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MULTIOMIC PATTERN OF TMPRSS2 (RS12329760) GENE REGULATORY EFFECTS: MQTL, PQTL AND TUQTL IN COVID-19

Sokolenko M.O., Sydorchuk L.P.

Bukovinian State Medical University, Chernivtsi, Ukraine

Key words: *COVID-19, TMPRSS2 gene (rs12329760), methylation quantitative trait loci (mQTL), protein quantitative trait loci (pQTL), transcript usage quantitative trait loci (tuQTL), serine protease, enzymes, mechanisms, inflammation, SARS-CoV-2.*

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E-mail: *sokolenko_maks@ukr.net lsydorchuk@ukr.net*

Objective *– of the study was to analyse multiomic characteristics of TMPRSS2 (rs12329760) gene regulatory effects – mQTL, pQTL and tuQTL and to explore its potential implications for COVID-19-related molecular pathways.*

Material and methods. *Genotyping of the TMPRSS2 gene (rs12329760) locus was performed in 48 patients with a mild course and 72 patients with moderate-to-severe COVID-19. Genomic DNA was extracted from peripheral blood leukocytes, and allelic discrimination of the target SNP was conducted using RT-PCR. mQTL, pQTL and tuQTL associations of rs12329760 (TMPRSS2) were identified using the QTLbase database within a ±10 Mb cis-window (chr21:42852497, hg19). Effect sizes (β), standard errors (SE), and p-values were extracted for each association. Gene and transcript annotation, including ENSG and ENST identifiers, were verified using the Ensembl (GRCh37/hg19 assembly) to ensure standardized gene nomenclature and accurate transcript-level mapping.*

Results. *In patiens blood, rs12329760 of the TMPRSS2 gene was found to be associated with methylation changes at the MX1 locus (cg10833439), where the effective T allele suppressed mQTL activity (β=-0.0086; p=1.680e-4). In the prefrontal cortex, rs12329760 showed a positive, although statistically insignificant, association with MX1 pQTL levels (β=0.0679–0.0588; p=0.0539–0.0871), indicating possible context-dependent proteomic modulation. In iPSC stem cells, rs12329760 of the TMPRSS2 gene was also found to be associated with 21 cis-regulatory transcript usage associations spanning 11 polymorphic loci. The variant demonstrated bidirectional tuQTL effects: suppression of BACE2 (ENST00000330333), MX1 (ENST00000486275; ENST00000619682) and C2CD2 (ENST00000482084) transcripts (β=-0.5201–-0.2906; p=0.0075–8.660e-5), while enhancing alternative isoforms of BACE2 (ENST00000491838), C2CD2 (ENST00000467074) and MX1 (ENST00000490220) transcripts (β=0.4545–0.2525; p=0.0065–9.260e-4).*

Conclusions. *Therefore, the multiomic analysis of mQTL, pQTL and tuQTL data confirms the functional significance of rs12329760 of the TMPRSS2 gene as a regulatory genetic modifier, and the detected bidirectional modulation of MX1 and other immune-related genes indicates its possible impact on antiviral response pathways associated with SARS-CoV-2 infection.*

МУЛЬТИОМНИЙ ПАТЕРН РЕГУЛЯТОРНИХ ЕФЕКТІВ ГЕНА TMPRSS2 (RS12329760): MQTL, PQTL ТА TUQTL ПРИ COVID-19

Соколенко М.О., Сидорчук Л.П.

