Variation of DAT1 VNTR Alleles and Genotypes Among Old Ethnic Groups in Mesopotamia to the Oxus Region

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Abstract Variation of a VNTR in the *DAT1* gene in seven ethnic groups of the Middle East was used to infer the history and affinities of these groups. The populations consisted of Assyrian, Jewish, Zoroastrian, Armenian, Turkmen, and Arab peoples of Iran, Iraq, and Kuwait. Three hundred forty subjects from these seven ethnic groups were screened for *DAT1*. *DAT1* VNTR genotyping showed 3, 6, 7, 8, 9, 10, 11, and 12 alleles in the samples. Analysis of these data revealed differentiation and relationship among the populations. In this region, which covers an area of 2–2.5 million km^2, the influence of geography and especially of linguistic characteristics has had potentially major effects on differentiation. Religion also has played a major role in imposing restrictions on some ethnic groups, who as a consequence have maintained their community. Overall, these ethnic groups showed greater heterogeneity compared to other populations.

The modern Mesopotamia (entire Tigris-Euphrates valley in southwest Asia) to Oxus (Amu Dar'ya) area includes many lands that are inhabited by miscellaneous ethnic groups with civilizations dating back 5,000–6,000 years. This region provides a unique opportunity to investigate the influence of geography, language, and possibly religion on the genetic structure of a human population. The studied area includes Iran, Iraq, and Kuwait, which can also act as representatives of other neighboring countries, such as Armenia, Turkmenistan, and Israel.

The *DAT1* VNTR is a minisatellite used to calculate differentiation and affinity from a population genetic aspect. This marker, which has a 40-bp variable

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number of tandem repeats (VNTR), exists in the noncoding region of exon 15 of the *DAT1* gene. Reported variability in the number of alleles at this locus ranges from 3 to 13 in white and black populations (Mitchell et al. 2000; Vandenbergh et al. 1992).

As to the role of *DAT1*, assessment of the *DAT1* VNTR polymorphism is a convenient way to clarify population relationships, and rare alleles at this locus may be particularly valuable in understanding the extent of genetic similarity among neighboring groups and in situations where admixture is suspected (DiMaio et al. 2003; Georgieva et al. 2002). The observed pattern of genetic variation could be a tool to assess linguistic-geographic barriers that increased genetic changes. On the other hand, probably genetic and linguistic diversity have often been shaped by demographic changes. Hence the *DAT1* VNTR was analyzed in the populations of this study because of the populations' high variation, different origins, languages, and demographic characters, and history going as far back as 6,000 years. Restrictions on some populations because of limitations imposed by their religions were also of special interest to this study.

Materials and Methods

Samples. Three hundred forty subjects were randomly selected from seven ethnic groups residing in Iran, Iraq, and Kuwait. These populations included Assyrians, Jews, Zoroastrians, Armenians, and Turkmen (representing ethnic groups from Iran, restricted by rules of their religion), and the Iraqi and Kuwaiti populations from Iraq and Kuwait. The first five ethnic groups are considered minority ethnic groups in Iran. These five ethnic groups make up less than 1% of the Iranian population. Table 1 shows more detailed information on each ethnic group.

The Assyrians are the main residents of present-day Mesopotamia. They speak Aramaic. Their religion, cultural, and linguistic characteristics are completely different from the Arabs, Kurdish, Persian, and Turkish ethnic groups. Currently, they are dispersed in Iraq, Iran, and North America. In Iran they are concentrated in the two cities of Tehran and Uremia, where sampling from this ethnic group was performed.

Jews who live in Iran are referred to as Persian Jews or Parsim; their presence in Iran dates back to approximately 600 B.C. As the second main community of Jews in the Middle East, this group speaks Persian as their maternal language, and their dispersion is limited to the main cities of Iran, such as Tehran in the north and Yazd and Isfahan in the center. Samples representative of this community were mostly taken from Tehran.

The Zoroastrians are believed to be one of the first residents of Iran whose religion and philosophy are based on the teachings ascribed to the prophet Zoroaster. They have kept their beliefs to this day, even after the Arab invasion of the Iran plateau. However, many Zoroastrians migrated to the Indian subcontinent as a result of pressure imposed on them by Islamic rulers. Sample collection from this

