EXPERIMENTAL ONCOLOGY

https://doi.org/10.15407/exp-oncology.2024.03.221

**G. GERASHCHENKO [1], R. GULKOVSKYI [1], N. MELNICHUK [1], N. HRYSHCHENKO [1], T. MARCHYSHAK [1], O. MANKOVSKA [1], A. BEZVERKHIY [1], I. KOTUZA [2], L. ROSHA [2], A. KOTUZA [2], Z. TKACHUK [1], V. KASHUBA [1], M. TUKALO [1]**

[1] Institute of Molecular Biology and Genetics NAS of Ukraine, Kyiv, Ukraine
[2] Feofaniya Clinical Hospital of the State Management of Affairs, Kyiv, Ukraine

* Correspondence: Email: g.v.gerashchenko@edu.imbg.org.ua

# IDENTIFICATION OF CLINICALLY RELEVANT GENE VARIANTS IN COLON ADENOCARCINOMA SAMPLES OF UKRAINIAN PATIENTS USING A COMPREHENSIVE CANCER PANEL: A PILOT STUDY

The study **aimed** to identify the clinically relevant gene variants in colon adenocarcinoma samples of Ukrainian patients using the NGS Comprehensive Cancer Panel (CCP) to implement them conveniently in clinical practice. **Methods.** We have studied 20 samples of Ukrainian patients with colorectal adenocarcinomas of various differentiation grades. To identify the clinically relevant gene variants, the CCP data were filtered using the Franklin by Genoox database. **Results.** A total of 79 clinically relevant gene variant alterations (SNVs, INDELs) were found in 28 of 409 genes. The largest number of mutations was found in 3 genes, *APC, TP53,* and *KRAS (*16, 14, and 8, accordingly). We revealed 4 variants in *PTEN* and *SMAD4*, 3 variants in *CHEK2, ERBB2,* and *PIK3CA* genes, and 2 variants in *AKT1, ATM, DST, IDH1,* and *TCF12*. Mutations for 7 genes, *KRAS, TP53, CHEK2, PTEN*, *AKT1, APC*, and *SMAD4,* were found in more than 1 tumor tissue sample. Tier 1—2 gene variants rate was about 50% of all genetic variants. The therapeutic significance was found in more than 55% of mutations. Additionally, 11 novel genetic mutations in 9 genes have been identified, including *G6PD, APC, DST, SINE1, SMAD2,* and *FLCN*. **Conclusions.** These data suggest a high level of clinical relevance of the NGS CCP approach. Further confirmation on a larger number of samples and using a deeper analysis by other approaches is required.

**Keywords:** NGS, gene variants, colon adenocarcinoma, clinical significance.

The development of modern anticancer drugs and targeted therapies is limited by drug resistance, and mutations in target genes are one of the main reasons for acquiring resistance [1]. It is increasingly clear that the mechanisms of acquiring pharmacological resistance are embedded in genetic, epigenetic, translational, and post-translational alterations in tumor cells [2]. Genetic analysis of the tumor-associated mutations in oncogenes and tumor suppressor genes, as well as mutations that lead

Citation: Gerashchenko G, Gulkovskyi R, Melnichuk N, Hryshchenko N, Marchyshak T, Mankovska O, Bezverkhiy A, Kotuza I, Rosha L, Kotuza A, Tkachuk Z, Kashuba V, Tukalo M. Identification of clinically relevant gene variants in colon adenocarcinoma samples of Ukrainian patients using a comprehensive cancer panel: a pilot study. *Exp Oncol.* 2024; 46(3): 221-227. https://doi.org/10.15407/exp-oncology.2024.03.221

to pharmacological resistance of tumors accompanying long-term treatment with anticancer drugs, provides the foundation for several promising methods for overcoming the resistance [3]. It would be beneficial to conduct a genetic analysis of the tumor to determine whether tumor cells have resistance to drugs at the beginning and during the treatment. This could be evidenced by the presence of certain mutations associated with resistance to drugs and therapy [4, 5].