Ключові слова: *COVID-19, ген TMPRSS2 (rs12329760), локуси кількісних ознак метилювання (mQTL), локуси кількісних ознак білка (pQTL), локуси кількісних ознак використання транскрипту (tuQTL), серинова протеаза, ферменти, механізми, запалення, SARS-CoV-2.*

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Мета роботи *– проаналізувати мультиомні характеристики регуляторних ефектів гена TMPRSS2 (rs12329760) – mQTL, pQTL та tuQTL, а також дослідити їх потенційний вплив на молекулярні шляхи, пов'язані з COVID-19.*

Матеріал і методи. *Генотипування локусу гена TMPRSS2 (rs12329760) проведено в 48 пацієнтів з легким перебігом та 72 пацієнтів із COVID-19 середнього та тяжкого ступеня. Геномну ДНК екстраговано з лейкоцитів периферичної крові, а алельну дискримінацію цільового SNP проведено за допомогою RT-PCR. Асоціації mQTL, pQTL та tuQTL гена rs12329760 (TMPRSS2) ідентифіковані за допомогою бази даних QTLbase у межах cis-вікна ±10 Мб (chr21:42852497, hg19). Для кожної асоціації були отримані розміри ефектів (β), стандартні помилки (SE) та p-значення. Анотацію генів та транскриптів, включаючи ідентифікатори ENSG та ENST, перевірено за допомогою Ensembl (GRCh37/hg19) для забезпечення*

стандартизованої номенклатури генів та точного картування на рівні транскриптів.

Результати. *Виявлено, що у крові хворих rs12329760 гена TMPRSS2 пов'язаний зі змінами метилювання в локусі MX1 (cg10833439), де ефективний алель T пригнічував активність mQTL (β=-0,0086; p=1,680e-4). У префронтальній корі rs12329760 показав позитивний, хоча й статистично незначущий, зв'язок із рівнями MX1 pQTL (β=0,0679–0,0588; p=0,0539–0,0871), що вказує на можливу контекстно-залежну протеомну модуляцію. У стовбурових клітинах iPSC також було виявлено, що rs12329760 гена TMPRSS2 пов'язаний з 21 асоціацією використання цис-регуляторного транскрипту, що охоплюють 11 поліморфних локусів. Варіант продемонстрував двонаправлені ефекти tuQTL: пригнічення транскриптів BACE2 (ENST00000330333), MX1 (ENST00000486275; ENST00000619682) та C2CD2 (ENST00000482084) (β=-0.5201–-0.2906; p=0.0075–8.660e-5), одночасно посилюючи альтернативні ізоформи транскриптів BACE2 (ENST00000491838), C2CD2 (ENST00000467074) та MX1 (ENST00000490220) (β=0.4545–0.2525; p=0.0065–9.260e-4).*

Висновки. *Таким чином, мультиомний аналіз даних mQTL, pQTL та tuQTL підтверджує функціональне значення rs12329760 гена TMPRSS2 як регуляторного генетичного модифікатора, а виявлена двонаправлена модуляція MX1 та інших імуноасоційованих генів вказує на її можливий вплив на шляхи противірусної відповіді, пов'язані з інфекцією SARS-CoV-2.*

Introduction. The COVID-19 pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has upended the daily life of individuals around the world, with historic numbers of cases, deaths and profound socioeconomic disruption worldwide [1, 2]. Among different genes potentially involved in COVID-19, the association between the polymorphisms of renin-angiotensin-aldosterone system-related genes, i.e., ACE2 and TMPRSS2 and the severity of COVID-19 disease have been the most investigated [3]. In fact, ACE2 rs2285666 and TMPRSS2 rs12329760 single nucleotide polymorphisms (SNPs) have been investigated in several studies, although their results are controversial. Some studies have correlated the T (A) allele of the TMPRSS2 rs12329760 [4] SNPs with lower severity of COVID-19 in an Indian population.

The rs12329760 variant of the TMPRSS2 gene is located in the coding part of the gene. According to the predictions of some bioinformatics software, this variant can decrease enzyme activity and stability; hence activation of the SARS-CoV-2 and its entry into host cells are diminished [5]. Therefore, this variant has been predicted to have a protective effect against SARS-CoV-2 infection. Interestingly, this variant has been proposed as one of the candidate gene variants in justifying the high incidence and COVID-19-related mortality rate in the Italian population compared to the other European and Asian countries [6]. The gene encoding TMPRSS2 is located on chromosome 21 at 21q22.3. It is highly polymorphic, considering the variations in frequencies according to the studied population, and the literature presents two SNPs as potential modulators of the infectious process caused by SARS-CoV-2: rs2070788 and rs12329760 [7]. rs12329760 is characterized by a substitution of the T allele for C at position 589 of the gene, a promoter region, and is therefore classified as a missense mutation, replacing a valine with a methionine at position 160, the T allele appears to be related to lower expression of the TMPRSS2 protein, with moderate catalytic activity [8].

