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The RAS/*BRAF* genes status in patients with colorectal cancer (review)

Ekaterina A. Kazachenko[1,2], Vitaly P. Shubin[3], Stanislav S. Otstanov[4], Alexey S. Tsukanov[3], Evgeny A. Khomyakov[3,5]

[1]Center for additional professional and online education "PUSK", Moscow Institute of Physics and Technology (MIPT, PhysTech) (Institutskiy lane, 9, Dolgoprudny, Moscow, 141700, Russia)
[2] M.V. Lomonosov Moscow State University (Lomonosov MSU) (Leninskie Gory st., 1, Moscow, 119991, Russia)
[3]Ryzhikh National Medical Research Center of Coloproctology (Salyama Adilya st., 2, Moscow, 123423, Russia)
[4]Laboratory for the Analysis of public Health indicators and Digitalization of Healthcare, Phystech school of biological and medical physics of Moscow Institute of Physics and Technology (MIPT, PhysTech) (Institutskiy lane, 9, Dolgoprudny, Moscow, 141700, Russia)
[5]Russian Medical Academy of Continuous Professional Education (Barrikadnaya st., 2/1, bld. 1, Moscow, 125993, Russia)

ABSTRACT *Colorectal cancer (CRC) is the third in prevalence among oncological diseases worldwide and second in the structure of oncological mortality. Genetic assessment of CRC is a necessary stage during selecting further treatment for patients. Many studies demonstrate a diverse distribution of mutations in the KRAS, NRAS, and BRAF genes in CRC. A critical literature review was conducted in order to systematize data on the mutational profile and genetic heterogeneity of these driver mutations in Russian patients with CRC. Articles were searched for in open databases. Totally 17 Russian studies and 3 English meta-analyses were analyzed for comparison with Russian data.*
Mutations in the KRAS, NRAS, and BRAF genes, according to Russian and international studies, are found in 40%, 4%, and 7% in CRC patients, respectively. The frequency and specific localization of mutations may depend on the geographical location and nationality of the cohort. High intertumoral and intratumoral heterogeneity in CRC, especially in KRAS gene mutations, significantly influences the choice of further therapy and underscores the need for more detailed study of the mutational profile of the primary tumor, affected lymph nodes, and distant metastases.
In Russia, several molecular genetic methods are used to determine somatic mutations in CRC with different sensitivity and specificity, the most common is real-time PCR. More accurate diagnostic methods include digital droplet PCR, Sanger sequencing, and next-generation sequencing, but each method has its limitations that must be considered when planning diagnostics and research. The promising directions in personalized oncology is the study of gene copy number variations, which may contribute to the development of new methods for treating CRC in the future.
Despite the large number of studies, some aspects of the mutational profile of CRC in Russian studies remain poorly understood, which is why further research is needed on patients with colorectal cancer in Russia.

KEYWORDS: *Colorectal cancer, mutation profile, heterogeneity, KRAS, BRAF, NRAS*

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ADDRESS FOR CORRESPONDENCE: *Ekaterina A. Kazachenko, I year student of the Master's degree in "Applied Data Analysis in the medical field", center for additional, additional professional and online education "PUSK", Moscow Institute of Physics and Technology (MIPT, PhysTech), 9 Institutskiy lane, Dolgoprudny, Moscow, 141700, Russian Federation; phone: +7 (926) 972-19-22; e-mail: ekaterina.k.97@mail.ru,*

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INTRODUCTION

Colorectal cancer (CRC) ranks third in prevalence among all oncological diseases in the world and second in the structure of oncological mortality [1]. Environmental factors, lifestyle, dietary patterns, as well as genetic predisposition and some concomitant diseases play a key role in the etiology of CRC [2–6]. According to world estimates, approximately 90–95% of cases of CRC occur in people without hereditary genetic mutations due to acquired somatic and epigenetic modifications [7]. The development of molecular genetic technologies and research methods has led to the fact that

today the assessment of the mutation profile of a tumor is standard clinical practice [8]. In the later stages of the disease, the choice of treatment regimen and further prognosis largely depend on the mutational status of the tumor [8]. In the vast majority of cases, colorectal tumors contain mutations in the genes *KRAS, BRAF, APC, TP53, PIK3CA, NRAS, SMAD4* [9,10]. Somatic mutations in such genes activate several signaling pathways, in particular RAS-RAF-MAPK and PI3K-PTEN-AKT, which lead to uncontrolled cell growth, proliferation and malignancy progression. According to obtained data, mutations in RAS oncogenes (*KRAS* and *NRAS* genes) are detected in about half of the cases of CRC, while the proportion of mutations in *KRAS* is 13–66% of cases, and *NRAS* is only 2–9.5% [10,11,20–24,12–19]. 1–17% of patients with CRC have a mutation in oncogenes of the RAF family [10,11,13–16,20,22–24]. Since 2004, when the "Food and Drug Administration (FDA) for Quality Control" has approved the use of the chemotherapeutic monoclonal drug cetuximab in patients with metastatic colorectal cancer, directed against the epidermal growth factor receptor (EGFR) [25], the era of targeted drugs has begun. The oncogenes *KRAS, NRAS* and *BRAF* play a crucial role in determining the sensitivity of a tumor to such therapy, while mutations in these genes lead to complete or partial resistance [2,13,20,26–29]. However, the presence of a wild type of gene is not always associated with a complete response, which may be due to the presence of additional genetic changes associated with resistance [30].