Therefore, there is a need to identify clinically relevant mutations in tumors, which could potentially serve as markers for effective diagnosis, prognosis, and therapy. Many genetic alterations were found in tumor tissues. However, it should be noted that up-to-date only a portion of these mutations have been established as clinically relevant. Definition of clinically relevant mutations is based on recommendations of the American College of Medical Genetics Genomics and the Association for Molecular Pathology (ACMG-AMP) [6]. In light of this, it seems that online resources for the analysis of clinically relevant alterations in human tumors, in particular, the Franklin by Genoox platform, are actively developed and used for genetic analysis of tumors [7, 8]. Our investigation aimed to identify clinically relevant gene variants in colon adenocarcinoma samples of patients treated in Ukraine using the NGS with open online resources analysis to implement cancer genetic alterations detection in further clinical practice.

## Materials and Methods

***Patient samples.*** The surgical samples of 20 patients (approximately equally men and women) aged 60—87 years treated at the Feofaniya Clinical Hospital of the State Management of Affairs of Ukraine were investigated in this research. All patients had colorectal cancer verified as adenocarcinomas of different locations and differentiation grades without metastasis. Six patients had concomitant pathologies. The Helsinki Declaration was followed in conducting human sampling. Ethical approval was obtained from the Ethics Commission of Feofaniya Clinical Hospital of the State Management of Affairs Number 3 on May 24, 2024.

***Colon cancer genotyping by Ion Torrent Gene Target Library preparation and NGS sequencing.*** The genomic DNA (gDNA) was extracted from cancer samples using the GeneJET FFPE DNA Purification Kit (Thermo Fisher, USA). 15 ng of gDNA was taken for library preparation. DNA was amplified using Ion AmpliSeq™ Comprehensive Cancer Panel Primer Pools and AmpliSeq HiFi mix (Thermo Fisher, USA). PCR pools for each sample were combined and subjected to primer digestion with the FuPa reagent (Thermo Fisher, USA). Libraries were indexed using the Ion Xpress Barcode Adapter Kit. After purification, the amplified libraries were quantified by qPCR with the TaqMan library quantification kit (Thermo Fisher, USA). All samples were diluted to a final concentration of 100 pM, then the amplicon libraries were pooled for emulsion PCR on an Ion OneTouch System 2TM using the Ion S5 Plus Template OT2 200 kit. Template-positive spheres from barcoded libraries were multiplexed, followed by next-generation sequencing on the Ion GeneStudio™ S5 System, all with the use of the Ion 540™ OT2-Kit and the Ion 540™ Chip Kit (Thermo Fisher, USA).

***Ion Torrent bioinformatics analysis, variant filtering, and assessment of pathogenicity (clinical significances).*** A primary sequence analysis was performed on Ion Torrent suite software v5.12 with TMAP alignment of sample reads against the hg19 genome assembly. The alignment step was limited to target gene regions only. The variant calling and the annotation were carried out by Ion Reporter version 5.20. The alignments and the presence of filtered-in variants were visually confirmed with Integrative Genomics Viewer (IGV v2.17). The sequence variants were first annotated by using Ion Reporter, where variants were screened using the Human Gene Mutation Database (HGMD; http://www.hgmd.cf.ac.uk/ac/index.php), dbSNP database (dbSNP; http://www.ncbi.nlm.nih.gov/SNP), gnomAD browser (http://gnomad.broadinstitute.org/), ClinVar database (ClinVar; http://www.ncbi.nlm.nih.gov/clinvar), *etc.* as public references. The deleterious effects of missense variants were predicted using the PolyPhen-2 [9] (http://genetics.bwh.harvard.edu/pph2/), and PROVEAN/SIFT [10] (http://provean.jcvi.org/index.php), MutationTaster [11] (https://www.mutationtaster.org), and Franklin by Genoox (https://franklin.genoox.com) algorithms. The Franklin by Genoox platform was used primarily for variant filtering, analysis, and identification of variants for reporting. The patho-

