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Research Article

Adaptive Changes of Chromosomal Q-Heterochromatin in The Human Population That Has Been Forced to Live in The Extreme Climatic Conditions

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Abstract

Homo sapiens is the only one of the animals that managed to populate the entire Earth while remaining a single tropical biological species. It period is noteworthy that modern man started and implemented this unprecedented biological experience in a very short of time (50,000-70,000 years). There are many hypotheses that try to answer this question: the role of reason, a large brain with neocortex, high physiological plasticity, bipedalism, the peculiarity of the forelimbs and many others. After the completion of the Human Genome Project, the possible role of genes in the adaptation to different ecological zones began to be studied purposefully. Yet many questions remain unanswered, including why exactly did man manage to master of the Earth, if there are no genes in his genome, which in one form or another would be absent in other animals? We believe that a part of the so-called 'excess' DNA in the human genome, which represents chromosomal heterochromatin regions (HRs), probably played and continues to play an important role in this process. In particular the chromosomal Q-heterochromatin regions (Q-HRs), which are present in the genome of only three higher primates (Homo sapiens, Pan troglodytes and Gorilla gorilla). The peculiarity of chromosomal Q-HRs is that they show wide heritable variability only in the human populations. We managed to obtain data indicating the adaptive changes in the chromosomal of Q-HRs in the human population, which due to circumstances beyond their control migrate to the territory with extreme climatic conditions.

Kew Words: human adaptation; high-altitude adaptation; constitutive heterochromatin; chromosomal Q-heterochromatin; 'excess' Dna.

Introduction

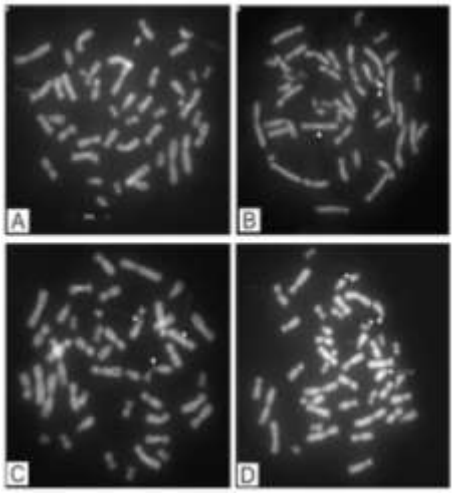
Homo sapiens is the only one of the animals that managed to populate the entire Earth while remaining a single tropical biological species. It is noteworthy that modern man started and implemented this unprecedented biological experience in a very short period of time (50,000-70,000 years). During this period, humans have managed to populate almost all ecological zones of the Earth's surface, including such extreme areas as the Far North and high-altitudes, which are particularly difficult for the existence of tropical animal species.Among the climatic conditions where man has to live, the most difficult is the high-altitude areas. Therefore, the study of men at highland is important because living at high-altitude has a significant impact on the human body, particularly on physiology, adaptation, health, and disease. To understand the impact of life at high-altitude on humans, it is important to know how many humans live at high-altitude. New research has shown that over 500 million humans live at ≥1,500 m, 219 million at ≥2,000 m, 81.6 million at ≥2,500 m, 25.2 million at ≥3,000 m, 14.4 million at ≥3,500 m, 6.4 million at ≥4,000 m, 2 million at ≥4,500 m), and 0.31 million at ≥5,000 m [1]. However, remains not fully known biological mechanisms that allowed man to adapt to such harsh climatic condition. There are many hypotheses that try to answer this question: the role of reason, a large brain with neocortex, high physiological plasticity, bipedalism, the peculiarity of the forelimbs and many others. After the completion of the Human Genome Project, the possible role of genes in the adaptation to different ecological zones began to be studied purposefully. In particular, it has been found that the genome of human populations living permanently in the conditions of Tibet, the Himalayas, the Andes, and the Ethiopian highlands contains genes that are presumably related to human adaptation to high-altitude. However, it turned out that, firstly, these hypothetical genes are not found in all highlanders and, secondly, their frequency(s) are different in different high-altitude populations [2-6]. Some authors even believe that there are different genes for adaptation to different

mountain provinces[7]. The problem is further complicated by the well-known circumstance that there are no genes in human genome, which in one form or another would be absent in other animals. Moreover, *H. sapiens* does not differ significantly from other higher eukaryotes in the number of genes, having about 22,000 in its genome. Nevertheless, it was man who managed to master and populate the entire earth's landmass.