Thus, the genetic assessment of large intestine tumors is a necessary condition at the stage of choosing further treatment for patients. Currently, there are many studies in the literature presenting the results of assessing the motivational profile and genetic heterogeneity of CRC in various countries, which demonstrate a diverse pattern of distribution of the studied mutations. In the Russian literature, unjustifiably little attention is paid to these fundamental issues. The available information on the mutational profile of the CRC is very heterogeneous and scattered. Moreover, the results of Russian research are not taken into account in most international meta-analyses. For these reasons, we conducted a critical review of the literature in order to systematize data on the assessment of the mutation profile and genetic heterogeneity of *KRAS, NRAS*, and *BRAF* gene mutations in patients with colorectal cancer.

MATERIALS AND METHODS

The search for Russian-language publications was carried out in the scientific electronic library eLIBRARY and the Cyberleninka database using the following keywords: 'colorectal cancer', 'KRAS', 'NRAS', 'BRAF'. Thus, 389 articles were found (264 in eLIBRARY, 125 in Cyberleninka), of which 18 studies were selected. The search was done by one researcher. The analysis included clinical studies performed in the territory of the Russian Federation, in which statistical data on the rate of mutations in the *KRAS, NRAS, BRAF* genes in patients with CRC at any stage, as well as depending on gender, localization of the primary tumor, and degree of differentiation were presented as results. The search was not limited to full-text journal articles: the search also looked at research results published as conference abstracts or available only as research summaries. If the necessary information was available (the occurrence rate of the studied mutations, other clinical and demographic characteristics), such studies were also included in the analysis. Thus, for each feature, the results were entered in summary tables. Duplicated studies (3 studies) were excluded from the analysis. A quantitative meta-analysis was not carried out, as it was not the purpose of this review. As a comparison of the occurrence rate of certain parameters and characteristics of mutations, international meta-analyses were analyzed. The search for English-language publications was carried out in the databases Scopus, Cochrane, EMBASE using the following keywords: 'colorectal cancer',

'meta-analysis', 'KRAS', 'NRAS', 'BRAF'. As a result of a search in English-language databases, 2 studies conducted in the territory of the Russian Federation were found, which were also included in the review. Thus, only 17 Russian studies were analyzed. For comparison with the Russian data, 3 meta-analyses were found, which were also included in the summary tables.

The Role of KRAS, NRAS, BRAF Genes in Carcinogenesis of Colorectal Cancer

There are 3 main pathways of CRC carcinogenesis: classical, dentate and inflammatory. The classical pathway is associated with chromosomal instability (CIN), the dentate pathway — with hypermethylation of CpG islands (CpG island methylator phenotype, CIMP) and Microsatellite instability, (MSI) [31]. The inflammatory pathway is the rarest of carcinogenesis, occurring in about 2% of all cases of CRC [32]. As a result of chromosomal instability, various quantitative and structural changes in chromosomes occur, which can affect proto-oncogenes and tumor suppressor genes. Mutations are most often found in the *APC* tumor suppressor gene (adenomatous polyposis coli), proto-oncogenes of the RAS family (*KRAS, NRAS* genes) and RAF (*BRAF* gene), and the *TP53* tumor suppressor gene [2]. The RAS family of proto-oncogenes plays the role of a regulator of the epidermal growth factor receptor (EGFR), limiting cell growth, proliferation, migration and differentiation. The proteins synthesized by them, as a product of transcription and translation of RAS family genes, in the cell play the role of a signaling mediator between the EGFR receptor and further signaling pathways inside the cell — RAF-MEK-ERK and PI3K-AKT-m TOR, which in turn activate further cell proliferation and differentiation. During the transmission of the signal from EGFR, RAS proteins are activated due to the addition of a guanosine triphosphate molecule. After performing their function, proteins lose one phosphate residue, which leads to their inactivation. Thus, natural control of growth factor signals occurs in the cell. When a mutation occurs in one of the genes of this family, the inactivation process is disrupted, which leads to the accumulation of proteins in the active form. This leads to excessive activation of the signaling cascade, and subsequently to unlimited cell division with loss of differentiation. At the same time, the site of the mutation in the gene (*KRAS* or *NRAS*) determines the further structure of the synthesized proteins, the nature of their influence and the properties of the tumor [33,34].