Existing systems science approaches allow us to consider genetic variants not only as structural changes in proteins, but also as regulatory factors that affect the multilevel organization of gene expression. Accordingly, the analysis of mQTL (methylation quantitative trait loci), pQTL (protein quantitative trait loci) and tuQTL (transcript usage quantitative trait loci) opens up the possibility of qualitatively assessing the impact of SNPs on epigenetic modifications and alternative transcript usage [9]. Despite the availability of data on eQTL effects of TMPRSS2 [10], a comprehensive multiomic characterization of mQTL, pQTL and tuQTL effects of rs12329760 in the context of COVID-19 is practically absent. Thus, there is a need for a systems study that will combine genetic, transcriptomic and proteomic levels to clarify the functional role of rs12329760.

The aim of the study was to analyse multiomic characteristics of TMPRSS2 (rs12329760) gene regulatory effects – mQTL, pQTL and tuQTL and to explore its potential implications for COVID-19-related molecular pathways.

Research materials and methods.

Identification of Genetic Polymorphisms. Genotyping of the TMPRSS2 (rs12329760) locus was performed in 72 patients with moderate to severe COVID-19 and 48 patients with mild disease. Genomic DNA was extracted from peripheral blood leukocytes using the Thermo Scientific™ GeneJET™ Whole Blood Genomic DNA Purification Mini Kit following the manufacturer's instructions, and allelic discrimination of the targeted SNP was performed using RT-PCR. mQTL, pQTL, and tuQTL associations of rs12329760 (TMPRSS2) were identified using the QTLbase database within a ±10 Mb cis-window (chr21:42852497, hg19). Effect sizes (β), standard errors (SE), and p-valueswere extracted for each association. Gene and transcript annotations, including ENSG and ENST identifiers, were verified using Ensembl (GRCh37/hg19 assembly) to ensure standardized gene

nomenclature and accurate mapping at the transcript level.

The study was conducted in accordance with international ethical and bioethical principles in accordance with ICH-GCP standards, the Declaration of Helsinki (1964 as amended), the Council of Europe Convention on Human Rights and Biomedicine (1997), and current Ukrainian legislation. The study protocol was reviewed and approved by the Bioethics Committee of the Bukovina State Medical University (Protocol No. 2, October 2025). Written informed consent was obtained from all participants before inclusion in the study.

Statistical Analysis. All statistical analyses were performed in accordance with contemporary biomedical research standards using Statistica 13.0 software (StatSoft Inc., USA; license No. JPZ804I382130ARCN10-J). The χ^2 (Pearson) test was employed to assess differences in genotype frequency distributions. The significance of differences between independent samples with approximately normal distributions was evaluated using Student's t-test, whereas the Wilcoxon–Mann–Whitney U test was applied for non-normally distributed data. Statistical significance was accepted at $p < 0.05$.

Results and their discussion. The heatmap of the methylation quantitative trait locus (mQTL) rs12329760 of the TMPRSS2 gene in blood, in interaction with the MX1 gene within a single association (cis-regulation) region (± 10M; chr21:42852497 (hg19)), is shown in Figure 1. The rs12329760 variant of the TMPRSS2 gene suppresses the mQTL activity of the MX1 gene (cg10833439 (chr21:42793818)) in blood through the effective T allele of interaction (β=-0,0086; SE= 0,0021; p=1,680e-4).