It is believed that a part of the so-called 'excess' DNA in the genome, which represents chromosomal heterochromatin regions (HRs), probably played and continues to play an important role in human adaptation to different climatic-and-geographical conditions, including high-altitude zones. In particular the chromosomal Q-heterochromatin regions (Q-HRs), which are present in the genome of only three higher primates (*Homo sapiens, Pan troglodytes* and *Gorilla gorilla*) [8-10]. The genome of higher eukaryotes is known to consist of two components: euchromatin and heterochromatin. The first component consists mainly of coding DNA (genes), whereas the second component represents short, repetitive sequences that do not code for specific polypeptides and are known by different names: 'excess', 'redundant', 'junk', 'selfish', etc. DNA. In humans, the genic portion makes up about 1.5-2.0% of the DNA of the genome, while the rest is in the form of short, repetitive nucleotides to varying degrees. About 15-20% of the 'excess' DNA in the human karyotype is organized in constitutive chromosomal HRs. Two types of constitutive heterochromatin are known: C- and Q-HRs. C-heterochromatin is present in the genome of all higher eukaryotes, whereas Q-heterochromatin is found in the karyotype of only three animals (humans, chimpanzees and gorillas). The peculiarity of chromosomal Q-HRs in the genome of these higher primates is that they show wide heritable variability only in the human population. It was found that human populations differ significantly from each other in the number of chromosomal Q-HRs [11-22]. Established that the number of Q-HRs in the genome of a population depends on the climatic-and-geographical peculiarities of the places of their permanent residence, but not on their racial and ethnic features [22]. In particular, it was shown that the largest number of chromosomal Q-HRs was found in the genome of indigenous people of Africa [12,19], and the smallest number of Q-heterochromatin materials was found in aborigines of high-altitudes and the Far North [20,21] [see Supplement Materials].

Now for the first time it has been possible to obtain evidence that chromosomal Q-HRs in the genome of a human population can change even over several generations. We managed to obtain data indicating the change in the number of chromosomal Q-HRs in the population, which due to circumstances beyond their control migrate to the territory with extreme climatic conditions. This is what happened to one Kyrgyz population after the Bolshevik Revolution in Russia in 1917. To avoid physical extermination and political persecution one Kyrgyz tribe fled to Afghanistan in the 1920s and appealed to give them asylum. The Afghan government offered them an almost uninhabited and inaccessible high-altitude area in the northeastern Pamirs, which is characterized by an extremely harsh climate [see Supplement Materials]. As the research shows, during more than five generations that the Kyrgyz have lived in complete genetic isolation in the extreme conditions of the Pamir high-altitudes (4200 m and higher above sea level), some changes have occurred at their chromosomes compared to those who continue to live in Kyrgyzstan.

Supplement – 2.



Karyotypes of four human samples stained (Q-staining) with quinacrine mustard. (a) Metaphase of female (46, XX) having no chromosomal Q-HR. (b) Metaphase of human male (46, XY) having one chromosomal Q-HRs. (c) Metaphase of man (46, XY) having three chromosomal Q-HRs. (d) Metaphase of man (46, XY) having four chromosomal Q-HRs.

Materials and Methods

The material of the study was Kyrgyz sample, who returned to his historic homeland a few years ago by consent of the governments of the two countries due to extremely difficult living conditions at the high-altitude of Afghanistan Pamirs: high morbidity and child mortality, short life expectancy (~35 yrs.), lack of school education, medical services and many others. The sample included 58 repatriates, who moved to live in their historical homeland, which their ancestors left more than 100 years ago (the Alay mountains of Kyrgyzstan). As a control, we used a sample of Kyrgyz (high school students) who are indigenous inhabitants of the Alay highland region (2,600 m and above sea level). For comparison, we studied a sample of students from the North India, who study at Bishkek, Kyrgyzstan. To study the variability of chromosomal Q-HRs, we used a short-term culture of peripheral blood lymphocytes. The sizes and fluorescence intensities of chromosomal Q-bands were evaluated using a 5-point system according to [24]. Q-bands with the 4th and 5th fluorescence intensities were accepted as chromosomal Q-HRs according to the recommendations of [25]. Identification and registration of Q-bands were carried out on the computer screen using a microscope of Carl Zeiss (Germany) model Axio Imager A2 with planapochromatic objective EC 'Plan-Neofluar' 100x with numerical aperture 1.40, which is equipped with a special computer system IKAROS (Carl Zeiss, Germany), which automatically analyses and document the entire human karyotype.

