Nationwide Study of Clinical and Molecular Features of Hereditary Non-polyposis Colorectal Cancer (HNPCC) in Latvia

ARVIDS IRMEJS[1], VIKTORS BOROSENKO[1], INGA MELBARDE-GORKUSA[1], ANDRIS GARDOVSKIS[1], MARIANNA BITINA[1], GRZEGORZ KURZAWSKI[2], JANINA SUCHY[2], BOHDAN GORSKI[2] and JANIS GARDOVSKIS[1]

[1]*Hereditary Cancer Institute, Riga Stradins University, Riga, Latvia;*
[2]*International Hereditary Cancer Center, Pomeranian Medical University, Szczecin, Poland*

Abstract. *Background: The mutational spectrum of mismatch repair (MMR) genes in the Baltic States has been reported to be quite similar to that in Poland; however during a country-wide study considerable differences in the population of Latvia were discovered. This study was undertaken to investigate the clinical and molecular features of HNPCC in Latvia. Materials and Methods: Family cancer histories were collected, from January 2000 until October 2003, for 702 consecutive hospital based colorectal cancer (CRC) cases. In families suspected of having a history consistent with hereditary non-polyposis colorectal cancer (HNPCC), DNA testing for MLH1, MSH2 and MSH6 genes was performed. Immunohistochemical examination of the normal and the cancer tissue from large bowel tumors was undertaken for MSH2 and MSH6 protein expression in 182 out of 702 (26%) of the cases. Results: Among the 702 CRC patients only 1 (0.14%) fulfilled the Amsterdam criteria. Thirteen (1.9%) cases matched the criteria for suspected HNPCC and 10 (1.4%) cases matched the late onset HNPCC criteria. Altogether in 7 out of 702 (1%) cases MMR gene mutations were detected: 2 in MLH1, 3 in MSH2 and 2 in MSH6 gene. Only one out of the seven mutations was registered in the Human Genome Mutation Database and the ICG (International Collaborational Group)-HNPCC mutation data base. Negative MSH2 and MSH6 protein expression was detected in 4 (2.2%) and 18 out of 182 (9.9%) cases respectively. Conclusion: The role of the classical Amsterdam criteria in diagnosing HNPCC in CRC patients from Latvia is very limited and diagnostic criteria for suspected HNPCC are the most effective. The frequency of constitutional mutations within the MMR genes is 1% of all newly diagnosed CRC cases and the spectrum of mutations is potentially characteristic.*

Correspondence to: Arvids Irmejs, Hereditary Cancer Institute, Riga Stradins University, Dzirciema Street 16, LV 1007, Riga, Latvia. Tel: +3717069973, Fax: +3717069973, e-mail: hci@stradini.lv

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There are approximately 1,000 new colorectal cancer (CRC) cases diagnosed every year in Latvia and there were 1,119 new cases detected in 2004, which is the highest frequency of the disease ever registered in Latvia. The CRC cancer mortality in Latvia was 619 and 673 in 2002 and 2003, respectively (1).

According to the available epidemiological data, familial CRC accounts for 10-15% of all colorectal cancers (3), and one would expect 90-130 familial cases in Latvia per year. Twenty to 60 of these should be associated with a hereditary condition [either hereditary non-polyposis colorectal cancer (HNPCC) or familial adenomatous polyposis (FAP)]. Important ethnic and geographical variations of both clinical and molecular features exist in the case of hereditary CRC in different populations. Knowledge of the clinical and molecular features is crucial for successful identification of the risk group of the hereditary CRC in a particular geographic area. There are several populations, *e.g.* from the neighboring Poland and Finland, where different diagnostic criteria and specific molecular strategies should be applied (4, 5). According to an earlier publication the mutational spectrum of mismatch repair (MMR) genes in the Baltic States showed a tendency to be quite similar to that in Poland (4). However the number of cases involved from the Baltic States was very small, and, therefore, it was necessary to perform a country-wide study of the clinical and molecular features of hereditary CRC in Latvia.