Table 1. Demographic and Geographic Data for the Studied Populations

Ethnic Group	*Religion*	*Language*[a]	*Main Geographic Distribution*	*Sampled Region (Country and Cities)*	*Population Size in Iran*	*Reference*
Assyrians	Christianity	Aramaic, Persian	Assyrian Diaspora[b]	Iran (Uremia, Tehran)	10,000–15,000	Eden (1979); Sanasarian (2000)
Jews	Judaism	Hebrew, Persian	Jewish Diaspora[b]	Iran (Tehran)	11,000–35,000	Sanasarian (2000); Shalom (2005)
Zoroastrians	Zoroastrianism	Old Persian	Zoroastrian Diaspora[c]	Iran (Yazd)	<20,000	Amighi (1991); Sanasarian (2000)
Armenian	Christianity	Armenian, Persian	Armenia	Iran (Tehran)	300,000–400,000	Bournoutian (2002)
Turkmen	Islam (Sunni)	Turkmen, Persian	Turkmenistan[d]	Iran (Bandar Turkmen)	1,500,000–2,000,000	Sirkeci (2005); Stansfield (2007)
Iraqis	Islam (Shiite)	Arabic	Iraq	Iraq (Karbala, Baghdad)	–	
Kuwaitis	Islam (Sunni)	Arabic	Kuwait	Kuwait (Kuwait City)	–	

a. Persian is used as a second language for Assyrians, Jews, Armenians, and Turkmen. In addition, nowadays, most Assyrians, Jews, Armenians, and Zoroastrians also speak Arabic, Turkish, and/or Kurdish, or the language(s) used in the countries where they live.

b. Currently the distribution of Jews is in many countries, such as North America, Russia, Western Europe, and the Middle East.

c. India, Pakistan, Iran, and North America are the main regions of Zoroastrian settlement.

d. Turkmen distribution is in Turkmenistan, Iran, Iraq, and Afghanistan.

community was limited to the city of Yazd, where the majority of Zoroastrians are concentrated.

The Armenians are a nation and an ethnic group originating from the Caucasus and eastern Anatolia, where a large concentration of this community has remained, especially in Armenia. However, many of them are also scattered elsewhere throughout the world, such as Iran, where the term *Parskahay* is used to refer to the Armenians who live in this country. Today the Armenians are Iran's largest religious minority. Tehran was the main area where samples for this ethnic group were collected.

The Turkmen are a Turkic people found primarily in the Central Asian states of Turkmenistan and Afghanistan and in northeastern Iran. They speak the Turkmen language, which is classified as a member of the Western Oghuz branch of the Turkic language family, which also includes Turkic Azerbaijan. This ethnic group is mostly limited to the northern province of Iran and neighboring Turkmenistan.

The Arabs are a Semitic people who originated in the Arabian Peninsula. The term *Arab* is also applied to those whose primary language is Arabic and who reside outside the Arabian peninsula in neighboring countries and North Africa.

Genotyping. The VNTR locus was analyzed in a sample population of 340 randomly selected healthy individuals. Amplification was performed in a 25-µl reaction volume using a DNA Thermal Cycler, as previously described by Vandenbergh (1992). The fragment sizes were 320 bp (6 repeats), 360 bp (7 repeats), 400 bp (8 repeats), 440 bp (9 repeats), 480 bp (10 repeats), 520 bp (11 repeats), and 560 bp (12 repeats) (Vandenbergh et al. 1992).

Statistical analysis was performed using the GenePop software. Hardy-Weinberg equilibrium was analyzed using Markov chain methods and Levene's correction. The inbreeding coefficient (F_{IS}) was computed according to Weir and Cockerham (1984) and Robertson and Hill (1984).

Results

Eight *DAT1* alleles were observed: **3* (200 bp), **6* (320 bp), **7* (360 bp), **8* (400 bp), **9* (440 bp), **10* (480 bp), **11* (520 bp), and **12* (360 bp). Table 2 shows that high frequencies have been observed for *DAT1*10* among all populations. Only *DAT1*10* and *DAT1*9* had similar frequencies in the Armenians population. The *DAT1*11* allele was observed at a relatively high frequency (0.2) among the Assyrian group, whereas the Kuwaiti and Turkmen groups showed frequencies of only 0.024 and 0.017, respectively, for this allele. Also, the *DAT1*12* allele had a frequency of 0.033 in the Assyrian group; however, this allele was not observed in other ethnic groups. The *DAT1*8* allele showed a high frequency of 0.092 in Zoroastrians compared to the frequencies observed in the Kuwaiti (0.018) and Turkmen (0.017) populations. Also, the *DAT1*3* allele, with a frequency of 0.012, was observed in the Kuwaiti group, and other rare alleles, such as *DAT1*6* and

Table 2. Frequencies of the *DAT1* VNTR Alleles Among the Studied Populations and in Comparison Populations