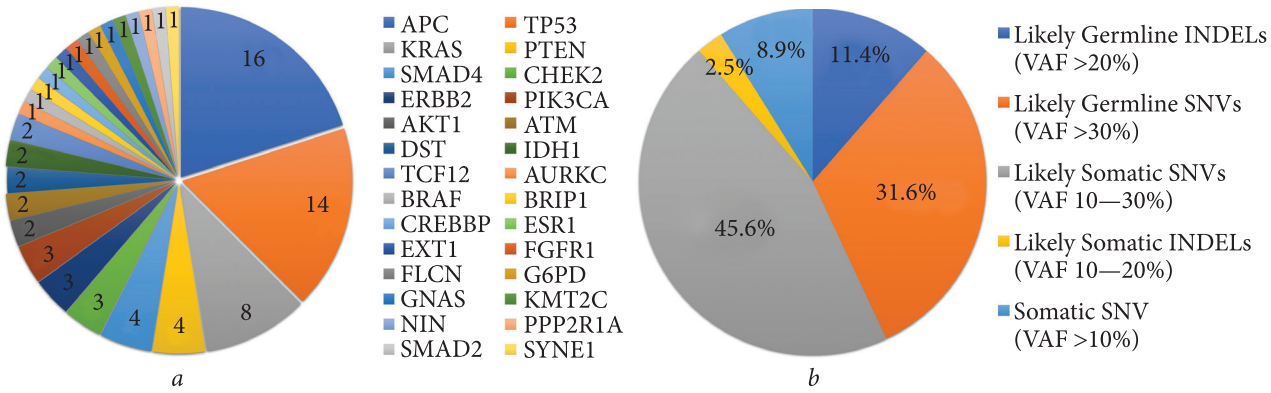


***Fig. 1.*** (*a*) Distribution of the number of identified altered genetic variants by gene; (*b*) Distribution of variant frequencies by variant type (SNVs, INDELs) and somatic or germline (constitutional) origin. VAF — variant allele frequency

genicity (clinical significance) of genetic mutations was evaluated according to the ACMG-AMP and Oncogenic classifications [12, 13].

## Results

Using targeted sequencing with the Ion AmpliSeq™ Comprehensive Cancer Panel of 409 tumor suppressor genes and oncogenes in 20 patients, we detected genetic variants that were classified by Franklin as clinically relevant in all 20 individuals. A total of 79 gene variants were identified in 28 genes (Fig. 1, *a*).

The largest number of mutations was found in 2 genes: 16 variants (20.3%) in the *APC* gene and 14 variants (17.7%) in the *TP53* gene. We detected 8 variants (10.1%) in the *KRAS* gene and 4 variants (5.1%) in each of the *PTEN* and *SMAD4* genes, 3 variants (3.8%) each in the *CHEK2*, *ERBB2,* and *PIK3CA* genes, and 2 variants (2.5%) — in *AKT1*, *ATM*, *DST, IDH1,* and *TCF12.* Only 1 variant (1.3%) was detected in each of the other 15 genes depicted in Fig. 1, *a*.

These detected genetic alterations refer to single nucleotide variants (SNVs) or insertions and deletions (INDELs) and were classified as potentially germline or somatic according to the European Society of Medical Oncology guidelines (Fig. 1, *b*) [14]. Likely somatic SNVs with VAF 10%—30% and likely germline SNVs with VAF > 30% were detected most frequently (45.6% and 31.6%, respectively).

Seven tumor-associated genes (*KRAS, TP53, CHEK2, PTEN, AKT1, APC,* and *SMAD4*) showed the presence of clinically associated mutations at a specific gene location or codon in different samples (Table 1). Gene variants in codon 12 of the *KRAS* gene were detected in 6 colon adenocarcinoma samples, and the variant *KRAS* c.38G>A in codon 13 was detected twice. The *CHEK2* c.470T>C variant was detected in 3 colon adenocarcinoma samples, and the *APC* c.3340C>T, *SMAD4* c.1082G>A, *PTEN* c.389G>A, *AKT1* c.49G>A, and *TP53* c.817C>T variants were each detected twice.

