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Mitochondrial DNA sequence diversity in two groups of Italian Veneto speakers from Veneto

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SUMMARY

Although frequencies of mitochondrial DNA (mtDNA) haplogroups in the different European populations are rather homogenous, there are a few European populations or linguistic isolates that show different mtDNA haplogroup distributions; examples are the Saami and Ladin speakers from the eastern Italian Alps.

MtDNA sequence diversity was analysed from subjects from two villages in Veneto. The first, Posina, is situated in the Venetian Alps near Vicenza. The second, Barco di Pravisdomini is a village on the plains near Venice. In spite of their common Veneto dialect, the two group populations have not preserved a genetic homogeneity; particularly, they show differences in T and J haplogroups frequencies. MtDNA diversity in these two groups seems to depend more on their geographic situation.

INTRODUCTION

In many studies of extant human populations, variations in gene frequency have been attributed to the geographic distance between populations (reviewed in Wijsman & Cavalli-Sforza, 1984). Although some extensive gene frequency gradients seem to be due to directional gene flow (Menozzi et al. 1978; Sokal & Menozzi, 1982; Ammerman & Cavalli-Sforza, 1984; Rendine et al. 1986), other gene frequency patterns simply cannot be explained by a major migratory movement. Different selective pressures or isolation factors must also be considered (Slatkin, 1975; Piazza et al. 1981; Slatkin, 1985; Piazza et al. 1988; Barbujani & Sokal, 1991a). In par-

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ticular, several authors have noted a positive correlation between genetic and linguistic characteristics for different groups (Livingstone, 1963). This phenomenon is found in Italy where a great genetic heterogeneity occurs throughout the various regions, and where genes and languages have a similar geographical distribution when taking into account the ten major Italian dialects (Barbujani & Sokal, 1991b). In fact, the genetic structure of the modern Italian population could probably be deduced from pre-Roman gene frequency data alone (Piazza et al. 1988). In some cases it has been suggested that history left tracks in different gene frequencies.

The vast majority of present-day European mitochondrial DNA (mtDNA) sequences, according to mtDNA markers, can be classified into ten haplogroups (H, I, J, K, M, T, U, V, W, X) (Torroni et al. 1996). In addition, mtDNA

sequences from Europe generally exhibit a very low diversity compared to sequences from other continents. There are some exceptions, however. High mtDNA sequence diversity has been observed in the Saami, and a few populations or groups that include the Ladin. The Ladinspeaking populations studied by Stenico et al. (1996) are linguistic isolates from the Italian Alps. German and Italian linguistic groups from the Italian Alps in the administrative region of Trentino-Alto Adige were also studied (Stenico et al. 1996). The mitochondrial sequence diversity exhibited by the two groups of Ladin speakers was different from that of other linguistic groups. The particular patterns of diversity observed in these Alpine isolates are probably due to their historical geographic isolation, the Ladin community of Val Gardena being the most differentiated.

In this study, we report on the distribution of mtDNA haplogroups in two groups of Venetospeaking Italians: the first inhabiting Posina and the second Barco, in Veneto. Posina is in an isolated mountainous area, between Trento and Vicenza, whereas Barco is situated on the plains, near Venice. This study reveals a difference in the mtDNA sequence diversity patterns of these two Veneto-speaking groups, manifested as a difference in the differential distributions of haplotypes belonging to the J and T haplogroups. The population from Posina presents a similar diversity to the Ladin-speaking populations from Val Gardena and Colle Santa Lucia.

HISTORY, SUBJECTS AND METHODS

History

Veneto owes its name to the Venetians, a population whose paleovenetian civilisation flourished on the territory of the three Venetia (Trentino-Alto Adige, Veneto and Friuli-Venezia Giulia) from the dawn of the Iron age in 1000 before Christ (B.C.) to the end of the second century B.C. Called Henetes by the Greeks and mentioned by Homer in the *Iliad*, this population is said to have come from Asia Minor after the Trojan war in the thirteenth century B.C., and to

have brought an iron age culture with them. The Venetians landed on the shores of the Adriatic where they drove out the Euganei, who then took refuge in the mountains. The Euganei were a group of people who could have belonged to the Rhaetii, an unclearly defined P.M. historic people co-existing with the Ligures in the western valleys (Chieco Bianchi, 1988).

