J. Biol. Res. 2009 n. I - Vol. LXXXII - Rubbettino - Soveria Mannelli

Study of genetic variation in three human populations in Piedmont (Italy)

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KEYWORDS: Gene Polymorphism, *DAT1*, *LRPAP1*.

Abstract

In the present study, we analyzed the VNTR polymorphism of DAT1 gene and the LRPAP1 I/D gene polymorphism, in a total of 250 individuals belonging to three population of North Western Italy (Biella, Cavaglià, Postua). Among these, Postua is particularly interesting, because its history has been characterized by partial geographic and social isolation and by a high degree of marriages between relatives. Results obtained for both the DAT1 and the LRPAP1 frequency distribution confirm those previously described for other Italian and European populations. In contrast to data obtained using Alu insertion loci, the Postua sample appeared not differentiated from the neighbouring towns Cavaglià and Biella. Indeed, no significant differences were found between studied populations in the frequency of the DAT1 VNTR alleles, showing an inter-populational homogeneity for this genetic marker. Despite the ubiquitary presence of the major alleles (*9 and *10 alleles), in our samples we found the rare allele DAT1 *3 not yet recorded among Europeans. For LRPAP1 gene polymorphism, Postua showed the higher frequency of I allele, although no significant differences were found between studied populations in the frequencies of I and D alleles.

Introduction

Several polymorphic genetic systems have been used to study genetic variation and evolutionary history of modern humans. In the present study, we compared within- and between-population genetic diversity at two autosomic loci in three populations of North Western Italy: Biella, Cavaglià and Postua. In particular, Postua history has been characterized by partial geographic and social isolation and by a high degree of marriages between relatives. Previous studies based on mtDNA, Y-chromosome markers analysis (Marin, 2004) and other eight human polymorphic Alu insertion loci (Santovito et al., 2007a) confirmed Postua as a moderately close community, partially unaffected by genetic flows in the past. In a multidimensional scaling analysis plot based on the Alu frequencies, Postua resulted to be isolated and well separated from Cavaglià and Biella, although its geographical distances are only 25 Km from Cavaglià and 40 Km from Biella, respectively. Considering the limited population size (570 inhabitants), hypothetical bottleneck effects and/or genetic drift events may have acted on the Postua group.

In this study, we analyzed the three populations using two different kinds of gene polymorphisms: the variable number of tandem repeats (VNTR) polymorphism of *DAT1* (dopamine transporter) gene, and insertion/deletion (I/D) polymorphism of the Low-density lipoprotein Receptor-related Protein-Associated Protein 1 (*LRPAP1*) gene. The *DAT1* gene plays a central role in the regulation of dopamine levels and neurotransmission, and it has been supposed as involved in many neurological diseases and psychiatric disorders (Li et al 2006; Ohadi et al., 2007). *DAT1* contains a VNTR of a 40-bp monomer in its 3' UTR, ranging from 3 (~200 bp) to 16 (~720 bp) copies of the core sequence. Alleles *DAT1*9* and *DAT1*10*, containing 9 and 10 repeats respectively, were found to be very common, while the other variants resulted much less frequent and were geographically restricted (Mitchell et al. 2000; Santovito et al., 2008). The *LRPAP1* polymorphism analyzed in this study consists in the presence (insertion, allele I) or absence (deletion, allele D) of a sequence of 37 bp in intron 5, and has been associated with the risk of developing late-onset Alzheimer's disease (LOAD) (Sanchez et al., 2001). The aims of this study were: 1) to provide new data about these polymorphisms in Italian populations; 2) to analyse the heterogeneity of the samples paying particular attention to the Postua situation; and 3) to compare the results with those available for other European populations. Knowledge of frequency distribution of these polymorphisms in different populations will help to elucidate the evolutionary pressures that shaped the variability among different human populations.

Materials and Methods

For *DAT1* polymorphism, a sample of 204 individuals was studied: 90 belonging to Postua, 43 to Cavaglià and 71 to Biella. For *LRPAP1* polymorphism, the studied subjects were 250: 102 belonging to Postua, 53 to Cavaglià and 95 to Biella. Postua is a little mountain village of 570 inhabitants characterized in the past by partial geographic and social isolation and by a high degree of marriages between relatives. Cavaglià and Biella are two neighbouring towns of 3600 and 40.000 inhabitants, respectively. All studied populations are located in Piedmont, a region of North Western Italy. All samples were obtained from native blood donors. Informed consent was obtained from all individuals participating to the study. Peripheral blood samples (5-10 ml venipuncture) were collected in heparinized vacutainers and stored at –20°C. To extract DNA we used Chelex^{â} solution protocol as described by Walsh et al. (1991). PCR amplifications of *DAT1* gene was performed using primers and reaction profiles as described by Vandenbergh et al., 1992. For *LRPAP1* gene amplification, we used primers and PCR reaction profile as described in Beneŝ et al. (2000). The amplified products for *LRPAP1* gene were 185 bp for the deletion (D) and 222 bp for the insertion (I) alleles. Amplified PCR products were run on 2% agarose gels, stained with ethidium bromide, and visualized under UV light. Allele and genotype frequencies of each gene polymorphisms were determined using the GENEPOP program.