*Table 1.* **Gene variants found in several colon adenocarcinoma samples**

| Gene | Coding | Amino Acid Change | VAF |
|---|---|---|---|
| KRAS | c.34G>A | p.Gly12Ser | 34.71% |
| KRAS | c.35G>A | p.Gly12Asp | 31.08% |
| KRAS | c.35G>A | p.Gly12Asp | 13.5% |
| KRAS | c.35G>A | p.Gly12Asp | 22.3% |
| KRAS | c.35G>T | p.Gly12Val | 31.07% |
| KRAS | c.35G>T | p.Gly12Val | 8.6% |
| KRAS | c.38G>A | p.Gly13Asp | 25.95% |
| KRAS | c.38G>A | p.Gly13Asp | 30.5% |
| APC | c.3340C>T | p.Arg1114* | 34.0% |
| APC | c.3340C>T | p.Arg1114* | 15.4% |
| SMAD4 | c.1082G>A | p.Arg361His | 34.49% |
| SMAD4 | c.1082G>A | p.Arg361His | 51.8% |
| CHEK2 | c.470T>C | p.Ile157Thr | 50.70% |
| CHEK2 | c.470T>C | p.Ile157Thr | 72.67% |
| CHEK2 | c.470T>C | p.Ile157Thr | 49.41% |
| PTEN | c.389G>A | p.Arg130Gln | 39.59% |
| PTEN | c.389G>A | p.Arg130Gln | 13.76% |
| AKT1 | c.49G>A | p.Glu17Lys | 38.82% |
| AKT1 | c.49G>A | p.Glu17Lys | 22.76% |
| TP53 | c.817C>T | p.Arg273Cys | 28.42% |
| TP53 | c.817C>T | p.Arg273Cys | 40.18% |
| TP53 | c.818G>A | p.Arg273His | 24.63% |

