**RECONSTRUCTION OF THE GENOFOND PECULIARITIES OF THE ANCIENT PAZYRYK POPULATION (I-II MILLENIUM BC) FROM GORNY ALTAI ACCORDING TO THE MTDNA STRUCTURE.**

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Structure of the control region of the mtDNA isolated form three representatives of Pazyryk Culture of Gorny Altai (IV-II century BC), one of the central cultures in the Scythian-Siberian world, was analyzed by molecular genetic techniques.

In addition to technical molecular biological problems connected with the specificity of the material under study, the following anthropogenetic questions were considered: (1) what information concerning the ethnogenetic composition of Pazyrykians can be obtained from mtDNA examination; (2) what is the relative informativeness of different methodical approaches to the estimation of polymorphism; (3) what is the correspondence between the molecular genetic and paleoanthropological data; and, finally, (4) to determine the role of Pazyrykians in the processes of evolution of the human population genofond in Eurasia.

In case of a woman from the burial mound Ak-Alakha-5 (sample 1), DNA was isolated from muscle tissue. In case of those buried in the mounds Nos. 1 and 3 of the monument Verkh-Kal'gzhin-II (samples 2 and 3), a light brownish mass extracted from the skull and identified macroscopically as a mixture of fatty and brain tissues was used. Isolation of DNA and removal of PCR-inhibiting activities was performed by the technique based on selective sorption of DNA fragments on SiO2particles.

The primers Thr (15898 - 5/ - TCAAAGCTTACACCAGTCTTGTAAACC - 3/ - 15926) and TDKD (16518 - 5/ - CCTGAAGTAGGAACCAGATG - 3/ - 16498) were used for amplification of the mtDNA control region.

The double-stranded PCR products obtained using samples 1 and 2 were cloned in the vector PCRTMII (Invitrogene, original TA cloning kit). The plasmid DNAs obtained were sequenced using automated sequencer and conventionally using commercial kits (USB). The primary sequence of the mtDNA control region of the sample 3 was determined by a direct sequencing of the asymmetric PCR product; the reaction conditions were similar to those used for production of double-stranded amplificates with one exception: the quantity of one primer was 50-fold lower than of the other.

Each step of DNA isolation was accompanied by controls; all components of the PCR reaction mixture were analyzed to exclude any possibility of contamination with the contemporary DNA. The processes were performed in two repeats with the DNA isolated from two tissue samples for each individual.

The primary DNA structure of the mtDNA control region ~ 400 bp long was determined for each mtDNA sample isolated of the three Pazyrykians. The mitotypes revealed were compared with the available mtDNA variants of the modern Eurasian representatives.

One transition at position 16,356 is characteristic of the mitotype of the sample 1. The completely identical variant occurs only in Europoids with a frequency of 1.2% and is distributed in them from the Near East to Iceland. Among other mitotypes of this group, 28.1% differs from the mitotype 1 by one substitution (located at different positions of the fragment compared); 36.5%, by two substitutions; and 16.2, by three substitutions. The rest mitotypes display a greater number of positions with substitutions. Virtually identical data were obtained for representatives of Western-Siberian Finno-Ugric peoples (Khanty, Mansi, Komi, and Mari), occupying an intermediate position between Europoids and Mongoloids in their anthropological characteristics. Of the Mongoloids involved in the comparison, the variants most close to the mitotype 1 occur most frequently in Altaians, although they are more rare than in Europoids. The groups of mitotypes with one–three substitutions in Mongoloids are represented by 1, 5.9, and 5.9%, respectively, the major part of the mitotypes displaying more than three substitutions. The Arctic Mongoloids of the Far East virtually lack the similar mitotypes. This fact allows this variant to be considered as the most specific mitotype of the Europoid pool of mtDNA. The mitotype 1 corresponds in its structure to the haplogroup 1, revealed by Richards et al. (1996) in the mitochondrial genofond of Europeans and occupying there the dominating position.

