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Frequency of rs35803318 single nucleotide polymorphism of ACE2 gene among the Kazakhs

The article presents the results of genotyping of DNA samples obtained from the study participants on the single nucleotide polymorphism (SNP) rs35803318 (C/T) of the ACE2 gene. Genotyping was carried out by real-time polymerase chain reaction (PCR) using the technique Amplification of the Refractory Mutation System (ARMS). The frequencies of rs35803318 (C/T) genotypes and alleles in 96 representatives of the Kazakh ethnic group living in the Karaganda region were analyzed. According to the results of our study, the CT genotype (63.5%) is the most common among Kazakhs, the CC genotype was 20.8% and the TT genotype was 15.6%. The distribution of polymorphism alleles is as follows: allele C – 52.6%, allele A – 47.4%.

Keywords: ACE2, receptor, gene, SNP, single nucleotide polymorphism, ARMS, genetic variants, Kazakhs.

Introduction

One of the most important proteins in the human body, angiotensin-converting enzyme 2 (ACE), plays a vital role in the regulation of biological processes. ACE2 was first described in 2000 [1] and is a glycoprotein mainly expressed in the lungs, intestines, kidneys and heart [2]. ACE2 primarily functions in the regulation of the renin-angiotensin system (RAS), which plays a role in controlling blood pressure, hydroelectrolyte balance and cardiovascular homeostasis [3].

The interest in ACE2 has surged with the emergence of COVID-19, which is caused by the SARS-CoV-2 coronavirus. Research indicates that SARS-CoV-2 utilizes ACE2 as an entry receptor to infect body cells [4]. This discovery led to the development of many studies aimed at studying the interaction of SARS-CoV-2 with ACE2 and the development of therapeutic methods and vaccines [5].

In addition, ACE2 has become an object of research in the context of other diseases. For example, its role in pathologies associated with the cardiovascular system has been studied in detail [6]. ACE2 has also been linked to infections such as severe acute respiratory syndrome (SARS) [7] and associated with metabolic [8], renal [9].

The ACE2 gene encodes the angiotensin-converting enzyme 2. The ACE2 gene (Gene ID: Gene ID: 59272) is located in the short arm of human X chromosome (Xp22.2) and consists of 22 exons (ACE2 Angiotensin Converting Enzyme 2 [Homo Sapiens (Human)] — Gene — NCBI, n.d.) [5].

These changes can affect the structure, function, or regulation of genes. Gene polymorphisms are a natural and important aspect of genetic diversity and can have a variety of effects on organisms and their phenotypes.

The ACE2 receptor is a surprisingly interesting object of research in the field of biology and medicine. Its role in the regulation of RAS, its effect on diseases and pathologies, as well as its association with SARS-CoV-2 infection make it one of the key proteins requiring further study to better understand its functions and possible medical applications.

Materials and Methods

This study includes participants who are over the age of 18 and are representatives of the Kazakh ethnic group. The total number of participants was 96, of which 27 (28.1 %) — men, 69 (71.9 %) — women. The age range is 18–78 (Mean±SD 43.44±14.22).

The study was conducted in accordance with the recommendations of Helsinki ethical principles and approved by the local bioethics committee non-commercial joint-stock company "Karaganda Medical University" (protocol No. 2 dated 11 October 2022). Every participant provided written consent after being informed.

"RIBO-prep" kit (AmpliSens, Russia) was used to extract genomic DNA from venous blood samples. The isolation procedure was conducted in accordance with the manufacturer's instructions. To identify the target single nucleotide polymorphism (SNP) rs35803318 C>T the isolated DNA was analyzed using real-time polymerase chain reaction (Real-time PCR) using the technique Amplification of the Refractory Mutation System (ARMS). Real-time PCR was conducted using a DTlite amplifier (DNA Technology, Russia). The real-time PCR conditions and sequences of four primers (Lumiprobe, Russia) are presented in Table.