Ethnic	*Allele*								
Group	**3*	**6*	**7*	**8*	**9*	**10*	**11*	**12*	*Reference*
Iraqis					0.228	0.771			Present study
Kuwaitis	0.012			0.018	0.323	0.622	0.024		Present study
Assyrians					0.244	0.511	0.200	0.033	Present study
Zoroastrians		0.026	0.013	0.092	0.316	0.553			Present study
Jews					0.462	0.537			Present study
Turkmen			0.012	0.012	0.250	0.700	0.012		Present study
Armenians					0.500	0.500			Present study
Iranians		0.003	0.023	0.068	0.309	0.579	0.018		Banoei et al. (2007)
Whites					0.350	0.700			Vandenbergh et al. (1992)
Blacks					0.240	0.700			Vandenbergh et al. (1992)
Mongolians			0.026		0.050	0.900	0.013		Nakatome et al. (1995)
Chinese				0.015	0.060	0.890	0.020		Nakatome et al. (1995)
Japanese		0.020	0.060		0.060	0.910	0.010		Nakatome et al. (1995)
Chileans					0.230	0.740			Vieyra et al. (2003)
Brazilians			0.0069	0.0075	0.270	0.700			Silva et al. (2005)
Omanis		0.018	0.009	0.005	0.332	0.609	0.018		Simsek et al. (2005)
Siberians		0.050	0.050						Mitchell et al. (2000)
Russians			0.100		0.215	0.784			Mitchell et al. (2000)
Europeans				0.010	0.210	0.770	0.010		Mercier et al. (1999)
French				0.0064	0.330	0.650			Mercier et al. (1999)
Italians					0.330				Mercier et al. (1999)
Greeks						0.520			Mercier et al. (1999)

*DAT1*7*, were seen in the Zoroastrian and Turkmen populations (Table 2). Table 3 shows the frequency of different *DAT1* genotypes among studied ethnic groups.

All seven populations were in Hardy-Weinberg equilibrium ($\chi^2 = \text{infinity}$). The heterogeneity coefficient was measured for each ethnic group by the F_{IS} using the methods of Weir and Cockerham (1984) and Robertson and Hill (1984). In general, the F_{IS} parameter was positive for the whole population, but negative results were observed for some ethnic groups where inbreeding was highly common.

Discussion

Table 2 shows frequencies of the different alleles for the studied ethnic groups and for other world populations. The results show similar frequencies of the 9-repeat allele for the studied populations compared to white (0.35) and black (0.24) populations (Vandenbergh et al. 1992). The frequency of this allele, however, is significantly different from the frequencies in the Mongolian, Chinese, and Japanese populations (Nakatome et al. 1995, 1996). The Zoroastrians showed a frequency of *DAT1*9* identical to those of other Muslim Iranian populations (Banoei et al. 2007). Regarding a common ancestry, the Zoroastrians have always

Table 3. Frequency of Different *DAT1* Genotypes Among the Studied Ethnic Groups

	Ethnic Group							
Genotype	*Iraqis (n = 46)*	*Kuwaitis (n = 82)*	*Assyrians (n = 43)*	*Zoroastrians (n = 39)*	*Jews (n = 40)*	*Armenians (n = 50)*	*Turkmen (n = 40)*	*Iranians (n = 449)*
**3/*3*		0.01						
**6/*6*				0.02				
**7/*7*								
**7/*9*				0.02				
**7/*10*							0.03	<0.01
**8/*8*		0.01		0.05				0.04
**8/*9*				0.02				0.01
**8/*10*				0.05				<0.01
**8/*11*							0.04	
**9/*9*	0.10	0.15	0.13	0.05	0.10	0.06	0.04	0.10
**9/*10*	0.24	0.31	0.16	0.10	0.72	0.88	0.42	0.40
**9/*11*		0.01	0.06					
**10/*10*	0.65	0.45	0.39	0.31	0.17	0.06	0.47	0.36
**10/*11*		0.01	0.06					
**11/*11*		0.01	0.13					
**12/*12*			0.02					

been considered a minority ethnic group among the present Iranian population and have maintained their community on the basis of religious beliefs. Other *DAT1* alleles in the Zoroastrians showed more similarity to ethnic groups who reside in eastern and central Iran and who mostly match the Persian-Aryan group.

Homozygosity for the 10-repeat allele (480 bp) was significantly different among the Armenians and Iranian Jews compared with other ethnic groups. Cavalli-Sforza et al. (1994) noted the distance of the Armenian genetic background from other populations; they also reported that the Jewish community has maintained considerable genetic similarity among themselves and with people from the Middle East, with whom they share a common ancestry. Although all Jewish people reside in different countries and are isolated from one another, most Jewish populations are not significantly different from one another at the genetic level (Carmelli and Cavalli-Sforza 1979).

