Brief Communication

DAT1 VNTR Polymorphisms in a European and an African Population: Identification of a New Allele

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Abstract Polymorphism frequencies of the dopamine transporter gene (*DAT1*) hypervariable region have been analyzed in a sample of Italian and Ivory Coast individuals. The 3′ untranslated region (UTR) of *DAT1* includes a variable number of tandem repeats (VNTR) of a 40-bp monomer, ranging from 3 to 13 repeats in Caucasian and African populations. In our sample we found alleles with 3 to 16 repeats, and the most common alleles were the 10-repeat (*DAT1*10*) and the 9-repeat (*DAT1*9*) alleles. We also found two rare alleles in the Italian population and four in the Ivory Coast population. For the first time the new allele *DAT1*16* is described in the Ivorians. The Ivory Coast population was not in Hardy-Weinberg equilibrium for the *DAT1* locus because of a deficit of heterozygote genotypes. The observed heterozygosity of the Ivorian population was half that of the Italians. The lower observed heterozygosity and deviation from Hardy-Weinberg equilibrium could be the result of microevolutionary trends, such as genetic drift and/or inbreeding, acting on the relatively small and isolated population sampled for this study, although some sort of selective pressures acting against the shorter alleles cannot be excluded. This evidence, in association with the reduced polymorphism shown by the *DAT1* VNTR compared to other VNTRs, seems to indicate that the *DAT1* locus may be under some selective pressure.

The human dopamine transporter gene (*DAT1*), located on chromosome 5p15.3 (Vandenbergh et al. 1992), plays a central role in the regulation of dopamine levels and neurotransmission and has been suggested to play a role in many neurological diseases and psychiatric disorders (Greenwood et al. 2006; Li et al. 2006; Ohadi et al. 2007). *DAT1* contains a variable number of tandem repeats (VNTR) of a 40-bp monomer in its 3′ untranslated region (UTR), ranging from 3 (about 200 bp) to 13 (about 600 bp) copies of the core sequence. The origin of this polymorphic

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variation may be relatively recent, as a VNTR homologue has been observed in other primates but not in lower mammals (Van Ness et al. 2005).

The VNTR polymorphism of *DAT1* has been analyzed in several human populations (Vandenbergh et al. 1992; Sano et al. 1993; Li et al. 1994; Cook et al. 1995; Doucette-Stamm et al. 1995; Nakatome et al. 1995; Persico et al. 1996; Gelernter et al. 1998; Kang et al. 1999; Mitchell et al. 2000; Galeyeva et al. 2001; Simsek et al. 2005). Results showed a limited degree of polymorphism (usually three or four alleles) in most human groups. Alleles *DAT1*9* and *DAT1*10*, containing 9 and 10 repeats, respectively, were found to be common, whereas other variants were much less frequent and were geographically restricted (Mitchell et al. 2000; Simsek et al. 2005). Various studies have demonstrated the presence of ethnic differences in the *DAT1* 3′ VNTR polymorphism frequency distribution (Kang et al. 1999; Mitchell et al. 2000; Simsek et al. 2005). For example, the rare allele *DAT1*5* was detected only in an African American population (Persico et al. 1996) with a very low frequency (1%), and another rare allele, *DAT1*12*, was observed in populations of African ancestry only (Kang et al. 1999). Recent investigations have revealed that this variation is not neutral but is most probably a functional polymorphism influencing the expression of the gene. The *DAT1*10* allele is associated with an increase in gene transcription, especially in **10/*10* homozygote genotypes (Michelhaugh et al. 2001; Mill et al. 2002).

Considering the implication of some *DAT1* polymorphisms in many neurological diseases (Ohadi et al. 2007; Greenwood et al. 2006; Li et al. 2006; Kang et al. 1999), such variation cannot be used in anthropological studies as a simple genetic marker subjected to stochastic processes. Conversely, knowledge of the frequency distribution of these alleles in different populations may help to elucidate the evolutionary pressures that shaped the variability among different human populations. In the present study we investigated the genetic variability at the *DAT1* locus in an African population from the Ivory Coast, for which no data are available in literature, and in an Italian sample for which only one study has been published. We also compared our data with data available from other populations sampled worldwide.

