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Original Research Article

Diversity and Relationship Between Iranian Ethnic Groups: Human Dopamine Transporter Gene (*DAT1*) VNTR Genotyping

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ABSTRACT The 40-bp VNTR polymorphism in the 3′ untranslated region of the human *DAT1* (dopamine transporter 1) was analyzed in the Iranian ethnic groups in order to examine the influence of geographical and linguistic affiliation on the genetic affinities among the Iranian population. A total of 449 subjects belonging to nine ethnic groups from the Iranian population were included in the study. The screening of 898 chromosomes showed five alleles (6, 7, 8, 9, 10, and 11), of which allele 10 revealed the highest frequency in most regions. Allele 8 was predominant in one ethnicity and occurred more frequently in the center of Iran. This study shows that the *DAT1* distribution in Iran has a different pattern from those in other studies, which can contribute to an understanding of differentiation and diversity of Iranian ethnic groups. This polymorphism could represent the genetic diversity among the various ethnic groups of Iran. Am. J. Hum. Biol. 19:821–826, 2007.

The *DAT1* gene coding the human dopamine transporter is located at chromosome 5 (5p15.3) (Vandenbergh et al., 1992). A 40-bp variable number of tandem repeats (VNTR) polymorphism resides in Exon 15 of this gene. Vandenberg et al. (1992) reported variability in the number of repeats within this region, ranging from 3 to 11 in white and black populations. Linkage and association between this VNTR and various neuropsychiatric disorders have been reported (DiMaio et al., 2003; Georgieva et al., 2002). The product of this gene transports released dopamine into the presynaptic terminals. This mechanism is based on regulation of the reuptake of released dopamine back into the presynaptic terminals after its synaptic release (Giros et al., 1992; Giros et al., 1993). Allele distribution of the *DAT1* VNTR has been studied in several groups, revealing a number of repeats ranging from 3 to 13 in a wide spectrum of population samples (Doucette-Stamm et al., 1995; Kang et al., 1999; Mitchell et al., 2000).

Assessment of the *DAT1* VNTR polymorphism is a convenient way of clarifying population relationships, and rare alleles at this locus may be particularly valuable in understanding the extent of genetic similarity among neighboring groups and situations where admixture is suspected (DiMaio et al. 2003; Georgieva et al., 2002). Distribution of the *DAT1* polymorphism was analyzed in the Iranian population in order to investigate the influence of geographical and linguistic affiliations on the genetic relationships between different ethnic groups in Iran.

MATERIALS AND METHODS

Samples

A total of 449 subjects were randomly selected from Iranian individuals throughout Iran. Sampling was performed in nine ethnic groups in Iran. This population included Pars (Persian), Azeri (Turk), Kurdish, Gilak, Mazandarani, Lur, Sistani and Balouchi, Bandari and Arab. These ethnic groups comprised the majority of the Iranian population (95–97%) that is distributed in Iran. The Pars ethnic group consisted of five clans or subgroups that included Shiraz, Esfahan, Kerman, Yazd, and Mashhad regions. Aryan conform the

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TABLE 1. Demographic and geographical data of the Iranian population

Region no.	Name of sampled region or city	Ethnic group	Geographical location	Language
1	Rasht	Gilaki	North	Gilaki
2	Babol	Mazandarni	North	Gilaki
3	Mashhad	Pars	Northeast	Parsi
4	Uromieh	Azeri–Turk	Northwest	Turki
5	Sanandajd	Kurdish	West	Kurdi
6	Khoramabad	Lur	West	Luri
7	Esfahan	Pars	Center	Parsi
8	Yazd	Pars	Center	Pasri
9	Kerman	Pars	South	Parsi
10	Shiraz	Pars	South	Parsi
11	Ahvaz	Arabian	Southwest	Arabian
12	Bandar e Abbas	Bandari	South	Parsi
13	Zahedan	Balouchi and Sisitani	East	Balouchi and Sistani

largest ethnicity in Iran. About 51% of Iran's current population is ethnically Persian; other estimations put this figure as high as 70%. Many other ethnic groups which exist in Iran are of non-Persian Aryan groups such as the Gilak and the Kurds, the Turkish Azerbaijanis, Turkmen, Arabs and other minorities.