The Venetian tribes spread out in the territory now known as Veneto, with the exception of the Veronese and the Trentino who were Rhaetii. The Friuli was less intensely populated by Venetians and most of this region was occupied by the Carni, of Celtic origin. There are other historic references to the name Venetian in places other than Veneto, for instance in Central Europe as far as the shores of the Baltic, and on the Atlantic coast of Brittany (in Breton dialect Vannes is *gwened* and means Venetian). For linguists and archaeologists (Prosdocimi, 1978), the word Venetian is synonymous with Indo-European. The Venetians of Veneto represent an Indo-European strain.

The Etruscans, from central Italy, comprised an advanced civilization which lasted from the twelfth to fifth centuries B.C. However, the great expansion of Etruscans, extending from southern Italy to the Po valley in the north, could not have reached the region inhabited by the Venetians (Livio). The Celts, whom the Romans called 'Gauls', arrived in Padania at the end of the fifth century B.C. These people occupied Central and Northern Europe before the Romans extended their domination further north, arriving from Switzerland, Austria and South-West-Germany. The Venetians, without submitting to them, were culturally and artistically influenced by the Gauls, and vice-versa. This progressive influence of Roman ways on the Venetians lasted from the second century B.C. until the fall of the Roman Empire (second-fifth century A.D.).

Numerous archaeological sites confirm the presence of paleovenetians in Veneto since around the end of the second millennium B.C. Many archaeological sites (Broglio, 1987) however, have revealed the existence of human



Fig. 1. Map of the three main regions in Venetia. Note: The shaded area in the map of Italy indicates the administrative region Veneto. The striped area in the map of Italy indicates the administrative region Tuscany. ■, Villages studied by Stenico et al (Stenico et al. 1998). Ladin-speaking samples: LG, Selva Val Gardena Wolkenstein, LC, Colle Santa Lucia; LF, Fassa and LB, Badia. ◆, The two Veneto-speaking samples, Posina and Barco.

habitations in the Euganei Venetian territory since the upper Paleolithic period (35 000–10 000 years).

Subjects

Subjects for this study were volunteers, coming from two villages in Veneto, Italy (Fig. 1). Herein, we report the distribution of mtDNA haplogroups in these two Italian Veneto-speaking groups: the first inhabiting Veneto at Posina in the west, and the second from Barco di Pravisdomini in the east. Veneto is located between the northern shores of the Adriatic sea and the foothills of the Dolomites in the Eastern Alps. It is bordered by Friuli-Venezia Giulia to the east, Lombardy to the west, Trentino-Alto Adige to the north west, Emilia Romagna to the

south and Austria to the far North. Venice is the capital of Veneto. Posina is situated in a valley at the foot of Pasubio in the Venetian Prealps, 50 km to the south of Trento. It is a village neighbouring the North Eastern valley of the Eastern Alps where Ladin (Stenico et al. 1996) is still spoken. The valley where Posina is found, in former times, provided the passage between Trento and the plain (Dalla Via, 1993). Barco di Pravisdomini and the neighbouring villages in the Veneto area (Meduna, Annone Veneto) are the villages of the plains, 50 km east of Venice, opposite Posina at the extremity of Veneto and adjoining Friuli-Venezia Giulia.

Sixty-eight individuals were studied, 38 coming from Posina and the 30 other individuals from Barco. Subjects were maternally unrelated individuals. All grandmothers of these indi-

