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Molecular-genetic profiling in patients with adenomatous polyps of the gastrointestinal tract

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ABSTRACT *AIM: to reveal hereditary mutations in patients with adenomatous polyps of the gastrointestinal tract*
PATIENTS AND METHODS: a retrospective cohort study included 8 patients with adenomatous polyps of the gastrointestinal tract (ranging from 4 to several hundred). The APC, AXIN2, BMPR1A, BRCA2, CDH1, CHEK2, EPCAM, GALNT12, GREM1, MLH1, MLH3, MSH2, MSH3, MSH6, MutYH, NTHL1, PMS2, POLD1, POLE, SMAD4, STK11 genes were studied using new generation sequencing.
RESULTS: five patients were found to have pathogenic mutations in the genes APC (3 patients with > 100 polyps), POLE (1 patient with < 10 polyps), MutYH (1 patient with 2 mutations with > 28 polyps; 1 patient with monoallelic mutation in combination with a mutation in the APC gene with a number of polyps > 100).
CONCLUSION: the probability of detecting a pathogenic mutation increases with an increase in the number of polyps in a patient.

KEYWORDS: *adenomatous polyps, gastric polyps, colon polyps, familial adenomatous polyposis, NGS, targeted sequencing*

CONFLICT OF INTEREST: *The authors declare no conflict of interest*

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INTRODUCTION

Colorectal polyps (CP) are neoplasms protruding above the surface of the mucous layer into the lumen of the bowel [1]. According to the classification of the World Health Organization (WHO), polyps can be divided into four types: adenomatous, inflammatory, hyperplastic and hamartomic [2].
According to the World Gastroenterological Society, during colonoscopy as part of screening, adenomatous polyps are diagnosed in 18–36% of patients [3].
Gastric polyps are found in 1–4% of patients after gastroscopy. Hyperplastic and adenomatous polyps are often found against the background of *H.Pylori* infection, but they can also manifest in hereditary tumor syndromes (HTS), such as Lynch syndrome, familial colon adenomatosis (FCA). The recognition of gastric syndromic polyps is important for the management of patients [4].
There are classical and attenuated FAP. The classical form of FAP is characterized by the development of one hundred to thousands of adenomas of the rectum and colon during the second to third decade of life. The incidence of FAP is approximately 1 in 8,300 cases. This disease is the cause of about 1% of cases of colorectal cancer (CRC) [5]. Classical FAP is inherited by autosomal dominant

type and occurs as a result of germinal mutation in the *APC* gene. APC protein is a classical tumor suppressor that plays a central role in the signaling of the Wnt cascade, partly by regulating the degradation of β-catenin. In about 10% of patients, mutations in the *APC* gene occur *de novo*, mosaicism is often found [5,6].

Attenuated FAP (AFAP) is characterized by a smaller number of adenomatous colorectal polyps (usually less than 100) and a later age of their appearance [7]. Patients with AFAP also have an increased risk of developing malignant neoplasms [8]. AFAP is caused by mutations in the *APC* gene in codons 1–157, 1595–2843 and exon 9 [9]. At the same time, a similar clinical picture is in the presence of mutations in the *MutYH* and *POLD1/POLE* genes.

MutYH -associated polyposis (MAP) is characterized by the development of 20 to 100 adenomatous colorectal polyps; however, hyperplastic, dentate polyps and mixed (hyperplastic and adenomatous) polyps can occur. Duodenal adenomas are common. In some cases, patients may have a phenotype similar to the classical form of FAP [10]. In patients with MAP, the risk of developing CRC at the age of 60 ranges from 43% to 100%. In some patients, CRC develops in the absence of polyposis [11,12].

The cause of MAP is the presence of a homozygous or compound heterozygous mutation in the *MutYH* gene, which encodes DNA glycosylase involved in excisional DNA repair.

Another form of familial polyposis associated with germinal mutations in the *POLE* and *POLD1* genes (Polymerase Proofreading-Associated Polyposis, PPAP), encoding the exonuclease domain of DNA polymerases epsilon and delta, respectively. In this case, the exonuclease activity of the DNA polymerase is lost, while the polymerase activity remains. Tumors of such patients have an MSS phenotype, but accumulate missense mutations [12].