Participating individuals were between 25 and 55 years of age. Peripheral blood samples were collected from individuals belonging to each ethnic group. Informed consent was obtained from donors who were checked and confirmed for the status of their healthy. Ethnic background was documented in each individual using a questionnaire (Table 1).

Genotyping

The VNTR locus was analyzed in a sample population of 449 randomly selected healthy Iranian individuals. Amplification was performed in a 25 μl reaction volume using a DNA Thermal Cycler as previously described by Vandenbergh (1992).

The amplification procedure included an initial denaturation step at 94°C for 3′, followed by 30 cycles of denaturation at 94°C for 1′, annealing at 65°C for 1′, extension at 72°C for 1′, and a final 5 min extension at 72°C. Amplified fragments were electrophoresed on a 6% polyacrylamide gel (PAGE) and visualized with silver nitrate staining. The fragment sizes were 320 bp (6-repeats), 360 bp (7-repeats), 400 bp (8-repeats), 440 bp (9-repeats), 480 bp (10-repeats), and 520 bp (11-repeats).

Data analysis

Statistical analysis was performed using the Genepop software. The Hardy Weinberg equilibrium was analyzed using the Markonov chain methods and Levene's correction. Inbreeding coefficient (Fis) was computed according to Weir and Cockerham (1984) and Robertson and Hill (1984).

RESULTS

A total of six *DAT1* alleles were observed: 6 (320 bp), 7 (360 bp), 8 (400 bp), 9 (440 bp), 10 (480 bp), and 11 (520 bp). Figure 1 shows that high frequencies have been observed for the *DAT1**10 among all populations, also Table 2 displays frequency of different *DAT1* alleles among some ethnic groups in comparison with all ethnic groups in Iran. Figure 2 shows the frequencies of the observed genotypes among the studied ethnicities, with genotype 9/10 representing the highest frequency, followed by high homozygosity of the 10/10 genotype. The *DAT1**8 allele was dominant among the Persian group who live in Mashhad in the Northeast of Iran (Table 3). This allele also occurred in two other ethnic groups of Persian descent, who belong to Yazd and Esfahan. *DAT1**7 allele has also been observed in the above three ethnic groups, as well as the Sistani and Balouchi group in the East with a 0.032 frequency. However, this allele has not been observed in other ethnic groups. High frequencies were observed for the two alleles *DAT1**10 and *9 in all groups except the Mashhad and Arab groups. In the latter, *DAT1**9 allele and *10 allele had the same frequency.

The population was in Hardy Weinberg equilibrium ($X2$ = infinity). Heterogeneity coefficient was measured for each ethnic

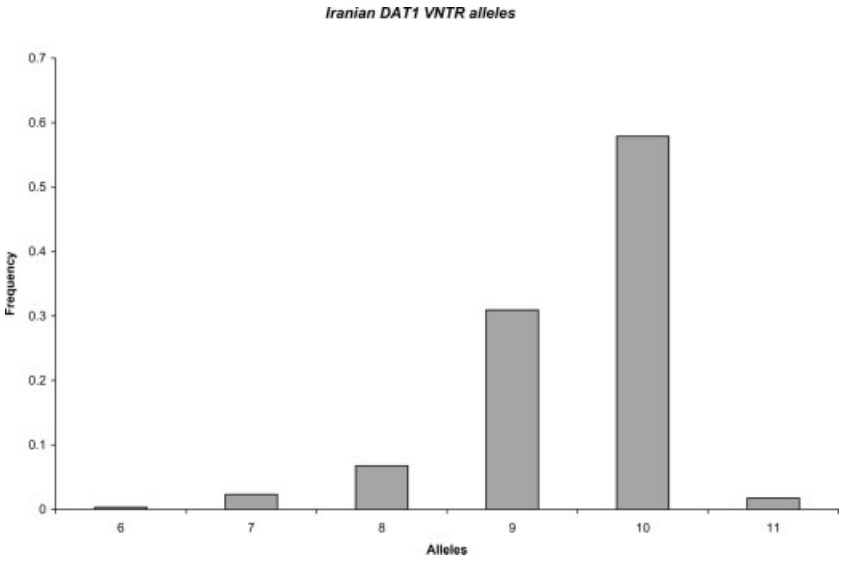

Fig. 1. Frequencies of the *DAT1* VNTR alleles among the Iranian population.

