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Analysis of Tat protein characteristics in human immunodeficiency virus type 1 sub-subtype A6 (*Retroviridae: Orthoretrovirinae: Lentivirus: Human immunodeficiency virus-1*)

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Introduction. Tat protein is a major factor of HIV (human immunodeficiency virus) transcription regulation and has other activities. Tat is characterized by high variability, with some amino acid substitutions, including subtype-specific ones, being able to influence on its functionality. HIV-1 sub-subtype A6 is the most widespread in Russia. Previous studies of the polymorphisms in structural regions of the A6 variant have shown numerous characteristic features; however, Tat polymorphism in A6 has not been studied.
Goals and tasks. The main goal of the work was to analyze the characteristics of Tat protein in HIV-1 A6 variant, that is, to identify substitutions characteristic for A6 and A1 variants, as well as to compare the frequency of mutations in functionally significant domains in sub-subtype A6 and subtype B.
Material and methods. The nucleotide sequences of HIV-1 sub-subtypes A6, A1, A2, A3, A4, subtype B and the reference nucleotide sequence were obtained from the Los Alamos international database.
Results and discussion. Q54H and Q60H were identified as characteristic substitutions. Essential differences in natural polymorphisms between sub-subtypes A6 and A1 have been demonstrated. In the CPP-region, there were detected mutations (R53K, Q54H, Q54P, R57G) which were more common in sub-subtype A6 than in subtype B.
Conclusion. Tat protein of sub-subtype A6 have some characteristics that make it possible to reliably distinguish it from other HIV-1 variants. Mutations identified in the CPP region could potentially alter the activity of Tat. The data obtained could form the basis for the drugs and vaccines development.

Keywords: *human immunodeficiency virus type 1 (HIV-1); sub-subtype A6; mutations; polymorphism; Tat protein*

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Анализ особенностей белка Tat вируса иммунодефицита человека 1 типа суб-субтипа А6 (*Retroviridae; Orthoretrovirinae; Lentivirus: Human immunodeficiency virus-1, sub-subtype A6*)

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Введение. Белок Tat вируса иммунодефицита человека (ВИЧ) является главным фактором регуляции транскрипции генома ВИЧ и имеет ряд дополнительных внутриклеточных и внеклеточных активностей. Как и другим белкам ВИЧ, Tat свойственна изменчивость, при этом некоторые аминокислотные замены внутри белка Tat, включая субтип-специфичные, способны влиять на его функциональность. В РФ наиболее широко распространён ВИЧ 1 типа (ВИЧ-1) суб-субтипа А6. Исследования полиморфизма структурных областей генома А6 выявили многочисленные характерные особенности этого варианта, однако изучение области генома, кодирующей Tat, у ВИЧ-1 суб-субтипа А6 не проводилось.
Цели и задачи: Основной целью работы был анализ особенностей белка Tat у ВИЧ-1 суб-субтипа А6. Задачами исследования были было выявление характеристических замен, сравнение полиморфизма белка Tat суб-субтипа А6 и наиболее близкого к нему суб-субтипа А1, а также определение статистически достоверных различий в функционально значимых доменах Tat суб-субтипа А6 и наиболее изученного субтипа В.
Материал и методы. Материалом для работы послужили нуклеотидные последовательности ВИЧ-1 суб-субтипов А6, А1, А2, А3, А4, субтипа В и референсная нуклеотидная последовательность, полученные из международной базы данных Los Alamos.
Результаты и обсуждение. Мутации Q54H и Q60H являются характеристическими заменами для А6. Продемонстрированы существенные достоверные различия в частоте естественных полиморфизмов белка Tat между суб-субтипами А6 и А1. В функционально значимом CPP-регионе выявлены мутации, достоверно различающиеся по частоте между суб-субтипом А6 и субтипом В ВИЧ-1 – R53K, Q54H, Q54P и R57G.
Заключение. Белок Tat варианта А6 ВИЧ-1 обладает особенностями, позволяющими достоверно отличить его от других генетических вариантов вируса. Выявленные в функционально значимом CPP-регионе мутации потенциально способны изменять активность белка Tat. Полученные данные могут составить основу для разработки лекарственных и вакцинных препаратов.

Ключевые слова: *вирус иммунодефицита человека 1 типа (ВИЧ-1); суб-субтип А6; мутации; полиморфизм; белок Tat*

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Introduction

While modern antiretroviral therapy (ART) has been highly successful, there is an urgent need for new therapeutic agents. One of the main reasons for this insistent need lies in the phenomenon of drug resistance in the human immunodeficiency virus (HIV), which limits the options for ART regimens, especially in patients having long treatment history. Another challenging task is development of effective drug combinations having a high level of safety and convenient dosage forms. New medicines are being developed, along with preventive and therapeutic vaccines against HIV.

Nonstructural HIV proteins can become a promising target of future therapeutic agents and vaccines [1–3]. In this article, we continue to discuss the specific characteristics of these proteins regarding HIV type 1 (HIV-1) sub-subtype A6, most prevalent in Russia [4, 5]; the Tat protein has become the focus of our studies.

