Dopaminergic D4 receptor polymorphism among 24 populations of India: an anthropological insight

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Abstract Various studies have tried to elucidate the genomics underlying the ethnic diversity of Indian populations. The 48-basepair variable number tandem repeat (VNTR) of the *DRD4* gene, besides its clinical importance, has been a subject of interest for understanding human evolutionary history worldwide. In spite of its highly polymorphic nature it has not been much explored by anthropologists, especially in the Indian context. In the present study, an attempt was made to infer the allelic variation of the repeat allele among 24 Indian ethnic groups belonging to different ethnic and linguistic groups along with different geographic affiliations. Only the 2R, 3R, 4R, 5R, 6R, and 7R repeat alleles were found in the studied populations, with 4R being the most frequent followed by 2R. All the other repeat alleles (3R, 5R, 6R, and 7R) occurred at very low frequency. Based on the 4R and 5R frequencies, the study reveals a common genetic substratum of the Indian population as already proposed by earlier studies. Further, the study also highlights the inflow of genes from the north.

Key words: *DRD4* gene, linguistics, tribe, caste, common genetic substratum

Introduction

India, with its wide range of climatic and environmental conditions and huge diversity in terms of language, culture, religion and ethnicity, must, like Africa, have played an important role in human evolutionary history. Evidence of fossilized Hominoidae (Pongidae and Protominidae) of late Miocene period, such as *Gigantopithecus*, *Brahmapithecus*, *Ramapithecus*, *Sivapithecus* from Siwaliks and *Homo erectus* from the Narmada basin of India, hints towards the role of the subcontinent in the origin of human species (Pilbeam, 1979; Benefit and McCrossin, 1995; Ruvolo, 1997; Trinkaus, 2005). This can be further supported by a recent report by Saraswathy et al. (2010), which proposed a common genetic substratum of India. To understand the peopling of India, and in turn the peopling of the world, researchers have used several genetic markers. Markers related to the dopaminergic system, specifically dopamine D4

receptor (*DRD4*), are now proving to be an effective tool for diversity studies. *DRD4* polymorphism is being extensively used to understand micro- and macro-migration, specifically for two reasons: (i) its highly polymorphic nature; and (ii) the simple technology involved in typing this gene polymorphism. The polymorphism of *DRD4* is revealed in the variable number of imperfect 48-basepair (bp) tandem repeats on exon 3, ranging from 2 to 11 repeat (R) units. The 4, 7 and 2 repeats are the most common ones (Chen et al., 1999).

DRD4 repeat alleles are reported to be implicated in various neuropsychiatric disorders/traits, such as novelty seeking (Malhotra et al., 1996; Jonsson et al., 1997; Gelernter et al., 1997; Sullivan et al., 1998; Ray et al., 2009), attention deficit hyperactivity disorder (ADHD) (LaHoste et al., 1996; Swanson et al., 1998; Swanson and Moyzis, 2003; Grady et al., 2003), and risk behaviors such as addiction and gambling (Comings et al., 1999; McGeary, 2009; Eisenegger et al., 2010). Apart from its clinical implications, *DRD4* offers possibilities for use in anthropological studies due its great variation among the world's populations (Chang et al., 1996).

Chen et al. (1999) also proposed that long repeat alleles of the *DRD4* gene (5–11 repeats) were associated with long-distance migrations. Only a few anthropological studies on *DRD4* polymorphism in India are available: just three papers have been published in which *DRD4* polymorphism was screened in Indian populations (Khurana and Seshadri, 2003; Ghosh and Seshadri, 2005; Bhaduri et al., 2007). Further, the populations studied in these publications were also not ethnically well defined; they were rather essentially

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geographically defined populations. Thus, in the present study an attempt is made to understand human evolutionary history of India with respect to *DRD4* repeat polymorphism among 24 ethnically, geographically, and linguistically well-defined populations.

Materials and Methods

Sample collection

Intravenous blood samples (5 ml) were collected from 894 individuals belonging to 24 different Indian communities from different geographical regions (Figure 1), after obtaining prior written consent. Each community is distinguishable ethnically, linguistically, and geographically. Details of these populations are given in Table 1. The present study was approved by the Ethical Committee of the Department of Anthropology, University of Delhi, India.