Materials and Methods

Samples and DNA Extraction. A sample of 277 individuals was analyzed: 204 individuals from Piedmont, a region in northwest Italy, and 73 individuals from Ouangolodougou, a rural village located in northern Ivory Coast. Informed consent was obtained from all individuals participating in the study. Peripheral blood samples (5–10 ml obtained through venipuncture) were collected in heparinized vacutainers and stored at −20°C. DNA extractions were performed using the Chelex protocol, as described by Walsh et al. (1991).

PCR Methods. Analysis of *DAT1* polymorphisms was performed by PCR amplifications using the following primers (Vandenbergh et al. 1992):

5′-TGTGGTGTAGGGAACGGCCTGAG-3′ and 5′-CTTCCTGGAGGTCACGGCTCAAGG-3′. PCR reactions were carried out in a total volume of 50 μl containing 10 ng of DNA (template), with a final concentration of 1× reaction buffer, 1.5 mM of $MgCl_2$, 5% DMSO, 250 μM of dNTPs, 0.5 μM of each primer, and 1 U/sample of *Taq* DNA polymerase (Fisher, Pittsburgh, Pennsylvania). The amplification profile was composed of an initial denaturation step at 94°C for 4 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 65°C for 1 min, and extension at 72°C for 1 min, and a final 10-min extension at 72°C. PCR products were run on 2% agarose gel stained with ethidium bromide and visualized under ultraviolet light.

Statistical Analysis. Allele and genotype frequencies as well as the heterozygosity were determined using the GenePop program (Raymond and Rousset 1995). Hardy-Weinberg equilibrium was evaluated with a chi-square test using a 95% confidence interval.

Results and Discussion

DAT1 polymorphisms differ among ethnic groups and residential populations in different parts of the world. The distribution of *DAT1* VNTR allele frequencies obtained in this study and of frequencies reported in the literature for other populations is shown in Table 1. Observed alleles ranged from 3 to 16 repeats (200–720 bp), but no previously described 4-, 6-, 12-, and 13-repeat alleles were observed in our sample. Notably, in one individual from the Ivory Coast sample we found a 16-repeat (720-bp) allele, previously undetected in other human populations.

Frequencies of *DAT1* polymorphisms observed in the northwestern Italian and Ivorian populations were similar to those previously described in other samples of Italian and West African populations (Yoruba), respectively (Persico et al. 1996; Kang et al. 1999). As reported in several studies, the most commonly observed alleles were the 10-repeat allele (*DAT1*10*) and the 9-repeat allele (*DAT1*9*). Even if both alleles show high frequencies worldwide, the *DAT1*10* allele appears to be heterogeneously distributed, whereas in Europe *DAT1*9* seems to follow a decreasing gradient from south to north (see Table 1).

Despite the ubiquitous presence of the major alleles, in our samples we found rare alleles not yet recorded in the same geographic areas. It has been suggested that shared rare *DAT1* alleles among populations are likely to reflect common ancestry (Mitchell et al. 2000). The *DAT1*3* allele recovered in our Italian sample with a frequency of 1.9% has not been previously reported in other European populations (Doucette-Stamm et al. 1995). This allele has been found only in some Middle Eastern, African American, Hispanic American, and African populations, among which the Ivorian population reported here showed the highest value (6.8%). Thus it is plausible that this allele originated in Africa and subsequently was diffused in the Mediterranean area and, more recently, in the Americas.