Intracellular activities of Tat

The Tat (trans-activator of transcription) protein, together with another protein, Rev, forms a group of regulatory proteins and plays an essential role in HIV transcription regulation [6]. This protein controls the transcription process by modulating the activity of the viral promoter and the cellular RNA polymerase II (RNAP II; RNA POL II) [7]. Based on the Tat involvement, the transcription of proviral DNA is split into 2 phases: Tat-dependent and Tat-independent.

Prior to the transcription, the proviral HIV DNA disengages from histones; the subsequent chromatin remodeling (reorganization) provides access to this molecule (**Fig. 1**). The first stage – initiation involving low-processive RNA POL II – starts with incorporation of NF-κB and SP1 factors, followed by forming of the so-called trans-activation (TAR) response element (reactivation response Tat-binding elements having a stem-loop structure and located at the 5' end of the viral transcript), and ends with production of short RNA fragments encoding primarily Tat and Rev regulatory proteins. Then, short transcripts translocate to the cytoplasm where they are translated directing the synthesis of the above proteins [6–8]. Through the nuclear localization signal, the newly translated Tat enters the nucleus [9] to launch the Tat-dependent transcription of HIV nucleic acid [6–8]. The Tat protein binds to the TAR-element and recruits the host positive transcription elongation factor – the P-TEFb complex consisting of CDK-9 kinase and cyclin T1 – to the initiation site. The above process results in increased processivity of RNA POL II and the subsequent synthesis of full-length RNA molecules [3, 7, 8, 10]. The Tat protein also participates in activation of acetylation of nucleosomal histones making the DNA accessible for RNA POL II and stimulating the initiation of viral RNA transcription [11, 12]. If any of the above Tat activities is absent, the transcription is aborted and the virus remains dormant in infected cells [13].

In addition to the above activities, the Tat protein can significantly interfere with mitochondrial functions in infected cells; by altering the transcription of mitochondrial DNA (mtDNA) and causing damage to its molecule, this protein probably mediates mitochondrial dysfunction observed in HIV infected patients [14, 15].

Another intracellular effect of the Tat protein involves its ability to inhibit cell proliferation by disrupting the formation of the mitotic spindle [16], contributing to HIV pathogenesis and reduction in the number of CD4+ T cells (**Fig. 2**).

Extracellular effects of Tat

During the intracellular HIV replication, the Tat protein is produced abundantly, and, although it does not have any signal peptide required for secretion, it can use unconventional pathways to exit the cell, which are available due to its affinity for phospholipids of the cell membrane. Alternatively, the Tat protein can be released during destruction of infected cells [17]. Then, the protein is secreted into the extracellular space to reach most of the host tissues and cells, interacting with their surface receptors and entering the cells by adsorptive endocytosis [17, 18].

The Tat protein does not have a distinct secondary structure, being sparsely ordered and flexible; however, this feature does not prevent it from binding to elements of the intracellular transcription system as well as to many “partner” molecules when it freely floats outside the cell [1, 17].

The central nervous system (CNS) is one of the best known targets of the adverse effect of the Tat protein. HIV is not able to invade neurons directly, as they do not have the respective surface receptors; therefore, all the effects of this protein are produced by its penetration into CNS cells from outside in a soluble form. The combined effect of Tat and other HIV proteins causes disorders known as HIV-associated neurocognitive disorders (HAND), which can be severe, escalating to dementia. The Tat impact is associated with the direct neurotoxic effect through impairment of the neuronal cytoskeleton

