Original Article

Dopamine receptor D4 exon 3 variable number of tandem repeat polymorphism: Distribution in eastern Indian population

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BACKGROUND: A 48bp variable number of tandem repeat (VNTR), in the dopamine receptor D4 (*DRD4*), has been extensively studied in association with a variety of traits and neuropsychiatric disorders in different ethnic groups; the VNTR has been found to affect receptor binding.
AIMS: This investigation, for the first time, compared distribution of *DRD4* VNTR in different Indian populations from the eastern part of the country, belonging to Indo-Caucasoid and Indo-Mongoloid ethnicity.
MATERIALS AND METHODS: 852 individuals were recruited and divided into six population groups; Brahmin, Kayastha, Scheduled Caste, Mahishya, Muslim and Manipuri (Meitei). Allele and genotype frequencies were compared among groups as well as with data available for south-western Indian population.
RESULTS: A total of six alleles (2-7-repeats) were observed, of which the 4-repeat (4R) was most frequent. Gross genetic dissimilarities were noticed between the Indo-Caucasoid and Indo-Mongoloid ethnic groups. Muslim group lacked 5R and 7R, while Manipuri group exhibited a very high frequency of 2R. Populations from eastern India revealed lower 7R frequencies as compared to the south-western populations.
CONCLUSIONS: The *DRD4* VNTR has been reported to play important role in cognition and alleles with higher repeats have been found to be associated with novelty seeking and personality traits. The present comparative analysis of different eastern Indian population would be helpful in extending our knowledge on this particular *DRD4* variant. It will also be useful in understanding the behavioural differences between populations in the light of their genetic make up.

Key-words: *DRD4*, exon 3 variable number of tandem repeat, eastern-Indian population

Introduction

Dopamine receptor D4 (*DRD4*) is found primarily in the limbic system and frontal cortex of the brain and display an unusual number of genetic polymorphisms with wide ethnic diversity.[1,2] Amongst these, a 48bp variable number of tandem repeat (VNTR) in the exon 3, which encodes for a proline-rich domain in the third intra-cytoplasmic loop of the protein, has attracted geneticists worldwide.[3] Studies in different ethnic groups have revealed 2 to 11 repeats of this VNTR with numerous single nucleotide polymorphisms giving rise to 67 different haplotypes.[2-8]

The 7 repeat (7R) variant has been found to have altered pharmacological properties as compared to variants with 2 or 4 repeats (2R or 4R).[9] The 2R, 4R and 7R variants were also reported to exhibit difference in response to antipsychotic drugs such as clozapine and spiperone.[3] Hence, the VNTR has been a much-desired target for association studies on numerous neuropsychiatric disorders and neurobehavioral disorder, like attention deficit hyperactivity disorder (ADHD).[6,10-12] The VNTR has also been investigated for association with novelty seeking, macro-migratory habits, risk taking behavioural attributes and impairment in executive function tasks.[13-17]

Although much work has been carried out on this VNTR in different ethnic groups, till date to the best of our knowledge, only three detailed analyses are available on the Indian population.[5,6,18] Consequently, it was felt necessary to investigate intra-population diversity of this VNTR in India, a country well known for its incomparable geographical and ethnic diversity. We have evaluated allelic and genotypic frequencies of this VNTR in five population groups from West Bengal, eastern India, anthropologically belonging to Indo-Caucasoid ethnicity (N= 814) and one Indo-Mongoloid group from Manipur in northeast India (N= 38). Attempts were also made to compare the genotypic frequencies of these six groups

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with data available for five groups from the south-western part of India.[5]

Materials and Methods

Subjects

Out of 852 individuals included in the study, 814 subjects are from the state of West Bengal in eastern India (23°N, 87°E), belonging to the Indo-Caucasoid ethnic category and remaining 38 subjects are from Manipur, a northeast Indian state (23.83°N to 25.68°N, 93.03°E to 94.78°E), belonging to the Indo-Mongoloid ethnic category. All 852 individuals are divided into the following groups:

(a) Bengali Brahmin: A general Hindu caste, traditionally residing in the Bengal region of the Indian subcontinent (West Bengal and Bangladesh). Historically, they have been the standard bearers of Madhyadeshiya (the historic-cultural region of the upper Ganga-Yamuna doab) Indo-Aryan culture in Bengal (http://en.wikipedia.org/wiki/Bengali_Brahmin).

