Initial Results of Colorectal Polyposis Research in Latvia

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Abstract. *Aim: Patients suffering from colorectal polyps are more likely to develop a malignant condition with poor prognosis. The aim of the study is to investigate clinical and molecular features of colorectal polyposis syndromes in Latvia in order to offer and provide predictive genetic testing for the affected families, as well as to evaluate the frequency of familial adenomatous polyposis (FAP) in Latvia. Patients and Methods: Six polyposis patients along with three of their relatives were included in this study. Two patients were selected from the colorectal cancer database (from a total of 2,552), and four patients not affected with colorectal cancer (CRC) were referred from the endoscopic facility of our hospital. All the patients were examined during the period from January 1st, 2000 until June 30th, 2007. Clinical data, histological examinations and family cancer histories of the respective patients were evaluated. Screening for germline APC mutations was performed in five patients and their relatives. In addition, all patients underwent genetic counseling. Results: Two patients out of 2,552 from the CRC Hereditary Cancer Institute database fulfilled the clinical criteria for FAP. Thus, the frequency of FAP is 0.08% (2/2,552) of all CRC cases, and comprises ~0.0003% of the population of Latvia (7/2.2 million inhabitants). Unknown polyposis was identified in two cases. Pathogenic APC gene mutations were detected in five out of seven examined patients and their relatives. Two of the mutations (c.3942delG;p.Arg1314SerfsX7 and c.3286C>T;p.Gln1096X) are novel. Conclusion: In this study, we report the first four APC mutation-positive FAP cases in Latvia. The present frequency of FAP is lower than that reported in Finland, Lithuania, and other neighbouring countries, but the numbers might increase if a more systematic identification approach is used. Initial molecular examinations reveal partially unique spectrum of APC gene mutations.*

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Key Words: Colorectal cancer, hereditary cancer, familial adenomatous polyposis, APC gene.

Familial adenomatous polyposis (FAP) accounts for less than 1% of the annual colorectal cancer (CRC) burden. There are about 1,000 new CRC cases every year in Latvia and one would expect up to 10 cases of suspected FAP- or MYH-associated polyposis (MAP), or other polyposis syndromes in Latvia annualy (1-4).

Taking into consideration the prevalence of FAP cases among the CRC patients in four main Latvian cancer hospitals, as well as among the patients referred from outpatient departments, we present the first data on the frequency of FAP in Latvia and show the first results of FAP molecular diagnostics in this country.

Patients and Methods

Patients. Six polyposis patients and three of their relatives constituted the study group. The inclusion criterion for patients was the presence of more than 50 polyps in the colon (FAP suspicious). Two patients were included from the Hereditary Cancer Institute CRC database (a total of 2,552) and treated in four main Latvian cancer hospitals from January 1st 2000 to June 30st 2007. Four patients not affected with colorectal cancer referred from endoscopic facility were selected during the same period of time. Clinical data, histological examinations, and family cancer histories of the respective patients were evaluated. Screening for germline *APC* mutations was only performed in five patients and their relatives. All patients underwent genetic counseling.

Mutation analysis. Blood samples were obtained from five out of nine polyposis patients and their relatives. All samples were tested for *APC* germline mutations at the Institute of Human Genetics, Bonn, as described elsewhere (5).

Genomic DNA was isolated from peripheral EDTA-anticoagulated blood using standard protocols. Screening for point mutations was performed on genomic DNA using the protein truncation test (PTT) for mutations in exon 15, and denaturing high performance liquid chromatography (DHPLC) for mutations in exons 1-14 and the first 400 base pairs of exon 15. Briefly, PCR was performed on 100 ng of genomic DNA using the HotStarTaq Master Mix Kit (QIAGEN, Hilden, Germany) and standard thermocycling conditions on a PTC-200 thermocycler: 5 min denaturation at 94˚C, followed by 33 cycles of denaturation for 30 s at 94˚C, annealing for 20 s at 56˚C and extension for 1 min at 72˚C, and final extension for 20 min at 72˚C. PCR fragments demonstrating an aberrant pattern by either method were sequenced on an ABI 3100 automated sequencer (Applied

Biosystems, Darmstadt, Germany) using the cycle sequencing procedure and the BigDye terminator kit version 1.1.