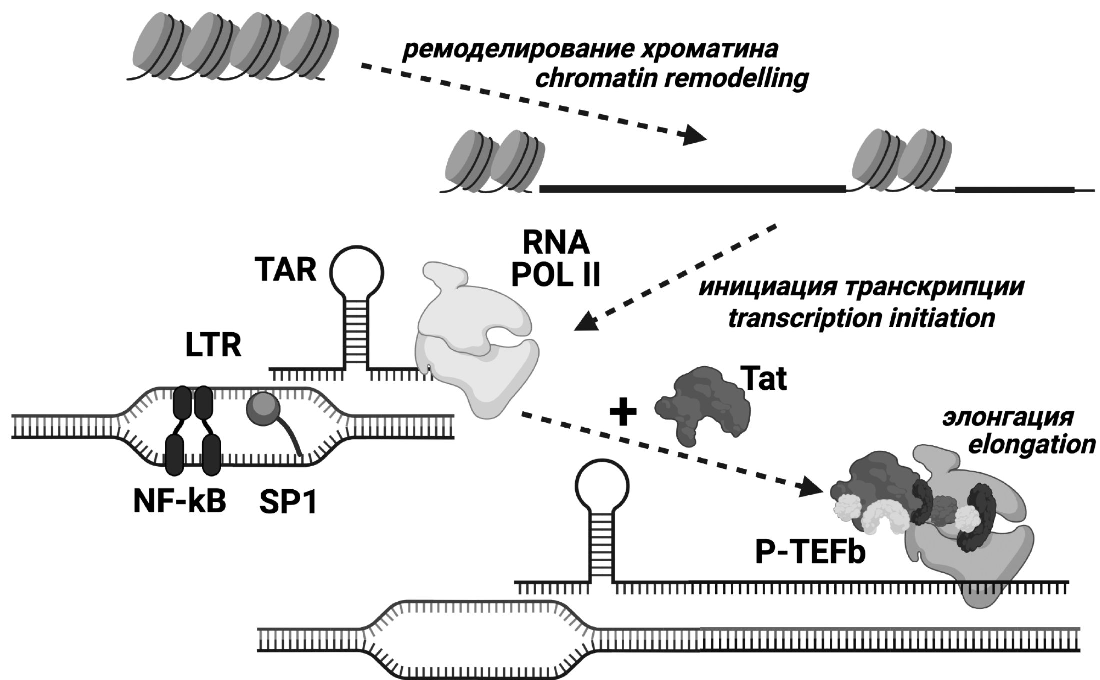

Fig. 1. Transcription of proviral human immunodeficiency virus DNA. RNA Pol II, cellular RNA polymerase II; TAR, transactivation response element; LTR, long terminal repeats; NF-κB and SP1, nuclear factors that regulate the rate of viral transcription initiation; Tat, Tat protein; P-TEFb, positive transcription elongation factor. The illustration was created using the graphics program BioRender, https://biorender.com/.

Рис. 1. Транскрипция провирусной ДНК вируса иммунодефицита человека. RNA Pol II – клеточная РНК-полимераза II, TAR – элемент ответа на трансактивацию; LTR – длинные концевые повторы; NF-κB и SP1 – ядерные факторы, регулирующие скорость инициации вирусной транскрипции; Tat – белок Tat; P-TEFb – позитивный фактор элонгации транскрипции. Иллюстрация создана с применением графической программы BioRender, https://biorender.com/.

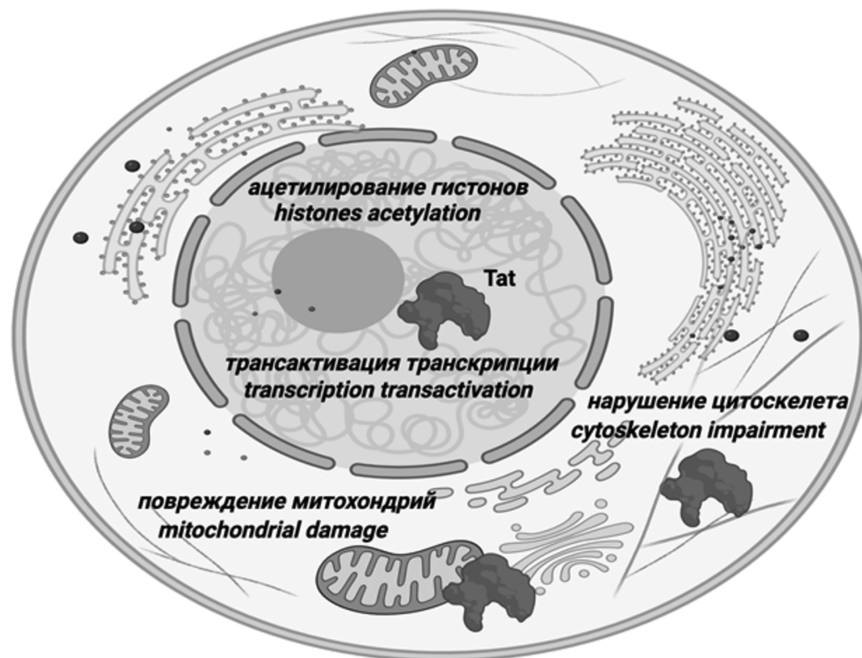

Fig. 2. Intracellular activities of Tat protein.
The illustration was created using the graphics program BioRender, https://biorender.com/.

Рис. 2. Внутриклеточные активности белка Tat.
Иллюстрация создана с применением графической программы BioRender, https://biorender.com/.

and through destruction of synaptic structures of CNS [1, 18, 19].

The impact of Tat on the cardiovascular system can be manifested in participation in development of pulmonary hypertension, which frequently accompanies HIV infection. In this situation, the pathophysiological mechanism presumably involves destruction of DNA endothelial cells, together with chronic oxidative stress caused by Tat penetration [20, 21]. Through its effect on cardiomyocytes and parasympathetic neurons, the protein can trigger bradycardia [22]. Besides, it is presumably associated with increased risk of developing atherosclerosis in HIV infected patients [23], which is usually explained by macrophage dysfunction and direct effect on endothelial cells.

The oncogenic potential of Tat can be seen as proven; being able to interact both with protein molecules and DNAs, it interferes in cell replication processes, affecting the cell cycle progression and inducing tumor angiogenesis [24]. Besides, Tat is known to prevent repairment of DNA double-strand breaks [24].