(b) Bengali Kayastha: Kayastha is a general Hindu caste, also believed to be the fifth 'varna' of the Hindu caste system. In the history of India, since pre-Mughal times, representatives of this caste were highly educated and engaged in administrative posts (http://www.kayastha.org/kayastha_samaj.html#origin). Bengali Kayasthas form one of the regional Kayastha communities residing in West Bengal and are also known as the 'writing caste' due to their good education, intellect and success in administrative occupations. Presently, they are involved in miscellaneous professions.

(c) Bengali Scheduled caste: Under-privileged Hindu castes residing in West Bengal, who have suffered from caste discrimination, but presently under government protection.

(d) Bengali Mahishya: According to historians, a child born of a 'Kshatriya' father and 'Vaisya' mother is called Mahishya. Presently, they are considered a general Hindu caste. Although they were traditionally labourers and tenant farmers, the Mahishyas have migrated to urbanized areas in and around Kolkata, the capital city of West Bengal (http://en.wikipedia.org/wiki/Mahishya#Origins), given up agriculture in large numbers and started to fluorish in the fields of engineering, skilled labour, and business.

(e) Bengali Muslim: A religio-ethnic group; many of them are religious converts from under-privileged Hindu castes and currently follows the Sunni sect of Islam.

(f) Manipuri population: 38 individuals from Manipur form the sixth population group and native inhabitants are of Indo-Mongoloid origin (Meitei).

Genotyping

Peripheral blood was collected from all the individuals after obtaining informed written consent. Genomic DNA was prepared from leukocytes using the protocol of Miller *et al*.[19] *DRD4* 48bp VNTR was analyzed using primer sets mentioned by Lichter *et al*. after minor modification.[2] PCR amplification was carried out in a final reaction volume of 20 µl containing 75-100 ng of genomic DNA, 10 mM Tris buffer with 50 mM KCl, 0.01% gelatin, 1.5 mM $MgCl_2$, 200 µM dNTP mix, 10 pmoles of each primer, 5% glycerol and 1U Taq polymerase (Bangalore Genei, India) using Perkin Elmer thermal cycler (Gene Amp #2400). The cycling conditions were (i) initial denaturation at 95°C for 5 min, (ii) 35 cycles of denaturation at 94°C for 40 sec, annealing at 65°C for 40 sec and extension at 72°C for 45 sec followed by (iii) a final extension at 72°C for 5 min. PCR products were separated by 12% polyacrylamide gel electrophoresis and visualized following ethidium bromide staining. For confirmation, sequence analysis was carried out in Applied Biosystems 3130 Genetic analyzer using Big Dye v 3.1 chemistry and by Sequencing Analysis Software, v 5.2. Nomenclature of alleles was based on number of repeat units. Human Ethical Committee of the Institute approved the study protocol.

Statistical analysis

The versatile program POPGEN32 (available at http://www.ualberta.ca/~fyeh/fyeh/) was used to determine (1) allele and (2) genotype frequencies.

Results

Allele frequency distribution

Table 1 shows allele frequencies of the VNTR in different groups. A total of six alleles were observed, 2R to 7R. The most frequent allele was 4R in all the groups;

Table 1: Allele frequencies of *DRD4* 48bp variable number of tandem repeat in different eastern Indian populations

Groups	2R	3R	4R	5R	6R	7R
Brahmin (n= 256)	0.07 (18)	0.02 (5)	0.82 (209)	0.008 (2)	0.051 (13)	0.035 (9)
Kayastha (n=442)	0.086 (38)	0.007 (3)	0.86 (379)	0.007 (3)	0.038 (17)	0.0045 (2)
Scheduled caste (n=424)	0.12 (50)	0.005 (2)	0.82 (346)	0.009 (4)	0.04 (17)	0.012 (5)
Mahishya (n=158)	0.13 (21)	0.006 (1)	0.78 (123)	0.013 (2)	0.06 (9)	0.013 (2)
Muslim (n=348)	0.095 (33)	0.003 (1)	0.84 (293)	-	0.06 (21)	-
Manipuri (Meitei) (n=76)	0.24 (18)	0.013 (1)	0.75 (57)	-	-	-

Numbers in parentheses denote observed number of each allele

frequency ranged between 0.75-0.86. The second most frequent allele was 2R. Frequency of 2R was highest in the Manipuri group (0.237) and lowest in Brahmins (0.07). 2R, 4R and 6R were present in all the Indo-Caucasoid groups, while 3R, 5R and 7R were rare. 5R and 7R were not detected in the Muslim and Manipuri populations. The Manipuri group also lacked the 6R allele.