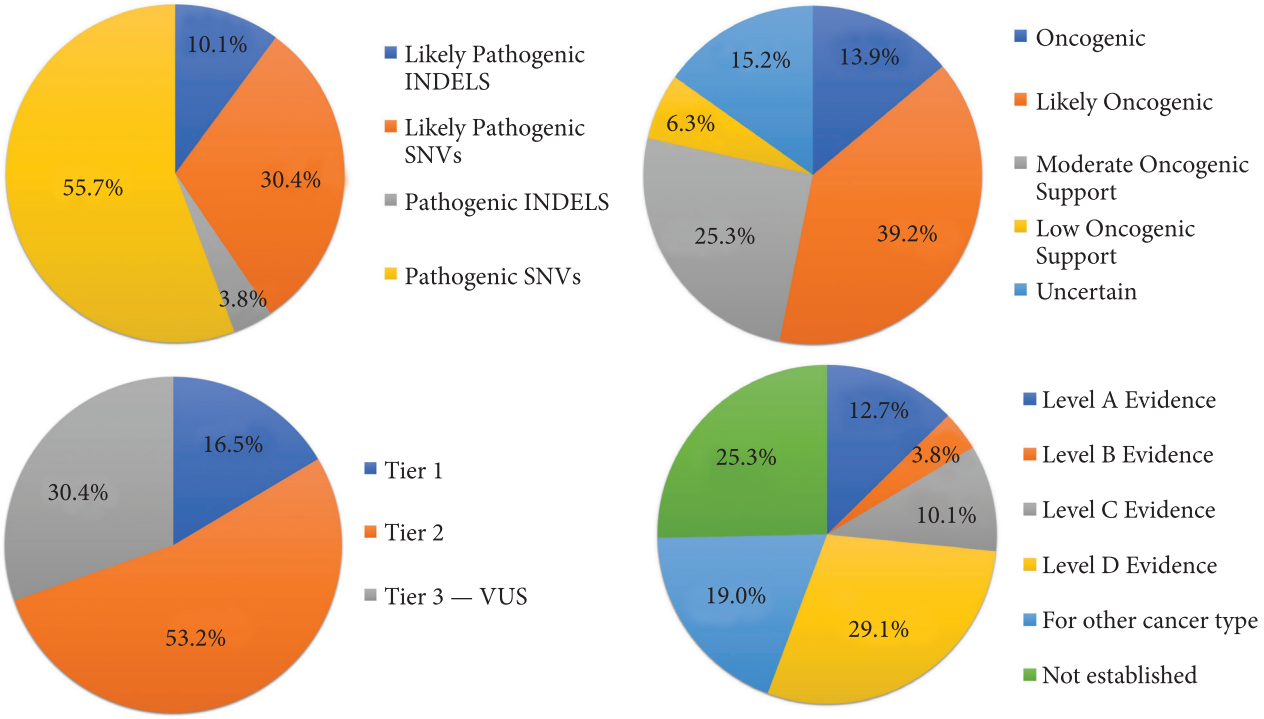


***Fig. 2.*** Distribution of clinically relevant gene variants (alterations) according to different clinical and pathological classifications in colon adenocarcinoma samples: *a* — ACMG classification; *b* — Oncogenic classification; *c* — AMP classification; *d* — Therapeutic significance

By the ACMG classification, the detected clinically relevant gene variants belong to 4 groups: pathogenic SNVs (the largest group), pathogenic INDELs, likely pathogenic SNVs, and likely pathogenic INDELs (Fig. 2, *a*). However, 53.2% of these variants classified as pathogenic or likely pathogenic by the ACMG fell into Tier 3 (a variant of uncertain significance) by the somatic AMP classification (Fig. 2, *c*). However, only 15.2% of these ACMG variants were classified as having uncertain oncogenicity, while 6.3% were assigned low oncogenic support under the established Oncogenic classification by Franklin (Fig. 2, *b*) [15].

We identified no genetic variants that are included in the professional guidelines as diagnostically significant in colon adenocarcinoma. However, 34 variants were identified as possessing diagnostic significance in other cancer types. In 10 of 20 samples, we identified 11 variants that were included in the professional guidelines as prognostic in colon adenocarcinoma: 2 at level A evidence, 3 at level B evidence, and 6 at level C evidence.

Most importantly, in 19 of 20 samples, we identified 44 variants included in professional guidelines as those that predict response or resistance to therapies in colon adenocarcinoma. These variants were classified by the level of evidence as follows: 10 at level A, 3 at level B, 8 at level C, and 23 at level D (Fig. 2, *d*). Overall, more than 55% of the mutations were found to have therapeutic significance.

## Discussion

This article describes the study of genetic alterations in colorectal adenocarcinoma samples of Ukrainian patients that were detected by the NGS Comprehensive Cancer panel and analyzed by three international classifications (ACMG — hereditary, AMP — somatic, Oncogenic — oncogenicity level) using Franklin by Genoox to identify (filter) clinically relevant variants. It should be noted that the number of filtered relevant variants was lower than the number annotated by Ion Reporter from the primary data by orders of magnitude (not shown in the report). Initial annotation of the sequencing results identified approximately 500 to 2,500 variants in each sample that differed from the reference genome. The primary dataset contains genetic alterations regardless of their clinical significance, i.e., both previously known and

described as well as those identified for the first time. This is because most of the mutations have not yet been clinically relevant, as this is a lengthy process for database analysis, but the number of such gene variants is growing every year due to new genetic studies of various types of cancer [16].

Clinically relevant variants of the *KRAS, APC,* and *TP53* genes found in several colon adenocarcinoma samples in this study (Table 2) are critical for the development of this type of cancer [17].

Of particular note are mutations classified as Tier 1—2 in the somatic classifications, which account for nearly 50% of all clinically relevant genetic variants detected (Fig. 2, *c*).

Even in this small sample, we detected 11 novel gene variants that have not been reported in the literature or databases. Nevertheless, they are clinically relevant if they pass the pathogenicity classifications according to the Franklin by Genoox analysis algorithms. As shown in Table 2, the sequencing results of these 11 novel genetic mutations in 9 tumor-associated genes were detected in 9 samples from patients with colon adenocarcinoma.

