Polymorphism of the Dopamine D4 Receptor (DRD4) and Serotonin Transporter (5-HTTL) Gene Promoter Regions in African Tribes of Hadza and Datoga


Abstract—Molecular genetic analysis of the allelic variants of the DRD4 and 5-HTTL gene promoter regions was performed in African tribes of Hadza and Datoga, characterized by different levels of socially acceptable aggression. It was demonstrated that Hadza and Datoga people differed in the structural organization of one of the 5-HTTL alleles (extra long allele xL). Analysis of the allele length polymorphism of both genes showed that in the Hadza and Datoga samples examined, variation parameters, as well as the genotype and allele frequency distribution pattern were almost the same. At the same time, analysis of the SNP polymorphism at the A/G substitutions of the 5-HTTL locus revealed a substantial decrease of the active allele L frequency in the population of Hadza compared to the population of Datoga ($\chi^2 = 3.77; d.f. = 1; p = 0.052$).

INTRODUCTION

Investigation of genetic polymorphism in the system of genes involved in the regulation of important behavioral characters is of considerable interest for cognitive biology, as well as medical and population genetics. The dopamine and serotonin neurotransmitter systems play an important role in the regulation of human behavior. At present, more than 15 candidate genes of neurotransmitter systems associated with different behavioral characters have been described [1–3]. Most of the genes playing the key roles in functioning of brain neurotransmitter systems are characterized by the presence of numerous polymorphic variants. Among these genes, the dopamine receptor D4 gene (DRD4), as well as the serotonin transporter gene (5-HTTL) received particular attention, because it was suggested that their promoter polymorphisms could be associated with the differences in the expression of these genes.

Promoter region of human dopamine receptor D4 gene, mapped to region p15.5 of chromosome 11, harbors a number of single nucleotide polymorphisms (SNPs). It was demonstrated that SNPs did not change transcriptional activity of the gene. In addition, in the DRD4 promoter region, silencer region containing the 120-bp insertion was localized. However, no direct influence of deletion/insertion polymorphism on the gene transcriptional activity was demonstrated so far [4].

The promoter region of serotonin transporter gene, mapped to human chromosome 17, region q11.2-q12, was shown to contain GC-rich, 20 to 23 bp-long repeated elements. In most of the populations examined, the two main alleles of this gene characterized by different transcriptional activity were revealed. Transcriptional activity of the short allele (S), containing 14 repeats is lower than that of the long allele (L), which contains 16 repeats [5]. In addition to these alleles, in African–American and Japanese populations rare alleles with a great number of repeats, specifically, very long alleles (xL) and extra long alleles (vL) were identified [6]. More recently, in the 5-HTTL gene the SNP polymorphism (A/G) lying in the L and S allele repeat 6, was identified. It was shown that this SNP influenced the level of gene expression, since it was located within the binding region of AP2 transcription factor, which suppressed transcription of allele L. Thus, it was demonstrated that genotypes $L_G L_G$, $L_G S$, and $SS$ were characterized by low transcriptional activity [7].

In many recent studies the distribution of the polymorphic loci at these genes were examined with the purpose of finding an association between certain alleles and some characteristics of deviant behavior (espe-
cially obsessive–compulsive disorder, hostility, and physical aggression), some psychiatric disorders, and alcohol addiction [2, 3, 6, 7]. In this respect, traditional tribal communities, characterized by different levels of socially acceptable aggression, deserve considerable interest. In particular, this concerns Hadza and Datoga tribes living in North Tanzania and still having no contacts with the Western civilization.

The Hadza people (also known as Hadzapi, Tindiga, Kindiga, Kangeju, and Wahi) are one of the few groups preserving traditional way of life. Their culture in many respects continues to be the same as that of the Bushmen from Namibia until the 1970s [8, 9]. The Hadza are known for their egalitarianism and peacefulness; the leadership is nominal. The Hadza live near the Lake Eyasi at the northwest of Tanzania, and their language (Hadzane, Hadzapi) is distantly relative to the Bushman language. At present, the Hadza number about 1000 individuals. Some 300 Hadza (part of eastern Hadza) still live as traditional hunters–gatherers [9].

