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NOVEL GERMLINE *MLH1* AND *MSH2* MUTATIONS IN LATVIAN LYNCH SYNDROME FAMILIES

D. Bērziņa[1,2,], A. Irmejs[1,2], D. Kalniete[1,2], V. Borošenko[1,2], M. Nakazawa-Miklaševiča[1], K. Rībenieks[1,2], G. Trofimovičs[1,2], J. Gardovskis[1,2], E. Miklaševičs[1,2]*

[1]*Hereditary Cancer Institute, Riga Stradins University, Dzirciema iela 16, Riga, Latvia, LV-1007*

[2]*Pauls Stradins Clinical University Hospital, Pilsonu iela 13, Riga, Latvia, LV-1002*

***Background/Aims*: Hereditary non-polyposis colorectal cancer or Lynch syndrome is an autosomal dominantly inherited disease with high penetrance, mostly due to mutations in the *MLH1* and *MSH2* genes. The aim of this study is to investigate the mutation spectrum of the *MLH1* and *MSH2* genes. *Methodology*: High risk colorectal cancer families were selected from overall 1053 consecutive patients. Screening of germline mutations in the *MLH1* and *MSH2* was performed by direct sequencing and multiplex ligation-dependent probe amplification. *Results*: Ten patients fulfilled the Amsterdam I/II criteria and Bethesda guidelines of the Lynch syndrome. Three novel mutations were identified in *MLH1* and *MSH2* genes, as well as two known mutations in the *MLH1* gene. Large rearrangements in the *MLH1* gene were found in two patients. *Conclusions*: The mutations in the *MLH1* and *MSH2* genes in Latvian high-risk families are highly heterogeneous. Combination of direct sequencing and MLPA is the most appropriate molecular method of detecting hereditary nonpolyposis colorectal cancer patients and family members at risk.**

***Key Words*: Lynch syndrome, mismatch repair genes, germline mutations, *MLH1*, *MSH2*.**

Approximately 3–5% of colorectal cancer cases belong to the hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome. HNPCC is an autosomal dominantly inherited disease with high penetrance due to germline mutations in mismatch repair genes. The increased overall mutation rate is associated with an elevated risk of developing early onset colorectal cancer as well as extracolonic tumors, such as endometrial and ovary cancer in women, stomach, small bowel, pancreas, and others [1]. Overall survival is better in patients with HNPCC compared to patients with sporadic cancer [2]. About 70% of HNPCC cases have developed due to the mutations distributed equally through the exons in the *MLH1* and *MSH2* genes [3, 4], and only some mutations have a proven founder effect [4]. HNPCC as a clinical syndrome is diagnosed according to the Amsterdam criteria and Bethesda guidelines and allow the identification of high risk families [5]. Family members with a confirmed mutation or at high risk, if the mutation is unknown but diagnosis is clinically proven, should take a screening colonoscopy every 1–2 years beginning at age 20–25 [5]. Endometrial sampling and transvaginal ultrasonography in women from HNPCC families is also considered to be useful starting at age 30–35 [1, 5], as the risk of developing endometrial cancer for a woman in a HNPCC family is 40–60% [6]. Still, due to HNPCC most endometrial cancer cases are diagnosed symptomatically, not by transvaginal ultrasound or biopsy. Transvaginal ultrasound can be more helpful in the case of ovarian cancer as the risk of developing it is about 6–12% [6]. Therefore, it is important to screen patients and their relatives for mismatch repair gene mutations in order to confirm the diagnosis of HNPCC and begin prevention measures for reducing the probability of developing cancer. This allows more accurate identification of patients from HNPCC families. In previous studies, it was concluded that the use of the Amsterdam criteria for HNPCC patient diagnosis in Latvia is limited and mutation spectrum differs from other neighboring countries [7, 8]. This study continues the research of mismatch repair gene mutations in the case of HNPCC.

The aim of this study is to investigate the mutation spectrum of *MLH1* and *MSH2* in high risk families and to accumulate information necessary for future diagnosis and consulting high risk patients and their family members.