The following quantitative characteristics of chromosomal Q-HRs in population were studied:

1. The Q-HRs frequencies in all seven Q-polymorphic autosomes (3, 4, 13-15, 21 and 22) and Y chromosome;
2. The distribution of individuals according to the number of Q-HRs in population (distribution of the numbers of Q \HRs);
3. The mean number of Q-HRs per individual as important quantitative characteristics of Q-heterochromatin variability in population.

The obtained data were processed by generally accepted statistical methods using the SPSS Statistics program. The Student's ***t***-test was used to compare the mean numbers of Q-HRs per individual in the population between samples. Empirical distributions were compared using the χ^2 criterion.

Results

The aim of the study is finding out whether the number of chromosomal Q-HRs in the genome of a human population may change if it is forced to exist in extreme climatic conditions of the high-altitude for several generations.

Location of Q-HRs	**Populations**		
	Kyrgyz repatriates (n = 58) I	Kyrgyz natives (n = 112) II	Indians (n = 97) III
3	50 (43,10)* 44,64**	68 (30.4) * 21.9**	93 (50.5) * 23.6**
4	7 (6,03) 6,25	12 (5.4) 3.9	17 (8.2) 4.1
13	31 (26,72) 27,68	84 (37.5) 27.1	128 (62.3) 30.7
14	8 (6,9) 7,14	29 (13.0) 9.3	27 (13.9) 6.9
15	6 (5,17) 5,36	34 (15.2) 10.9	39 (19.6) 9.6
21	3 (2,59) 2,68	42 (18.7) 13.5	52 (26.8) 13.2
22	7 (6,03) 6,25	41 (18.3) 13.2	48 (24.2) 11.9
Total Q-HRs	112 (96,52) 100.0	310 (138.5) 99.9	404 (203.1) 100.0
Mean number of Q-HRs	1.9±0.123	2.78±0,083	4.12±0.149
Statistics	$\chi^2_{I, II} = 67.3$; df = 168; P = 0.002;	$\chi^2_{I, III} = 115.2$; df = 155; P < 0.001;	$\chi^2_{II, III} = 90.56$; df = 207; P < 0.001;

* Q-HR frequency of the chromosomes analyzed.

** Q-HR frequency as a percentage of the overall number of chromosomal Q-HRs.

Table 1: Frequency of Q-HRs in seven Q-polymorphic autosomes among Kyrgyz and Indians.

As shown in Table 1, in the studied samples chromosomal Q-HRs were detected on all seven Q-polymorphic autosomes, where Q-heterochromatin can potentially be detected in the human karyotype. The previously established regularity in the distribution of Q-HRs at the population level [11-24] are observed in all samples studied, namely: (a) more than half of Q-HRs are localized on autosomes 3 and 13, and the rest are more or less evenly distributed on other Q-polymorphic chromosomes; b) the studied samples are statistically significantly different from each other in the distribution of the absolute frequencies of Q-HR on Q polymorphic chromosomes; (c) however, the samples did not differ from each other in terms of the relative

frequencies of Q-HRs expressed as a percentage of the total number of chromosomal Q-HRs, i.e. each Q-polymorphic autosome at the population level contains a comparable portion of the total number of Q-heterochromatin irrespective of their places of permanent residence or race-ethnic origin; (d) when the mean number of Q-HRs per individual in the population increases, the absolute frequency of Q-HRs increases simultaneously on all Q-polymorphic chromosomes and *vice versa.*