Structure and polymorphism of Tat

Tat is a small nuclear protein (from 86 to 106 amino acids (AAs) in length, usually 101); it is encoded by 2 exons and contains several domains (**Fig. 3**). The first exon includes AAs in positions from 1 to 72 and serves as a template to synthesize the Tat72 transcriptionally active protein playing the key function in transcription elongation. The second exon (73–101 AAs) overlaps with the *env* gene and performs mostly additional functions of Tat, possibly mediating the expression of human chromosomal genes associated with activation of T cells and their apoptosis [8, 25, 26].

The Tat72 protein site is composed of five functional domains (**Fig. 3**):

1) The first (I) N-terminal domain (1–21 AAs) includes proline-rich (Pro, P) region and conserved tryptophan residue (Trp, W) in position 11; the first and second domains are responsible for interaction with cyclin T1;

2) The second (II) domain (22–37 AAs) includes 7 highly conserved cysteine residues (Cys, C) in positions 22, 25, 27, 30, 31, 34, and 37;

3) The third (III) domain (38–48 AAs) includes a hydrophobic core sequence – 43LGISYG48; it is responsible for the contact with histone acetyltransferases and the SP1 protein;

4) The fourth (IV) is a positively charged domain (49–57 AAs) containing a highly conserved arginine-rich (Arg, R) motif, 49RKKRRQRRR57; it is the main TAR binding region. This domain has an increased tendency to nuclear localization, participating in secretion and adsorption of the Tat protein;

5) The fifth domain (V) is a glutamine-rich (Gln, Q) region (58–72 AAs) exhibits the greatest degree of genetic variability; together with the fourth domain, it plays a key role in Tat nuclear localization [1, 9, 13, 25].

The sixth (VI) domain (73–101 AAs) encoded by the second exon contains an RGD motif acting as a ligand for some integrins, and a highly conserved motif 86ESKKKVE92, which may be related to optimal HIV-1 replication *in vivo* and Tat-mediated protection from apoptosis of CD4+ T cells [25, 26].

Despite the high mutational variability of HIV-1, Tat is a relatively conserved protein. Foremost, this refers to its first 56 AAs, implying their important functional role in the activity of this molecule. Among the conserved residues, there are all the Cys residues (except for C31), most of the Pro residues, the conserved Trp residue in position 11 and the basic domain (lysine (Lys,K) and Arg residues in positions 49–57) [17]. Nevertheless, similar to other HIV proteins, the Tat protein has a variability. Therefore, the existing, though limited studies tend to focus on the analysis of sequences of this protein, on assessment of the impact of the specific Tat structure on its properties as well as on clinical manifestations of HIV infection, including the analysis of subtype-specific substitutions. Most of the studies explored Tat domains II, IV, and V, with 2 subtypes compared – subtype B, as most extensively studied, and subtype C as most aggressive; some of the obtained data are given below.

The experiments on cell culture [27] and animal models [28], the analysis of clinical isolates [29], and clinical and epidemiological data of patients [30] demonstrated that the C30S31 mutation in the Tat dicysteine motif (C30C31) can have an effect on the pattern of HIV-infection neuropathogenesis and, specifically, on the likelihood of development of cognitive disorders (HAND). The performed studies also evaluated the significance of the basic CPP (cell penetrating peptide) region comprising 10 AAs (48–57), which is associated with the Tat entry into cells. Tat internalization is mediated by its binding to heparan sulfate proteoglycans (HSPGs) expressed on cell surfaces of all tissues, including brain tissue; negatively charged HSPGs interact with positively charged Arg and lysine (Lys, K) residues in the CPP region [31–33]. CPP polymorphisms can change the Tat adsorption by cells, affecting the propagation of a “secondary” inflammatory signal in CNS. The *in vitro* studies using confocal microscopy with fluorescently labeled peptides showed that R57S or R57G substitutions significantly decreased Tat CPP uptake [18].

The study of polymorphisms in functionally significant Tat domains of different HIV subtypes showed that in HIV-1 subtypes B, D, and F, Tat CPP largely retains a complete set of 8 Arg/Lys residues required for optimal Tat uptake; however, 3 subtypes – A, C, and G – demonstrated the presence of R57S or R57G substitutions [18]. The recent study of polymorphisms in Cys, Arg, and Gln-rich domains of HIV-1 Tat subtype C in Southern Brazil demonstrated that C31S, R57S, Q63E AA substitutions in subtype C were found in 82, 74, and 80% of cases, while in subtype B, they were found in 10, 20, and 20% of cases, respectively. The P60Q mutation was more often found in subtype B than in subtype C (in 55 and 6% of cases, respectively) [34].