Data analysis

Genomic DNA was extracted from the collected blood samples using the salting-out procedure (Miller et al., 1988). The polymerase chain reaction (PCR) of the *DRD4* 48-bp VNTR locus was performed using a protocol previously described by Eisenberg et al. (2008) with some modifications. The 15 µl PCR reaction mixture contained 1 × PCR buffer, 1.5 µM forward primer (5'-GCGACTACGTGGTCTAC TCG-3'), 1.5 µM reverse primer (5'-AGGACCCTCATGG CCTTG-3'), 200 µM dATP, dTTP, dCTP and 100 µM dITP and dGTP, 3% dimethyl sulfoxide, 1.5 units Taq DNA polymerase and 50 ng DNA template. Thermocycling conditions were: 15 min at 95°C to activate the enzyme and denature the DNA; 40 cycles of 1 min denaturation at 94°C, 1 min annealing at 64.4°C, 1 min 30 s extension at 72°C; followed by

Figure 1. Outline map of India showing the geographical locations of the sampled areas of the 24 populations.

one cycle of 10 min final extension at 72°C. These primers yielded a 4R PCR product of 475 bp. Samples were run with a 100-bp ladder stained with ethidium bromide and visualized under UV light on 2.0% agarose gel. To avoid genotyping error, the technique was first standardized on

Table 1. Name of the population, state, geographical location, category, their linguistic affiliation and number of samples analyzed

State	Population	Geographical location	Category	Linguistic group	No. of samples		
Himachal Pradesh	Jat	North India	Caste	Indo-European	33		
	Rajput		Caste	Indo-European	33		
	Brahmin		Caste	Indo-European	44		
Delhi	Punjabi	North India	Caste	Indo-European	29		
	Sindhi		Caste	Indo-European	33		
	Aggarwal		Caste	Indo-European	121		
Manipur	Kabui	North-east India	Tribe	Tibeto-Burman	37		
	Aimol		Tribe	Tibeto-Burman	43		
	Kom		Tribe	Tibeto-Burman	24		
	Thadou		Tribe	Tibeto-Burman	37		
	Meitei		Non-tribe	Tibeto-Burman	38		
	Paite		Tribe	Tibeto-Burman	41		
	Brahmin		Caste	Tibeto-Burman	27		
	Muslim		Religious group	Tibeto-Burman	31		
	Jharkhand		Oraon	East India	Tribe	Dravidian	29
			Munda		Tribe	Austro-Asiatic	25
Rajasthan	Patelia	West India	Tribe	Indo-European	20		
	Naikda		Tribe	Indo-European	22		
	Damor		Tribe	Indo-European	25		
	Kathodi		Tribe	Indo-European	35		
Andhra Pradesh	Brahmin	South India	Caste	Dravidian	34		
	Kolam		Tribe	Dravidian	48		
	Thoti		Tribe	Dravidian	36		
	Nayakpod		Tribe	Dravidian	49		
Total					894		

polyacrylamide gel and shifted to agarose gel for convenience, only after tallying the genotyping results of around 25 samples from both the gels. Allele frequencies were calculated using the statistical tool POPGENE1.31 (Yeh and Yang, 1999). Hardy–Weinberg equilibrium was determined using the χ^2 goodness-of-fit test.

Results

Of all the repeat polymorphic variants of *DRD4* 48-bp VNTR reported among the world populations, only six variants/alleles were found in the present study. The two most commonly found alleles in the studied Indian populations are the 4R and the 2R allele. These two alleles are shared by all the studied populations, while the other alleles observed, i.e. 3R, 5R, 6R, and 7R, were distributed to varying extents. The 2R allele varies from 0.02 (Oraon of east India and Kolam of south India) to 0.29 (Kom of north-east India), while the 4R allele ranges from 0.69 among Punjabis of north India to 0.94 among Manipur Muslims of north-east India. However, the 3R, 5R, 6R, and 7R alleles are found to be distributed in a low frequency among the studied populations. The other higher-repeat alleles, e.g. 8R, 9R and 10R, have been reported among other world populations but none of these higher repeats is observed in the present study. The average allele frequencies of 2R, 3R, 4R, 5R, 6R, and 7R are 0.138, 0.007, 0.827, 0.010, 0.011, and 0.007, respectively. Most of the population groups under study (15 populations) deviate from Hardy–Weinberg equilibrium (Table 2). This deviation from Hardy–Weinberg equilibrium might be due to reasons such as small sample size, small population size, consanguinity, or low frequency of the minor repeat alleles.

When the studied populations are grouped on the basis of geography, the south Indian populations have the highest frequency of 4R allele (0.852), followed by east Indian (0.845), west Indian (0.843) north-east Indian (0.814), and north Indian populations (0.811), respectively (Table 2). This is in contrast to the 2R and 7R alleles, for which north-east Indian and north Indian populations, respectively, exhibit higher frequencies. The south Indian populations have the lowest frequencies of 2R while the 7R is absent.