The *BRAF* gene encodes a protein of one of the representatives of the serine/threonine protein kinases of the RAF family, which also plays a key role in the carcinogenesis of CRC. Similar to the RAS family of proteins, it performs a signaling function and is a downstream mediator after KRAS/NRAS. After activation, BRAF triggers a further cascade of MEK-ERK signal transmission, as a result of which the processes of proliferation, differentiation and inhibition of apoptosis are activated in the cell [35]. For the natural regulation of the signaling pathway, there are biofeedback mechanisms, as well as a limited lifetime of the BRAF protein in its active form [36]. As a result of mutation in the *BRAF* gene, new structural and functional forms of the synthesized enzyme appear that do not respond to the processes of natural regulation, which leads to excessive and uncontrolled processes of malignant progression. Thus, KRAS, NRAS and BRAF proteins are key links in the signaling pathway of epidermal growth factor. Mutations in the corresponding genes lead to loss of regulation, uncontrolled activation of the processes of growth, division and differentiation, and increased cell survival, which leads to further malignant transformation. Such genetic modifications can occur both in the early (key or driver mutations) and in the late (III-IV) stages of CRC with progression and metastasis. In addition, mutations in the *KRAS* gene are considered to be leading in the process of adenoma-to-adenocarcinoma transition [2].

The Mutation Rate in the KRAS, NRAS and BRAF Genes in Patients with CRC

Despite the large amount of data on the incidence of mutations in the *KRAS, NRAS* and *BRAF* genes in

Table 1. *The mutation rate in the KRAS, NRAS, BRAF genes in colorectal cancer according to Russian studies*

Russian Studies	The number of patients in the study	*KRAS*	*NRAS*	*BRAF*
Telysheva E.N. (Moscow) [40]	355	40.6%	1.4%	6.2%
Shubin V.P. (Moscow) [41] *IV stage of CRC	45	53.3%	6.7%	6.7%
Ognerubov N.A. (Tambov) [42]	153	39.2%	4.6%	3.9%
Kudryashova E.M. (Irkutsk) [43]	325	44.3%	–	–
Oganyan K.A. (Saint-Petersburg) [44]	400	45%	2.5%	5.8%
Pisareva, E.E. (Novosibirsk) [45]	80	46%	–	3.8%
Belyaeva A.V. (Saint-Petersburg) [46]	135	35.6%	–	–
Vodolazhskiy D.I. (Rostov-on-Don) [47]	800	38.6%	–	–
Fedyanin M.Yu. (Moscow)[48]	65	43.1%	3.1%	3.1%
Bogomolova, I.A. (Ulyanovsk) [49]	37	37.8%	5.4%	8.2%
Fedorova, P.A. (Saint-Petersburg) [50]	321	43%	9%	14%
Brezhnev, D.G. (Kursk) [51]	25	28%	8%	8%
Musaelyan, A.A. (Saint-Petersburg) [52]	200	44%	1.5%	9%
Sakaeva D.D. (Ufa, Kazan) [53] *IV stage of CRC	317	29.9%	2.6%	–
Martyanov A.S. (Saint-Petersburg) [39]	8355	49.5%	4.7%	6.7%
Loginova A. (Moscow) [54]	489	–	–	7%
Average indicator rate		**41.2%**	**4.5%**	**6.9%**
International meta-analysis data				
Levin-Sparenberg E. [37] *IV stage of CRC	77104	35.9%	4.1%	7.1%

CRC, some populations have not been sufficiently studied so far. This is due to the use of different methods and approaches of molecular diagnostics. In the Russian population, the mutation rate in the *KRAS* gene varies from 28% to 53% among all patients with CRC (Table 1). The mutation rate of the *NRAS* and *BRAF* genes varies between 1.4–9% and 0.04–14%, respectively. In the literature, one can also find an analysis of the mutation rate in CRC, depending on the country or geographical location and nationality. Such a meta-analysis was conducted based on data from Asia, Europe, America and Australia, but without taking into account Russian data [37]. According to the results, it was found that the mutation rate in the *KRAS* and *BRAF* gene varied significantly depending on the geographical location ($p = 0.025$ and $p = 0.002$, respectively) [37]. Another study did not reveal significant differences in the mutation rate in the *KRAS* gene when analyzing different nationalities (Europeans, South Americans, the population of the Middle East and Asia) ($p = 0.34$). However, statistically significant differences were found in the mutation rate in the *BRAF* gene ($p = 0.025$) [38]. According to the results of the study by Martyanov A.S. et al., mutation in the *BRAF* gene was statistically significantly less common in residents of the southern regions of the Russian Federation and the North Caucasus ($p = 0.0007$) [39].