Table 1. Primers used for partial PCR amplification of Veneto mtDNAs

	Amplified region position	
Haplogroup	(primers position) ^a	Polymorphic restriction site
Н	06890 - 07131	7025~Alu I
	(06890 - 06910; 07131 - 07110)	
U	12104–12342	$12308~Hin{ m fI}$
	(12104-12127; 12342-12315)	[nt 12308G]
K	08829-09184	9052 HaeII
	(08829 - 08845; 09184 - 09163)	
K	12104-12342	$12308~Hin{ m fI}$
	(12104-12127; 12342-12315)	[nt 12308G]
T	15409-15701	$15606 \; Alu \mathrm{I}$
	(15409-15428; 15701-15682)	
V	04308-04739	4577~Nla III
	(04308-04225; 04739-04719)	[nt 4580A]
PreV, V	14668-14919	$14766\ MseI$
	(14668-14687; 14919-14900)	$[\mathrm{nt}14766\mathrm{C}]$
W	08829 – 09184	8994 HaeIII
	(08829 - 08845; 09184 - 09163)	
X	01615 - 01894	$1715~Dde{ m I}$
	(01615 - 01643; 01894 - 01874)	
X	14430-14614	$14465\ AccI$
	(14430 - 14452; 14614 - 14590)	
J	10270–10579	10394~DdeI
	(10270-10290; 10579-10557)	
I	01615 – 01894	1715~DdeI
	(01615 – 01643; 01894 – 01874)	
I	09910 – 10107	10032~AluI
	(09910-09932; 10107-10089)	
L	03388 – 03717	$3592 \; HpaI$
	(03388 - 03408; 03717 - 03701)	

^a Primers are numbered according to Anderson *et al.* (1981) with 5'-3' coordinates before the comma corresponding to the forward primers and those after the comma to the reverse primers.

viduals were born respectively in Posina or in Barco.

DNA extraction

DNA was extracted from Peripheral Blood Mononuclear Cells (PBMC) or hair roots. The entire DNA was extracted by QIAamp DNA Blood Mini Kit (QIAGEN).

mtDNA analysis

Hypervariable segments (HVS-1 and HVS-2) of the Control Regions (CR) of mtDNAs were directly amplified by polymerase chain reaction (PCR). HVS-1 was amplified from np 16025 to np 16431. The primers used are L16025 5'-TCTTT-CATGGGGAAGCAGATTT-3' (light chain, nps 16025–16046) and H16410 5'-GTGCGGGATATTGATTTCAC-3' (heavy chain, nps 16431–16411). PCR was conducted as follows: denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 1 min,

repeated for 35 cycles. HVS-2 was amplified as described elsewhere (Macaulay et al. 1999) from np 008 to np 248. The primers used are HV2 S: 5'-GGTCTATCACCCTATTAACCAC-3' (light chain, nps 00008–00029) and HV2 AS: 5'-TCAATTGTTATTATTATGTCCTACAA-3' (heavy chain, nps 00248–00223), with identical PCR conditions.

After purification of PCR products (QIAquick PCR Purification Kit, QIAGEN), only light strands of HVS-1 and HVS-2 were sequenced by use of ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer) using respectively primers L16025 and HV2 S: denaturation at 98 °C for 10 s, annealing at 55 °C for 10 s and extension at 60 °C for 10 s, repeated for 35 cycles.

According to Torroni *et al.* (1996) and Macaulay *et al.* (1999), the relevant polymorphic restriction sites analysed were reported in Table 1.

Database analysis

Sequence polymorphisms in regions HVS-1 and HVS-2 of the D-Loop from the Barco (Table 2) and Posina (Table 3) samples were compared to other samples from the HVR base (Burkhardt et al. 1999), including samples from seven villages from Venetia and Trento-Alto Adige (Stenico et al. 1996) and samples from Tuscany (Francalacci et al. 1996). Haplogroups were determined using classifications described by Torroni (Torroni et al. 1996; Macaulay et al. 1999) and Richards (Richards et al. 1996) (Table 4). In some populations from the HVR base, differences between I and W haplogroups, as well as differences between H and U haplogroups, were unresolvable due to the absence of RFLP analysis in reference data.

Statistical analysis

The DNA sequences were aligned using Clustal X ver. 1.81. (Thompson *et al.* 1994). MEGA program was employed to identify each haplotype using the complete deletion option with no assumption of traditional haplotypes definition. By this method, 309 sites were seen to be conserved (for the 167 individuals), representing 119 different haplotypes.

The population genetic structure was analysed using AMOVA (Excoffier et al. 1992) and F statistics (Weir & Cockerham, 1984) of six various populations using Arlequin 1.1 program (Schneider et al. 2000). An exact test of population differentiation was performed using a Markov chain with 1000 iterations. Tamura and Nei distance option was selected because of the differences in the transition and transversion rates and the bias in nucleotide composition (C, 34.4%; T, 21.3%; A, 34.9%; G, 9.4%).