The protein quantitative trait locus (pQTL) rs12329760 of the TMPRSS2 gene in the prefrontal cortex, in interaction with the MX1 gene within two associations via cis-regulation (± 10M region; chr21:42852497 (hg19)), is shown in Figure 2. The rs12329760 variant of the TMPRSS2 gene enhances the activity of the pQTL locus of the MX1 gene (P20591 (chr21:42792231-42831141)) in the prefrontal cortex through the effective T allele of interaction; however, the association is not statistically significant (β=0,0679-0,0588; SE= 0,0344; p=0,0539-0,0871).

The transcript usage quantitative trait loci (tuQTL) rs12329760 of the TMPRSS2 gene in iPSC stem cells, in association with 11 gene polymorphisms through 21 cis-regulatory interactions (± 10M region; chr7:150690079 (hg19)), are presented in Figure 3.

The list of Ensembl genes (ENSG), including merged transcripts (ENST) with coding sequences within the tuQTL gene loci interacting with TMPRSS2 via rs12329760 in iPSC stem cells, is provided in Table 1. Transcripts belonging to the same gene identifier (MX1,

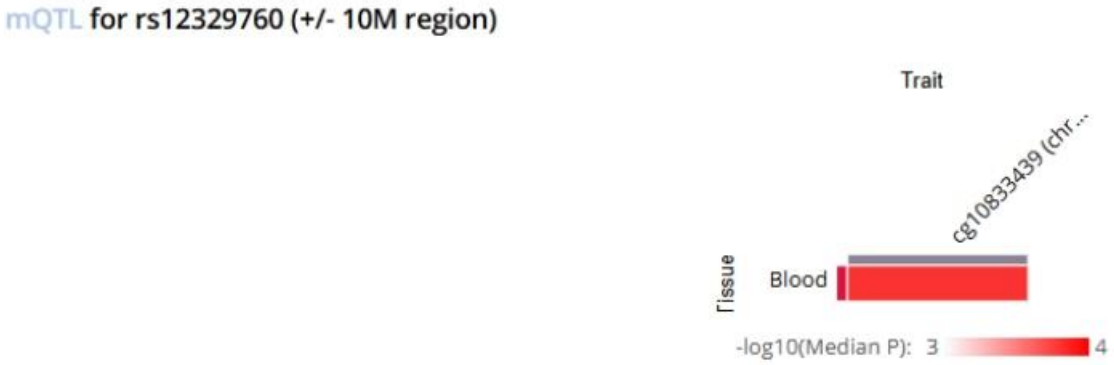

Fig. 1. Heatmap visualization of the methylation quantitative trait locus (mQTL) for dbSNP: rs12329760 of the TMPRSS2 gene in blood, in interaction with the MX1 gene within a single association (cis-regulation) region (± 10M; chr21:42852497 (hg19)). The color intensity of each cell corresponds to the mQTL median -log10 (Median P)

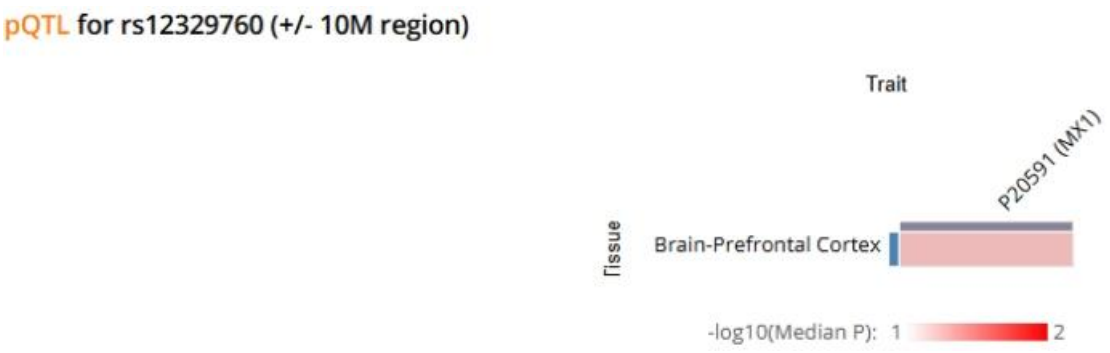

Fig. 2. *Heatmap of the protein quantitative trait locus (pQTL) for dbSNP: rs12329760 of the TMPRSS2 gene in the prefrontal cortex, in interaction with the MX1 gene within two associations via cis-regulation (± 10M region; chr21:42852497 (hg19)). The color intensity of each cell corresponds to the pQTL median -log10 (Median P)*