Discussion

Proto-Australoids could be, as proposed by Guha, the original inhabitants of India (Guha, 1944). Almost all the tribes, except for those of the north-eastern region, are found to have Proto-Australoid ethnic elements, though in varying degrees. Specifically, Austro-Asiatic and Dravidian-speaking tribal groups of India are reported to be modern representatives of the earliest settlers of the subcontinent (Kumar et al., 2008), who would possibly have had the so-called Proto-Australoid ethnic elements. The evolution of the *DRD4* 48-bp repeat polymorphism among the human lineage is not very clear. Earlier studies on chimpanzees, gorillas, and orangutans revealed that the ancestral hominoid gene had the 5R allele (Livak et al., 1995). However, a recent study among other primate members observed the highly polymorphic nature of the repeat gene: high repeat alleles (7, 8, and 9) are observed among spider monkeys (The DB-*DRD4* Database Project: http://www.ibibibase.com/projects/db-drd4/what_is_drd4.htm). Another hominoid ape *Hylobates* shows regional variation and a complex nature of sequence repeat (Inoue-Murayama et al., 2000).

Table 2. Distribution of allele frequencies of *DRD4* polymorphism among 24 population groups of India

State	Population	Allele frequencies						HWE (<i>P</i> value)	
		2R	3R	4R	5R	6R	7R	χ^2	<i>G</i> ²
Himachal Pradesh	Jat	0.24	0	0.76	0	0	0	0.039*	0.050
	Rajput	0.05	0	0.88	0.03	0	0.05	0.002*	0.372
	Brahmin	0.07	0.01	0.86	0.02	0.03	0	0.000*	0.010*
Delhi	Punjabi	0.22	0.02	0.69	0	0.04	0.03	0.000*	0.040*
	Sindhi	0.06	0	0.91	0	0	0.03	0.965	0.919
	Aggarwal	0.21	0.02	0.76	0	0.01	0.004	0.000*	0.077
Manipur	Kabui	0.15	0.01	0.82	0.01	0	0	0.413	0.625
	Aimol	0.24	0.01	0.74	0	0	0	0.203	0.220
	Kom	0.29	0.00	0.71	0	0	0	0.039*	0.046*
	Thadou	0.15	0.03	0.81	0.01	0	0	0.160	0.305
	Meitei	0.08	0.00	0.87	0.01	0.03	0.01	0.016*	0.274
	Paite	0.17	0.01	0.81	0.01	0	0	0.121	0.221
	Brahmins	0.15	0	0.82	0	0	0.04	0.000*	0.016*
	Muslims	0.07	0	0.94	0	0	0	0.002*	0.048*
Jharkhand	Oraon	0.02	0	0.93	0.04	0.02	0	0.000*	0.003*
	Munda	0.24	0	0.76	0	0	0	0.065	0.082
Rajasthan	Pateliya	0.18	0	0.83	0	0	0	0.018*	0.039*
	Naikda	0.14	0	0.86	0	0	0	0.503	0.373
	Damor	0.10	0.04	0.84	0	0.02	0	0.000*	0.003*
	Kathodi	0.13	0	0.84	0	0.01	0.014	0.043*	0.167
Andhra Pradesh	Brahmin	0.12	0.03	0.85	0	0	0	0.000*	0.002*
	Kolam	0.02	0	0.93	0.04	0.01	0	0.999	0.998
	Thoti	0.14	0	0.86	0	0	0	0.598	0.618
	Nayakpod	0.09	0	0.77	0.05	0.09	0	0.000*	0.003*

* *P* < 0.05.

However, among the human population groups world-wide, the 4R allele is present with relatively higher frequency, consisting of only one particular form: $\alpha\beta\theta\zeta$ (Lichter et al., 1993); this hints at the possibility of this allele being of ancestral human origin. This hypothesis is further confirmed based on limited sequence data reported for primate *DRD4* 4R allele (Livak et al., 1995), the lower level of linkage disequilibrium (LD) for polymorphisms surrounding this allele, and the sequence motif arrangements of the non-4R alleles (Ding et al., 2002).

The presence of the human ancestral 4R allele at significant frequencies in all Indian populations, irrespective of their tribe and caste status, supports the common genetic substratum of the Indian population proposed previously (Saraswathy et al., 2010). Moreover, the high frequency of progenitor 4R allele among the south Indian and east Indian populations belonging to Proto-Australoid ethnic elements and Dravidian and Austro-Asiatic speakers, respectively, is strongly supportive of the view that Proto-Australoid groups were the earliest settlers in India and might have diversified into two groups in the course of human evolution in the region (Kumar et al., 2008). Furthermore, studies conducted on different population groups of eastern India (Bhaduri et al., 2007), and southern India (Ghosh and Seshadri, 2005) also found the 4R allele to be the most prevalent. However, they also reported very low frequencies of 8R and 9R alleles in some populations. In the present studied populations, long repeat alleles, i.e. 8–11R, are found to be absent.