Materials and Methods

A consecutive group of 702 hospital based CRC cases from three hospitals were selected, from January 2000 to October 2003, for the study. Cases were considered as consecutive, if at least 70% of newly diagnosed patients were involved in the study from the particular hospital and department in the given period of time. In the study 365 cases were included from the Oncology Center of Latvia, 173 cases from the Paula Stradins University Hospital and 164 cases from the regional Oncology Hospital of Liepaja.

Table I. *Diagnostic criteria of hereditary colorectal cancer used in the study.*

No.	Hereditary syndrome or clinical subgroup	Diagnostic criteria
1.	Definitive HNPCC (HNPCC) (6-8)	Amsterdam I and II criteria.
2.	Late onset HNPCC (HNPCC LO)	Amsterdam II criteria without age limitation
3.	Suspected HNPCC (HNPCC SUSP) (4, 9)	Nuclear pedigree criteria of suspected HNPCC: 1) at least 2 first degree relatives with HNPCC associated cancer (colorectal, endometrial, small bowel, ureter, renal pelvis); 2) at least one cancer should be diagnosed before age 50.
4.	Extended HNPCC (HNPCC EXT) (10)	1) At least 2 first degree relatives with HNPCC associated cancer (colorectal, ovarian, stomach, hepatobiliary, pancreatic, urinary bladder, brain, unknown gynecological); 2) at least one cancer should be diagnosed before age 50.
5.	Familial colorectal cancer (FCC) (3)	1) Colorectal cancer in at least 2 first or second degree relatives; 2) in at least one colorectal cancer diagnosed before age 60; 3) HNPCC and FAP should be excluded.
6.	Definitive or suspected Hereditary breast cancer (HBC)	1) At least 2 breast cancers or 1 breast and 1 ovarian cancer among first degree (or second degree through male) relatives; 2) at least 1 breast cancer in a family diagnosed under age 40.
7.	Cancer familial aggregation (CFA)	At least 3 first degree blood relatives with malignancy of any localization.
8.	Three cancers in blood relatives (3CA)	At least 3 blood relatives with malignancy of any localization.

In the study group 365/702 (52%) were females and 337/702 (48%) males. The age group distribution was 7 (1%) cases 30-39 years; 32 (4.56%) cases 40-49 years; 110 (15.67%) cases 50-59 years; 251 (35.75%) cases 60-69 years; 261 (37.18%) cases 70-79 years; 40 (5.7%) cases 80-89 years and 1 (0.14%) case of more than 90 years.

In order to evaluate the representativity of the study group, it was compared to all newly diagnosed colorectal cancers diagnosed between the years 1999 and 2002 in Latvia. According to data from the Cancer Patients Registry of Latvia (CPRL) during those 4 years (1999 - 2002) there were 3615 new CRC cases diagnosed in Latvia.

To compare the two groups the Chi-square (χ^2) test was used. The values $p=0.30$, (Yates corrected χ^2) for sex distribution; $p<0.68$, for age distribution and $p<0.97$ for distribution of cancer localization in the large bowel showed that there were no differences between the groups for these parameters.

The methodology of the study has been accepted by the Commission of Ethics, Riga Stradins University.

The cancer family history. All patients completed a family cancer history questionnaire. Family cancer histories were analyzed according to the diagnostic criteria of hereditary CRC, which are summarized in Table I. If there were no malignancies among blood relatives of the proband, the family was classified as negative. If there was at least one malignancy among blood relatives, but the family did not match diagnostic criteria of any hereditary CRC syndrome, then the family was classified as others.

If the pedigree fulfilled the criteria of any hereditary CRC syndrome, the proband was invited to the Hereditary Cancer Institute of Riga Stradins University. All of our patients received counseling with written information about the clinical aspects of the cancer family syndrome in their family including recommendations/options concerning prophylactics, surveillance and treatment. After getting the written informed consent of the patient, 6 ml of peripheral blood was taken.