PATIENTS AND METHODS

Patients with colorectal cancer corresponding to the Amsterdam criteria or Bethesda guidelines were selected from 1035 consecutive colorectal cancer patients at the Pauls Stradins Clinical University Hospital or counseled at the Hereditary Cancer cabinet during 2005–2009. Approval of Riga Stradins University Medical ethics committee was obtained and all patients who participated in this study signed an informed consent form. Patients or their relatives who participated in previous studies [7, 9] were excluded.

DNA was extracted from whole blood by the QIAgen FlexiGene DNA Kit. All DNA samples were subjected to whole sequencing of *MLH1* and *MSH2* as described earlier [10, 11]. Mutations were confirmed by sequencing both DNA strands on an independent PCR product. Samples with no mutation detected by sequencing were subjected to the multiplex ligation-dependent probe amplification (MLPA) analysis. MLPA and sequencing reactions were performed using the SALSA MLPA P003 MLH1/MSH2 kit (MRC-Holland, the Netherlands). MLPA reactions were analyzed using the Applied Biosystems genetic

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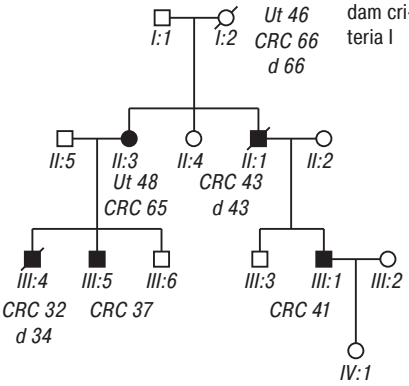
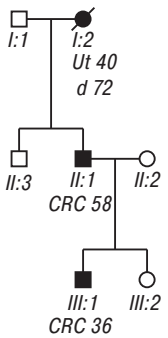
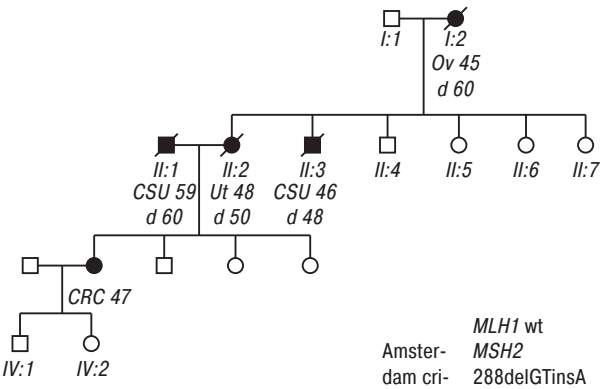
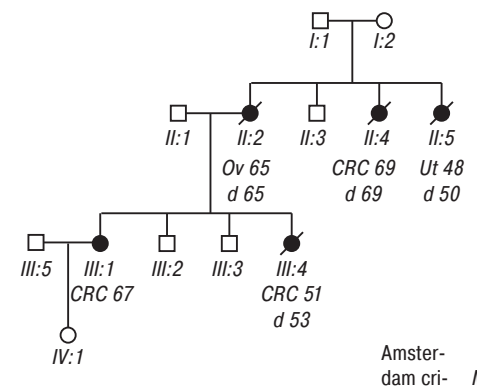
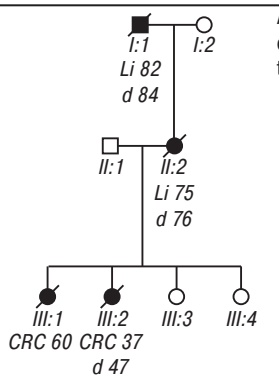
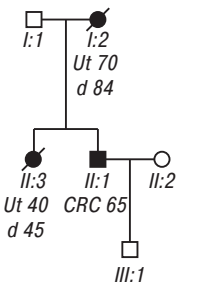
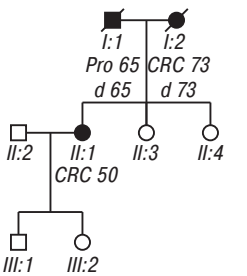
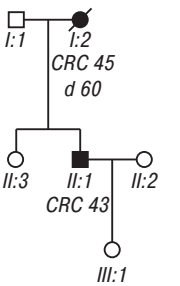
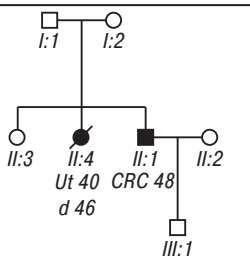
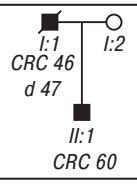
*Correspondence: E-mail: dace.berzina@rsu.lv