Number Of Q-HRs	Populations		
	Kyrgyz repatriates (n = 58) I	Kyrgyz natives (n = 112) II	Indians (n = 97) III
0	3	1	
1	15	6	1
2	25	35	7
3	13	48	25
4	2	20	32
5		2	14
6			13
7			3
8			1
9			1
Total Q-HRs	112	310	404
Distribution of Q-HRs	$\chi^2_{I, II} = 34.7$; df = 169; P = 0.002;	$\chi^2_{I, III} = 115.2$; df = 155; P < 0.001;	$\chi^2_{II, III} = 59.7$; df = 208; P < 0.001;
Mean number Of Q-HRs	1.9±0.123	2.78±0.083	4.12±0.149
	$t_{I, II} = 6.07$; df = 169; P < 0.001;	$t_{I, III} = 10.38$; df = 155; P < 0.001;	$t_{II, III} = 8.16$; df = 208; P < 0.001;

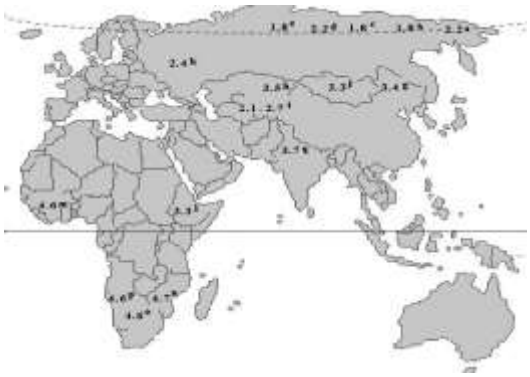
Table 2: The distribution and the mean numbers of chromosomal Q-HRs per individual in Kyrgyz and Indians.

Table 2 shows the distributions of the numbers of Q-HRs in individuals at the population. The studied samples differ significantly from each other both in the distribution of the numbers of Q-HRs and in the mean numbers of Q-HRs. The highest number of Q-HRs is found in the genome of Indians, and the lowest in Kyrgyz repatriates. At the same time, the Kyrgyz repatriates differ with the predominance of individuals with a low number of Q-HRs and a very narrow range of Q-HRs amount variability in the population (from 0 to 4).

Discussion

The distribution of chromosomal Q-HRs at the level of human populations are known. In particular, it has been established that: a) Q-heterochromatin can be detected only on seven human autosomes (3, 4, 13, 14, 15, 21 and 22) and on the Y chromosome; b) at the population level the distribution of Q-HRs on the seven Q-polymorphic autosomes is uneven, the greatest number of Q-HRs is found on chromosomes 3 and 13 (over 50%), the rest of them are distributed more or less evenly on the other autosomes. However, populations do not differ from each other in the relative content of Q-HRs on seven autosomes; c) the number of Q-HRs in individuals in a population varies from 0 to 10, although there are 23 loci in the human karyotype where Q-HRs can potentially be detected; d) distribution of the numbers of Q-HRs in individuals of a population is almost normal; e) populations differ from each other in the mean numbers of Q-HRs (from 1.8 to 4.7). These differences proved to be related to features of the climatic-and-geographic conditions of the place of permanent residence, and not to racial and ethnic composition of the population. The highest number of Q-HRs is present in the genome of populations living at low geographical latitudes (from 3.7 to 4.7), and the lowest at high geographical latitudes and high-altitude mountains (from 1.8 to 2.7) [see Supplement Materials]; f) individuals capable of successfully adapting themselves to the extreme high-altitude climate (e.g. mountaineers) and of the Far North (e.g. oil industry workers of polar Eastern Siberia) are characterized by extremely low amounts of Q-HRs in their genome [11-24,31].

Supplement – 3.



The mean number of Q-HRs per individual in the native populations of Eurasia and Africa: a = Chukchi of Chukotsk (n = 132); b = Yakuts of the Yakut ASSR (n = 127); c = Selkups of Eastern Siberia (n = 90); d = Nenets of Eastern Siberia (n = 117); e = Khants of Eastern Siberia (n = 54); f = Mongolians of the MPR (n = 72); g = Chinese of northern China (n = 124); h = Kazakhs of southern Kazakhstan (n = 101); i = Kirghiz of the Pamirs and Tien Shan (n = 603); k = Russians of Bishkek (n = 200); l = Ethiopians of the Ethiopian Highlands (n = 52); m = Negroids of Guinea-Bissau (n = 13); n = Negroids of Mozambique (n = 148); o = Negroids of Zimbabwe (n = 34); p = Negroids of Angola (n = 132); q = Indians of northern India (n = 58) [Ibraimov, 2003; with permission].

Supplement 4.

