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

In the article 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 were presented. 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. The ACE2 gene (angiotensin-converting enzyme 2) was extensively investigated due to its role in susceptibility to infection by the SARS-CoV-2 virus causing COVID-19. The ACE2 gene encodes a protein that serves as a receptor for the virus, and its variations can affect a person's susceptibility to infection. The ACE2 protein plays a role in the regulation of angiotensin and the effect on blood pressure. Therefore, there is a link between ACE2 gene polymorphisms, including rs35803318, and the development of cardiovascular diseases. 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). “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). We analyzed the distribution of rs35803318 alleles in relation to the sex of Kazakh representatives. Among men, the C allele dominates (59.3 %), the T allele (40.7 %). Women have an equal distribution between the C allele (50 %) and the T allele (50 %).

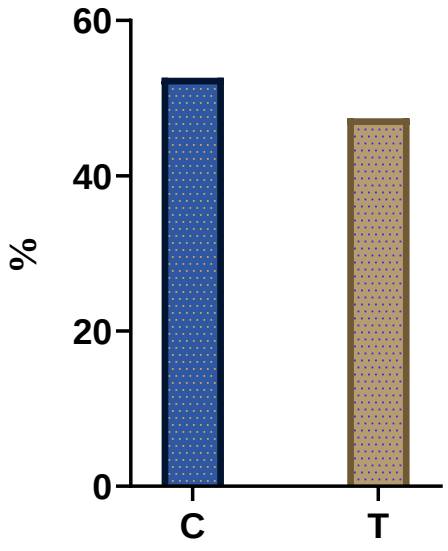

Figure 1. Frequency of rs35803318 alleles among Kazakhs

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). In the group of Kazakh men, the frequency of rs35803318 genotypes was CC — 59.25 %, TT — 40.7 %, CT — 0 %; among women CC — 5.8 %, TT — 5.8 %, CT — 88.4 %.

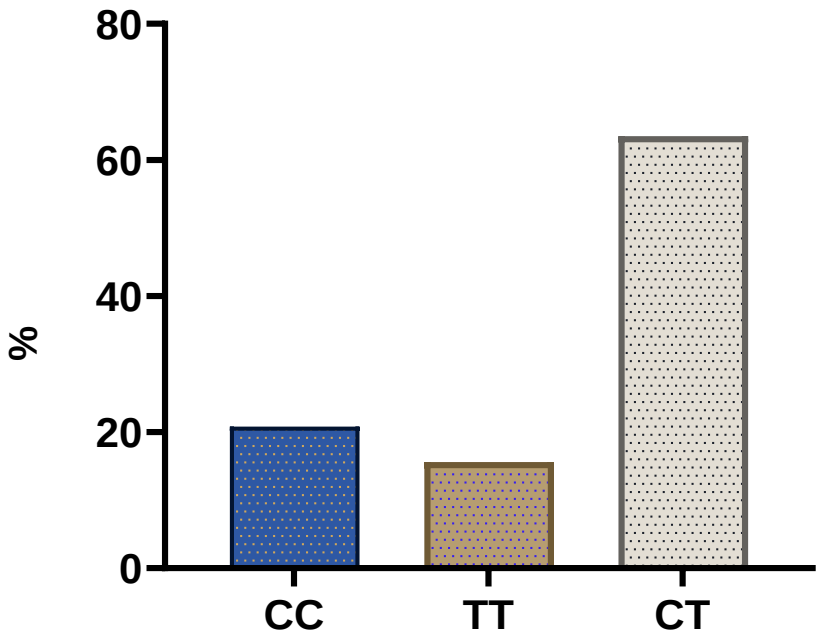

Figure 2. Frequency of rs35803318 genotypes among Kazakhs

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.

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.

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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 аллельдері жиіліктерінің таралуы талданды. ACE2 гені (ангиотензинді түрлендіретін 2 фермент) оның COVID-19 тудыратын SARS-CoV-2 вирусының инфекцияға шалдыққыштық рөліне байланысты кеңінен зерттелді. ACE2 гені вирустың рецепторы ретінде қызмет ететін ақуызды кодтайды және ACE2 ақуызы ангиотензинді реттеуде және қан қысымына әсер етуде маңызды рөл атқарады. ACE2 гені вирустың рецепторы ретінде қызмет ететін ақуызды кодтайды және оның өзгеруі адамның инфекцияға шалдыққыштығына әсер етуі мүмкін. ACE2 ақуызы ангиотензинді реттеуде және қан қысымына әсер етуде маңызды рөл атқарады. Сондықтан ACE2 генінің нұсқалары, соның ішінде rs35803318 және жүрек-қан тамырлары ауруларының дамуы арасында байланыс бар. Біздің зерттеу нәтижелеріне сәйкес қазақтар арасында CT генотипі (63.5 %) ең көп таралған, CC генотипі 20.8 % және TT генотипі 15.6 %-ды құрады. Полиморфизм аллельдерінің таралуы мынадай: 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 (ангиотензин-конвертирующий фермент 2). Генотипирование осуществлялось методом полимеразной цепной реакции 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. Ген ACE2 был широко исследован в связи с его ролью в восприимчивости к инфекции вирусом SARS-CoV–2, вызывающим COVID–19. Ген ACE2 кодирует белок, который служит рецептором для вируса, и его

вариации могут влиять на восприимчивость человека к инфекции. Белок ACE2 играет роль в регуляции ангиотензина и влиянии на кровяное давление. Поэтому имеется связь между вариантами гена ACE2, включая rs35803318, и развитием сердечно-сосудистых заболеваний. 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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