Table

rs35803318 primers list for ARMS-PCR

Direction	Primer Sequence	Real-time PCR conditions (denaturation, annealing cycles)
FIP (T allele)	5′-CAATGCCAACCACTATCACTCCCCTT-3′	94 °C/3 min (94 °C/15 sec, 68 °C/30 sec) × 40
RIP (C allele)	5′-CCATATGGCTGATTGTTTTTGGAGTTTTG-3′	
FOP	5′-AAGTCTAGGAAAGGCCACTTACTTCTTCCG-3′	
ROP	5′-TTTCTGGGGATACAGCAACACTTGGAC-3′	

The percentages were used to characterize the categorical variables. The $\chi 2$ test was employed to assess the Hardy-Weinberg equilibrium (HWE), and deviations from HWE were deemed significant at a p-value threshold of less than 0.05. The statistical analysis of the research results was conducted using the GraphPad Prism 8 program.

Results and Discussion

rs35803318 genotypes frequency did not correspond to the Hardy-Weinberg equilibrium (p=0.0271). According to the database dbSNP NCBI, the frequency of allele C (0.95043) is higher than allele T (0.04957) [10]. We identified alleles C and T, the frequency of their distribution was 52.6 % and 47.4 %, respectively (Fig. 1).

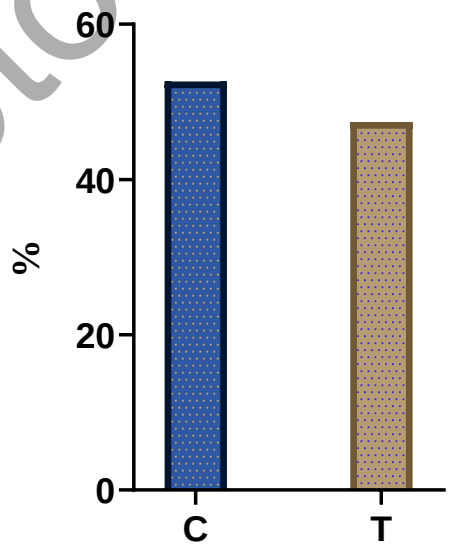

Figure 1. Frequency of rs35803318 alleles among Kazakhs

According to studies [11, 12], the rs35803318 genetic variant exhibits a higher frequency in Italian, European and American populations compared to the extremely low frequency seen in African and Asian groups.

The results of the study showed that among Kazakhs, the most common polymorphism genotype was CT, making up 63.5 % of the total number of examined participants, the CC genotype was 20.8 % and the TT genotype was 15.6 % (Fig. 2).