In terms of distribution, the 7R allele stands next to the 4R allele in the majority of the world's populations. Moreover, available biochemical data indicate that both the 2R and 7R proteins have blunted responses to dopamine as compared to that of the 4R protein (Leung et al., 2005). Despite such similarity in their biochemical manifestation, the 2R and 7R alleles show remarkable differences in their distribution patterns among various world populations: the 2R allele is more abundant among south and east Asian populations, including Indian populations, while the 7R allele is more widely distributed among the New World populations.

In the Indian context the 2R allele is the second most common allele after the 4R allele. The 7R allele, which is the second most common allele in other world populations, is found to be the least frequent in Indian populations along with the 3R allele. The 7R allele is found at very low frequencies among the caste populations of northern, north-eastern and north-western parts of India, which were the most vulnerable regions in terms of the inflow of genes from the northern part of the globe, specifically Europe, where 7R is found in relatively high frequencies, ranging from 0.06 to 0.27 (http://alfred.med.yale.edu/alfred/SiteTable1A_working.asp?siteuid=SI000224I). However, such high frequencies of 7R are also found in some population groups of Africa (http://alfred.med.yale.edu/alfred/SiteTable1A_working.asp?siteuid=SI000224I). In contrast to this, all the tribes of India and all the caste groups of south India considered in the present study are found to evidence a complete absence of 7R allele.

Most of the presently studied populations, in spite of being from different geographical regions and belonging to different linguistic families, such as Indo-European (north-

western—Patelia, Naikda, Damor), Dravidian (southern—Kolam, Thoti, Nayakpod), Dravidian (east—Oraons), and Austro-Asiatic (east—Mundas), show absence of the 7R allele. All these populations, being tribal populations of India, are supposed to be the autochthones of the subcontinent.

Ding et al. (2002) proposed two possible hypotheses regarding the evolution of the *DRD4* gene: (i) recent mutational origin of 7R allele from the 4R allele, subsequently followed by positive selection of the same; and (ii) importation of the 7R allele from a closely related Hominid, most probably Neandertals. As the proposed age of the 7R allele (30000–50000 years) coincides with the existence of Neandertals and the archaic type of *Homo sapiens sapiens*, they also related the 7R allele to major expansions, radical new technologies (Upper Palaeolithic) and the development of agriculture. Further, Wang et al. (2004) proposed that the recent origin of the 7R allele and its high frequencies in some world populations could be attributed to a strong positive selection. However, Naka et al. (2011) argued that *DRD4* 7R is not extraordinarily young, and as such is not subjected to positive selection, and proposed that the increase in its frequencies in some populations could be attributed to genetic drift.

However, one cannot rule out the possibility of the earlier presence of the 7R allele in Indian populations as they possess high frequencies of the 2R allele, and this, according to Ding et al. (2002), is a derivative of 7R allele. But as the 2R allele is supposed to have come into existence after the 7R allele, one cannot rule out the possibility that the populations of India had the 7R allele in the initial stages of their inhabitation of the Indian subcontinent, either by origin or by migration, and later there could have been a selective disadvantage of the 7R allele, as they opted for settled lifestyles as proposed by Chen et al. (1999). This hypothesis is to some extent supported by studies of Wang et al. (2004) who proposed that the 7R allele decreased after its migration into India. Furthermore, the presence of the 7R allele at negligible frequencies in some populations could possibly be because of recent gene flow from Eurasia where its frequency is reported to be high (Basu et al., 2003).

Conclusion

The presence of the 4R and 2R alleles in all the populations studied here at relatively high frequencies, and the presence of the 5R allele (Hominoid specific) in at least one population from each geographical region—i.e. Rajput and Brahmin from north India; Kabui, Meitei, Thadou, and Paite from north-east India; Oraon from east India; Kolam and Nayakpod from South India—suggests a common, primitive, autochthonous genetic substratum of India. Further, restriction of the 7R allele to a few north and north-east Indian populations indicates that the populations of these geographical regions have experienced a recent gene flow from the north, where the frequency of 7R is reported to be relatively high. Even though the present study results are based on a single gene, the highly polymorphic nature of the gene with repeat alleles ranging from 2R to 11R makes it a reliable marker for anthropological studies. However, more detailed studies, including the sequencing data of the repeat regions

of *DRD4* gene along with haplotype analysis, are likely to give additional insight into the peopling of India.

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