According to the meta-analysis by Bylsma et al. [55], mutations in the *KRAS* gene occur with approximately the same rate in the right and left halves of the large intestine in patients with metastatic CRC, but other data exist. Thus, in a study of more than 19 thousand patients with CRC in the USA, mutations in the *KRAS* gene were significantly more common in right-sided localization of the primary tumor ($p < 0.01$) [18]. According to a Chinese study, statistically significant differences were also obtained in the occurrence rate of mutations in the *KRAS* gene with a predominance in the right half of the colon compared with the left half ($p < 0.0001$) [10]. The opposite trend was revealed in a Russian sample of patients. In more than half of the cases, the *KRAS* gene mutations were detected in patients with left–sided tumor localization ($p < 0.05$) [42] (with the exception of patients with the p.Gly13Asp mutation — 60–83% of patients with right-sided cancer) [42,43,46,56]. When studying the mutation in the *NRAS* gene, no significant relationship with tumor site, depth of

invasion and other oncological parameters was revealed [10,44,55,57]. Due to the relatively low mutation rate in the *NRAS* gene both in Russia and in the world (about 4%), it is difficult to assess individual parameters in patients with this mutation. Moreover, there is no data in the Russian literature on the relationship between the primary tumor site and mutations in the *NRAS* gene. More patients and meta-analysis results are needed to obtain more reliable data. The results of some Russian and international studies on the relationship between the *BRAF* gene mutations in CRC and the localization of the primary tumor demonstrate challenging data. So, according to Loginova A. et al. [54], among all patients in whose tumors mutations in the *BRAF* gene were detected, the proportion of tumors in the right half of the colon was 61.8%, in the left half and rectum — 17.6%, respectively. According to another Russian study, the proportion of tumors with this mutation was only 14.6% in the right colon and 3% in the left one [44], which roughly corresponds to the results of the Chinese study (8.4% in the right colon, 1.9% in the left colon, 1.3% in the rectum) [10]. In addition, mutations of the *BRAF* gene in many studies were also significantly associated with a lesion of regional lymph nodes, deeper invasion of the primary tumor (T3-4), perineural invasion and the presence of distant metastases [44,50,57].

Ambiguous data are also presented in the literature regarding the degree of differentiation of the primary tumor and the presence of a particular mutation. In the Chinese population, in patients with CRC, tumors with a mutation in the *KRAS* gene are more likely to have high or moderate tumor differentiation than low differentiation (48.3% vs 46.1% vs 31.3%, respectively, $p = 0.023$) [10]. The retrospective study did not show a significant difference between the incidence of the *KRAS* gene mutations in patients with different degrees of tumor differentiation ($p = 0.17$) [57]. According to other Chinese studies, it was found that mutations of the *BRAF* gene were more common in low-grade tumors than in highly and moderately differentiated ones ($p < 0.001$) [10].

The data on the mutation rate, depending on the degree of differentiation of the primary tumor in the Russian population, are quite heterogeneous and contradict each other, which may be due to the small number of analyzed patients and the use of different research methods, which emphasizes the need for further research. According to a Russian study [41], the proportion of mutations in the *KRAS* gene with a low degree of differentiation (G3) was 83%, with a moderate degree (G2) — 50%. According to other data, the proportion of mutations in the *KRAS* gene with a high-moderate degree of differentiation was 48.9%, and with a low degree — 33.3% with a statistically significant difference ($p = 0.0047$) [44]. No statistically significant differences in differentiation were found for tumors with mutations in the *BRAF* and *NRAS* genes [44].

Regarding the mutation rate in the *KRAS, NRAS* and *BRAF* genes by age and gender, there is also no uniform trend in all the studies.

According to some data, the *KRAS* gene mutations are more common in women and especially women aged over 55 years [37,39,42,47,53,56,57], while other studies demonstrate no difference in relation to female gender and older age (Table 2) [10,37,44]. In a Russian multicenter study, interesting data were obtained based on the results of an analysis of the incidence of the *KRAS* gene mutations in 3 cities: a higher rate was in women in Kazan, while in Novgorod and Ufa — in men [53].

The incidence of the *BRAF* gene mutations is significantly higher in women ($p = 0.018$ [37], $p = 0.024$ [58], $p = 0.001$ [57]), according to Ognerubov N.A. [42] and Martyanov A.S. [39], and according to three international studies.

Regarding mutations of the *NRAS* gene, there are also contradictory data regarding gender: according to some studies, the incidence is up to 2 times higher in women [42,47], and in some studies, it is significantly more common in men [39]. According to the results of other studies, including international ones, the relationship with gender and other demographic parameters and mutation there is no *NRAS* gene [10,37,44,49,57].

Table 2. *Mutation rate in KRAS, NRAS, BRAF genes depending on gender in patients with colorectal cancer*

	The number of patients with mutations	Gender		*p*-value
		Male	Female	
KRAS				
Ognerubov N.A. (Tambov) [42]	60 / 74 (81%)	28 (46.7%)	32 (53.3%)	
Kudryashova E.M. (Irkutsk) [43]	144 / 325 (44.3%)	65 (45.1%)	79 (54.9%)	
Mazurenko N.N. (Moscow) [56] *	208 / 573 (36.3%)	122 (58.7%)	86 (41.3%)	0.017
Vodolazhskiy D.I. (Rostov-on-Don) [47]*	309 / 800 (38.6%)	128 (41.4%)	181 (58.6%)	
Martyanov A.S. (Saint-Petersburg) [39]	4137 / 8335 (49.6%)	1949 (47.1%)	2188 (52.9%)	< 0.0001
NRAS				
Ognerubov N.A. (Tambov) [42]	7 / 74 (9.5%)	2 (28.6%)	5 (71.4%)	–
Martyanov A.S. (Saint-Petersburg) [39]	389 / 8335 (4.7%)	221 (56.8%)	168 (43.2%)	0.004
BRAF				
Ognerubov N.A. (Tambov) [42]	6 / 74 (8.1%)	1 (16.7%)	5 (83.3%)	–
Martyanov A.S. (Saint-Petersburg) [39]	556 / 8335 (6.7%)	204 (36.8%)	352 (63.2%)	< 0.0001
Loginova A. (Moscow) [54]	34/489 (7%)	11 (32.4%)	23 (67.6%)	–