A Phenogram based on the six populations using Fst values and the Neighbour-Joining (NJ) (Saitou & Nei, 1987) clustering algorithm was done. A European control population (Bulgarian) was included as an outgroup to remove the possible artefacts due to the very closed location (the maximum distance between the populations was 200 km) and the small size of Ladin-speaking

populations (LC, LG). The tree was rooted on the Bulgarian population. An UPGMA tree was also done using the same matrix, but the topology was not statistically different: therefore, only the NJ tree was considered.

RESULTS

Control region data

Sequences for the two segments of the hypervariable region of the mtDNA D-loop were determined for all the 68 Veneto-speaking individuals who participated in this study. Nucleotide differences relative to the reference sequence (CRS, Anderson et al. 1981) were identified for all individual samples. Fifty variable positions were detected in the 380 sites sequenced in HVS-1 and 18 variable positions in the 240 sites sequenced in HVS-2. All nucleotide changes were transitions, except for three transversions at nps 16097 (T to A in haplotype VB 11), 16327 (A to T in haplotype VB 28) and 217 (T to A in haplotype VB12) in haplotypes from Barco, and six transversions in haplotypes from Posina, one G-C at position 16129 (haplotype VP 30), one A-C at position 16182 (haplotype VP6) and the A-C transversion at position 16183 in four haplotypes (VP6, VP17, VP37 and VP30).

Diversity of haplotypes

Most of the haplotypes observed in this study were unique. There were twenty-three different haplotypes among the 38 subjects from Posina and 24 different haplotypes were found in the 30 Barco individuals. An identical haplotype was observed in seven individuals, six from Posina (VP4, VP12, VP27, VP29, VP32, VP34) and one from Barco (VB27). This haplotype was characterized by an HVS-1 sequence identical to the CRS, and an HVS-2 sequence with only one difference (152C) relative to the CRS.

Haplotypes contained within haplogroups H, K, U and V did not show any particular polymorphisms capable of characterizing one population group or another. In contrast, haplotypes within haplogroups J and T showed between group differences. 90% of Posinian

1 1 1 1 1 1 1 1 1 2 2 2 2 $6 \; 8 \; 9 \; 1 \; 2 \; 2 \; 4 \; 1 \; 6 \; 6 \; 6 \; 8 \; 8 \; 8 \; 9 \; 1 \; 2 \; 2 \; 3 \; 3 \; 4 \; 5 \; 6 \; 7 \; 9 \; 9 \; 9 \; 0 \; 1 \; 1 \; 2 \; 2 \; 5 \; 6 \; 9$ 7 7 4 5 5 8 8 8 9 9 9 0 0 1 1 $9\ 8\ 7\ 1\ 6\ 9\ 5\ 8\ 2\ 3\ 9\ 5\ 6\ 9\ 2\ 1\ 3\ 4\ 1\ 4\ 9\ 6\ 1\ 0\ 4\ 8\ 8\ 4\ 1\ 9\ 4\ 7\ 2\ 2\ 1$ 2 3 6 0 2 5 8 9 3 5 9 4 7 5 7 CRS a C T T C T G G C A A C C C T C A C T T C T C C C C C T T T G T A T T G Hap T A T C T G A A A T T T G A T ? - - - - - T - - - - - - H - - - - - - G G - - - - -? - - - - - - - - - - - - C - H VB16 ? - - - - - - - - - - H VB18 * ------VB23 * - - - - - - - - - - - H VB25 ? - - - - - - G - - - - - - - - - - - H VB27 ? - - - - - - - - - H -----VB30 ? - - - - - - - - C - - - - - - - C - - - - - H VB19 ? - - - - - - - - - C - H - G - - C - - - - C C A - A - G - - - - - - C C - - -VB8 T - - - C - - - - - - - - J - G - - - A - - - - - - -VB10 T - - - C - - - - - - - - - - - - J - G - - - - A - - - - - -- G - - - - A - - - - - -VB26 T - - - C - - - - - - - - J - G - - - - A - - - - - -- G - T C - - - C - - - G -- - G - - - A - - - - - -VB3 ---- T T - C ---- T - G - - - - - - - - - -- G - - C - - - - C - - - --- G -- C -- -- C -- -- $VB21 \,$ - - - - C - - - - G - - T C - - - - - - - - T - - - - - - T1 - G - - C - - - - C - - - -- G - - G - - - - - - - -VB14 - - - - - - - C - - - U - - G - - - - - C - - - -VB6 ? - - - - - - - - - - - - V C - - - A - - - - - - -VB29 1 ----- T ----- V C - - - - - - - - - - -VB20 ? - - - - - - C - other - G - C - - - C - - - - C