Table II. *Clinical frequency of the different subgroups of hereditary colorectal cancer.*

Diagnosis	All cases	(%)
HNPCC	1	0.14
HNPCC SUSP	13	1.9
HNPCC LO	10	1.4
HNPCC EXT	15	2.1
FCC	20	2.9
CFA	53	7.6
HBC	12	1.7
3CA	26	3.7
Others	211	30.0
Negative	341	48.6
Total	702	100.0

Genetical examinations. In individuals from the families matching definitive, suspected or extended diagnostic criteria of HNPCC, DNA testing for the *MLH1, MSH2* and *MSH6* genes was performed (8-13). Complete DNA testing for the *MLH1* and *MSH2* genes was conducted using the "exon by exon" DHPLC/sequencing technique (2). If no mutations were found in the *MLH1* and *MSH2* genes, in families matching criteria of definitive or suspected HNPCC, DNA sequencing of four fragments of the *MSH6* gene (ex5 and three fragments of ex4), where mutations have been most frequently described in the literature, were performed. In the study group 22/29 (76%) HNPCC suspected patients, from whom it was possible to obtain a blood sample, underwent the examinations.

Table III. *Results of the genetic examinations in Amsterdam and suspected HNPCC families.*

Diagnosis	All cases	Examined cases	%	MLH1, MSH2		MSH6 (4 fragments)	
				Pos	Neg	Pos	Neg
HNPCC	1	1	100%	–	1	–	1
HNPCC susp CRC	6	6	100%	2 (MSH2 ex3 (c.508C>T; Gln170Stop)) (MSH2 ex12; c.1786-1788delAAT; 596delAsn)	4	–	6
HNPCC susp Ut	7	5	71%	1 (MLH1 ex12 (G/A 1409+1 caused out of frame del)	4	–	5
Total	14	12	85%	3	9	–	12

Pos-mutation detected; Neg-no mutation; HNPCC susp. CRC-at least 2 colorectal cancers in nuclear pedigree; HNPCC susp. Ut-at least 1 endometrial cancer in nuclear pedigree.

In individuals from the families matching the criteria of HNPCC late onset and FCC, DNA sequencing only for the mutations in four fragments of the *MSH6* gene was performed. 8/10 (80%) HNPCC late onset cases and 5/20 (25%) FCC cases from the study group were investigated.

Additionally normal and cancer tissue samples from the large bowel for 182/702 (26%) of the unselected CRC patients underwent immunohistochemical (IH) examination for the *MSH2* and *MSH6* genes protein expression. This is a group of cases for whom normal and cancer tissue samples were obtained from the large bowel during surgical resection and the tissue samples were available in the pathology centers of the hospitals involved in the study. If homogenous lack of protein expression in the tumor tissue was detected, complete DNA sequencing for the gene involved was carried out, if a DNA sample was available.

All of the molecular analyses were performed in collaboration with the International Hereditary Cancer Center, Szczecin, Poland.

Results

Clinical features. In 341 (48.6%, CI=45-52.3%) out of the 702 CRC cases no malignancies were recognized among the relatives. In 361 (51.4%, CI=47.7-55%) of the CRC cases there was at least one malignancy among blood relatives. The clinical frequency of the different subgroups of hereditary CRC are summarized in Table II.

Molecular features. In the study group altogether seven mismatch repair gene mutations were detected: 2/7 (29%, CI=8-64%) in *MLH1*, 3/7 (43%, CI=15-75%) in *MSH2* and 2/7 (29%, CI=8-64%) in the *MSH6* gene. Overall molecular frequency of mismatch repair gene mutations is 7/702 (1%, CI=0.5-2%): 0.72% (CI=0.3-1.7%) for the *MLH1*/*MSH2* gene mutations and 0.28% (CI=0.08-1%) for the *MSH6* gene mutation. In 3/7 (43%, CI=16-75%) cases mutations were of the missense type, in 1/7 (14%, CI=3-51%) case of the nonsense type and in another 3/7 (43%, CI=16-75%) cases of the frameshift (deletion) type. Only one out of the seven mutations was registered in the Human Genome Mutation Database and ICG (International Collaborational Group)-HNPCC mutation database.

Molecular features of definitive and suspected HNPCC. In this group altogether three MMR gene mutations were detected. In the patient with a mutation in the *MSH2* gene ex12, a missense change of unknown significance in the *MLH1* gene ex17 (1964T/C, I655T) localized in region PMS2 also was found. No mutations were detected in the one Amsterdam I positive family.