Allele distribution and genotypes were highly similar between the Armenians and Iranian Jews (see Table 3). This could be an indication of a relationship between the two groups. It is also clear from history that these two groups had close communication over the centuries. The Khazar Empire (located northeast of the Black Sea, near present-day Armenia), which had adopted Judaism in the last quarter of the first millennium C.E., was an important constituent of the nascent Ashkenazi community, the most renowned Jewish branch in Europe (Behar et al. 2003). Also, historical evidence shows an Armenian kingdom founded by displaced Armenians along the Mediterranean coast between modern Turkey and Syria. *HLA* haplotype analysis implies a relationship between the Armenians

and the people of this region, who belong to the older Mediterranean substratum (Arnaiz-Villena et al. 2002).

HLA typing analysis has also shown that Turks, Kurds, and Armenians are close genetically; these three groups seem to have been living in the area for many millennia (Arnaiz-Villena et al. 2001). However, the *DAT1* allele and genotype distributions displayed different patterns among the Armenian, Azeri (Turk), and Kurdish ethnic groups in Iran (Banoei et al. 2007). Also, Arnaiz-Villena et al. (2002) reported that the present-day Turkish *HLA* profile reflects an older Mediterranean substratum that is not that different from the profile of the Persian Jews (Arnaiz-Villena et al. 2002). We have observed in this study that the *DAT1* genotypes among the Jewish population are less similar to those of other ethnic groups in the region. Based on earlier studies, using classical genetic methods, Carmelli and Cavalli-Sforza (1979) came to the conclusion "that Jews have maintained considerable genetic similarity among themselves and with people from the Middle East, with whom they share a common origin." In fact, new molecular studies have confirmed this conclusion (Hammer et al. 2000).

*DAT1*12*, which has a frequency of 0.033 in the Assyrians, was never seen in the other six ethnic groups or in the nine Iranian ethnic groups studied by Banoei et al. (2007). The relationship probability was lowest between Assyrians and other communities. Endogamy was found to be high for this population through determination of the heterogeneity coefficient (+0.6867). Our study supports earlier findings indicating the relatively closed nature of the Assyrian community as a whole, which as a result of their religious and cultural traditions, have had little intermixture with other populations (Elias 2000).

The frequency of the *DAT1*8* allele in the Zoroastrian ethnic group (0.092) was higher than the frequencies for the other ethnic groups studied here and higher than frequencies reported for others populations (see Table 2) (Muramatsu and Higuchi 1995; Silva et al. 2005; Simsek et al. 2005). The frequency is matched with values found among the ethnic groups in central Iran, which is nowadays considered the main residence of the Zoroastrians. Thus a similar genetic background is proposed for the Zoroastrian community and their compatriots who have a different religion. Despite the small number of Zoroastrians in Pakistan, who have similarities to their counterparts in Iran, the amount of diversity in this population is high (Mohyuddin et al. 2003). Mohyuddin and Mehdi (2005), performing an *HLA* analysis, showed that the Parsis (descendants of Zoroastrians from Iran) of Pakistan and India descended from the same stock and may have the closest ancestry with Jewish and Italian populations. Our data show high genetic differentiation for these population pair groups (relationship probability was estimated to be 0.0097 for the Zoroastrian and Jewish groups).

Although the Turkmen's religion has practically been used as a deterrent to mixing with other ethnic groups in Iran, similarity in allele variation between Turkmen and the Iranian population is evidence that genetic admixture has occurred throughout the centuries with neighboring ethnic groups (Banoei et al. 2007). The results also show a high relationship probability between the Turkmen and the Iraqi

population, which could explain the reason for genetic admixture between them; the Iraqi population consists of a community of approximately 1.5 to 3 million (11–13%) Turkmen (Sirkeci 2005).

DAT1 VNTR alleles were similar among two Arab groups (the Kuwaiti and the Iraqi populations). Migration to Kuwait has repeatedly been occurring from different neighboring nations over the past decades, which is reflected in the variation of alleles and genotypes in this ethnic group. The *DAT1*3* allele has been observed at a frequency of 0.012 only in the Kuwaiti population. In addition, we have observed a relative relationship between the Kuwaiti and Turkmen ethnic groups.

The patterns of distribution and genotypes for the *DAT1* VNTR in Kuwaitis and Iraqis were significantly different from the patterns in the Arab ethnic group in Iran, even though they all apparently belong to the same Arab ethnicity (see Table 3). Therefore a separate origin or genetic background of intermixture for the Arab community in Iran is proposed.

In summary, regarding the complicated history of the Middle East and wide-scale immigration, many believe that the heterogeneity of the Middle East area is due to the intermixture of populations. In contrast, this study and similar results from other researchers have shown that the multiplicity of religions, languages, customs, and creeds related to ethnicity have been responsible for the heterogeneity among the populations.

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