Unique Variant of Insertion Polymorphism of Mitochondrial DNA in an Olkhon Buryat

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A unique variant of mitochondrial DNA polymorphism was detected in an Olkhon Buryat living in the Irkutsk region. The main characteristic of this variant is the presence of a long insertion ANATTGAGA in the hypervariable segment I of the control region of mitochondrial DNA. Taking into account multiple nucleotide substitutions and 2 insertions in the mitochondrial genome fragment, we hypothesize that intense rearrangements in the mitochondrial DNA can be caused by ecological factors, e. g. high background radiation of a technogenic origin.

Key Words: *human mitochondrial DNA; insertion polymorphism*

Mitochondrial DNA (mtDNA) polymorphism in native population of various geographic zones became now a potent tool of evolutional, population, medical, and ecological genetics [7-9,11,15]. Polymorphism of mtDNA has never been studied in the majority of ethnic groups of Northern Asia [3,4].

Highly polymorphous genetic system of mitochondria attracts special interest since the moment when the humanity entered the atomic era, as an object of studies of the genetic effects of technogenic radiation.

We investigated the nucleotide organization of hypervariable segment (HVS I) of mtDNA control region in a male Olkhon Buryat, living during recent decades in a region with high background radiation [1,2].

MATERIALS AND METHODS

Total DNA from the whole blood was isolated by a modified method [10]. After purification of DNA on a Chelex 100, polymerase chain reaction was carried out for direct sequencing of HVS I fragment of mtDNA control region. Primers L15929 5' TCAAAGCTTA CACCAGTCTTGTAAACC 3' and H16498 5' CCT GAAGTAGGAACCAGATG 3' were chosen on the basis of the Cambridge sequence [6]. The reaction was carried out in a medium containing: 60 mM Tris-HCl, 25 mM KCl, 10 mM 2-mercaptoethanol, 10 mM Triton X-100, 3 mM $MgCl_2$, 160 μg/ml bovine serum albumin, 20 nM each primer, 50 mM each dNTP, and 2 units Tag polymerase. Thirty-five cycles were performed at the following temperatures: denaturation at 95°C for 1 min; annealing of primers at 55°C for 1.5 min, and polymerization at 72°C for 1.5 min. The duration of primary denaturation and final polymerization was 5 and 10 min, respectively. Sequencing was carried out using [$g^{32}P$]-dATP [14].

RESULTS

Nucleotide sequence of the control region of mtDNA in a Buryat living in Buryatia (Ulan-Ude) has been described only once [3]. We studied the nucleotide sequence of HVS I fragment in the control region of mtDNA in a resident of the island of Olkhon, a representative of Olkhon Buryats living near the Baikal lake. The studied mtDNA sequence considerably differed from the reference Cambridge sequence of human mtDNA [6]. Various transitions were detected in 13 nucleotide positions of the 156-bp mtDNA fragment (Fig. 1). Four of them were purine-purine (A→G) or pyrimidine-pyrimidine (T→C) substitutions and others were transversions: 3 A→T, 4 A→T, and 2 C→A. The ratio of transversions to transitions in the studied variant of mtDNA was notably higher than in populations investigated before [9].

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B1 GTATATAGTNCATTACAGTCAAATCCCTTCAAGTNCCCATGGATGACC
KP GTACATAGCACATTACAGTCAAATCCCTTCTCGTCCCCATGGATGACC
16 329
        *      *           *                  * *
B1 CCCCTAAGATACGGGGTCCCATGACCACCATCCTACATGAAATCAATA
KP CCCCTCAGATA-GGGGTCCCTTGACCACCATCCTCCGTGAAATCAATA
         *   *                              ********* *
B1 TCNNGAACACGAGNNNNACTCTCCTNGCTCCANATTGAGAGAGCCCAT
KP TCCCGCACAAGAGTGCTACTCTCCTCGCTCC--------- GGCCCAT
    *   *
B1 ACCACATGGGGG
KP AACACTTGGGGG
             16 474
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Fig. 1. Nucleotide sequence of a fragment of hypervariable segment I of the control region of mitochondrial DNA. B1 is the sequence of a Buryat resident of the Olkhon island. Here and in Fig. 2: N: nucleotide. Numbers indicate nucleotide positions, asterisks show non-coinciding positions. Cs: Cambridge sequence [6].

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16 329
KP GTACATAGCACATTACAGTCAAATCCCTTCTCGTCCCCATGGAT
B 1 ***T****TN********************AA**N*********
B 2 ***********************************************
16 399
KP GACCCCCCTCAGATA-GGGGTCCCTTG
B Б 1 *********A*****C********A**
B 2 ***************-***********
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Fig. 2. Fragment 16,329-16,399 bp in the hypervariable segment I of control region of mitochondrial DNA (mtDNA) in representatives of two populations of Siberian Buryats. B1) mtDNA fragment of Olkhon resident (Irkutsk region); B2) mtDNA fragment of an Ulan-Ude resident (Republic of Buryatia).

Two insertions represent the principal difference of the studied variant of mtDNA from the control sequence: insertion of a cytosine residue at 16,387 bp position and 9-bp insertion at 16,454 bp position (unique for all previously studied variants of this mtDNA region). By its bases, this insertion sequence belongs to the AT type of DNA.

Comparative analysis of 16,329-16,399 bp fragment in HVS I of control region of mtDNA in representatives of 2 different geographic populations of Siberian Buryats showed that the studied mtDNA variant is characterized by more numerous differences from the control variant than the mtDNA sequence of an Ulan-Ude resident (Fig. 2).

The origin of polymorphism of HVS I of mtDNA control region in this representative of Olkhon Buryats is not clear. Tating into account a much higher (in comparison with previously studied sequences) percentage of nucleotide substitutions and the presence of a unique long insertion in the hypervariable region of mtDNA we hypothetize that high variability of mtDNA in this geographic zone can be caused by special ecological factors, in particular high radiation because of ozone "hole" above the Baikal lake and global atmospheric transfer of radioactive trace elements [1,2]. One of the most important aftereffects of radiation is oxidative stress which induces mutations in the mitochondrial and nuclear DNA [5,13]. Mutations in mtDNA can be caused by disorders in DNA synthesis due to effects of oxygen radical forms on the genetic material of organelles [12].

Therefore, the unique insertion polymorphism of HVS I in the control region of mtDNA in an Olkhon Buryat may be a result of total activation of recombinant processes in the mitochondrial genome of the Olkhon islanders because of increased technogenic radiation [1,2]. To verify this hypothesis, we now investigate the variability of coding and non-coding sequences of mtDNA in Buryat and Russian populations of the Olkhon island.

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