Screening for large genomic deletions or duplications was performed by MLPA (multiplex ligation-dependent probe amplification) using the SALSA P043 APC exon deletion test kit (MRC Holland) according to the manufacturer's protocol. Data were analysed by use of GeneMapper, version 4.0 software (Applied Biosystems, Darmstadt, Germany) and gene frequency was calculated using the Coffalyser V4 program (MRC Holland).

Results

Two patients out of 2,552 CRC patients fulfilled the clinical criterion for FAP (>100 polyps). (Table I, patients 1(I) and 4(I)). Thus, the frequency of FAP is 0.08% (2/2,552) of all CRC cases. The pedigree of patient 1(I) is presented in Figure 1.

Of four polyposis patients referred from the endoscopic department, two had more than 100 polyps (3 and 2(I)) and, consequently, matched the clinical criterion for FAP. In the other two cases [5(I) and 6(I)] more than 50 polyps were detected and these cases were classified as unknown polyposis.

Of four FAP cases, three family members with clinical signs of FAP were identified and included in the study group. Thus, the present clinical prevalence of FAP in Latvia is 7/2.2 million inhabitants (0.0003%).

Four different germline mutations were detected in five polyposis cases examined for *APC* mutations (Figure 2). Two of the mutations (c.3942delG;p.Arg1314SerfsX7 and c.3286C>T;p.Gln1096X) are novel. The other two mutations in exon 11 c.1438C>T; p.Gln480X and exon 15 c.2510C>G;p.Ser837X were reported in other populations. Clinical and molecular data are summarised in Table I.

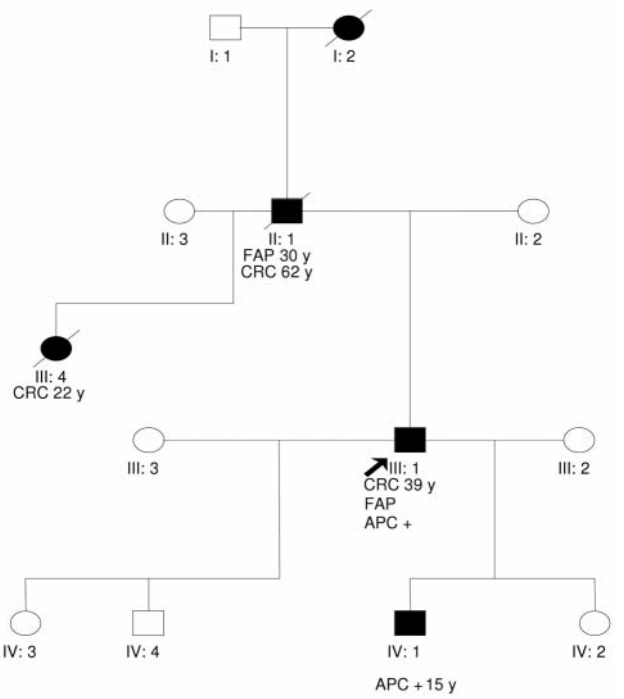

Figure 1. *Pedigree of patient 1(I) The mutation c.3942delG; p.Arg1314SerfsX7 in exon 15G of the APC gene was identified.*

Discussion

Our data include 36% of all CRC cases in Latvia in the last seven years, and all polyposis patients that were referred to us from the endoscopic department of our hospital. Not all patients from all of Latvia's endoscopic facilities were included.

The incidence of FAP/CRC is 0.08% (2/2,552), and the prevalence in the population of Latvia is 7/2.2 million - 0.0003% or 3 per million. The frequency is lower of that reported in the neighbouring countries, as compared with Finnish (2, 3) and Lithuanian populations (unpublished data). Obviously, the FAP frequency in Latvia might increase if a more systematic identification approach is used.

The reports from Finland show the incidence of FAP ranging from 0.62 to 2.38 per million, and the prevalence from 0.88 to 26.3 per million during the study period.

Unpublished data from Lithuania report FAP prevalence rate at 25 cases per million in Lithuania (about 3,500,000 inhabitants). The FAP/CRC ratio was 0.2% overall for colorectal cancer patients diagnosed in Lithuania during 1995-2002. FAP frequencies in different populations are summarized in Table II.

We report the first results of mutation analysis of the *APC* gene in Latvian FAP patients. We have identified 4 *APC* gene mutations. According to the Human Gene Mutation Database at the Institute of Medical Genetics in Cardiff (http:// www.hgmd.cf.ac.uk/ac/all.php), the mutations in codon 1096 and 1314 have not been described before, while mutation in codon 480 was found in an Argentinian population. Two of the most frequent mutations (c.3927_3931delAAAGA and c.3183_3187delACAAA) were not detected in Latvia (5, 8, 12, 13). Thus, initial molecular examinations reveal unique aspects of *APC* gene mutations in the Latvian population.