Genotype frequency distribution

A total of 15 genotypes were observed [Table 2] of which the 4-4, followed by the 2-4 and 4-6 combinations were most frequent in all the groups. Kayastha group had the highest variation in genotypes, a total of ten combinations, including two rare and exclusive genotypes, 5-6 and 7-7. Brahmins exhibited two exclusive genotypes, 3-3 and 6-7 and shared another rare 2-7 genotype with the Kayastha group.

Comparison with south-western population groups

Distribution of allele frequencies in different population groups from eastern and south-western parts of India is depicted in Figure 1. It is evident that the 2R allele is exceptionally high in the Indo-Mongoloid Manipuri group, while frequency of 7R is higher in the south-western Marathas (0.11), Ezhavas (0.07) and Nairs (0.16) as compared to the eastern groups (0.01).

Discussion

Developments in the field of molecular genetics suggest that a thorough knowledge of genetic variations, particularly at functional domains, may endow us with a better understanding of individual differences in behavioural attributes, disease susceptibility and drug response.

Ghosh and Seshadri made a comparative analysis of allele and genotype distribution of the *DRD4* 48bp VNTR in five Indian populations from the south-western region of the country; namely, Konkanastha Brahmins, Marathas, Ezhavas, Nairs and Muslims.[5] Eight alleles

Table 2: Genotype frequencies of *DRD4* 48bp variable number of tandem repeat in different eastern Indian populations

Genotype	Brahmin (N=128)	Kayastha (N=22 1)	Scheduled caste (N=212)	Mahishya (N=79)	Muslim (N=174)	Manipur (N=38)
2-2	-	0.0045 (1)	0.014 (3)	0.013 (1)	0.017 (3)	0.105 (4)
2-3	-	-	-	0.013 (1)	-	0.026 (1)
2-4	0.13 (17)	0.14 (31)	0.193 (41)	0.2 (16)	0.14 (25)	0.237 (9)
2-5	-	0.0045 (1)	0.0047 (1)	0.013 (1)	-	-
2-6	-	0.018 (4)	0.0047 (1)	0.013 (1)	0.011 (2)	-
2-7	0.008 (1)	-	0.0047 (1)	-	-	-
3-3	0.008 (1)	-	-	-	-	-
3-4	0.023 (3)	0.0136 (3)	0.009 (2)	-	0.006 (1)	-
4-4	0.66 (84)	0.75 (166)	0.66 (140)	0.61 (48)	0.71 (124)	0.667 (24)
4-5	0.016 (2)	0.0045 (1)	0.014 (3)	0.013 (1)	-	-
4-6	0.094 (12)	0.054 (12)	0.075 (16)	0.1 (8)	0.11 (19)	-
4-7	0.055 (7)	-	0.019 (4)	0.025 (2)	-	-
5-6	-	0.0045 (1)	-	-	-	-
6-7	0.008 (1)	-	-	-	-	-
7-7	-	0.0045 (1)	-	-	-	-

N represents number of samples, Numbers in parentheses denote the observed number of each genotype