Comparative analysis of the relative similarity of the mitotype 2 with the variants occurring in the contemporary populations failed to reveal any evident differences in the mean number of substitutions between the representatives of three groups–Europoids, Mongoloids, and intermediate ethnic groups. The fraction of the variants most close to mitotype 2 was demonstrated to be relatively higher in virtually all the Mongoloid groups, however, remaining lower than the fraction of mitotype 1 observed in Europoids. Marked ethnospecific characteristics were found in the sequence of mitotype 2. Three of the four substitutions distinguishing this mitotype from the Cambridge mitotype (16223: С ® Т; 16298: Т ® С, and 16327: С ® Т) correspond to the substitutions that are likely to be characteristic of the mitotypes belonging to the haplogroup C, identified by Wallace and Torroni in the human population basing on total restriction analysis of the entire mitochondrial genome. This haplogroup is widespread among the aboriginal population of Northern Asia and America, and its frequency decreases considerably in other regions. The high frequency of the haplogroup C (~70%) in Evenki was also confirmed by direct mtDNA sequencing of a sample of representatives of this group. These data allows the mitotype 2 to be considered as a specific Mongoloid variant occurring predominantly in contemporary ethnic groups of the Northern Asia (mainly Evenki, Even, and Selkup peoples) and to a lesser extent in Central-Asian populations.

Comparative analysis of the mitotype 3 demonstrated that the level of average differences between the mitotypes of the contemporary populations is higher than that for the first two variants. In addition, a lower values in case of comparison with Europoids are distinctly seen. The frequency of related variants is also relatively low, and they occur predominantly in the European populations. Qualitatively, the mitotype 3 corresponds to the European haplogroup 5. The data presently available suggest that this haplogroup is characterized by a low frequency all over the Europe and occur in different countries of the continent and in the Near East. An increase of its frequency in Finnish people was reported. Thus, the analysis performed indicates, on the one hand, a relatively high specificity of the mitotype 3 for the contemporary European populations and, on the other, its low representation in all the examined samples of the representatives of contemporary Eurasian populations. As it was noted above while discussing the results of restriction analysis, discovery of the mitotype 3 suggests an essentially higher occurrence of the haplogroup 5 mitotypes in the mitochondrial genofond of Pazyrykians than the frequency observed in the contemporary ethnic groups examined of both Europoid and Mongoloid origins.

Of special interest was to compare the results of molecular genetic studies to the modern paleoanthropological characteristics of Pazyryk population. Craniological studies of all presently available materials on burials of this culture have demonstrated both Mongoloid and Europoid components in the anthropological composition of Pazyrykians. Craniological variant occurring in the cattle-breeding tribes in the II millenium BC on the territories of the Southern Tadzhikistan, Southern and Southwestern Turkmenistan, and Northern Iran represents the Europoid component. In the epochs followed, this variant disappears from the territory of the Western and Middle Asia. It was suggested that the carriers of this morphological complex have been few and gradually assimilated into the mass of Eastern-Mediterranean Europoids. The Mongoloid component includes two anthropological types. One type, autochthonous, has been found on the Altai territory on the boundary of Neolithic and Eneolithic periods in the people buried in the Nizhnetitkeskenskaya and Kaminnaya caves and in the second half of the II millenium BC in Karakol Culture population. The typical combination of anthropological parameters characteristic of this type is similar to Southern-Siberian race complex, met currently in Kazakh, Kirgiz peoples, and certain groups of Khakas and Southern-Altaian peoples. The second type, Paleosiberian, dominated on the territory near Baikal during the Neolithic period. Currently, it occurs only in Evenki people in Northern Baikal region.

Comparison of the above-described data with the results of molecular genetic analysis demonstrates that it is currently impossible to give a comprehensive interpretation of all aspects of Pazyryk people anthropogenesis related with paleoanthropological consideration, mainly due to limited molecular genetic data on the majority of contemporary populations of the Northern, Middle, and Central Asia. However, certain questions, in particular the question connected with the Paleosiberian component, received a convincing confirmation and development. The data currently available also suggest certain genetic connections of Pazyrykians with the contemporary population of Gorny Altai. Discovery of the mitotype 2, possessing a marked similarity with the Northern-Asian Mongoloid mitotypes, in the individual with Europoid anthropological characteristics is one more essential finding. This fact demonstrates the absence of an absolute parallelism in anthropological and molecular-genetic characteristics. Note in conclusion that the interpretation potential of the molecular genetic data obtained will increase considerably with the accumulation of the information concerning the contemporary populations geographically and historically related with Pazyryk people.

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