The lives of Pamir Kyrgyz living at 4,200 m and above sea level in northeastern Afghanistan. There are also dozen videos on You Tube about Kyrgyz living in the Afghan high-altitude Pamirs, filmed by documentary filmmakers.

So far, the lowest amount of the chromosomal Q-HRs in the genome of human populations was found in the natives of Eastern Siberia, Pamir and Tien-Shan. In the aborigines of the Far North of Eastern Siberia, the mean number of Q-HR in the samples was: Chukchi - 2.2; Nenets - 2.2; Khanty - 1.8; Yakuts - 1.8; Selkups - 1.8 [21,26,31], and in the highlanders of the Pamirs and Tien-Shan (2.7 and 2.1, respectively) [16,21,31]. Among non-indigenous people, such low mean numbers of Q-HRs have been found in mountaineers and oil workers working in polar Eastern Siberia (1.60 and 1.72**,** respectively). It is noteworthy that in the genome of newcomers, but successfully adapted individuals the number of Q-HRs was lower than in the native populations of the high mountains Pamir and Tien-Shan and the Far North of Siberia [20,21].

The peculiarity of this study is that, for the first time, a decrease in the number of Q-HRs was found in a population that lived for a short time (about 100 years) in extremely harsh climatic conditions at high-altitude. The lower number of Q-HRs in mountaineers is explained by the fact that they have undergone, among other things, strict sports selection. To be enrolled in the section they had to have climbed at least 4,000 m above sea level without compromising their health]20\. The sample of oil workers were individuals who had worked in the polar conditions of Eastern Siberia for at least 3 years [21]. The point is that despite high salaries, good medical care and social benefits (e.g. early retirement) not all newcomers stay to live and work in this extreme climate. In other words, apparently individuals with very few Q-HRs in their genome could become mountaineers or drillers in the Siberian Arctic Circle, among all those willing to do so. Therefore, the low content of Q-HRs in the genome of the Kyrgyz from the high-altitude Afghanistan Pamirs can more reasonably be explained by the same reasons, i.e. individuals who were born with a smaller number of Q-HRs in their karyotype survived there more often.

The fact of a broad variability in the content of chromosomal Q-HRs in the human genome can be explained within the framework of cell thermoregulation (CT) hypothesis [27,28]. The essence of the hypothesis is that chromosomal HRs, as the densest structure around the interphase nucleus, known as condensed chromatin, participate in CT by eliminating of excess metabolic heat from the nucleus into the cytoplasm due to its highest heat conductivity. The phenotypic manifestation of CT on the organism level is human body heat conductivity (BHC), the magnitude of which depends on the number of chromosomal C- and Q-HRs in his genome. Since human populations do not differ significantly from each other in the number of C-HRs [29,30] it is obvious that the differences of individuals in the population at the level of BHC depends on the number of Q-HRs in their genome. It has been shown that the more Q-HRs in the karyotype of an individual, the higher the level of his BHC and *vice versa* [22,31.]

We do not yet know how the number of Q-HRs in the genome or its physiological phenotype (BHC) affects human adaptation to high-altitude climate. Nevertheless, we consider as highly probable that humans in the struggle with cold and high-altitude hypoxia, in addition to the known morphological and physiological mechanisms use low BHC as a means of defense. Perhaps individuals with low BHC are relatively better at maintaining temperature homeostasis, by more effectively retaining metabolic heat in the body under conditions of permanent high-altitude cold and limited food resources in high altitude regions. Our data support the assumption that: (1) the bodies of individuals in a population differ from each other, among other things, in heat conductivity; (2) apparently, the level of human BHC depends on the density of the peripheral condensed chromatin layer around the interphase nucleus, in the compaction of which the amount of Q-HRs in its genome plays an important role; (3) organ-based physiological thermoregulation, which is the same in all normal people, is realized under different physical conditions because individuals in the population differ in BHC [22,27,31]**.** The main conclusion of the study is that apparently chromosomal HRs, which represent the higher form of organization of short, highly repetitive DNA, are not 'excess' neutral material, but have selective value the amount of which in the human genome is under the control of natural selection [31].

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We apologize to that authors whose work is not cited or is cited only through reviews. The reason for this is only the space limitations of the publication.

Conflicts of interest: none.

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Statement of Consent/Ethical approval: Not required.

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