The detection of Turcot's syndrome in one of the patients (patient 2(II)) is particularly notable due to its relatively rare occurrence.

All the polyposis patients and their relatives underwent genetic counseling. The patients with positive mutation have been evaluated for prophylactic surgical procedures. Only one patient with FAP and rectal cancer (stage IA) underwent total proctocolectomy with ileostoma. However, the other patients have refused preventative surgery.

Table I. *Clinical and molecular data of the six patients with clinical diagnosis of FAP.*

Pt. no./ Age at Dgn	Histology	No. of polyps	Family cancer history	DNA exam of APC	Status	FAP Patient/relative
1(I) 40 y	AdenoCa of the rectum, II stage and adenomatous polyposis	>1,000	Father, polyposis 28 y, CRC 62 y; Father's mother, CRC 72 y; half-sister, CRC 22 y; Son, -FAP 16 y 1(I)	Exon 15 (c.3942delG; p.Arg1314SerfsX7)	Total procto-colectomy, follow up	FAP index patient
1(II) 16 y	Adenomatous polyposis colonis	>1,000	Son of 1(I)	Exon 15 (c.3942delG; p.Arg1314SerfsX7)	Follow up	FAP relative
2(I) 33 y	Adenomatous tubular polyps with dysplasia	>100	Daughter 2(II) - Medulloblastoma 13 y (Turcot's syndrome)	Exon 11 (c.1438C>T; p.Gln480X)	Left hemi-colectomy follow up	FAP index patient
2(II) 13 y	Brain medulloblastoma	unknown	Daughter of 2.1	Unknown	Cranial surgery	FAP relative
3(I) 36 y	Multiple tubulo-villous adenomatous polyps in colorectum	>100	Mother, CRC 55 y, mother's sister, breast Ca 70 y; Mother's father, abdominal Ca 80 y; Mother's mother head & neck cancer 80 y	Exon 15 (c.3286C>T; p.Gln1096X)	Follow up	FAP index patient
4(I) 57 y	Rectal Ca and adenomatous polyps with dysplasia grade I-II in upper and lower GI, abdominal wall desmoid suspected 6 years after colon surgery	>100	Sister CRC 48 y; daughter 4(II) – polyposis 25 y Mut APC+	No DNA sample available	Died at age 57	FAP index patient
4(II) 25 y	Rectosigmoidal adenomatous polyposis	<50	Daughter of 4(I)	Exon 15 c.2510C>G; p.Ser837X	Follow up	FAP relative
5(I) 24 y	Hyperplasio-geneous polyps and tubular adenomas with dysplasia grade I	50-100	Mother CRC 45 y; mother's sister, uterine Ca 33 y	APC-neg MYH-neg	Follow up	Unknown polyposis
6(I) 28 y	Tubulo-villous adenomatous polyps	50-100	Mother, stomach cancer 38 y	APC-neg MYH-neg	Follow up	Unknown polyposis

Ca, carcinoma; y, years (Age at diagnosis).

Table II. *European data for FAP.*

Country	Incidence FAP/CRC	Prevalence FAP	Author, Year of reference
Denmark	0.7%	70 Families	Bisgaard *et al*., 2004 (6)
Poland	Nd	280 FAP families	Plawski *et al*., 2007 (7)
Germany	Nd	1248 Unrelated patients 680 FAP families	Aretz *et al*., 2007 (5, 8)
Finland	Nd	81 Families; 251 affected patients	Jarvinen *et al*., 1992 (3)
Netherlands	Nd	315 FAP families	Nielsen *et al*., 2007 (9)
Greece	Nd	25 Families	Mihalatos *et al*., 2005 (10)
Lithuania	0.2%	17 Patients	Samalavičius and Kilims, 2005 (II)
Latvia	0.003%	4 Families (7 patients) 2 Families with unknown polyposis	Unpublished data

Nd, No data.