TABLE 2. Frequency of different DAT1 alleles among some ethnic groups

Ethnic groups	Allele 6	Allele 7	Allele 8	Allele 9	Allele 10	Allele 11	References
Iranian		0.023	0.067	0.310	0.531		Present study
Arab	0.000	0.000	0.015	0.050	0.471	0.015	
Gilak	0.000	0.000	0.000	0.338	0.647	0.015	
Lur	0.000	0.000	0.000	0.318	0562	0.030	
Mazandarani	0.000	0.000	0.000	0.370	0.630	0.000	
Esfahan	0.000	0.039	0.078	0.216	0.667	0.000	
Kerman	0.016	0.000	0.000	0.242	0.694	0.048	
Mashhad	0.000	0.106	0.652	0.121	0.106	0.015	
Yazd	0.000	0.100	0.133	0.350	0.400	0.017	
Shiraz	0.000	0.000	0.000	0.360	0.640	0.000	
Bandari	0.000	0.000	0.025	0.225	0.725	0.025	
Azari	0.000	0.000	0.000	0.294	0.676	0.029	
Kurd	0.000	0.000	0.020	0.347	0.592	0.041	
Sistani and Balouchi	0.032	0.032	0.000	0.306	0.629	0.000	
White	–	–	–	0.35	0.70	–	Vandenberg et al. (1992).
Black	–	–	–	0.24	0.70	–	Vandenberg et al. (1992).
Mongolian	–	0.026	–	0.05	0.90	0.013	Nakatome et al., 1995
Chinese	–	–	0.015	0.06	0.89	0.020	Nakatome et al., 1995
Japanese	0.02	0.06	–	0.06	0.91	0.010	Nakatome et al., 1995
Chilean	–	–	–	0.23	0.74	–	Vieyra et al., 2003
Brazilian	–	0.0069	.0075	0.27	0.70		Silva et al., 2005
Omani	0.018	0.009	0.005	0.332	0.609	0.018	Simsek et al., 2005
Siberian	0.05	0.05	–	–	–	–	Mitchell et al., 2000
Russian	–	0.1	–	0.215	0.784	0.0	Mitchell et al., 2000
	–	–					Mercier et al., 1999
European			0.010	0.210	0.770	0.010	
French			0.0064	0.33	0.65	–	
Italian				0.33	–	–	
Greek			–		0.52	–	

group by the Fis using two methods of Weir and Cockerham (1984) and Robertson and Hill (1984). In general, the "Fis" parameter was positive for the whole population, but negative results were observed for some ethnic groups where inbreeding was highly common.

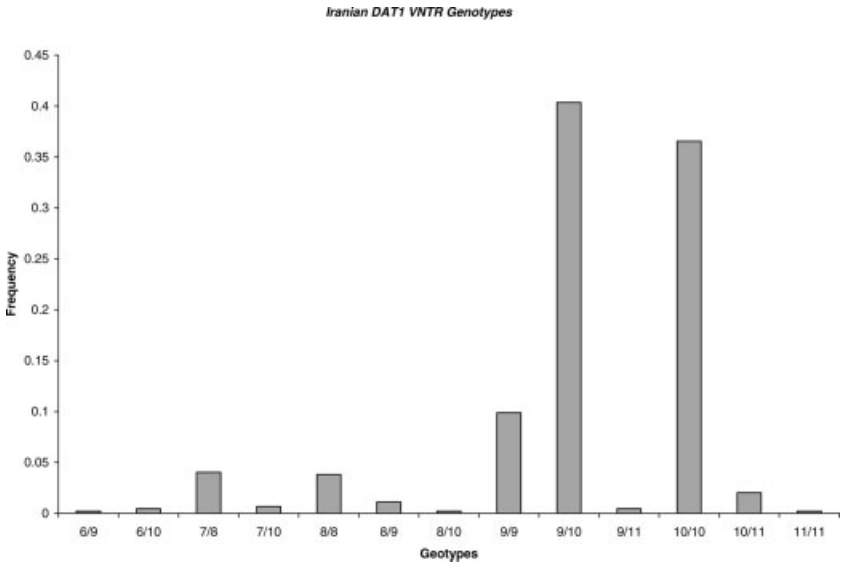

Fig. 2. Genotype frequencies of the Iranian population.