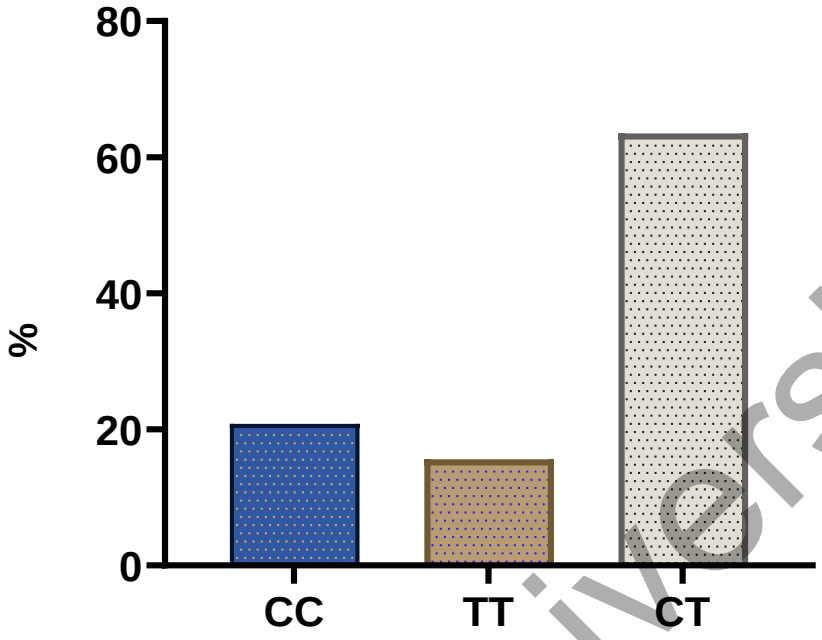

Figure 2. Frequency of rs35803318 genotypes among Kazakhs

The authors of this study [13] showed that the rs35803318 genetic variant is absent in Asian and practically absent in African populations, however, the frequency of polymorphism is higher in the indigenous peoples of the Amazon than in other populations. The data presented in this study [14] on the genetic variant rs35803318 in the synonymous coding region indicate interesting patterns of polymorphism distribution in different populations. Based on this data, it can be concluded that this variant is more polymorphic among American and European populations. Interestingly, in contrast to American and European populations, South Asians show less genetic variability in this synonymous coding region. This observation emphasises genetic variability and diversity between ethnic groups.

Conclusions

As a result of our study of the frequencies of rs35803318 genetic polymorphism in the Kazakh population, we have made an important conclusion. Among Kazakhs, the CT genotype was the most common, accounting for 63.5 %, while the CC genotype was 20.8 %, and the TT genotype was 15.6 %. In the Kazakh population, the C allele predominates at 52.6 % and the T allele at 47.4 %. These results provide valuable information about the genetic diversity in this population and can serve as a basis for further research on the relationship of this polymorphism with various diseases.

References

1 Donoghue, M., Hsieh, F., Baronas, E., Godbout, K., Gosselin, M., Stagliano, N., … & Acton, S. (2000). A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1–9. *Circulation Research*, *87*(5), E1–9. https://doi.org/10.1161/01.res.87.5.e1

2 Baig, A.M., Khaleeq, A., Ali, U., & Syeda, H. (2020). Evidence of the COVID-19 Virus Targeting the CNS: Tissue Distribution, Host–Virus Interaction, and Proposed Neurotropic Mechanisms. *ACS Chemical Neuroscience*, *11*(7), 995–998. https://doi.org/10.1021/acschemneuro.0c00122

3 Bader, M. (2010). Tissue Renin-Angiotensin-Aldosterone Systems: Targets for Pharmacological Therapy. *Annual Review of Pharmacology and Toxicology*, *50*(1), 439–465. https://doi.org/10.1146/annurev.pharmtox.010909.105610

4 Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., … & Pöhlmann, S. (2020). SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell*, *181*(2), 271–280.e8. https://doi.org/10.1016/j.cell.2020.02.052

5 Medina-Enríquez, M.M., Lopez-León, S., Carlos-Escalante, J.A., Aponte-Torres, Z., Cuapio, A., & Wegman Ostrosky, T. (2020). ACE2: the molecular doorway to SARS-CoV-2. Cell & Bioscience, 10(1), 148. https://doi.org/10.1186/s13578–020–00519–8

6 Crackower, M.A., Sarao, R., Oudit, G.Y., Yagil, C., Kozieradzki, I., Scanga, S.E., Penninger, J.M. (2002). Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature*, *417*(6891), 822–828. https://doi.org/10.1038/nature00786

7 Yang, X.-H., Deng, W., Tong, Z., Liu, Y.-X., Zhang, L.-F., Zhu, H., … & Qin, C. (2007). Mice transgenic for human angiotensin-converting enzyme 2 provide a model for SARS coronavirus infection. *Comparative Medicine*, *57*(5), 450–459.