HIV-1 sub-subtype A6 is most widespread in Russia, causing infection in more than 70% of patients [4, 35, 36]. No studies of Tat polymorphism in HIV-1 sub-subtype A6 have been conducted so far. At the same time, the ear-

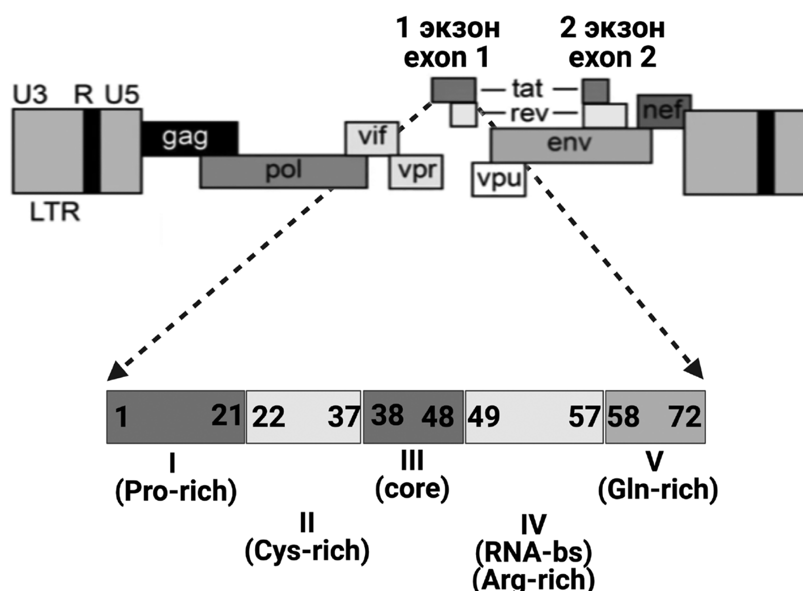

Fig. 3. *Tat* gene and functional domains of Tat72 protein. Tat is encoded by two exons. The first five domains are encoded by the first exon. I (Pro-rich), first domain, proline-rich region; II (Cys-rich), second domain, cysteine-rich region; III, third domain (core); IV (RNA-bs) (Arg-rich), fourth domain, arginine-rich region, binds to the TAR element of HIV RNA; V (Gln-rich), fifth domain, glutamine-rich region. The illustration was created using the graphics program BioRender, https://biorender.com/.

Рис. 3. Ген *tat* и функциональные домены белка Tat72. Tat кодируется двумя экзонами. Первые пять доменов (Tat72) кодируются первым экзоном. I (Pro-rich) – первый домен, пролин-богатая область; II (Cys-rich) – второй домен, цистеин-богатая область; III (core) – третий домен (коровый); IV (RNA-bs) (Arg-rich) – четвёртый домен, аргинин-богатая область, связывается с TAR-элементом РНК ВИЧ; V (Gln-rich) – пятый домен, глутамин-богатая область. Иллюстрация создана с применением графической программы BioRender, https://biorender.com/.

lier studies of other regions in the virus genome found A6-specific differences from other subtypes and recombinant forms [37–40]. The data on the Tat structure in sub-subtype A6 can be useful at different stages of development new medications for treatment and eradication of HIV infection, including vaccines and diagnostic tools.

The aim of this work was to study specific characteristics of the HIV-1 sub-subtype A6 Tat region encoded by the first exon and required for transactivation; identification of characteristic substitutions, comparative analysis of A6 polymorphisms and those of A1, being closest to it, as well as detection of statistically significant differences in the dicysteine motif and the CPP region in sub-subtype A6 and subtype B whose biological and clinical properties are well known. The findings will provide an insight into the specific features of the Tat structure in HIV-1 sub-subtype A6, will help predict both biological properties of this variant and patterns of development of the associated infection. Besides, they can give an impetus to further research on properties of the Tat protein, both on cell culture and in clinical practice.

Material and methods

The international Los Alamos HIV sequence database (Los Alamos National Laboratory; Main Search Interface of HIV Sequence Database; www.lanl.gov) was used to select 142 nucleotide sequences of the first exon of HIV-1 sub-subtype A6 *tat* gene, 50 nucleotide sequences of sub-subtype A1, 6 nucleotide sequences of sub-subtype A2, 4 sequences of A3, 3 ones of A4, and 50 nucleotide sequences of subtype B. Wherein, for sub-subtypes A2, A3 and A4 there were analyzed all sequences presented in database. The nucleotide sequence of wild-type HIV-1 strain HXB2 (K03455) was used as the reference ones. All sequences were aligned to the reference HXB2 using MEGA v.10.2.2 software (www.megasoftware.net). The size of the analyzed fragment was 213 bp of the first *tat* exon (71 of 72 AAs encoded by the *tat* first exon). After the alignment, non-multiples three nucleotide insertions including repetitive nucleotide sequences were interpreted as read errors and removed.

Then, the nucleotide sequences were subjected to quality check and subsequent rejection of the sequences containing: a) substitutions at the start codon; b) stop codons; c) missing nucleotides; d) twice in succession N degeneracies.