NTHL1, as well as *MutYH*, is a DNA glycosylase gene underlying autosomal recessive polyposis with high penetrance. *NTHL1* encodes DNA glycosylase of the excision repair pathway [13]. Recessive inheritance of adenomatous polyposis associated with mutations in the *MSH3* gene has been described in a number of patients [14].

AIM

Identification and analysis of molecular genetic characteristics of patients with adenomatous polyps of the gastrointestinal tract (GIT).

PATIENTS AND METHODS

The study included peripheral blood samples from 8 patients who underwent check-up and treatment in 2020–2021. Half of the patients (n = 4) had no gastrointestinal complaints; polyps were diagnosed during a routine check-up. In 4 patients, polyposis was accompanied by pain in the epigastric region, bloating, frequent liquid stools or constipation. The average age at the time of diagnosis of polyps was 39 ± 13 years. In most cases (n = 7) isolated colorectal polyposis was detected, one patient had synchronous gastric lesion.

Total polyposis was diagnosed in 2 patients. Four patients had a history of malignant neoplasms (MN) of various locations. Detailed clinical data of patients are described in Table 1. This study was approved by the local Ethics Committee of the SBHI LMCSC; and informed consent was obtained from each patient.

DNA isolation from peripheral blood lymphocytes was carried out using the QIAamp DNA Blood Mini Kit (Qiagen), on the QIAcube automated DNA, RNA and protein isolation system.

DNA libraries were prepared using the KAPA Hyper Prep Kit (Roche) according to a standard protocol with enzymatic fragmentation of nucleic acids. Hybridization selective enrichment was carried out using a custom panel of probes using the standard Hyper (Roche) protocol. The design of the probe panel was carried out using the Hyper Design (Roche) online service, included coding sites, splicing sites and UTR regions related to genes associated with the development of the HTS, including *APC, AXIN2, BMPR1A, BRCA2, CDH1, CHEK2, EPCAM, GALNT12, GREM1, MLH1, MLH3, MSH2, MSH3, MSH6, MutYH, NTHL1, PMS2, POLD1, POLE, SMAD4, STK11,* disorders in which are associated with the development of gastrointestinal polyps, as well as gastric cancer and CRC.

The MiSeq system (Illumina) was used as a sequencing platform. The sequencing data were

Table 1. *Clinical characteristics of the examined group of patients*

№	Gender	Clinical symptoms	Age	Location of polyps	Number of polyps	MN in history (AD)	Familial history (relatives of I/II degree of kinship)
1	Female	None	39	Colon	4	GC(39)	GC/none
2	Male	Bloating, frequent, loose stools	23	Colon + Gastric	Totally	None	None/ None
3	Female	Epigastric pain	21	ТК Colon	< 10	PTK (21) CC(21)	None/ None
4	Female	None	59	ТК Colon	> 15	None	PC/PC, GITC
5	Male	None	60	Colon	> 100	None	AP/none
6	M Male	Blood impurities in the stool	50	Colon	> 28	Synchronous CC (50)	None/ None
7	M Male	None	23	Colon	Totally	Hepatoblastoma (1)	AP/none
8	M Male	Blood impurities in the stool	37	Colon	> 10	None	Leukemia/none

Abbreviations: AD — age at the time of diagnosis, GC — gastric cancer, PC — prostate cancer, GITC — cancer of the gastrointestinal tract, AP — adenomatous polyposis, CC — colon cancer, MN — malignant neoplasm, C — colon, G — gastric.

analyzed in accordance with the recommendations of GATK Best Practices (Broad Institute) to search for germinal and somatic mutations according to the algorithm we described earlier [15].

RESULTS AND DISCUSSION

The hereditary nature of the disease was established in 5 patients (Table 2). In one patient with a clinical picture of total colorectal polyposis and synchronous gastric polyposis and another patient with total colorectal polyposis and hepatoblastoma, the previously described germinal pathogenic variants of the nucleotide sequence (VNS) were identified c.3927_3931del, p.Glu1309 AspfsTer, rs121913224 and c.3183_3187del, p.Gln1062Ter, rs587779352 in the *APC* gene in heterozygous form accordingly, associated with the classical form of FCA (OMIM# 175100). A 60-year-old patient with more than 100 adenomatous polyps, who has a relative with adenomatous polyposis, revealed the previously described VNS c.1192_1193del, p.Lys398GlufsTer, rs387906238 in the *APC* gene in heterozygous form, as well as c.1187G > A, p.Gly369Asp, rs36053993 in the *MUTYH* gene in heterozygous form.