Table 2. Variable sites in the control region HV1 and HV2 of 30 mtDNA from Veneto of Barco

Sub, subjects; Hap, haplogroups; (cluster membership was determined as described in the text); VB, Veneto from Barco.

HV 1 haplotypes, nps between 16055 and 16410 that are different from CRS (Anderson *et al.* 1981). (-) denotes that there is no difference from the CRS. HV 2 haplotypes, nps between 00008 and 00240 that are different from CRS (Anderson *et al.* 1981). (-) denotes that there is no difference from the CRS. ?, not determined.

a, CRS is Cambridge Reference Sequence (Anderson et al. 1981).

Table 3. Variable sites in the control region HV1 and HV2 of 38 mtDNA from Veneto of Posina

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For key see Table 2.

Table 4. Haplogroup-specific	polymorphic sites in Posina	and Barco mtDNA
Polymorphic sites	Polymor	rphic sites

					Poly	moı	rphic	e site	es							Po	lym	orpl	hic s	ites					
	1 7 1 5	3 5 9 2	4 5 8 0	7 0 2 5	9 0 5 2	1 0 3 9 4	1 2 3 0 0 8	1 4 4 6 5	1 4 7 6 6	1 5 6 0 6			1 7 1 5	3 5 9 2	4 5 8 0	7 0 2 5	9 0 5 2	1 0 0 3 2	1 0 3 9 4	1 0 3 9 7	1 2 3 0 8	1 4 4 6 5	1 4 7 6 6	1 5 6 0 6	
Pos VP3 VP4 VP5 VP8 VP11 VP12 VP13 VP16 VP25 VP26 VP27 VP29 VP32 VP34 VP15 VP11 VP14 VP1 VP2 VP2 VP20 VP22 VP24 VP33 VP36 VP33 VP36 VP38 VP38 VP39 VP39 VP39 VP39 VP39 VP39 VP39 VP39		b	+ -	c		+ + +	+ + + + + + + + + + + + + + + + + + +	+ + + +	C	+ + + + + + + + + + + + + + + + + + +	$egin{aligned} \mathbf{Hap} \\ \mathbf{H} \\ H$	Bar VB1 VB4 VB5 VB7 VB9 VB16 VB18 VB23 VB25 VB27 VB30 VB19 VB12 VB28 VB10 VB17 VB26 VB24 VB11 VB3 VB13 VB15 VB21 VB22 VB28 VB20 VB29 VB20 VB20 VB20 VB20 VB20 VB20 VB20 VB20		- -		c + + + + + + + + + + + + + +	- -	+ + +	+ + + + + + + + + + + + + + + + + + +	- -	A	e	C	+ + + + + + + + + + + + + + + + + + +	Hap H H H H H H H J J J J1a K T T T T U V V Other

The restriction enzymes used in the analysis are designated by the following single-letter codes: $^a=DdeI$; $^b=HpaI$; $^c=AluI$; $^d=HaeII$; $^c=AccI$. Polymorphisms 4580A, 12308A and 14766C are determined by direct sequencing. Sites are numbered from the first nucleotide of the recognition sequence. A sign (+) indicates the presence of a restriction site and a sign (–) indicates the absence of the polymorphic site. VP, Veneto from Posina; VB, Veneto from Barco.

haplotypes studied from haplogroup T have 296T, a mutation in the HVS-1 region, and 70 % have the 143A mutation in HVS-2. One haplotype (VB3) from Barco exhibited the 296T mutation, and no haplotype from Barco has the 143A mutation in HVS-2. There is no disequilibrium linkage between the 296T an 143A

mutations. The 163G polymorphism in HVS-1 was observed in two haplotypes (VP15 and VP21) from the J haplogroup and in one haplotype (VP7) from the T haplogroup of Posina. Whereas this same 163G polymorphism was found in all haplotypes, except one from a T haplogroup, from Barco. The 185A poly-