Table 1. Distribution of *DAT1* VNTR Allele Frequencies in Studied and Previously Analyzed Populations

Population	Number Tested	DAT1 Allele Frequency											Reference
		*3	*5	*6	*7	*8	*9	*10	*11	*12	*13	*16	
Africa													
Ivory Coast	73	0.068	0.007		0.007	0.027	0.144	0.739				0.007	Present study
North Africa	10						0.200	0.750	0.050				Mitchell et al. (2000)
Mbuti	39				0.330	0.010	0.280	0.360					Kang et al. (1999)
Biaka	65	0.020			0.150	0.050	0.220	0.560					Kang et al. (1999)
Ethiopian Jews	33					0.020	0.200	0.790					Kang et al. (1999)
Yoruba	49	0.050			0.010	0.050	0.130	0.730		0.020			Kang et al. (1999)
Europe													
Northwest Italy	204	0.019					0.350	0.610	0.019				Present study
Italians	348						0.350	0.630	0.010				Persico et al. (1996)
Greeks	21						0.381	0.524	0.095				Mitchell et al. (2000)
Danes	51						0.220	0.760	0.020				Kang et al. (1999)
Finns	35						0.100	0.900					Kang et al. (1999)
Irish	102						0.300	0.690					Kang et al. (1999)
Mixed Europeans	88						0.310	0.690					Kang et al. (1999)
Mixed Europeans	443						0.270	0.720					Doucette-Stamm et al. (1995)
Russians	46						0.200	0.800					Kang et al. (1999)
Russians	56					0.010	0.150	0.830	0.010				Galeyeva et al. (2001)
Chuvash, Russia	33						0.106	0.879	0.015				Mitchell et al. (2000)
Mordavians	58				0.010		0.290	0.690	0.010				Galeyeva et al. (2001)
Adygei	54					0.020	0.230	0.740	0.010				Kang et al. (1999)
Middle East													
Omanis	110	0.009		0.018	0.009	0.005	0.332	0.609	0.018				Simsek et al. (2005)
Druze	93	0.020					0.420	0.550					Kang et al. (1999)
Yemenite Jews	37	0.030					0.500	0.450	0.030				Kang et al. (1999)
Asia													
Ami	38						0.130	0.870					Kang et al. (1999)
Atayal	42						0.060	0.940					Kang et al. (1999)
Cambodians	25						0.200	0.800					Kang et al. (1999)
Chinese, San Francisco	58				0.020	0.010	0.060	0.910					Kang et al. (1999)

Chinese, Taiwan	48					0.100	0.840	0.050		Kang et al. (1999)
Chinese Han	203				0.015	0.060	0.905	0.020		Li et al. (1994)
Hakka	39				0.010	0.090	0.870	0.030		Kang et al. (1999)
Mongolians	78			0.026		0.051	0.904	0.013	0.006	Nakatome et al. (1995)
Ket	16			0.031		0.031	0.938			Mitchell et al. (2000)
Sel'Kup	29			0.086		0.207	0.707			Mitchell et al. (2000)
Altai-Kizhi	48			0.031		0.052	0.865	0.031	0.021	Mitchell et al. (2000)
Evenki	61			0.057		0.033	0.787	0.123		Mitchell et al. (2000)
Japanese	107			0.009		0.042	0.930	0.019		Sano et al. (1993)
Japanese	176			0.017		0.063	0.912	0.009		Nakatome et al. (1995)
Japanese	45			0.010		0.010	0.980			Kang et al. (1999)
Kachari (Assam)	14						1.000			Kang et al. (1999)
Yakut	51				0.010	0.050	0.940			Kang et al. (1999)
Americans										
U.S. Native Americans	19					0.240	0.760			Mitchell et al. (2000)
Cheyenne	56					0.020	0.980			Kang et al. (1999)
Jemez Pueblo	42					0.010	0.990			Kang et al. (1999)
Amerindians, Colombia	54						1.000			Mitchell et al. (2000)
Maya	57					0.070	0.920	0.010		Kang et al. (1999)
Rondonian Surui	47					0.010	0.990			Kang et al. (1999)
Prima, Arizona	45						1.000			Kang et al. (1999)
Prima, Mexico	96					0.050	0.950			Kang et al. (1999)
Karatiana	54						1.000			Kang et al. (1999)
Ticuna	65					0.070	0.930			Kang et al. (1999)
Hispanics	141	0.010				0.260	0.710	0.010	0.010	Doucette-Stamm et al. (1995)
Oceania										
Australian Aborigines	18			0.028			0.972			Mitchell et al. (2000)
Nasioi	24				0.060		0.940			Kang et al. (1999)
Mixed populations										
U.S. mixed	29	0.008		0.012	0.019	0.240	0.700	0.016		Vandenbergh et al. (1992)
U.S. mixed	84	0.012				0.226	0.762			Cook et al. (1995)
African Americans	446	0.030		0.020	0.030	0.170	0.730	0.010		Doucette-Stamm et al. (1995)
African Americans	82	0.010	0.010	0.020	0.030	0.220	0.700	0.010		Persico et al. (1996)
African Americans	40	0.030		0.030	0.030	0.150	0.760	0.010		Gelernter et al. (1998)