The mutation detection rate in the suspected HNPCC group was 3/13 (23%, CI=8-50%). The results of the genetic examinations of the clinical subgroups of HNPCC can be seen in Table III.

Molecular features of extended HNPCC. In 1/10 families matching the extended HNPCC criteria, 1 mutation in the *MLH1* gene ex16 (c.1745 T/C (Leu582Pro) was found. The *MLH1*/*MSH2* mutation detection rate in the extended HNPCC group was 1/15 (7%, CI=1.2-29.8%).

Molecular features of HNPCC late onset. In this group one mutation in the *MSH6* gene ex4 (c.1580delT,frameshift) was detected. The *MSH6* mutation detection rate in the group was 1/10 (10%, CI=1.8-40.4%).

Two HNPCC late onset families with considerably earlier average cancer diagnosis age (52 years in comparison to 60 years and more in the other eight cases) were selected for *MLH1*/*MSH2* gene sequencing and in one case mutation in the *MSH2* gene ex15 (c.2529-2530 del TG (frameshift)) was found. Accordingly the *MSH2* detection rate in the group was 1/10 (10%, CI=1.8-40.4%). The overall mutation detection rate in the HNPCC late onset group was 2/10 (20%, CI=6-51%).

Molecular features of the IH examined group of colorectal cancers. In 182 cases the *MSH2* and *MSH6* protein expression in normal and tumor tissue of the CRC was evaluated. The frequency of *MSH2* protein expression negative cases in the CRCs was 4/182 (2.2%, CI=0.9-5.5%), the frequency of *MSH6* protein expression negative cases in the CRCs was 18/182 (9.9%, CI=6.4-15.1%). In 2/182 (1.1%, CI=0.3-3.9%) tumors both *MSH2* and *MSH6* negative protein expression was observed. Altogether in 20/182 (11%, CI=7.2-16.4%) patients negative protein expression of either or both of the *MSH2* or *MSH6* genes was observed.

In 15/20 (75%) patients with negative protein expression it was possible to obtain blood samples. No mutations 0/3 (0%, CI=0-56%) were detected in complete *MSH2* gene sequencing of the negative *MSH2* gene protein expression cases. In 1/14 (7.1%, CI=1-31%) cases of negative *MSH6* gene protein expression, a missense mutation in the *MSH6* gene (ex4 Phe985Leu(C/G)) was found. In this case negative expression of the *MSH2* gene protein was also detected. In 1/2 (50%, CI=9-91%) cases with negative protein expression of both the *MSH2* and *MSH6* genes, a mutation in the MSH6 gene was found.

Discussion

The results of this study have shown that the frequency of Amsterdam positive families in Latvia is 0.14% of all newly diagnosed CRC cases. The closest frequency of Amsterdam positive families has been reported from the United Kingdom, where 0.3% of all newly diagnosed CRC cases matched those diagnostic criteria (11). In other studies from Sweden, Denmark, Finland, Italy, USA and Israel, the incidence of Amsterdam positive cases is between 0.5% and 1.5% of all newly diagnosed CRC cases (12-17). Raedle *et al*. have reported a 3.2% frequency of Amsterdam positive cases in their series in Germany; however, the number of CRC patients involved in that study was only 145 (18). According to the available data it is possible to conclude that the Amsterdam positive families frequency of 0.14% in our study is smaller than in all other previously studied Western populations. However, this difference is statistically significant only in comparison with the frequencies reported from Denmark and Germany (13, 18), and not to all the other cases reported in our discussion. Unfortunately, it is not possible to exclude bias related to the poor reliability of collected pedigree information. Many families have small numbers of relatives and they have very poor medical information about one another for several different historical reasons. Also another study from Eastern Europe has reported on the limitations of the application of pedigree/clinical data in the diagnosis of HNPCC due to poor availability of medical documentation of patients' relatives and the small size of contemporary families (20).