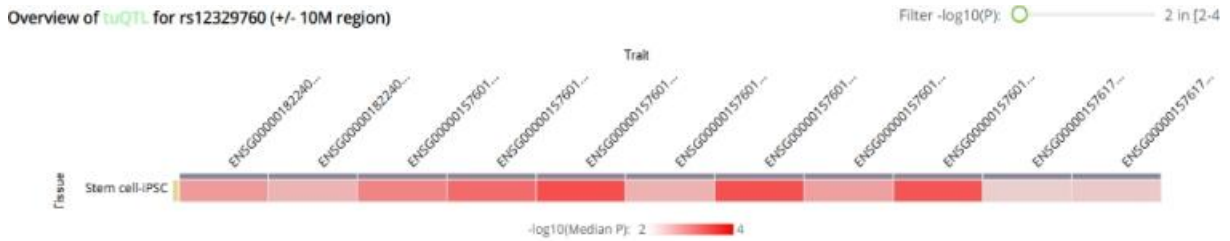

Fig. 3. Heatmap of transcript usage quantitative trait loci (tuQTL) for dbSNP: rs12329760 of the TMPRSS2 gene in iPSC stem cells, in association with 11 gene polymorphisms and 21 associations (via cis-regulation) (± 10M region; chr7:150690079 (hg19)). The color intensity of each cell corresponds to the tuQTL median -log10 (Median P)

BACE2) differ in transcription start and end sites, splicing events, and exon composition, and may give rise to substantially different proteins. Clusters of overlapping transcripts lacking coding sequences are annotated as separate genes. Transcripts overlapping within non-coding sequences (i.e., intronic regions or UTR – UnTranslated Region) are classified as distinct genes. The interaction of rs12329760 with transcripts of the MX1 gene (ENST00000486275; ENST00000619682; ENST00000486275), BACE2 (ENST00000330333), and C2CD2 (ENST00000482084) was associated with suppression of their activity (β=-0,5201-/-0,2906/; p=0,0075-8,660e-5). In contrast, interaction of rs12329760 with other transcripts of the MX1 gene (ENST00000490220; ENST00000490220) and the genes C2CD2 (ENST00000467074) and BACE2 (ENST00000491838) was accompanied by increased tuQTL activity (β=0,4545-0,2525; p=0,0065-9,260e-4).

Table 1

Transcript usage quantitative traits (tuQTL) of the TMPRSS2 gene (dbSNP: rs12329760) in iPSC stem cells

Gene influencing transcript usage	Effective allele of interaction.	Effect size β.	SE	P
MX1 (ENSG00000157601.grp_1.contained.*ENST00000486275*; chr21:42792232-42831141)	-	-0,4996- /-0,2906 /	0,1231- 0,1467	8,660e^{-5}- 0,0052
MX1 ENSG00000157601.grp_1.contained.*ENST00000490220*; chr21:42792232-42831141	-	0,4057- 0,2830	0,1161- 0,0874	6,590e^{-4}- 0,0015
MX1 ENSG00000157601.grp_1.contained.*ENST00000619682*; chr21:42792232-42831141	-	-0,5201	0,1473	5,860e-4
MX1 ENSG00000157601.*grp_2.contained.ENST00000486275*; chr21:42792232-42831141	-	-0,4982-/- 0,4162/	0,1213- 0,1462	7,240e^{-5}- 0,0052
MX1 ENSG00000157601.*grp_2.contained.ENST00000490220*; chr21:42792232-42831141	-	0,4345-0,2525	0,1280- 0,0892	9,260e^{-4}- 0,0054
C2CD2 ENSG00000157617.grp_1.contained.*ENST00000467074*; chr21:43305222-43373999	-	0,4250	0,1535	0,0065
C2CD2 ENSG00000157617.grp_1.contained. *ENST00000482084*; chr21:43305222-43373999	-	-0,4222	0,1552	0,0075
BACE2 ENSG00000182240.grp_2.contained.*ENST00000330333*; chr21:42539729-42654445	-	-0,4722	0,1529	0,0025
BACE2 ENSG00000182240.grp_2.contained. *ENST00000491838*; chr21:42539729-42654445	-	0,4545	0,1559	0,0042