The Datoga people (also known as Tatog, Mang’ati, and Barabaig) are the Nilotes speaking the language of Chari–Nile group, Nile-Saharan language family. The main occupation of Datoga is cattle husbandry. The Datoga currently number about 200000. Colonial authorities, as well as post-colonial independent government were suspicious of all herdsmen, considering them as aggressive and poorly administered [10]. At present, the Datoga are marginal, stigmatized group, definitely suffering from social and political discrimination. The Datoga keep patriarchal extended families and polygamous patrilocal marriages [11]. In the Datoga society young men play an important role of guardians and warriors. They are responsible for watching the cattle and keeping them from being stolen.

In the frames of the project on the investigation of candidate genes associated with deviant aggressive behavior, the present study was focused on molecular genetic analysis of the DRD4 and 5-HTTL promoter polymorphisms in the Hadza and Datoga populations.

MATERIALS AND METHODS

Field material in the form of buccal epithelium was collected from 2006 through 2009 in the United Republic of Tanzania. Genomic DNA of Hadza (n = 74) and Datoga (n = 74) was isolated with the help of the Diatom™ DNA Prep 200 extraction kit (Izogen Laboratory, Russia), applied for isolation of DNA from different biological materials, according to the protocol of the manufacturer. The DRD4 and 5-HTTL promoter polymorphisms were typed using polymerase chain reaction, which was performed with the GenePak® PCR MasterMix Core (Izogen Laboratory, Russia) reagent kit according to the recommendations of the manufacturer. The primer pairs and reaction conditions used were as follows.

The DRD4 locus: primers 5′-tgcgcagacatcggggt-3′ and 5′-atttcctttaaagcgcct-3′. The amplification conditions included initial denaturation at 94°C for 4 min, followed by 30 cycles consisted of three steps, comprising denaturation for 1 min at 94°C; primer annealing for 1 min at 56°C; and extension for 1 min at 72°C. Final extension was carried out at 72°C for 10 min.

The 5-HTTL locus: primers 5′-tgatccagacgctaac-3′ and 5′-cggagcggggagact-3′. The amplification conditions included initial denaturation at 94°C for 4 min, followed by 30 cycles consisted of three steps, comprising denaturation for 1 min at 94°C; primer annealing for 1 min at 59°C; and extension for 1 min at 72°C. Final extension was carried out at 72°C for 10 min.

To identify the SNP polymorphism (A/G), the 5-HTTL amplification product was subdivided into 10 µl aliquots with one of these treated with the MspI restriction endonucleases at 37°C overnight.

Amplification and restriction products were separated by electrophoresis in 2% agarose gel and stained with ethidium bromide. The results were photographed and analyzed using a BioDocAnalyze device.

Cloning of amplified 5-HTTL DNA fragments was performed using the pGEM-T easy Vector System kit (Promega), according to the recommendations of the manufacturer. Sequencing of the cloned fragments was performed using an ABI PRISM BigDye Terminator v. 3.1 kit, and the reaction products were analyzed using anABI PRISM 3100-Avant automated DNA sequencer.

Statistical treatment of the data was performed using the POPGENE v.1.31 and GDA software programs. The differences were considered statistically significant at p < 0.05.

RESULTS AND DISCUSSION

In the populations of Hadza and Datoga, two DRD4 alleles, described earlier in many ethnic populations were identified. These allelic variants were represented by the long (L) and short (S) alleles of the del/ins 120-bp polymorphism. The data on the genotype and allele distributions at this locus are demonstrated in Table 1.

The distribution of DRD4 genotypes in the populations was in Hardy–Weinberg equilibrium (G2 = 1.397; df, = 1; and p = 0.237, for Hadza; and (G2 = 0.910; df, = 1; and p = 0.920, Datoga). The test for heterogeneity between two samples in the genotype distribution showed no statistically significant differences between Hadza and Datoga (G2 = 0.573; df, = 2; and p = 0.751). The absence of the between-population differences was also confirmed by the FST index value (FST = 0.0000).

Analysis of the 5-HTTL length polymorphisms showed that both populations examined carried three