Molecular results from this study revealed that all seven families with MMR gene mutations did not fulfill the Amsterdam criteria. In addition, a case published earlier by our group with a mutation in the *MSH6* gene ex4 also did not fulfill the Amsterdam criteria (19). This confirms the other reports that in certain populations the use of the Amsterdam criteria is very limited and alone they are not sufficient to identify the majority of families with MMR gene mutations (20). Such a conclusion is further confirmed by finding that in the majority of the mutation positive families only two or fewer cancers were revealed in the pedigree. This is in contrast with a report of Park, where only a small proportion of the patients had *MLH1*/*MSH2* mutations in families not matching the Amsterdam criteria (8). Thus, the use of particular HNPCC diagnostic criteria should be adapted for different geographical regions.

Additionally, it should be mentioned that the first reported HNPCC family in Latvia with a mutation in the *MSH2* gene fulfilled the classical Amsterdam I criteria (21). But since 1997 it remains the only case of a "good" Amsterdam family in Latvia.

There are only a few reports on the incidence of suspected HNPCC criteria in other populations and nuclear pedigree criteria for suspected HNPCC were not always used. The 1.7% frequency of suspected HNPCC cases reported from Finland is very similar to the 1.9% in our study (14). The incidence reported from the United Kingdom is slightly lower at 1.4% (11). A considerably higher proportion, using nuclear pedigree criteria has been reported from Poland with 6.84% (CI=5.1-9.1%) of all newly diagnosed CRCs (20), as well from Italy with 4.6% (CI=3.7-5.7%) (22); this difference is statistically significant (20, 22).

The mutation detection rate in the suspected HNPCC group was 23% in the present study which is comparable to other reports, where the rate was between 29% and 34% (8, 20, 23). Such a relatively high mutation detection rate confirms the efficacy of the suspected HNPCC criteria identifying the families with MMR gene mutations and diagnosing HNPCC. Fifty percent (3/6) of the mutations in our study were detected in the suspected HNPCC group. Although the suspected HNPCC criteria are less specific than the Amsterdam criteria for the detection of families with MMR gene mutations (25-34% *vs.* 50-60%), our results in agreement with several other reports have clearly shown that the use of the suspected HNPCC criteria allows the identification of the majority of HNPCC cases. 1.4% of the cases in our study fulfilled the HNPCC late onset criteria. There are no reports of the frequency of those criteria in other populations. The frequency of *MSH6* gene ex4 mutations in the group was 10% (1/10) in our study.

Another *MSH6* gene ex4 mutation published earlier by our group was also detected in a family matching the

HNPCC late onset criteria (19). This finding is in agreement with other reports on the later onset of cancer with the *MSH6* phenotype compared to *MLH1*/*MSH2* cancers (24-27). More than 40 *MSH6* mutations have been reported in the literature and the average age of onset for colorectal and endometrial cancer was 53 and 56 years, respectively (24). The average colorectal and endometrial cancer onset age in the two *MSH6* families in our study was 62.5 (5 cases) and 57.5 (2 cases), respectively. The average CRC onset age is considerably later, but the number of cases is not sufficient to draw any conclusions on the *MSH6* phenotype. However, the importance of recognizing the HNPCC late onset group in order to diagnose this subgroup of HNPCC and to select patients for *MSH6* gene ex4 testing has been confirmed by this study and other reports.

The molecular frequency of HNPCC was 1% in our study, which was very similar to the 0.86% reported from the USA (28) and 0.8% in Denmark, (13) while a lower incidence of 0.3% has been reported in Italy (29). Foulkes *et al.* reported 0.44% molecular frequency of HNPCC in Israel, but only the most frequent found mutation was tested in that study (17). A considerably higher incidence of 2.0-2.7% has been reported in Finland (30), 2.7% in Germany (31) and Poland (20, 30, 31).

Seven out of the nine (78%) mutations detected in Latvia so far have not been reported previously in either the Human Genome Mutation Database or in the ICG-HNPCC mutation database. According to our results, the MMR mutation spectrum in HNPCC patients in Latvia is different from the neighboring countries, such as Poland, Finland, Estonia and Lithuania. No significant effect in HNPCC cases has been detected in Latvia so far, and the mutation spectrum has to be considered as potentially unique.

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