For each of sub-subtypes A – A6, A1, A2, A3, A4, as well as for subtype B, consensus sequences were received in 2 stages. At first, a nucleotide consensus sequence was formed for each variant of the virus using the Advanced Consensus Maker software tool on the website of Los Alamos (https://www.hiv.lanl.gov/content/sequence/CONSENSUS/AdvCon.html). The algorithm implies that the most common nucleotide at the specified position should be included in the consensus. As each AA is encoded by nucleotide triplet and during the consensus-making process, each nucleotide is analyzed individually, there is a chance of error if two nucleotides in one position have equiprobable finding. Therefore, the second stage included MEGA-based “manual” check and correction of

the consensus nucleotide sequences assembled with the Advanced Consensus Maker tool.

Then, the resulting sequences were cross-compared and checked against the reference sequence to identify characteristic substitutions for sub-subtype A6; characteristic substitutions meant mutations found in the virus genome with frequency >50%.

Consensus sequences and reference HXB2 (K03455) sequence were compared using MEGA 10.2.2 software. At first, there were defined the positions which were different between consensus of sub-subtype A6 and the reference strain HXB2. Then, the positions with differences in AAs were compared with the respective positions in the consensus of sub-subtypes A1, A2, A3, A4, and subtype B.

When comparing the natural polymorphisms of A6 and A1, initially using the MEGA v.10.2.2 program, natural polymorphisms of both sub-subtypes were detected relative to the reference strain HXB2 polymorphisms meant mutations – single substitutions found in ≥1% of observations [41]. The Nonparametric Statistics module from STATISTICA 8.0 software (StatSoft Inc., United States) was used to check for sites with statistically significant differences ($p < 0.05$ using the χ^2 test). In the further analysis, out of all found positions with statistically significant differences, only the positions where the polymorphism frequency for one of the sub-subtypes was ≥20% were assessed for the sake of clarity.

Separately, there were compared mutations - single substitutions - in the functionally significant dicysteine motif and CPP region of the Tat protein in sub-subtype A6 and in subtype B.After identifying all available substitutions in the analyzed regions in both variants of the virus there were identified the sites with significant differences ($p < 0.05$ in the case of using the $\chi 2$ test) with the using the Nonparametric Statistics module (STATISTICA 8.0 software package).

Results

During our study, we analyzed 71 out of 72 AAs located in the Tat region encoded by the first *tat* exon. After the quality control, 1 nucleotide sequence of sub-subtypes A6 (AF193275) and A1 (FJ388942) as well as 3 sequences of subtype B (AB097870, A07867, AY819715) were excluded from the further process. As a result, the final analysis included 141 sequences of sub-subtype A6, 49 sequences of sub-subtype A1, 6 sequences of sub-subtype A2, 4 sequences of sub-subtype A3, 3 ones of A4, and 47 nucleotide sequences of subtype B.

After consensus nucleotide sequences assembled using the Advanced Consensus Maker tool software had been checked and corrected with MEGA v.10.2.2, the following substitutions were made in the respective AA sequences:

- in the consensus of sub-subtype A6: in position 36, isoleucine (Ile, I) was substituted with valine (Val, V);
- in the consensus of sub-subtype A2: in position 36, methionine (Met, M) was substituted with R/L/V; in position 69 I – with I/V;

in the consensus of sub-subtype A3: in position 12, histidine (His, H) was substituted with H/Q; in position 23, serine (Ser, S) was substituted with asparagine (Asn, N)/S; in position 24, K was substituted with N/K; in position 53, K was substituted with K/R;

- in the consensus of sub-subtype A4: in position 36, I was substituted with V/M/S; in position 60, H was substituted with H/R/tyrosine (Tyr, Y);
- in the consensus of subtype B: in 39 position, I was substituted with threonine (Thr, T).

The results of comparing the consensus sequence A6 with the reference HXB2 and subsequent comparison with those of subtypes A1, A2, A3, A4 and subtype B obtained the data given in **Table 1**. Marked positions of AK in which the consensus of A6 subtype was different from the reference HXB2 strain and the consensuses of subtype B, sub-subtypes A1, A2, A3 or A4.

As seen from the **Table** and as was assumed, subtype B (positions 42, 59, 61, 67) had the smallest number of differences from HXB2. For other variants of virus, all Tat domains demonstrated differences both from the reference strain HXB2 and from subtype B.

Based on the analysis of the data presented in **Table 1**, we singled out 2 groups of AA substitutions in sub-subtype A6: a) ones typical solely of A6 (Q54H, Q60H); b) substitutions common both in A6 and in other sub-subtypes A (E2D, R7N, K12N, T23S, F32W, I39L, T40K, R57G, A58T, N61S, Q63K, T64D, A67N, S68P, L69I).

Having compared natural polymorphisms in the region encoded by the *tat* first exon, sub-subtype A6, and phylogenetically close sub-subtype A1, we found significant differences in all analyzed domains (**Table 2**).

The analysis of mutations – single substitutions in the dicysteine motif (C30C31) of the Tat protein did not demonstrate any significant differences between HIV-1 sub-subtype A6 and subtype B, while in the CPP region (AA positions 48–57), these virus variants had 4 substitutions with statistically significant differences – R53K, Q54H, Q54P, R57G (**Table 3**).