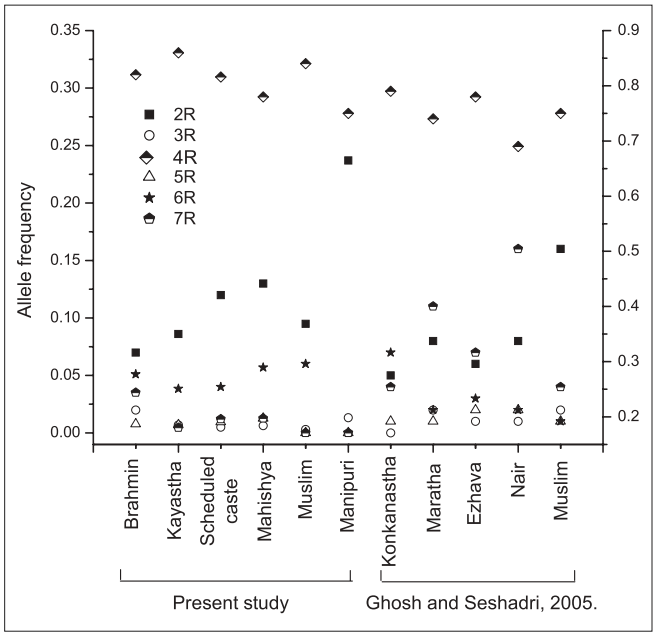

Figure 1: Allelic distribution of *DRD4* exon 3 48bp VNTR in 11 populations from India (present study, Ghosh and Seshadri, 2005). The left-hand Y-axis denotes position of 2R, 3R, 5R, 6R and 7R alleles, while the right-hand Y-axis is for the 4R allele

were reported, of which the 4R allele was predominant in all the groups, whereas a rare 9R allele was observed in the Maratha group only. They also observed presence of 8R allele for the first time in Asia.[18]

In the present investigation, not much difference in allele frequencies was noted within the Indo-Caucasoid group. Further, we did not notice any rare allele (8R, 9R or 10R) in these populations. In comparison to the findings of Ghosh and Seshadri, frequency of 7R allele in eastern Indian populations [Figure 1] was found to be low (0.01 in eastern groups, 0.08 in south-western groups). Combined frequency of the higher repeat alleles (5R-7R) was also low in the eastern populations (0.062) as compared to the south-western populations (0.124). The 7R allele was not at all detected in Muslim individuals from eastern India, whereas it was detected in Muslims from south-western part (0.04). Study samples further divided into male and female groups, to check if there is any contribution of sex, showed no significant differences in allele and genotype frequencies (data not shown).

A worldwide study by Chang *et al.* revealed presence of 7R (frequency = 0.11) in Kachari individuals from the northeast Indian state of Assam.[4] Chang *et al.* also reported presence of 7R in other Asian populations like Japanese (0.01), Malay (0.17) and Yakut (0.04). However, in the present investigation Manipuri group, from northeast India, revealed a total absence of 5R, 6R and 7R alleles [Table 1]. Interestingly, Leung *et al.* have also reported an absence of the 7R allele in Han Chinese children suffering from ADHD and observed an increased prevalence of the 2R allele in Han Chinese as compared to other ethnic groups.[20] Ethnically Han Chinese population is of Mongoloid origin. In the present investigation, for the Indo-Mongoloid Manipuri population, we have also noticed similar type of allelic distribution with an exceptionally high frequency of 2R (0.237) along with absence of the other higher repeat alleles [Figure 1]. Whether this resemblance could be attributed to a common Mongoloid ancestry of Han Chinese and Manipuri populations, is yet to be proved.

A very high frequency of 7R was reported in populations from Europe (0.21) and South America (0.63).[4] There have been a lot of speculations as to why the 7R has been positively selected during evolution in these populations in spite of its sub-optimal activity.[21] A meta-analysis on a common neurobehavioral disorder ADHD have shown significant association between DRD4 7R variant and the disorder.[22] Association between the higher repeat alleles (5-7R) and ADHD was also reported from India.[6] On the other hand, in Han Chinese subjects, a total absence of 7R along with high frequency of 2R were observed and the authors have proposed that since both 2R and 7R exhibit sub-optimal activity in comparison to that of 4R, the 2R probably have substituted 'role of 7R' in Asian populations.[20] Whether this raise the possibility that ADHD subjects have some evolutionary advantage, for example the disorder increases the reproductive vitality of affected individuals, is yet to be proved. It was hypothesized that faulty groups of genes, which cause multigenic disorders, can bear certain benefits for mankind and hence, can be positively selected during evolution.[23]

Though this particular polymorphism has been studied extensively worldwide in various ethnic groups, not much information was available from India. This is the first population-based investigation from eastern India comparing distribution of *DRD4* 48bp VNTR in subjects from different geographical locations. Whether transmission of these alleles "confers susceptibility to neuropsychiatric illnesses" or "may help in better survival" is still a matter of conjecture.[7] However, the present

comparative analysis on *DRD4* exon 3 VNTR in eleven population groups from India will assist in expanding our knowledge. Lower frequencies of the higher repeat alleles of the VNTR partly answer why neurobehavioral disorders like ADHD are not so common in the Indian population as compared to the European and American populations. Further explorations, at a much larger scale, would help us in understanding the implication of these observations.

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