219-224 (1997).
34. Narayan S., Roy D. Role of APC and DNA mismatch repair genes in the development of colorectal cancers. *Mol Cancer*, **2**: 41 (2003).
35. Cui L., Jin H.Y., Cheng H.Y., Yan Y.D., Meng R.G., Yu D.H. Genetic detection of Chinese hereditary non-polyposis colorectal cancer. *World J Gastroenterol,* **10**: 209-213 (2004).
36. Wagner A., Barrows A., Wijnen J.T., Van der Klift H., Franken P.F., Verkuijlen P., Nakagawa H., Geugien M., Jaghmohan-Changur S., Breukel C., Meijers-Heijboer H., Morreau H., Van Puijenbroek M., Burn J., Coronel S., Kinarski Y., Okimoto R., Watson P., Lynch J.F., De la Chapelle A., Lynch H.T., Fodde R. Molecular analysis of hereditary non-polyposis colorectal cancer in the United States: high mutation detection rate among clinically selected families and characterization of an American founder genomic detection of the MSH2 gene. *Am. J Hum Genet*, **72**: 1088-1100 (2003).
37. Wei S.C., Yu C.Y., Tsai-Wu J.J., Su Y.N., Sheu J.C., Wu C.H., Wang C.Y., Wong J.M. Low mutation rate of MSH2 and MLH1 in Taiwanese hereditary non-polyposis colorectal cancer. *Clin Genet,* **64**: 243-51 (2003).
38. Irmejs A., Borosenko V., Melbarde-Gorkusa I., Gardovskis A., Bitina M., Kurzawski G., Suchy J., Gorski B., Gardovskis J. Nationalwide study of clinical and molecular features of hereditary non-polyposis colorectal cancer (HNPCC) in Lativa. *Anticancer Res,* **27**: 653-8 (2007).
39. Shin Y.K., Heo S.C., Shin J.H., Hong S.H., Ku J.L., Yoo B.C., Kim I.J., Park J.G. Germline mutations in MLH1,

MSH2 and MSH6 in Korean hereditary non-polyposis colorectal cancer families. *Hum Mutat*, **24**: 351-8 (2004).
40. Hutter P., Couturier A., Membrez V., Joris F., Sappino A.P., Chappuis P.O. Excess of hMLH1 germline mutations in Swiss families with hereditary non-polyposis colorectal cancer. *Int J Cancer*, **78**: 680-4 (1998).
41. Dieumegard B., Grandjouan S., Sabourin J.C., Le Bihan M.L., Lefrère I., Bellefqih, Pignon J.P., Rougier P., Lasser P., Bénard J., Couturier D., Bressac-de Paillerets B. Extensive molecular screening for hereditary non-polyposis colorectal cancer. *B J Cancer*, **82**: 871-880 (2000).
42. Li J.H., Shi X.Z., Lü S., Liu M., Cui W.M., Liu L.N., Jiang J., Xu G.W. HMLH1 gene mutation in gastric cancer patients and their kindred. *World J Gastroenterol,* **11**: 3144-3146 (2005).
43. Wolf B., Henglmueller S., Janschek E., Ilencikova D., Ludwig-Papst C., Bergmann M., Mannhalter C., Wrba F., Karner-Hanusch J. Spectrum of germ-line MLH1 and MSH2 mutations in Austrian patients with hereditary non-polyposis colorectal cancer. *Wien Klin Wochenschr* , **117**: 269-77 (2005).
44. Casey G., Lindor N.M., Papadopoulos N., Thibodeau S.N., Moskow J., Steelman S., Buzin C.H., Sommer S.S., Collins C.E., Butz M., Aronson M., Gallinger S., Barker M.A., Young J.P., Jass J.R., Hopper J.L., Diep A., Bapat B., Salem M., Seminara D., Haile R. Colon cancer family registry. Conversion analysis for mutation detection in MLH1 and MSH2 in patients with colorectal cancer. *JAMA*, **293**: 799-809 (2005).