Table 5. Distribution of mtDNA haplogroups in the two villages of Veneto speakers

	Veneto from	Veneto from
Haplogroups	$\mathrm{Barco^a}$	Posina ^a
H	12 (40.0)	16 (42.1)
I	2(6.7)	0 (0)
J + J1a	5 (16.7)	2(5.3)
J	4	2
J1a	1	2
T + T1	5 (16.7)	10 (26.3)
${f T}$	3	10
T1	2	0
U + U3 + U5 + K	2(6.7)	5(13.2)
\mathbf{U}	1	1
U3	0	1
U5	0	1
K	1	2
HV	1 (3.3)	0 (0)
V	2(6.7)	1(2.6)
PreV	0 (0)	1(2.6)
X	0 (0)	3(7.9)
Other	1 (3.3)	0 (0)
Total	30 (100)	38 (100)

All haplogroups are according to Torroni et al. 1996 and Macaulay et al. 1999.

Table 6. Fst analysis

(a) Computing conventional F-Statistics from haplotype frequencies for the six populations studied

	TO	VB	OUT	$\overline{\mathrm{VP}}$	\mathbf{LG}
VB	0.00382				
OUT	0.00175	0.00727			
VP	0.00760	0.02259	0.01140		
LG	0.00138	0.01235	0.00492	0.0062	
LC	0.01152	0.00943	0.00863	0.01104	0.00112

(b) Matrix of significant Fst p values based on 100 permutations (with a p value < 0.05)

VВ	_				
OUT	_	_			
VP	+	+	+		
LG	_	_	_	_	
LC	+	_	_	_	_

 $^{+\,,\,}significant\,;\,-,\,not\,\,significant.$

Table 7. Fixation indices

FSC: -0.01704* FST: 0.00660* FCT: 0.02325*

morphism in HVS-2 was observed in all haplotypes from haplogroup J, from Barco, except in haplotype VB24. One haplotype from Posina, corresponding to individuals VP2, VP20, VP24 and VP33, was retrieved in the Colle Santa Lucia group (haplotype 1986), and one haplotype from Barco corresponding to individuals VB13, VB21

and VB22, was retrieved in the Colle Santa Lucia group (haplotype 1991, 1992). Haplotypes from Barco were distributed into ten haplogroups or sub-haplogroups: H, HV, I, J, J1a, K, T, T1, U, V, and haplotypes from Posina were distributed into ten haplogroups or sub-haplogroups H, J, K, T, U, U3, U5, preV, V and X.

Haplogroup distribution in Posina and Barco people

The main haplogroups observed in the Barco group were H (40%), J (16.7%), T (16.7%), V (6.7%) and I (6.6%). In the Posina group, the

^a Values are numbers of subjects with percentage in parentheses.

^{*} Significant F statistics p values (with a p value < 0.05).

VPTO VBOUT LG VB130.49040 OUT 284.58249 68.2978VP21.62931 65.2688443.36451 LG361.85214 40.0000 101.07143 805.14850 LC42.89640 52.54717 57.44898 44.79803 444.9997

Table 8. Estimated number of migrants every generation for haploid data using the Fst value

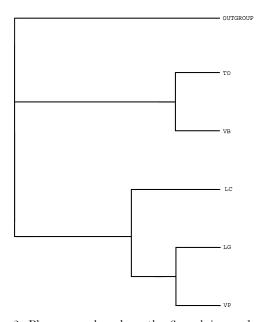


Fig. 2. Phenogram based on the five alpine and plain populations and one out-group using Fst values and the Neighbour-Joining (NJ) clustering algorithm. Phenogram based on the five alpine and plain population and one out-group using Fst values and the Neighbour-Joining (NJ) (Saitou & Nei, 1987) clustering algorithm was done. The tree was rooted on the Bulgarian population. TO, Tuscanian population; VP, Posina population (Veneto-speaker); VB, Barco population (Veneto-speaker); LC, Colle Santa Lucia population (Ladin-speaker); and LG, Val Gardena population (Ladin-speaker).

following distribution was found: H (42.1%), T (26.3%) and U (7.9%) and X (7.9%), while the T frequency was 5.3% (Table 5).