Note. SE (standard error) – standard error.

Based on the results obtained, it can be stated that various forms of interaction between genes that determine the individual immune response and the TMPRSS2 gene in patients with COVID-19 are capable of forming complex

networks of regulatory connections, which in turn affect the clinical outcome of the disease. Specific changes in DNA methylation can qualitatively modify both the level of gene expression and the stability of transcripts and alternative splicing [11]. The diverse use of transcripts (tuQTL) determines the spectrum of protein isoforms that significantly differ in functional activity [12]. At the same time, proteomic variations (pQTL) directly demonstrated the level of functionally active molecules in target tissues quite clearly.

By conducting a study of the tissue-specific effects of rs12329760 in blood, brain and pluripotent stem cells (iPSC), we were able to characterize the systemic nature of the regulatory changes that occur during coronavirus disease. Taking into account the known neurotropic nature of SARS-CoV-2 and the numerous neurological complications that have been recorded in COVID-19 patients [13], analysis in brain tissue certainly has additional pathogenetic value. At the same time, the systemic immune response was reflected in the results of our study in the blood, and the identified iPSC models open up prospects for modern functional interpretation of genetic variants that can determine individual characteristics of the course of COVID-19, as well as contribute to the formation of the basis for the creation of personalized approaches in predicting the risk of developing severe forms of the disease and effective therapeutic management.

Conclusions. Therefore, the multiomic analysis of mQTL, pQTL and tuQTL data confirms the functional significance of rs12329760 of the TMPRSS2 gene as a regulatory genetic modifier, and the detected bidirectional modulation of MX1 and other immune-related genes indicates its possible impact on antiviral response pathways associated with SARS-CoV-2 infection.

Prospects for further research. It is promising to combine multiomic data with clinical phenotypes of COVID-19 in order to assess the prognostic significance of this variant for the severity of the disease and to develop personalized approaches to risk stratification. An important direction also remains the analysis of interpopulation differences in allele frequency and its regulatory effects.

Conflict of interest. The authors declare no conflict of interest.

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Information about the authors

Sokolenko M.O. – Candidate of Medical Sciences, Associate Professor of the Department of Infectious Diseases and Epidemiology, Bukovinian State Medical University, Chernivtsi, Ukraine. e-mail: sokolenko_maks@ukr.net.
ORCID ID: https://orcid.org/0000-0002-7150-7146

Sydorchuk L.P. – Doctor of Medical Sciences, Professor, Head of the Department of Family Medicine, Bukovinian State Medical University, Chernivtsi, Ukraine. e-mail: lsydorchuk@ukr.net
ORCID ID: https://orcid.org/0000-0001-9279-9531

Відомості про авторів
Соколенко М.О. – канд.мед.наук, доцент кафедри інфекційних хвороб та епідеміології Буковинського державного медичного університету, м. Чернівці, Україна. e-mail: sokolenko_maks@ukr.net.
ORCID ID: https://orcid.org/0000-0002-7150-7146
Сидорчук Л.П. – д-р мед. наук, професор, завідувачка кафедри сімейної медицини, Буковинського державного медичного університету, м. Чернівці, Україна. e-mail: lsydorchuk@ukr.net
ORCID ID: https://orcid.org/0000-0001-9279-9531

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