8 Niu, M.-J., Yang, J.-K., Lin, S.-S., Ji, X.-J., & Guo, L.-M. (2008). Loss of angiotensin-converting enzyme 2 leads to impaired glucose homeostasis in mice. *Endocrine*, *34*(1–3), 56–61. https://doi.org/10.1007/s12020–008–9110-x

9 Yang, X., Wang, Y., Wang, J., Liu, Y., Deng, W., Qin, C., … & Zhang, L. (2012). Role of angiotensin-converting enzyme (ACE and ACE2) imbalance on tourniquet-induced remote kidney injury in a mouse hindlimb ischemia-reperfusion model. *Peptides*, *36*(1), 60–70. https://doi.org/10.1016/j.peptides.2012.04.024

10 (2022). National Library of Medicine. Retrieved from https://www.ncbi.nlm.nih.gov/snp/rs35803318#frequency_tab

11 Strafella, C., Caputo, V., Termine, A., Barati, S., Gambardella, S., Borgiani, P., … & Cascella, R. (2020). Analysis of ACE2 Genetic Variability among Populations Highlights a Possible Link with COVID-19-Related Neurological Complications. *Genes*, *11*(7), 741. https://doi.org/10.3390/genes11070741

12 Asselta, R., Paraboschi, E.M., Mantovani, A., & Duga, S. (2020). ACE2 and TMPRSS2 variants and expression as candidates to sex and country differences in COVID-19 severity in Italy. *Aging (Albany NY)*, *12*(11), 10087–10098. https://doi.org/10.18632/aging.103415

13 Khayat, A.S., de Assumpção, P.P., Meireles Khayat, B.C., Thomaz Araújo, T.M., Batista-Gomes, J.A., Imbiriba, L.C., … dos Santos, S.E.B. (2020). ACE2 polymorphisms as potential players in COVID-19 outcome. *PLoS ONE*, *15*(12), e0243887. https://doi.org/10.1371/journal.pone.0243887

14 Srivastava, A., Pandey, R. K., Singh, P. P., Kumar, P., Rasalkar, A. A., Tamang, R., Driem, G. van, Shrivastava, P., & Chaubey, G. (2020). Most frequent South Asian haplotypes of ACE2 share identity by descent with East Eurasian populations. PLOS ONE, 15(9), e0238255. https://doi.org/10.1371/journal.pone.0238255

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Қазақтардағы ACE2 генінің бір нуклеотидті полиморфизмнің rs35803318 жиілігі

Мақалада ACE2 генінің rs35803318 (C/T) бір нуклеотидті полиморфизмі бойынша зерттеуге қатысушылардан алынған ДНҚ үлгілерін генотиптеу нәтижелері келтірілген. Генотиптеу полимеразды тізбекті реакция (ПТР) әдісімен нақты уақыт режимінде «Рефракторлық мутациялық жүйені күшейту» әдісін қолдана отырып жүзеге асырылды. Қарағанды облысында тұратын қазақ этникалық тобының 96 өкілінде rs35803318 (C/T) генотиптері мен аллельдерінің жиіліктерінің таралуы талданды. Біздің зерттеу нәтижелерімізге сәйкес қазақтар арасында CT генотипі (63.5%) ең көп таралған, ал CC генотипі 20.8% және TT генотипі — 15,6% құрады. Полиморфизм аллельдерінің таралуы: C аллелі — 52,6%, A аллелі — 47,4%.

Кілт сөздер: ACE2, рецептор, ген, SNP, бір нуклеотидті полиморфизм, ARMS, генетикалық нұсқалар, қазақтар.

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Частота однонуклеотидного полиморфизма rs35803318 гена ACE2 среди казахов

В статье представлены результаты генотипирования образцов ДНК, полученных от участников исследования по однонуклеотидному полиморфизму rs35803318 (C/T) гена ACE2. Генотипирование осуществлялось методом полимеразной цепной реакции (ПЦР) в режиме

реального времени с использованием методики «Амплификация рефракторной мутационной системы». Проанализировано распределение частот генотипов и аллелей rs35803318 (C/T) у 96 представителей казахской этнической группы, проживающих в Карагандинской области. Согласно результатам нашего исследования среди казахов генотип CT (63,5 %) — наиболее распространенный, генотип CC составил 20,8 % и генотип TT — 15,6 %. Распределение аллелей полиморфизма выглядит следующим образом: аллель C — 52,6 %, аллель A — 47,4 %.

Ключевые слова: ACE2, рецептор, ген, SNP, однонуклеотидный полиморфизм, ARMS, генетические варианты, казахи.

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