Discussion

As noted previously, the Tat protein is a trans-activator of the transcription of HIV genome; it has a number of additional intra- and extracellular functions. Mutations (AA substitutions) inside its molecule can affect the performance efficiency of this protein; some substitutions, which have an effect on Tat functions, can be subtype-specific. Furthermore, the Tat protein is an inviting target incorporated in strategic developments aimed at functional cure for HIV infection [2]. Currently, there are 2 anti-HIV-proteins, Nullbasic (a mutant of Tat) and HT1 (a fusion of HEXIM1-Tat domains), inhibiting viral transcription by affecting the interaction of Tat and Rev with cellular factors [42, 43]. There are also 2 small molecules – didehydro-cortistatin A (dCA) and triptolide, which directly inhibit the Tat activity or enhance its degradation, respectively [44, 45]. In addition, 2 Tat-based vaccines are being developed; they induce production of Tat-neutralizing antibodies, increase the number of CD4+ T cells and reduce the viral load in HIV-infected patients [46, 47]. Our study addressed the region encoded by the

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tat first exon in HIV-1 sub-subtype A6, which is most prevalent in Russia.

Based on the results of the study, Q54H and Q60H mutations were interpreted as characteristic substitutions for sub-subtype A6 (**Table 1**). Interestingly, F32W and T40K mutations detected in consensus sequences of sub-subtype A6 and sub-subtypes A3 and A4, respectively, are not found in consensus sequences of subtype B, sub-subtypes A1 and A2 (**Table 1**). Considering small number of samples of sequences A3 and A4, positions F32W and T40K should be seen as candidates for characteristic substitutions for HIV-1 sub-subtype A6.

The comparison of profiles of natural Tat polymorphisms in sub-subtypes A6 and A1 in each domain encoded by the *tat* first exon demonstrated significant differences (**Table 2**). Special attention should be paid to F32W, V36I, T40K, Q54H, Q54P, Q60H, S68L substitutions, which were found in A6 3.5 times more frequently.

The analysis of frequency of occurrence of mutations in functionally significant domains did not reveal any significant differences in the dicysteine motif in sub-subtype A6 and subtype B, while in the CPP region, there were substitutions having statistically significant differences: R53K, Q54H, Q54P, and R57G (**Table 3**). Currently, it is known that Tat internalization is mediated by the interaction of positively charged Arg and Lys residues in the CPP region with negatively charged HSPGs on cell surfaces, and the R57G substitution significantly decreases cellular uptake of Tat [17, 23]. In sub-subtype A6, the R57G substitution was found in 92.2% of cases (in 130 of 141 sequences), while in subtype B, it was found in 6.4% of cases (3 of 47 sequences). R57G substitution has a frequent occurrence (92.2%) in the Tat sub-subtype A6; it can induce a decrease in inflammatory potential of this protein; however, the possible impact of other substitutions in Tat A6 in the CPP region should not be neglected: R53K (9.9% of observations), Q54H (63.1%), and Q54P (22.7%), which can intensify or weaken the effect of R57G mutation.

Conclusion

The performed analysis of the Tat region encoded by the first exon demonstrated the presence of characteristic substitutions in sub-subtype A6 as well as significant differences in natural polymorphisms between A6 and A1; in the CPP region, mutations with statistically significant

Table 1. The amino acid positions in which the consensus of sub-subtype A6 were different from the reference strain HXB2, and amino acids in consensus of subtype B and sub-subtypes A1, A2, A3, A4 human immunodeficiency virus type 1 in the corresponding positions

Таблица 1. Аминокислотные позиции, в которых АК консенсуса суб-субтипа А6 отличались от АК в референсном штамме HXB2, и АК в соответствующих позициях консенсусов субтипа В и суб-субтипов А1, А2, А3, А4 вируса иммунодефицита человека 1 типа

Domain Домен	Amino acid position number Номер позиции АК	HXB2 strain Штамм HXB2	B subtype Субтип В	A6 sub-subtype Суб-субтип А6	A1 sub-subtype Суб-субтип А1	A2 sub-subtype Суб-субтип А2	Суб-субтип А3 A3 sub-subtype	A4 sub-subtype Суб-субтип А4
I	2	E	E	D	D	E	E	E
	7	R	R	N	N	K	N	N
	12	K	K	N	N	N	H/Q	K
II	23	T	T	S	S	N	S/N	N
	32	F	F	**W**	Y	Y	W	Y
III	39	I	I	L	L	L	L	L
	40	T	T	**K**	N	N	N	K
	42	A	G	G	G	G	G	G
IV	**54**	Q	Q	**H**	Q	P	Q	L
	57	R	R	G	G	G	G	G
V	58	A	A	T	T	P	T	T
	59	H	P	P	P	S	P	P
	60	Q	Q	**H**	Q	Q	Q	H/R/Y
	61	N	D	S	S	S	S	S
	63	Q	Q	K	K	K	K	K
	64	T	T	D	D	D	D	D
	67	A	V	N	N	N	N	N
	68	S	S	P	P	P	P	P
	69	L	L	I	I	I/V	V	I

Note. Positions with characteristic substitutions and candidates for characteristic substitutions in sub-subtype A6 are shown in bold.