Results and Discussion

Distribution of *DAT1* VNTR allele frequencies obtained in this study and those reported in literature for other European populations are shown in Table 1. Observed alleles ranged from 3 to 11 repeats (200-520 bp), but the previously described 5, 6, 7, and 8 alleles were not observed in our sample. No significant differences were found between studied populations in the frequencies of

			DAT1 Allele Frequency					
Populations	N	*3	*7	*8	*9	*10	*11	References
Postua	90				0.388	0.588	0.022	Present Study
Biella	71	0.056			0.338	0.584	0,021	Present Study
Cavaglià	43				0.290	0.698	0.012	Present Study
North-Western Italy	204	0.020			0.350	0.610	0.020	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	443				0.270	0.720		Doucette-Stamm et al., 1995
Russians	56			0.010	0.150	0.830	0.010	Galeyeva et al., 2001
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

Table 1 - *Distribution of DAT1 VNTR allele frequencies in the studied and previously analysed European populations.*

the *9, *10 and *11 alleles, showing an inter-population homogeneity for this genetic marker. Moreover, frequencies of *DAT1* polymorphisms observed in the whole North Western Italy sample resulted to be very similar to those previously described in other Italian and European samples, representing another proof of this homogeneity. As reported in several studies, the most common alleles were the 10-repeat (*DAT1*10*) and the 9-repeat (*DAT1*9*). Along with the ubiquitary major alleles, in our samples we found the rare allele *DAT1*3*, not yet recorded in the same geographical areas. The *DAT1*3* allele was only found in some Middle East, African American, Hispanic American, and African populations (Doucette-Stamm et al., 1995; Santovito et al., 2008). It is plausible that this allele originated in Africa, subsequently spreading in the Mediterranean area and, more recently, in the Americas. The genotype frequency distribution in the studied populations is shown in Table 2. In our sample, the most frequent genotype was *9/*10. We assessed the existence of an unusual *3/*3 genotype, which was found in four individuals from Biella. The genotype and allele frequencies of *LRPAP1* gene are shown in Table 3. Postua showed the higher frequency of I allele, although no significant differences were found in the frequencies of I and D alleles, again confirming the genetic homogeneity between populations. There are only limited information available in literature about *LRPAP1* gene polymorphism distribution in worldwide populations (Table 3). Among Europeans, Bene et al. (2000) reported frequencies of 0.263 for the I allele and 0.737 for the D allele in a sample constituted by Czech subjects, while Sanchez et al. (2001), in a Spanish sample, reported frequencies of 0.270 and 0.730, for I and D allele, respectively. These values significantly differ ($p<0.001$) from those found in our North-Western Italian sample, for which the frequency of I and D alleles were 0.176 and 0.824, respectively (Table 3), suggesting high frequencies of the deleted allele in European populations.
In summary, the results obtained for both the *DAT1* and the *LRPAP1* frequencies distribution conform to those previously described for other Italian and European populations. In contrast to data obtained using Alu insertion loci (Santovito et al., 2007a), the Postua sample do not differentiate from the neighbouring towns (Cavaglià and Biella).

Acknowledgments

This work was supported by grants from "Fondazione Sella O.N.L.U.S." and Italian M.I.U.R.

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Genotypes	Observed Number (Frequency)			
	Postua		Biella	Cavaglià
3/3			4 (5.60%)	
9/9	11	(12.20%)	4 (5.60%)	1 (2.3%)
9/10	46	(51.10%)	38 (53.5%)	23 (53.5%)
9/11	2	(2.20%)	2 (2.80%)	
10/10	29	(32.20%)	22 (31.0%)	18 (41.9%)
10/11	2	(2.20%)	1 (1.4%)	1 (2.3%)

Table 2 - *Observed number of genotypes for the DAT1 VNTR polymorphism in the studied populations.*

Population	N	Genotypes			Alleles		References
		I/I	I/D	D/D	I	D	
Postua	102	0.019	0.363	0.618	0.200	0.800	Present Study
Biella	95	0.684	0.305	0.011	0.163	0.837	Present Study
Cavaglià	53	0.755	0.188	0.057	0.151	0.849)	Present Study
North-West Italy	250	0.024	0.304	0.672	0.176	0.824	Present Study
Ivory Coast	133	0.015	0.353	0.632	0.192	0.808	Santovito et al., 2007b
Czech Republic	515	0.078	0.371	0.551	0.263	0.737	Benes et al., 2000
Spain	300	0.070	0.400	0.530	0.270	0.730	Sanchez L. et al., 2001

Table 3 - *Genotype and Allele Frequencies of LRPAP1 gene in studied and other populations worldwide distributed*

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