The most clinically important gene variants are those that belong to Tier 1—2. There are 4 gene variants. 1 of them is in the *G6PD* gene and 3 are in the *APC* gene, whose genetic changes are critical for the development of colon cancer [17—19]. It should be noted that the *G6PD* gene variant c.895C>T, although low in oncogenic classification, has a high therapeutic value and influence on FDA-approved treatment protocols (level A).

Among 7 gene variants that have a somatic classification of Tier 3 (VUS), the most interesting are the *SMAD2* c.821delGinsTT and *FLCN* c.879del gene variants, which have high rates of oncogenicity classification as oncogenic and potentially oncogenic, respectively [15].

A comparison of the results of the clinically relevant genetic alterations with the COSMIC database for colon adenocarcinomas (https://cancer.

*Table 2.* **Newly identified clinically relevant gene variants in colon adenocarcinomas**

| Gene | Nucleotide dot | Protein dot | VAF | Effect | Oncogenic classification | AMP classification | Diagnostic significance | Therapeutic significance |
|---|---|---|---|---|---|---|---|---|
| G6PD | c.895C>T | p.Gln299Ter | 7.03% | Stop Gain | 2 Low Oncogenic Support | Tier 1 | 0 | A |
| APC | c.1453del | p.Met485CysfsTer13 | 28.15% | Frameshift | 9 Likely Oncogenic | Tier 2 | medulloblastoma | D |
| APC | c.4480del | p.Glu1494LysfsTer13 | 20.59% | Frameshift | 5 Moderate Oncogenic Support | Tier 2 | 1 | D |
| APC | c.3988_3989del | p.Pro1330Ter | 43.48% | Frameshift | 5 Moderate Oncogenic Support | Tier 2 | medulloblastoma | D |
| DST | c.4074del | p.Lys1358AsnfsTer11 | 29.17% | Frameshift | 1 Uncertain | Tier 3 - VUS | 0 | 0 |
| SYNE1 | c.7350+2T>C | | 27.47% | Splice Donor | 1 Uncertain | Tier 3 - VUS | 0 | 0 |
| DST | c.17707A>T | p.Lys5903Ter | 23.77% | Stop Gain | 1 Uncertain | Tier 3 - VUS | 0 | 0 |
| NIN | c.2399+1G>A | | 23.81% | Splice Donor | 1 Uncertain | Tier 3 - VUS | 0 | 0 |
| AURKC | c.584+1G>A | | 24.55% | Splice Donor | 1 Uncertain | Tier 3 - VUS | 0 | 0 |
| SMAD2 | c.821delGinsTT | p.Trp274PhefsTer8 | 24.00% | Frameshift | 10 Oncogenic | Tier 3 - VUS | 0 | 0 |
| FLCN | c.879del | p.Glu294ArgfsTer29 | 41.31% | Frameshift | 9 Likely Oncogenic | Tier 3 - VUS | 0 | 0 |

sanger.ac.uk/cosmic) showed that a number of genes with the highest percentage of genetic changes in tumors were found to be common among 20 genes in this database, namely *APC, TP53, ATM, PIK3CA, KRAS, BRAF,* and *SMAD4*. The lack of matches for other genes and variants may also be due to the absence of these genes in the panel under study and the presence of all types of disorders in the database, including those with unspecified clinical significance. This will be verified and completed in further studies.

The pilot study of genetic alterations of gene variants using the NGS Comprehensive Cancer Panel in colon cancer samples from Ukrainian patients allowed us to identify a number of known clinically relevant variants with high therapeutic value in known genes, including *APC, TP53, ATM, PIK3CA, FBXW7, KRAS, BRAF,* and *SMAD4*. In addition, some variants with high clinical significance were detected for the first time in 9 genes, including *G6PD, APC, SMAD2,* and *FLCN*. Most importantly, variants with high therapeutic significance were detected in 19 of 20 colon adenocarcinoma samples. This demonstrates the extremely high clinical power of the Comprehensive Cancer Panel applied even to small-sized samples. These results require further confirmation in a larger number of samples and by deeper analysis using other approaches.