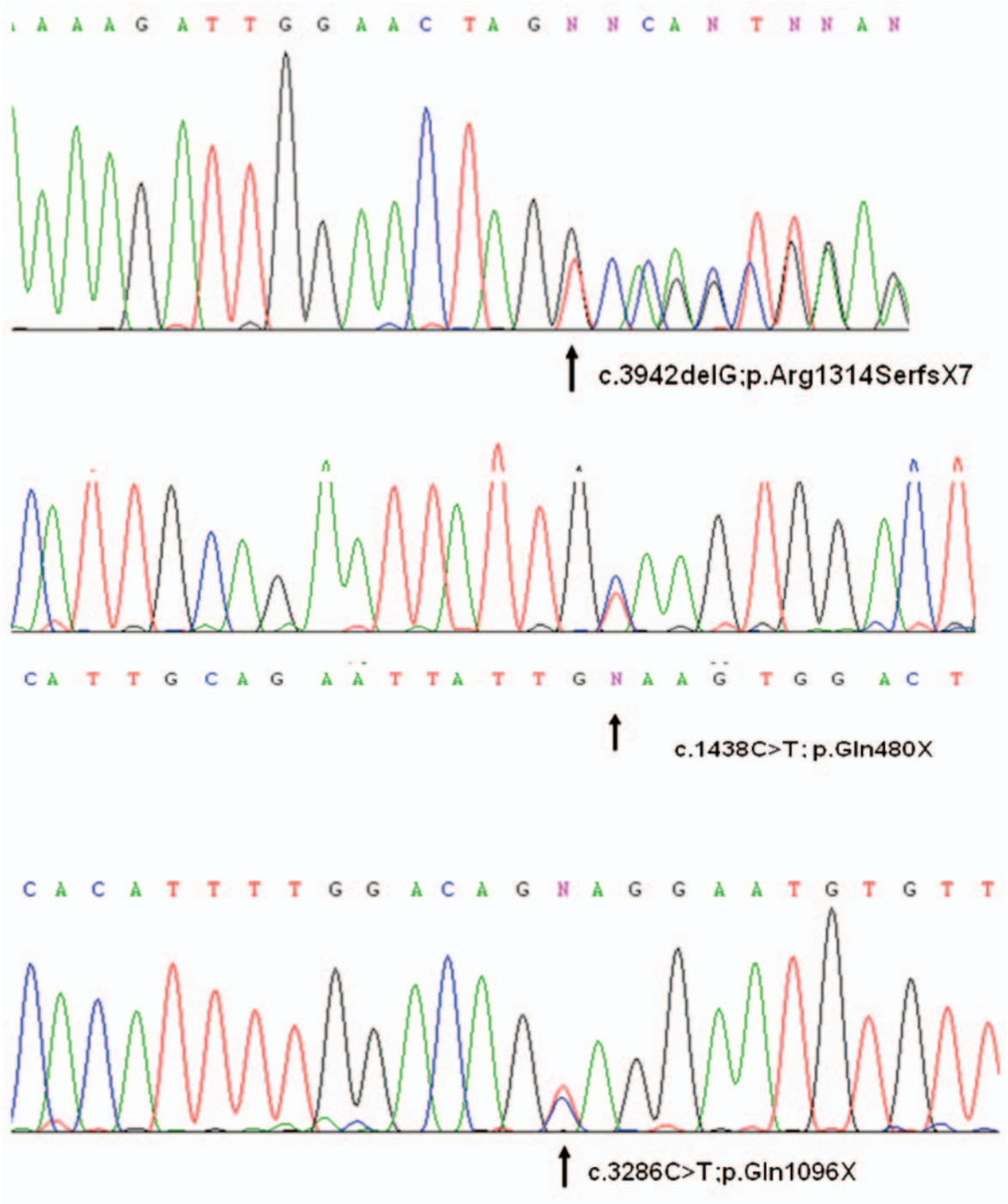

Figure 2. *Sequencing pattern of the mutations identified in the three Latvian patients affected with familial adenomatous polyposis.*

Conclusion

In this study, we report the first four *APC* mutation-positive FAP cases in Latvia. The present frequency of FAP is lower of that reported in Finland, Lithuania and other neighboring countries, but the numbers might increase if a more systematic identification approach is used. Initial molecular examinations reveal unique aspects of *APC* gene mutations.

Acknowledgements

The authors would like to acknowledge the help of Dr. sc. hum. Waltraut Friedl and Dr. Stefan Aretz from the Institute of Human Genetics, University of Bonn, Germany for advice, collaboration and cooperation in mutation detection and results interpretation.

We also expressed many thanks to the colleagues from the Latvian cancer hospitals for their help in establishing the registry.

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Received April 11, 2008
Revised November 19, 2008
Accepted December 2, 2008