*Note: *Only the 2nd exon of the KRAS gene was analyzed in the studies*

Thus, the incidence of occurrence of mutations of the *KRAS, NRAS* and *BRAF* genes in Russia corresponds to a similar incidence worldwide. However, when taking into account such parameters as the primary tumor site, the stage of the disease, the degree of differentiation, gender and age of patients, Russian and international data have some differences. Moreover, when comparing Russian studies, some results also differ. According to some parameters, it is impossible to analyze patients with the studied mutations in Russian studies. Presumably, this may be due to the small number of patients included in the analysis. To obtain more reliable data, multicenter studies with a large sample are required.

Heterogeneity of Mutations in KRAS, NRAS, BRAF Genes

The concept of tumor heterogeneity implies that at the stage of initiation of the carcinogenesis process, key mutations can occur in various genes. In addition, it was found that even the specific localization of a mutation within a single gene may also differ. For example, according to the results of a study of the Chinese population, mutations in the *KRAS* gene affect the second, third and fourth exons in 40%, 1.4%, 4.1%, respectively [10]. These data are confirmed by other studies, both international and Russian, with the highest incidence of lesion of the second exon [19,40,42–44,47,52,53,56]. In the second exon, the mutations most often affected codons 12 and 13 [10,11,16,19,42,58,59]. According to studies of the Chinese and Malaysian populations, the mutation rate in the *KRAS* gene in codon 12 was about 80%, in codon 13–21% [10,19].

According to a study of the European population, mutations in codon 13 are slightly more common (32% of all mutations in the *KRAS* gene) [16]. Among Russian studies (Table 3) the mutation rate in codons 12 and 13 corresponds to international data. The most common mutations in codons 12 and 13 are c.35G>A (p.Gly12Asp), c.38G>A (p.Gly13Asp) and c.35G>T (p.Gly12Val). The literature presents rather heterogeneous data with a large variation in the occurrence rate. Thus, in separate independent studies, the following data are provided on the localization of mutations in the *KRAS* gene: c.35G>A (p.Gly12Asp) — 35–57.9%, c.35G>T (p.Gly12Val) — 20–25%, c.38G>A (p.Gly13Asp) — 13–57.9%) [10,11,19,59]. However, according to meta-analysis data, the incidence of mutations c.35G>A (p.Gly12Asp) and c.38G>A (p.Gly13Asp) is still lower than in separately presented studies (27.2% and 16.8%, respectively) [58].

The occurrence incidence of certain *KRAS* gene mutations may also depend on the location of the primary tumor. At the same time, according to a Russian study, the c.35G>A (p.Gly12Asp) mutation

Table 3. *Mutation spectrum of KRAS, NRAS and BRAF genes*

Russian Studies	p.Gly12 Asp	p.Gly13 Asp	p.Gly12 Val	p.Gly12 Ala	p.Gly12 Cys	p.Gly12 Ser	p.Gly12 Arg
KRAS							
Telysheva E.N. (Moscow) [40]	39.7%	22.6%	17.1%	8.2%	6.2%	4.8%	1.4%
Kudryashova E.M. (Irkutsk) [43]	25.7%	20.1%	20.8%	8.3%	3.5%	6.25%	1.4%
Mazurenko N.N. (Moscow) [56]	33.7%	12.5%	32.7%	8.7%	3.4%	7.2%	0.9%
Pisareva E.E. (Novosibirsk) [45]	13%	15%	6%	4%	5%	3%	1%
Ognerubov N.A. (Tambov) [42]	20%	8.3%	25%	16.6%	1.7%	–	3.3%
Vodolazhskiy D.I. (Rostov-on-Don) [47]	44.3%	17.4%	16.5%	8.7%	7.1%	3.8%	2.2%
Martyanov A.S. (Saint-Petersburg) [39]	28.8%	17.6%	21.1%	5%	6.7%	4.8%	1%
Average indicator rate	**29.3%**	**16.2%**	**19.9%**	**8.5%**	**4.8%**	**5%**	**1.6%**
International meta-analysis data							
Peeters M. [58] *IV stage of CRC	27.2%	16.8%	24.1%	6.6%	7.6%	5.3%	1%
NRAS	**p.Gly12Asp**	**p.Gly13Arg**	**p.Gly12Cys**	**p.Gln61Arg**	**p.Gln61Lys**	**p.Gln61His/ Leu**	**p.Ala146Thr**
Martyanov A.S. (Saint-Petersburg) [39]	17.2%	4.6%	3.6%	15.2%	24.42%	9.8%/ 4.9%	–
International meta-analysis data							
Peeters M. [58] *IV stage of CRC	18.3%	8.7%	4.8%	14.4%	32.7%	5.8%	1.9%
BRAF	**p.Val600Glu**	**D594G**	**D594N**	**G596R**	**F595L**	**K601N**	**L597R**
Martyanov A.S. (Saint-Petersburg) [39]	91.7%	4.3%	1.3%	0.5%	0.4%	0.4%	0.4%
Loginova A. (Moscow) [54]	82.4%	17.6%					