Abbreviations used: HNPCC – hereditary non-polyposis colorectal cancer, MLPA – multiplex ligation-dependent probe amplification, FAP – family adenomatous polyposis.

analyzer ABI3130. The following databases were used for mutation analysis: INSIGHT-group database (http://www.insight-group.org/mutations/) and NCBI SNP database (http://www.ncbi.nlm.nih.gov/snp/).

Table. Patients and their families data

(CRC – colorectal cancer, Ut – uterine cancer, Ov – ovarian cancer, Li – liver cancer, Pro – prostate cancer, CSU – cancer site unknown, d – died)

Patient, age at CRC diagnosis	Pedigree	Diagnosis according to	Gene status
D321, 41 y		Amsterdam criteria I	*MLH1* wt *MSH2* wt
J236, 58 y		Amsterdam criteria II	*MLH1* 1340delTGinsC L447*fs*M490X *MSH2* wt
C321, 47 y		Amsterdam criteria II	*MLH1* wt *MSH2* 288delGTinsA R96*fs*L173X
C450, 67 y		Amsterdam criteria II	*MLH1* wt *MSH2* wt
C152, 60 y		Amsterdam criteria II	*MLH1* 1546C>T Q516X *MSH2* wt
D500, 65 y		Amsterdam criteria II	*MLH1* 6th exon duplication *MSH2* wt
A538, 50 y		Bethesda criteria	*MLH1* 12th exon deletion *MSH2* wt
E430, 43 y		Bethesda criteria	*MLH1* 37G>T E13X *MSH2* wt
D583, 48 y		Bethesda criteria	*MLH1* 1959G>T *MSH2* wt
E595, 60 y		Bethesda criteria	*MLH1* wt *MSH2* wt

RESULTS

Amsterdam I criteria define HNPCC families according to family colorectal cancer history and the age of onset; at least two successive generations and three patients should be involved, at least one of which is a first degree relative to the other two, one of the cancers should be diagnosed before age 50 and family adenomatous polyposis (FAP) should be excluded. Amsterdam II criteria also include cancers that are associated with the HNPCC, such as endometrial, small intestine, stomach, and others. Bethesda guidelines are used to test colorectal cancers for microsatellite instability, and it is proven, that these are more applicable for detecting patients who should undergo genetic testing [1]. In this study, the main criteria used from the Bethesda guidelines were the young age of onset (before 50) in one of the affected family members.

Ten index patients were identified from 1035 consecutive colorectal cancer patients in Latvia by family history according to the Amsterdam I/II criteria or Bethesda guidelines. Among them 1 patient fulfilled the Amsterdam I criteria (0.1%), 5 fulfilled the Amsterdam II criteria (0.57%) and 4 fulfilled the Bethesda guidelines (0.38%). Medical and family histories are summarized in the Table.

Seven patients out of 10 were found to harbor mutations in the *MLH1* or *MSH2* genes including large rearrangements. Four out of 5 patients meeting the Amsterdam II criteria were harboring mutations. Three out of 4 patients meeting the Bethesda guidelines were harboring mutations. No mutation was detected in the only patient meeting the Amsterdam I criteria.

DNA sequencing revealed *MLH1* and *MSH2* mutations in five index patients. Four of those mutations including two nonsense mutations (*MLH1,* 37G>T and 1546C>T) and two frameshift mutations (*MLH1*, 1340delTGinsC and *MSH2*, 288delGTinsA) are clinically significant, as they result in a truncated protein. One nonsense mutation in the first exon of the *MLH1* gene 37G>T (E13X) was discovered in patient E430. Patient C152 carried the nonsense mutation 1546C>T (Q516X) in the *MLH1* exon 16. Patient C321 had a mutation in the *MSH2* exon 2 288delGTinsA which leads to a premature stop codon at the amino acid position 173. Mutation in the *MLH1* exon 12 1340delTGinsC, discovered in patient J236, truncates protein, leading to premature stop at codon 490. Patient J236's family members were available for analysis: his son was diagnosed with colorectal cancer at age 36, and daughter (40 years old at present) is not diagnosed with any cancer. Both siblings carry the 1340delTGinsC mutation in the *MLH1* gene. In patient D583, the *MLH1* gene mutation 1959 G>T was found in exon 17.