Примечание. Жирным шрифтом выделены позиции с характеристическими заменами и кандидатами на характеристические замены для суб-субтипа А6.

Table 2. Frequency of mutations (single substitutions) in the region coded by the first exon of *tat* gene of human immunodeficiency virus type 1 sub-subtypes A1 and A6

Таблица 2. Частота встречаемости мутаций (единичных замен) в области. кодируемой первым экзоном гена *tat*. вариантов суб-субтипов А1 и А6 вируса иммунодефицита человека 1 типа

Domain Домен	Mutation Мутация	Frequency of occurrence. percentage Частота встречаемости (%)		Domain Домен	Mutation Мутация	Frequency of occurrence. percentage Частота встречаемости (%)		Domain Домен	Mutation Мутация	Frequency of occurrence. percentage Частота встречаемости (%)	
		A1	A6			A1	A6			A1	A6
I	E2D	87.8	99.3	III	I39L	73.5	99.3	V	A58T	67.3	85.8
	R7N	81.6	93.6		I39Q	24.5	0.0		**Q60H**	**14.3**	**75.9**
	K19T	77.6	0.7		**T40K**	**20.4**	**92.2**		T64D	79.6	98.6
	A21P	57.1	0.7		T40N	59.2	6.4		A67N	87.8	62.4
II	T23S	53.1	85.8	IV	R53K	26.5	9.9		S68P	91.8	59.6
	T23N	44.9	11.3		**Q54H**	**0.0**	**63.1**		**S68L**	**4.1**	**28.4**
	N24K	77.6	13.5		**Q54P**	**6.1**	**22.7**		L69I	59.2	89.4
	F32W	**6.1**	**95**		R57G	63.3	92.2		L69V	32.7	5.0
	F32Y	85.7	0.0						S70P	83.7	7.8
	Q35P	28.6	0.0						T64D	79.6	98.6
	V36I	**0.0**	**36.9**						A67N	87.8	62.4

Примечание. Мутациями считали замены в указанных позициях в сравнении с референс-штаммом HXB2. Жирным шрифтом выделены позиции. в которых замены у суб-субтипа А6 встречались более чем в 3.5 раза чаще.

Note. Mutations were defined as the substitutions in the indicated positions in comparison with the reference strain HXB2. The positions in which substitutions in the A6 subtype were found more than 3.5 times often are shown in bold.

Таблица 3. Мутации в дицистеиновом домене и CPP-регионе белка Tat вируса иммунодефицита человека 1 типа суб-субтипа А6 и субтипа В

Table 3. Mutations in the dicysteine domain and CPP region of Tat protein in human immunodeficiency virus type 1 subtype A6 and subtype B

	Mutation Мутация	A6 sub-subtipe Суб-субтип А6 *n* = 141	B subtipe Субтип В *n* = 47	*p* value *p*-критерий
Dicysteine domain (C30C31) Дицистеиновый домен (C30C31)	C30R	2	0	0.4150
	C31S	7	1	0.4207
	C31V	5	0	0.1986
CPP– region (48–57 aa positions) CPP-регион (48–57 позиции АК)	R49S	0	1	0.0857
	R52Q	1	0	0.5641
	R52W	1	1	0.4183
	R53K	14	0	**0.0327**
	R53G	0	1	0.0857
	Q54H	89	0	**0.0000**
	Q54P	32	2	**0.0134**
	Q54R	9	0	0.0856
	Q54S	2	0	0.4150
	Q54N	1	0	0.5641
	R56C	1	0	0.5641
	R56H	1	0	0.5641
	R57G	**130**	**3**	**0.0000**
	R57E	1	0	0.5641
	R57A	1	0	0.5641

Note. Substitutions with statistically significant differences between sub-subtype A6 and subtype B are shown in bold. The analysis was carried out using the χ^2 test.

Примечание. Жирным шрифтом выделены замены со статистически значимыми различиями между суб-субтипом А6 и субтипом В. Анализ проводился при использовании критерия χ^2.

differences for sub-subtype A6 and well-known subtype B were found. By and large, the findings show that Tat in sub-subtype A6 has distinctive characteristics and contains AA substitutions, which are capable of affecting the performance of the protein. The data obtained during our study provide information about Tat characteristics in HIV-1 sub-subtype A6, which are highly important for developing therapeutic agents and vaccines. The results of the experiments can give an impetus to further studies of the combined effect produced by the substitutions in

the A6 Tat CPP region on pathogenic capabilities of this protein in cell culture. These results can be instrumental for analyzing clinical patterns of HIV infection in patients with HIV-1 sub-subtype A6.

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