Statistical analysis

Amova and F statistics results were very similar, so only the last one was reported and discussed herein. The Fst values were low for the different samples of populations (Table 6a). The range of the Fst value (0.00062 < Fst < 0.02259) indicated few local genetic factors (such as founder effect or consanguinity). Among popu-

lations with a low Fst value, a significant Fst value (Table 6b) was found for the Posinian population (VP), Tuscanian population (TO) and Barco population (VB) and for the Colle Santa Lucia population (LC) and Tuscanian population (TO). The Fct indicated no Whalung effect (Table 7) for all of the populations.

The Nm values (Number of migrants every generation) were reported in Table 8. Higher Nm values were observed between the VP and Val Gardena populations (LG) (805.15), LG and LC (444.99) and between LG and TO (361.85). Whereas, lower Nm values were observed between VB and VP (21.22), VB and LG (40.00), TO and LC (42.14) and between VP and LC (44.79). The Neighbour-joining tree (Figure 2) (as UPGMA one (data not shown)) defined two groups. The first one constituted mountainous populations (VP, LG, LC) and the second one constituted the plain populations (VB, TO). This topology completely fitted the geographical pattern.

DISCUSSION

This study described mtDNA diversity in two Italian Veneto-speaking groups from Veneto: Posina and Barco. The two groups exhibited a quite similar distribution of haplotypes when haplogroup H (representing 40% of haplotypes from Barco and 42.1% of haplotypes from Posina) and haplogroup 2 or JT (31.6% of haplotypes from Barco and 33.3% of haplotypes from Posina) were considered. While in almost all Europeans, the frequencies of haplogroup H and haplogroup 2 or JT are in the ranges of 40–50% and 10–20% respectively, few European populations, communities or linguistic isolates show a higher value of the haplogroup 2

or JT frequency. Specifically, this haplogroup encompasses 63% of Val Gardena haplotypes and 51% of Middle Eastern population haplotypes, while it only represents 35.7% of the haplotypes in Fassa, 27% in Turkey, 25% in Tuscany and 24.9% in Badia. Samples from Ladin-speaking populations (Val Gardena, Colle Santa Lucia, Badia, Fassa) located in the North of the historical Venetia region in the southern Alps (Stenico et al. 1998), show similarity to the Posina and Barco samples. Other populations of northern Italy (Stenico et al. 1996) such as German-speakers (Hafling and Jenesian) and populations from Trento-Adige show a different pattern of mtDNA diversity.

These observations should reflect one of the two Paleolithic migrations from the Middle East, probably the one that occurred along the Mediterranean coast (Richards et al. 1996). The physical barrier formed by the Alps would have prevented people from migrating southward to Tuscany and then westward. Some people may have stayed in the mountainous area and become the founders of future Alpine populations. Nm values (calculated here from our two groups of Veneto-speakers, from Ladin-speakers (Stenico et al. 1996) and from the Tuscanian population (Francalacci et al. 1996)) (Table 8) sustained the hypothesis that it was not an isolation process for Alpine populations regarding plain populations, but a difference in the rate of the number of migrants between the two geographical areas. The beginning of genetic drift, suspected by the Fst, was due only to this phenomenon.

The Posina and Barco groups were distinguished by their haplogroup J and haplogroup T distributions. The Barco population, with a higher J value, is more like the neighbouring Tuscanians than Posina (16.6% in Barco, 16.6% in Tuscany and 5.3% in Posina). This differentiation is also shown by statistical analysis (Table 6a, b and Fig. 2). Haplogroup J represents about 7.1–11% of European haplotypes (Richards et al. 1998; Torroni et al. 1994, 1997), becoming more frequent in Central and West Europe. It was probably introduced into Europe from the Near East about 10000 years ago during the Neolithic

expansion. This Neolithic migration could have left tracks in the Barco and Tuscan groups more than in most alpine groups.