was more common in the right half of the colon (up to 83%), c.35G>T (p.Gly12Val) was more common in the left half, and both mutations with the same incidence in the rectum were about 30% [56].

The incidence of various mutations in the *BRAF* gene according to Russian and international studies could not be fully estimated, since in most of the studies found, only the most common localization of mutations in the *BRAF* gene was determined (p.Val600Glu) [40], and only a couple of studies presented the entire spectrum of localizations indicating the incidence of occurrence [39,54] where the p.Val600Glu mutation occurs in more than 80–90% of cases. The full range of mutation localizations in the *NRAS* gene is described in a single study [39], while other studies describe only single localizations of this mutation (p.Gly12Asp [52], p.Gln61Lys) [40].

Depending on which mutation is present in the gene, it is possible to determine the degree of aggressiveness of the tumor. Thus, according to the results of an experimental trial, it was found that the mutation of p.Gly12Asp in the *KRAS* gene leads to excessive MEK-dependent cell proliferation. The same mutation (p.Gly12Asp), but in the *NRAS* gene, has a lesser effect on cell growth and mainly provides tumor cells with resistance to apoptosis [60, 61]. Another mutation (p.Gln61Lys) of the *NRAS* gene promotes independent proliferation, which leads to the facilitation of the formation of metastatic foci, and generally has similar properties with canonical mutations of the *KRAS* gene [61].

There are some contradictions regarding the clinical features of the course of the disease in certain mutations. In general, it was found that mutations in 12 and 13 codons of the *KRAS* gene increase the incidence of primary generalized forms of CRC (stages III–IV) and worsen the prognosis compared with the wild type [33,37,42,45,46,52]. At the same time, it was found that the p.Gly12Asp mutation in the *KRAS* gene is associated with a significantly lower risk of metastasis [47]. In many studies, it has been shown that the p.Gly12Val mutation of the *KRAS* gene was significantly more often associated with a lesion of regional lymph nodes and a negative prognosis compared with other mutations [17,62]. This is due to the higher activity of GT-phase in this mutation [17]. According to other international studies, it was shown that, in general, *KRAS* gene mutations were not reliably associated with either lesion of distant lymph nodes or distant metastases [10], which clearly contradicts the data of meta-analysis [37].

The presence of a mutation in the *BRAF* gene significantly increases the risk of tumor metastasis and progression and is also associated with a worse prognosis in patients with CRC [10,26,35,37]. The relationship between the presence of a mutation in the *NRAS* gene and the number of affected lymph nodes and distant metastases has not been established [10,26,37,63].In patients with metastatic lymph node lesion (l/n), the *KRAS* gene mutations may be present in both the primary tumor and the lymph nodes. According to the results of a study of patients with l/n lesion in CRC, it was shown that the discordance in the mutation status of the *KRAS* gene in the analysis of the primary tumor and randomly selected l/n with metastasis was 31% among all patients and 55% among patients with a mutation in the *KRAS* gene [16].

With a mutation in the *BRAF* gene, the discordance between the primary tumor and the affected l/n was 4%. Thus, the researchers demonstrated a sufficiently large heterogeneity between the primary focus and metastasis in l/n by mutation in the *KRAS* gene, while such heterogeneity is less common by mutation in the *BRAF* gene [16].

It is worth noting that these studies have a relatively small sample of patients (41 patients with a mutation in the *KRAS* gene), but even so, the results emphasize the need for a close study of the affected lymph nodes and distant foci of metastasis. According to Russian studies, heterogeneity between the primary tumor and metastases by mutations in the *KRAS* gene occurs in approximately 9–16.9% of patients, and by mutations in the *NRAS* and *BRAF* genes in 3% of patients [41,48]. At the same time, in 18% of patients with wild type in the primary tumor, a mutation in the *KRAS* gene was detected only in the metastatic focus [48]. However, other studies have not demonstrated differences in the mutation incidence in the primary tumor and metastatic foci [43].

In addition to the inter-tumor heterogeneity of mutations in the *KRAS, NRAS*, and *BRAF* genes, there is also an intra-tumor one, which implies the presence of two different mutations in one tumor. When studying this phenomenon in patients with colorectal cancer, Normanno N. et al. [20] found that out of 182 tumor samples, 2 different locations of the *KRAS* gene mutation were identified in one sample (there is no data on the exact localization). The phenomenon of intra-tumor heterogeneity is also described in Russian studies. In a study by Telysheva E.N. et al. [40] in 1/144 patients with a mutation in the *KRAS* gene, 2 different mutation localities (p.Gly12Ala and p.Gly12Ser) were detected in a tumor tissue sample, as well as one case of simultaneous detection of a mutation in the *KRAS* gene (p.Gly12Ala) and the *NRAS* gene (p.Asn61Gln).