TABLE 3. Frequency of different DAT1 allele's genotyping among Iranian ethnic groups

	Genotype										
Ethnicities	6/10	7/8	7/10	8/8	8/9	8/10	9/9	9/10	9/11	10/10	10/11
Arab					0.029		0.176	**0.617**	0.029	0.147	0.029
Bandari						0.016	0.1	0.333		**0.533**	0.05
Lur							0.030	**0.575**		0.333	0.060
Gilaki							0.117	0.411		**0.441**	
Mazandarani							0.217	0.304		**0.478**	
Easfahan		0.098	0.019				0.039	0.352		**0.470**	
Kerman	0.032						0.064	0.3225	0.032	**0.516**	0.032
Mashhad		0.225		**0.516**			0.064	0.032		0.064	0.033
Yazd		0.2		0.033			0.066	**0.566**		0.1	0.033
Shiraz							0.129	**0.451**		0.419	
Kurdish					0.040		0.102	**0.448**	0.020	0.326	0.061
Azeri							0.088	0.411		**0.441**	0.058
Sistani Va Balouchi							0.129	0.322		**0.419**	

DISCUSSION

The most common allele (480 bp) was observed at a frequency of 0.5313, followed by the 9-repeats allele (440 bp). Results showed similar frequencies of 0.3105 for the 9-repeat allele in comparison with the white (0.35) and black (0.24) populations reported by Vandenberg et al. (1992). The frequency of this allele in the Iranian population, however, was significantly different from frequencies in the Mongolian, Chinese, and Japanese populations displaying values of 0.05 and 0.06, respectively (Nakatome et al. 1995; Nakatome et al. 1996).

The frequency of the 10-repeat allele in the Iranian population (0.5313) was substantially lower than that in the Mongolian (0.90), Chinese and Japanese (0.89–0.91) populations, it was also lower than that in the white and Black populations (0.7) (Vandenbergh et al. 1992). Also, Vieyra et al.(2003) reported *DAT1**10 with a frequency of 74%, followed by *DAT1**9, with a frequency of 23% in the Chilean population similar to those in the European populations. The two alleles 9 and 10 repeats had frequencies of ~0.27 and 0.70 among the São Paulo people of Brazil (Silva et al., 2005; Vieyra et al., 2003). In France,

these two alleles had frequencies of 0.65 and 0.33, respectively (Mercier et al., 1999).

Homozygosity for the 10-repeat allele (480 bp) was 0.3188 in the Iranian subjects (Fig. 2). Vandenberg et al. (1992) and Nakatome et al. (1996) reported that heterozygosity was 45% in whites, 42% in blacks, 14% in Mongolians, 12% in the Chinese, and 13% in the Japanese population, whereas heterogeneity was estimated to be 49% in the Iranian population. The *DAT1**7 allele has been observed in four regions (Esfahan, Yazd, Sistan and Balouchestan and Mashhad) with a frequency of 0.023. The frequency of the *DAT1**7 in Iran was higher than that reported for the Omani people (0.018) and Brazilian (0.0069) populations and was lower than that in the Japanese population (0.20) (Muramatsu et al., 1995; Silva et al., 2005; Simsek et al., 2005). Mitchell et al. (2000) reported the presence of a relatively high frequency of 0.05 for the rare *DAT1* alleles (*DAT1**7 and *6) among all Siberian groups, suggesting a close similarity to other North Asian groups, especially the Mongolians. The *DAT1**8 allele was the dominant allele among one ethnic group (Mashhad), with relatively higher frequencies in two other groups (Yazd and Esfahan). This allele had a frequency of 0.067, which was higher than that in the Brazilian (0.0075) and French populations (0.0064) (Mercier et al., 1999; Silva et al., 2005).