The T haplogroup is the major non-H haplogroup in the Posina population (26.3%), encompassing 16.7% of haplotypes from Barco. Haplogroup T may have come from the Near East, as had haplogroup J (Torroni et al. 1997; Richards et al. 1998). In Europe, haplogroup T exhibits a global frequency equal to 8%: in Spanish (including Catalonia and Andalusia) (Corte-Real et al. 1996; Pinto et al. 1996; Parsons et al. 1997) and Portuguese (Corte-Real et al. 1996) populations, the T haplogroup represents 7% and 11% respectively. In different Mediterranean populations (listed in Torroni et al. 1996), including Greeks, Albanians, Sardinians, Apulians, Sicilians, Arabs from Palestine and Jews, the T haplogroup has been spotted by RFLP analysis. None of the populations show more than 14.6% (in Sicilia) of T haplogroup.

From our data (Table 9) populations exhibiting a T haplogroup frequency equal to or higher than that of Middle Eastern populations (16.6%) are mainly situated in the Alpine area with the exception of Fassa, which is distinguished by a very low value. T haplogroup encompasses 53.8% of haplotypes from Colle Santa Lucia and Val Gardena populations (Ladin speakers) and only 16.7% of haplotypes from Barco. In the Posina population, nine out of ten (90%) of the T individuals have both mutations 16294T and 16296T, as noticed for the Val Gardena and Tuscan populations. These two mutations are considerably less frequent in T individuals from Barco (one out of five, 20%), and from Colle Santa Lucia (two out of seven, 27%). Therefore, the T haplogroup members of Posina, Val Gardena and Tuscany can be distinguished from the T haplogroup members from Barco and Colle Santa Lucia.

Despite this point, haplogroup composition of the Posinians in this study appeared to be different from that of the Tuscans, with no I or W haplogroups in the Posinians, and a small J haplogroup frequency (5.3% vs. 14.6% in Tuscans). Considering Barco and Posina mtDNA

Table 9. Distribution of JT, J and T haplogroups in European populations

		Haplogroup 2		
Population	n	or JT (%)	Haplogroup J (%)	Haplogroup T (%)
Colle Santa Lucia ^a	10	70	10	60
$Gardena^b$	16	63	3.8	50
Middle East ^c	42	51	19.4	16.6
Fassa ^b	14	35.7	28.5	7
Posina (this study)	38	31.5	5	26.5
Barco (this study)	30	30	17	16.6
$\mathrm{Turkey^{d-e}}$	74	27	16	9.3
Tuscany	49	25	12.5	10.2
Badia	13	24.9	7.7	15
$Germany^g$	107	21.5	nd	10.3
Bulgaria ^d	30	20	nd	10
Sardinia ^c	69	19	5.8	11.6
Great Britain ^{h-i}	106	18.8	11.8	6.9
Andalusia ^j	16	18.8	12.5	6.3
Portugal ^j	54	18.1	nd	10.9
$Russia^k$	117	17.9	6.8	8.5
$Estonia^k$	28	17.9	7	10.7
$\mathrm{Spain}^{\mathrm{j-l}}$	71	16	nd	8.5
$Austria^m$	16	12.5	12.5	0
$Switzerland^{h-n}$	77	11.7	9.1	2.6
$\operatorname{Finland}^{k-n}$	142	11	2	3.5
Basque Counrty ^{j-o}	106	7.5	nd	4.6
Catalunia ^j	15	6	nd	0
$Norway^{k-p}$	82	3.5	0	0
$Sweden^k$	25	0	0	0

Haplogroup JT is defined as Simoni et al. 2000. Haplogroup J and T are defined as Torroni et al. 1996. n means the numbers of individuals studied in each population. ^a From Stenico et al. 1996; ^b From Stenico et al. 1998; ^c From Di Rienzo et al. 1991; ^d From Calafell et al. 1996; ^e From Comas et al. 1996; ^f From Francalacci et al. 1996; ^g From Richards et al. 1996; ^h From Horai et al. 1990; ^f From Piercy et al. 1993; ^f From Corte-Real et al. 1996; ^g From Sajantila et al. 1996; ^f From Pinto et al. 1996; ^m From Handt et al. 1994; ^g From Piercy et al. 1995; ^g From Delghandi et al. 1998.

diversity, Veneto-speakers appear heterogeneous. MtDNA diversity in these two groups seems to depend more on the geographic distribution of the population than on their common dialect.

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