In a study by Kosmidou V. et al. [59], similarly, data are provided on the detection of several mutations in the *KRAS* gene (in codons 12 and 13) in one tumor sample (24 cases). In another Russian study, a sample was found simultaneously containing mutations in the genes *KRAS* p.Gly13Asp and *BRAF* p.Val600Glu [45].

In a study by Normanno N. et al., the proportion of tumors with a particular mutation and the

heterogeneity index were studied [20]. Thus, as a result, it was found that 60% of colorectal tumors with a mutation in the *KRAS* gene and 77% with a mutation in the *NRAS* gene have a heterogeneity of more than 70 (more than 70% of tumor cells have a mutation). However, only 26.7% of tumors with a mutation in the *BRAF* gene have a score of more than 70 with an average heterogeneity index of 54.8 [20].

In a study by Baldus et al., the intra-tumor heterogeneity of the *KRAS* gene mutation in the primary tumor was 8% (wild type vs *KRAS* gene mutation), and with the *BRAF* gene mutation was only 1% [16].

Both Russian and international studies show rather heterogeneous results with a wide range of data, while the issue of the influence of racial, ethnic and geographical characteristics of populations remains controversial and debatable. There are no generalizing studies in Russian databases, including those taking into account the venue. Therefore, the topic of studying the mutational profile of colorectal cancer remains relevant.

The Applied Significance of Mutations in the KRAS, NRAS, and BRAF Genes

Significant success in the treatment of colorectal cancer has been achieved with the help of targeted drugs [64]. Currently, two drugs (cetuximab and panitumumab) are actively used in clinical practice in the treatment of CRC [65].

It has been demonstrated that a tumor with the p.Gly13Asp mutation in the *KRAS* gene responds to cetuximab therapy [28,29]. But later studies have proved that anti-EGFR drugs are also ineffective with this mutation [66].

Another possible reason for the ineffectiveness of EGFR inhibitor therapy may be the receipt of false negative sequencing results due to intra tumor heterogeneity, which was described in detail above. A particularly high level was observed in the *KRAS* gene mutations both inside a single tumor and between the primary focus and lymph node metastases. Moreover, insufficient diagnosis or an inaccurate method of mutation verification may also be a predictor of the ineffectiveness of anti-EGFR therapy [16]. The *BRAF* oncogene is another predictor of the response to EGFR inhibitor therapy. Mutations of the *BRAF* gene are found in about 7–10% of patients with CRC and also reduce the effectiveness of anti-EGFR therapy [67,68]. The issue of studying the heterogeneity index of the *KRAS* gene mutation in order to identify the threshold of tumor sensitivity to monoclonal anti-EGFR therapy is actively discussed in the literature. According to the results of some studies, it was found that tumors in which the incidence of the *KRAS* gene mutation in tumor cells was less than 33% demonstrated a positive response to FOLFIRI therapy with cetuximab (total response rate of 70%). In the group of patients with the*KRAS* gene mutation rate of over 33%, the response rate corresponded to the response to FOLFIRI without cetuximab (45.7%). However, when assessing long-term cancer outcomes, there was no difference between the two groups in terms of disease-free survival (7.97 vs 8.37 months) [20].

Such data may indicate that the low content of the mutant *KRAS* allele is sufficient to develop resistance to anti-EGFR drug therapy. In the study mentioned above, it was also shown that the presence of mutations in other genes (*PIK3CA, TP53, BRAF*, etc.) with a mutation in the *KRAS* gene of less than 33% is significantly higher than in tumors with mutations in the *KRAS* gene of more than 33%. Thus, the presence of even a small proportion of cells with mutations will hinder the response to the selected therapy. In relation to colorectal tumors with a mutation in the *BRAF* gene, it is known that with the *BRAF* V600E mutation, the tumor is associated with resistance to therapy [69]. Currently, research is underway to find drugs that inhibit the activity of signaling pathways in mutations in the *KRAS, NRAS* and *BRAF* genes, but none of the drugs are currently used in practice. In experimental trials, the high efficiency of the allele-specific inhibitor of the p.Gly12Cys mutation of the *KRAS* gene has been shown [33].

Methods of Diagnosis of Mutations in KRAS, NRAS, BRAF Genes

Currently, several molecular genetic methods with different sensitivity and specificity indicators, as well as their requirements for the minimum content of tumor cells in the sample, are used in our country to determine somatic mutations in CRC. The most common in Russia is the real-time PCR method for diagnosing the most common somatic variants in the *KRAS* gene. The advantages of this technique include the lowest cost and the possibility of using samples with a tumor cell content of 10%, as well as diagnostic sensitivity of more than 90%. At the same time, such test systems make it possible to identify only 7 known variants in exon 2 of the *KRAS* gene, respectively, without studying mutations in exons 3 and 4 of this gene. Thus, in the case when the mutation is not detected in the patient, it is necessary to do further research of the tumor sample to determine the presence of those variants that are not included in the test system [8,70].