Taking into account the clinical picture and the presence of pathogenic VNS in the 398 codon of the *APC* gene, we can talk about the presence of an attenuated form of AFCA in this patient.

In a 50-year-old patient with the presence of more than 28 adenomatous polyps, synchronous CRC and the absence of MN in a family history in the *MUTYH* gene, VNS c.1187G > A, p.Gly369Asp, rs36053993 in heterozygous form, described in the Varsome, InSight databases as pathogenic, and c.548G > A, p.Gly183Asp, rs587781864 is registered in the Varsome database as probably pathogenic.

Taking into account the characteristic clinical picture and autosomal recessive type of inheritance, this disease should be regarded as *MUTYH*-associated colon polyposis (OMIM # 608456).

The most interesting case is a 21-year-old patient with less than 10 polyps and adenocarcinoma of the rectosigmoid part with liver metastases. The patient revealed a previously undescribed VNS

Table 2. *Germline pathogenic variants of nucleotide sequence*

№	Gene	VNS	rsID	Pathogenic variant class
1	Not revealed			
2	*APC*	chr5:112839515delAAAAG c.3927_3931del, p.Glu1309AspfsTer	rs121913224	Class 5 (Pathogenic)
3	*POLE*	chr12:132676655T > C c.802-2A > G	–	Class 5 (Pathogenic)
4	Not revealed			
5	*MutYH*	chr1:45331556C > T c.1187G > A, p.Gly369Asp	rs36053993	Class 5 (Pathogenic)
	APC	chr5:112819224 delAA c.1192_1193del, p.Lys398GlufsTer	rs387906238	Class 5 (Pathogenic)
6	*MutYH*	chr1:45331556C > T c.1187G > A, p.Gly369Asp	rs36053993	Class 5 (Pathogenic)
	MutYH	chr1:45332791C > T c.548G > A, p.Gly183Asp	rs587781864	Class 4 (LikelyPathogenic)
7	*APC*	chr5:112838774delAAAAC c.3183_3187del, p.Gln1062Ter	rs587779352	Class 5 (Pathogenic)
8	Not revealed			

c.802-2A>G in the *POLE* gene, which is located in the highly conserved region of the gene and is represented by the replacement of one nucleotide in the canonical splicing site. According to ACMG criteria [16], this VNS can be regarded as pathogenic clinically significant, associated with PPAP and predisposition to CRC, type 12 (OMIM # 615083). According to the study of Bellido et al, in patients with pathogenic VNS in the *POLE* gene, more than 2 colon adenomas occur in 81.8% of cases, the average number of colorectal adenomas is 19.3 (1–68), and the incidence of CRC in carriers of the *POLE* mutation is 63.8%, the average age at the time of diagnosis is 40.7 years. It should be noted that in this study only patients with the missense variant c.1270C > G, p.Leu424Val, rs483352909 were considered, and for patients with nonsense variants or mutations of the splicing site, the clinical picture may differ. The authors propose criteria for selecting patients for genetic testing for the presence of mutations in the *POLE* gene: the presence of 20–100 adenomatous colorectal polyps, or compliance with the criteria of Amsterdam I, or the presence of CC and 5–20 adenomatous colorectal polyps under the age of 50, or the presence of CRC and 5–20 adenomatous polyps and a relative of the 1st degree of kinship with CC diagnosed at the age of 50, or the presence of CRC and 5–20 adenomatous colorectal polyps and more than two relatives of 1–2 degrees of kinship with CRC diagnosed at any age [17].

CONCLUSION

Recently, several dominant and recessive HTS have been described, the clinical manifestation of which are adenomatous colorectal and/or gastric polyps.

In this regard, there is a need to work out a strategy for molecular genetic testing, approve the necessary minimum list of genes and the sequence of tests. Also, one of the important steps towards determining the optimal treatment approach,

prevention, early diagnosis, further support of patients with polyposis may be the organization of follow-up of patients carrying the mutation.

AUTHORS CONTRIBUTION

Sample collection and clinical and anamnestic characteristics: *Anastasia M. Danishevich, Alexandra E. Valeeva*

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