MLPA analysis revealed two large rearrangements. In patients A538 and D500, large rearrangements of the *MLH1* gene were found using MLPA. Patient A538 had the deletion of exon 12. Patient D500 has the duplication of exon 6.

None of all the mutations that were found in this study coincided with the previously reported mutations in Latvia [7, 9].

DISCUSSION

About 1000 new colorectal cancer cases are diagnosed in Latvia every year and approximately 100 of them at the Pauls Stradins Clinical University Hospital. Less than 1% are FAP cases [12]. As concluded before, the HNPCC rate from consecutive colorectal cancer patients in Latvia is about 2% [7] and about 20 primary diagnosed HNPCC patients can be expected in Latvia per year. The HNPCC is estimated at about 0.34% within the population of Latvia [13]. In other studies, hereditary colorectal cancer is estimated at 3–5% from all the colorectal cancer cases [14, 15] and 0.41% from the total population [16]. It is possible that the number of HNPCC cases in Latvia is underestimated due to a lower reliability of patients' family data or the lack of full information about the medical history of a family. It has been described that finding hereditary cancer families in Latvia is a common problem because of small families, as there is small number of first degree relatives and not all patients cooperate with the doctors [17]. Families with hereditary cancer syndrome are more easily detected if the family is large. Previously in Latvia a statistically significant difference was observed between the size of the family diagnosed with hereditary cancer, according to defined criteria, and families with non-diagnostic findings. The mean numbers of blood relatives within the families with hereditary cancer syndromes were 13.6 and 12.2, while the mean number of blood relatives for the families not diagnosed with hereditary cancer syndrome was 9.5 [17]. As proven by case of hereditary breast cancer families in Latvia during population screening, the results of clinical screening and mutation screening do not overlap and molecular screening reveals more mutation carriers as clinical criteria [17]. Similar results were observed in the case of HNPCC from patients corresponding with the Amsterdam criteria — mutations were found in some of the patients, and mutation screening in consecutive patients revealed patients without familial cancer history [18]. In this study, only one patient is diagnosed according to the Amsterdam I criteria and the patient did not harbor any mutation in the *MLH1* and *MSH2* genes. Five patients were diagnosed according to the Amsterdam II criteria, which also included cancers that were associated with the HNPCC syndrome, not only colorectal cancer. Out of those five patients, four of them had mismatch repair gene mutations. From four patients who corresponded with the Bethesda guidelines, three patients had germline mutations in mismatch repair genes. Three families did not have any mutation in the *MLH1* and *MSH2* genes. The syndrome of those patients could be due to the mutations in other mismatch repair genes or associated with an unknown susceptibility locus or epimutations [19–21]. Up until now, several *MLH1*, *MSH2* and

MSH6 gene mutations in the case of HNPCC have been found in Latvia [7, 9], but none of these mutations were found in our research. None of the mutations had a proven founder effect in Latvian colorectal cancer patients, although the mutation *MLH1* 1409+1 A>G that was found in Latvia [7] is described in Polish and Finnish populations [8, 22].

Information about the *MLH1* 1959G>T mutation is not consequential and there is a possibility that the exact mutation does not affect mismatch repair. The mutation is predicted to form alternative splice site, resulting in exon skipping [23], although information available in the INSIGHT-group database does not conclude pathogenesis of this mutation in all cases. However, this mutation can be considered a rare polymorphism, as there is no phenotypic consequence [18, 24]. We concluded that 6 mutations out of 7 were pathogenic, as they resulted in altered protein, thus affecting mismatch repair and resulting in the development of cancer. The mutation *MLH1* 37G>T (E13X) has been reported in the INSIGHT-group database.

Mutations in the *MLH1* and *MSH2* genes are highly heterogeneous in Latvia. Combination of direct sequencing of the *MLH1* and *MSH2* genes and MLPA is the most appropriate molecular method of detecting HNPCC patients and family members at risk.

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