## Funding

This work was supported by a grant from the National Research Foundation of Ukraine [grant number 2021.01/0024] and a Simons Support Grant [grant number 1290589].

## Conflict of interest

The authors declare no competing interests.

REFERENCES

1. Fontana F, Anselmi M, Limonta P. Molecular mechanisms of cancer drug resistance: emerging biomarkers and promising targets to overcome tumor progression. *Cancers (Basel)*. 2022;14(7):1614. https://doi.org/10.3390/cancers14071614
2. Mansoori B, Mohammadi A, Davudian S, et al. The different mechanisms of cancer drug resistance: A brief review. *Adv Pharm Bull*. 2017;7(3):339-348. https://doi.org/10.15171/apb.2017.041
3. Waarts MR, Stonestrom AJ, Park YC, et al. Targeting mutations in cancer. *J Clin Invest*. 2022;132(8):e154943. https://doi.org/10.1172/JCI154943.
4. Konda P, Garinet S, Van Allen EM, et al. Genome-guided discovery of cancer therapeutic targets. Cell Rep. 2023;42(8):112978. https://doi.org/10.1016/j.celrep.2023.112978
5. Gammall J, Lai AG. Pan-cancer prognostic genetic mutations and clinicopathological factors associated with survival outcomes: a systematic review. *NPJ Precis Oncol*. 2022;6(1):27. https://doi.org/10.1038/s41698-022-00269-5
6. Richards S, Aziz N, Bale S, et al. ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424. https://doi.org/10.1038/gim.2015.30
7. Carvalho NA, Santiago KM, Maia JML, et al. Prevalence and clinical implications of germline pathogenic variants in cancer predisposing genes in young patients across sarcoma subtypes. *J Med Genet*. 2023;61(1):61-68. https://doi.org/10.1136/jmg-2023-109269
8. Di Rado S, Giansante R, Cicirelli M, et al. Detection of germline mutations in a cohort of 250 relatives of mutation carriers in multigene panel: impact of pathogenic variants in other genes beyond BRCA1/2. *Cancers (Basel)*. 2023;15(24):5730. https://doi.org/10.3390/cancers15245730
9. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods*. 2010;7(4):248-249. https://doi.org/10.1038/nmeth0410-248.
10. Choi Y, Sims GE, Murphy S, et al. Predicting the functional effect of amino acid substitutions and indels. *PLoS One*. 2012;7(10):e46688. https://doi.org/10.1371/journal.pone.0046688
11. Schwarz JM, Cooper DN, Schuelke M, et al. MutationTaster2: mutation prediction for the deep-sequencing age. *Nat Methods*. 2014;11(4):361-362. https://doi.org/10.1038/nmeth.2890
12. Amendola LM, Jarvik GP, Leo MC, et al. Performance of ACMG-AMP variant-interpretation guidelines among nine laboratories in the clinical sequencing exploratory research consortium. *Am J Hum Genet*. 2016;98(6):1067-1076. https://doi.org/10.1016/j.ajhg.2016.03.024
13. Nykamp K, Anderson M, Powers M, et al. Sherloc: a comprehensive refinement of the ACMG-AMP variant classification criteria. *Genet Med*. 2017;19(10):1105-1117. https://doi.org/10.1038/gim.2017.37