Table 2. Observed Number of Genotypes for the *DAT1* VNTR Polymorphism in the Italian and Ivory Coast Populations

	Observed Number (and Frequency, %)	
Genotype	*Italy*	*Ivory Coast*
**3/*3*	4 (1.96)	5 (6.85)
**5/*16*	0 (0.00)	1 (1.37)
**7/*10*	0 (0.00)	1 (1.37)
**8/*8*	0 (0.00)	1 (1.37)
**8/*10*	0 (0.00)	2 (2.74)
**9/*9*	16 (7.84)	3 (4.11)
**9/*10*	107 (52.45)	15 (20.55)
**9/*11*	4 (1.96)	0 (0.00)
**10/*10*	69 (33.82)	45 (61.64)
**10/*11*	4 (1.96)	0 (0.00)

The Ivorian population showed allele frequencies similar to those of the Yorubas, but they also differed in the presence of the rare alleles *DAT1*5* and *DAT1*16*. The *DAT1*5* allele has been reported only in an African American sample (Persico et al. 1996), whereas the *DAT1*16* allele is reported here for the first time.

The genotype-frequency distribution in the studied populations is shown in Table 2. The most frequent genotypes were **9/*10* for the Italians and **10/*10* for the Ivorians. We assessed for the first time the existence of an unusual **5/*16* genotype, which was found in one individual in the African population. According to many global surveys of genetic markers indicating that African populations have consistently greater diversity than other populations (Goldstein et al. 1995; Garrigan and Hammer 2006), our African sample showed a higher allele assortment with respect to the Italians. However, the observed heterozygosity of the Ivorian population (26%) fell about the lower extreme of the value range reported by other investigators for Africans (24–63%) (Kang et al. 1999) and was only half that of the Italian sample (56.3%). Moreover, the Ouangolodougou population was not in Hardy-Weinberg equilibrium (chi-square test) at the *DAT1* locus, because of a deficit of heterozygote genotypes, or at other loci, namely, glutathione S-transferase T1 and M1 (Santovito et al., unpublished data). The lower observed heterozygosity and deviation from Hardy-Weinberg equilibrium could be the result of microevolutionary trends, such as genetic drift and/or inbreeding, acting on the relatively small and isolated population sampled for this study, although some sort of selective pressure against the shorter alleles cannot be excluded (Michelhaugh et al. 2001; Mill et al. 2002). This latter possibility is consistent with the observation that in all human populations analyzed for this polymorphism, the number of alleles is invariably low (Mitchell et al. 2000), in contrast to data reported for other VNTRs. Further studies are needed to address the impact of selective constraints on the variability associated with this gene in different populations

and ethnic groups. Alternatively, it has been suggested that this *DAT1* VNTR polymorphism could be in linkage disequilibrium with other nearby polymorphisms, for example, with the polymorphism in exon 15 (Thompson et al. 1997).

In conclusion, we have provided new *DAT1* VNTR frequency data for a European and an African population previously not described, and we reported a new allele (*DAT1*16*) characterized in the Ivory Coast population.

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