Digital droplet PCR is a more accurate method than real-time PCR, since mutation detection is possible even with less than 1% of tumor cells in the sample [33]. That is why digital droplet PCR is used in the diagnosis of circulating tumor DNA in patients with colorectal cancer. According to the results, this method of preoperative diagnosis of mutations in the *KRAS* gene demonstrated sensitivity up to 83% and specificity up to 91%. However, only 73% of patients subsequently confirmed the presence of a mutation in the tumor [71,72]. This method is also limited by a small range of mutations under study and is significantly more expensive than the real-time PCR method [33].

The next option for diagnosing the status of RAS/*BRAF* genes is Sanger sequencing [10,40,41,43,45,48,49,57,58]. The advantage of this method is the ability to recognize all available point mutations in RAS/*BRAF* genes [8,70].

The sensitivity and specificity of Sanger sequencing exceed those of PCR test systems used in Russia. At the same time, the negative aspects include the higher cost and the requirement for the sample — at least 50% of the tumor cells in the sample [8]. To increase the content of tumor cells in the studied material, additional stages of preparation of the specimen (laser microdissection of the tumor specimen) can be used [45]. The next-generation sequencing method (NGS) is another method for detecting mutations [10,20,33,73].

The method is not limited to the use of standard sets. Therefore, it can be used for diagnosis, including rare mutations. To achieve maximum accuracy of the method, a tumor cell content of over 1% is required [33]. However, the main limitation of the method is the highest cost compared to other methods [33]. The NGS method can also be used in the diagnosis of circulating tumor DNA, and its effectiveness is not inferior to digital droplet PCR [74].

One of the promising areas in the field of personalized oncology is the study of gene copy number variation (CNV).

The number of copies can be calculated based on the results of NGS or digital droplet PCR. Currently, within the framework of experimental trials, various variants of mutation replication are being studied to classify tumor subtypes, determine the effect of these changes on the tumor phenotype and sensitivity to therapy [75–77]. For example, it was found that the presence of the CNV *KRAS* gene in pancreatic cancer, as well as mutations in the *KRAS* gene, worsens the prognosis and reduces the sensitivity of the tumor to chemotherapy (MEK inhibitors) [75]. The role of CNV in the development of colorectal cancer has not been fully determined. Current research suggests that this phenomenon may play a role in a certain cohort of patients with hereditary CRC [78]. In another study, possible mechanisms of resistance of mucinous colorectal tumors to therapy with 5-fluorouracil, oxaliplatin and irinotecan associated with CNV were identified [76]. Other studies emphasize that much more complex interaction mechanisms may play a role in the development of colorectal cancer, including CNV and aberrant expression of mRNA and long non-coding RNA [79]. The study

of CNV to determine genetic patterns and classify tumor subtypes will help in the further development and search for possible ways to treat oncological diseases, including colorectal cancer.

CONCLUSION

A review of the literature showed that driver mutations in the *KRAS, NRAS*, and *BRAF* genes, according to Russian and international studies, occur in patients with colorectal cancer with an average rate of about 40%, 4%, and 7%, respectively. At the same time, mutations in the Russian population are more prevalent in tumors of the left half of the colon and rectum. The occurrence rate of certain mutations, as well as its specific localization, may depend on the geographical location and ethnicity of the cohort being studied. The high inter-tumor and intra-tumor heterogeneity of CRC, especially for the *KRAS* gene mutations, has a significant impact on the choice of further therapy and emphasizes the need for a more detailed study of the mutational profile of the primary tumor, affected lymph nodes and distant foci of metastasis. Despite the large number of studies, some aspects of the mutational profile of colorectal cancer within the Russian population are still poorly understood, and therefore further studies of patients with large intestine cancer in Russia are required. The development of new promising methods for studying the carcinogenesis of colorectal cancer is necessary to further determine the relationship of genetic changes and search for new directions for personalized medicine.

AUTHORS CONTRIBUTION

Concept of the study: *Vitaly P. Shubin, Evgeny A. Khomyakov*

Collection and processing of the material: *Ekaterina A. Kazachenko*

Writing of the draft: *Ekaterina A. Kazachenko*

Editing: *Vitaly P. Shubin, Aleksey S.Tsukanov, Evgeny A. Khomyakov, Stanislav S. Otstanov*

INFORMATION ABOUT THE AUTHORS (ORCID)

Ekaterina A. Kazachenko — 0000-0001-6322-7016

Vitaly P. Shubin — 0000-0002-3820-7651

Stanislav S. Otstanov — 0000-0003-2043-495X

Aleksey S. Tsukanov — 0000-0001-8571-7462

Evgeny A. Khomyakov — 0000-0002-3399-0608

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