14. Mandelker D, Donoghue M, Talukdar S, et al. Germline-focused analysis of tumour-only sequencing: recommendations from the ESMO Precision Medicine Working Group. *Ann Oncol*. 2019;30(8):1221-1231. https://doi.org/10.1093/annonc/mdz136
15. Horak P, Griffith M, Danos AM, et al. Standards for the classification of pathogenicity of somatic variants in cancer (oncogenicity): Joint recommendations of Clinical Genome Resource (ClinGen), Cancer Genomics Consortium (CGC), and Variant Interpretation for Cancer Consortium (VICC). *Genet Med*. 2022;24(5):986-998. https://doi.org/10.1016/j.gim.2022.01.001
16. Tang J, Lam GT, Brooks RD, et al. Exploring the role of sporadic BRAF and KRAS mutations during colorectal cancer pathogenesis: A spotlight on the contribution of the endosome-lysosome system. *Cancer Lett*. 2024;585:216639. https://doi.org/10.1016/j.canlet.2024.216639
17. Okimoto K, Hirotsu Y, Arai M, et al. Validity of pathological diagnosis for early colorectal cancer in genetic background. *Cancer Med*. 2023;12(7):8490-8498. https://doi.org/10.1002/cam4.5596
18. Zabeti Touchaei A, Vahidi S, Samadani AA. Immune landscape in APC and TP53 related tumor microenvironment in colon adenocarcinoma: A bioinformatic analysis. *Eur J Microbiol Immunol (Bp)*. 2024;14(2):154-165. https://doi.org/10.1556/1886.2024.00015
19. Zhang L, Shay JW. Multiple roles of APC and its therapeutic implications in colorectal cancer. *J Natl Cancer Inst*. 2017;109(8):djw332. https://doi.org/10.1093/jnci/djw332

Submitted: June 18, 2024

*Г. Геращенко [1], Р. Гульковський [1], Н. Мельничук [1], Н. Грищенко [1],*
*Т. Марчишак [1], О. Маньковська [1], А. Безверхий [1], І. Котуза [2],*
*Л. Роша [2], А. Котуза [2], З. Ткачук [1], В. Кашуба [1], М. Тукало [1]*

[1] Інститут молекулярної біології і генетики НАН України, Київ, Україна
[2] Клінічна лікарня «Феофанія» Державного управління справами, Київ, Україна

## ІДЕНТИФІКАЦІЯ КЛІНІЧНО-ЗНАЧУЩИХ ВАРІАНТІВ ГЕНІВ У ЗРАЗКАХ АДЕНОКАРЦИНОМИ ТОВСТОЇ КИШКИ УКРАЇНСЬКИХ ПАЦІЄНТІВ ЗА ДОПОМОГОЮ КОМПЛЕКСНОЇ ОНКОЛОГІЧНОЇ ПАНЕЛІ: ПІЛОТНЕ ДОСЛІДЖЕННЯ

**Метою** дослідження було виявити клінічно-значущі варіанти генів у зразках аденокарциноми товстої кишки українських пацієнтів за допомогою NGS Comprehensive Cancer Panel (CCP) для впровадження їх у зручній для клінічної практики формі. **Методи**. У дослідженні використано 20 зразків пацієнтів віком 60—87 років, усі з української популяції, які хворіли на рак товстої кишки різної локалізації та ступеня диференціювання. Для виявлення клінічно-значущих варіантів генів дані секвенування CCP панелі були відфільтровані за допомогою бази даних Franklin by Genoox. **Результати**. Усього виявлено 79 клінічно-значущих варіантів генів (SNV, INDEL) у 28 з 409 генів. Найбільша кількість мутацій виявлено в трьох генах — APC, TP53 і KRAS (16, 14 і 8, відповідно); по чотири варіанти — в генах *PTEN* і *SMAD4*, по три варіанти — в генах *CHEK2*, *ERBB2* і *PIK3CA* і по два варіанти — в генах *AKT1, ATM, DST, IDH1* і *TCF12*. Для семи генів — *KRAS, TP53, CHEK2, PTEN, AKT1, APC* і *SMAD4* — мутації виявлено більш ніж в одному зразку. Частота варіантів генів 1-2 рівня клінічної значущості (Tier 1—2) становила близько 50% від усіх виявлених генетичних варіантів. Терапевтичне значення виявлено у понад 55% мутацій. Крім того, ідентифіковано 11 нових генетичних мутацій у 9 генах, зокрема *G6PD, APC, DST, SINE1, SMAD2* і *FLC*N. **Висновки**. Ці дані свідчать про високий рівень клінічної ефективності підходу секвенування CCP панелі. Потрібне подальше підтвердження на більшій кількості зразків та глибший аналіз з використанням інших підходів.

**Ключові слова**: NGS, варіанти генів, аденокарцинома товстої кишки, клінічне значення.