The *DAT1**11 allele has been observed in 10 out of the 13 Iranian regions, indicating similar frequencies, while the *DAT1**6 allele has seen in other two regions (Kerman, Sistan, and Balouchestan), which are neighboring ethnic groups.

Heterozygosity at the *DAT1* locus in all Asian populations was significantly lower than that in the Iranian population. In fact, the frequency of heterozygosity in Iran was higher than that in the white and black populations, indicating high diversity among Iran's population.

There is as yet no clear definition of the ethnicities in Iran. However, there are a number of national and ethnic groups living in various parts of Iran, whose background and anthropological origins have been the subject of numerous researches; however, investigators are divided concerning many of the questions posed.

The Azeri or Turkish group is a mixed population of Oghuz origin. Others believe that this ethnic Azeri group has close affinities with the Aryan race, but their language has been changed, through invasions by non-Aryan groups (such as the Mongols), who imposed their rule and culture on them. In fact, they show identical patterns of *DAT1* VNTR frequencies with other neighboring ethnic groups.

Arabs from Ahvaz and Bandari regions have been identified as a mixed population of Iran's resident people and those from the neighboring Arab regions. Arab groups also show some relationship with other Iranian groups, especially with the Mazandaran and Shiraz region showing frequencies of $\sim$0.23 and 018, respectively. These affinities are perhaps due to the establishment of Arabic clans in the Mazandaran area (referred to as Tabarestan in the past) in 800 A.C. In the case of Shiraz, previous Arab migration to this city as the nearest main city is another explanation for such relationship, albeit at low level. The *DAT1**10 is the dominant allele among all populations except those from Ahvaz and Mashhad that show high frequencies for alleles *DAT1**9 and *8, respectively. The *DAT1**7 allele has been observed in four regions, which are separated from other areas by the Alborz and Zagros Mountains, and today classify the Persian ethnicities. On the other hand, people who live in the Northeast with a high *DAT1**8 allele frequency have distinct demographic and linguistic characters compared to the neighboring areas. In fact, their language is a form of western middle Persian language named "Dari". Today Dari is a dialect of the Zoroastrians who live in Yazd and Kerman and are often called Yazdi. The Mashhad group has showed no relationship with other groups (0.0000 in all cases). Based on these data, the above two possible hypotheses may be related to the limitation in sampling with respect to quantities and qualities and ancestral origin of Mashhad ethnicities. Their ancestors belong to the *Merv* area, located beyond the (Oxus) river and central Asia. But no accurate information is available regarding people of *Merv* and central Asia. Maybe they migrated separately from the other Aryan races, stemming from a different progenitor.

A rare allele such as he *DAT1**7 was observed by Mitchell et al. (2000) in many of the Siberian populations (Mitchell et al., 2000) This rare allele has also been observed in the African, American, and Omani populations (Simsek et al., 2005; Vandenberg et al., 1992), the presence or absence of some rare alleles at this locus has been particularly useful in com-

paring populations. Hence, it is suggested that a close genetic relationship exists between central and northeast of Iran. This may be the result of invasion and migration from the outside to the Iranian interior, via the East. Persian ethnic groups (comprising five clans) displayed significant differences in their *DAT1* VNTR alleles. It demonstrates that people who live in the two southern regions (Shiraz and Kerman) differ from other Pars groups. They display alleles which have more similarity with samples from the non-Persian ethnic groups of the southern and southwestern regions, especially the Bandari group. This similarity is reflected in the demographical and linguistic characters, such as accent and pronunciation among these people. It is strongly suggested that they have been racially mixed with their neighbors in the south.

Another two groups closely related to the Persians, both ethnically and linguistically, are the Kurds and the Lurs in the west. Distribution of *DAT1* VNTR is identical in both groups, as well as the two other ethnic groups, the Gilak and Mazandarani who also showed similar patterns. According to our data, the *DAT1* 3′ VNTR is more polymorphic in the Iranian population in comparison with other world populations. The frequencies of the observed alleles at this study are also different in comparison with the frequencies observed in other populations. These findings reflect high heterogeneity among different ethnicities in Iran.

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