Frequency distribution analysis of a mtDNA pseudogene in some populations of Northern Italy

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Abstract

In this study, the frequency of this mtDNA pseudogene was analyzed in 236 individuals coming from 3 North-West Italy populations: Postua (n = 102), Cavaglià (n = 53) and Biella (n = 51). The insertion frequency and the heterozigosity values vary significantly among populations, with Postua showing the lower insertion frequency value (0.172, vs 0.225 for Cavaglià and 0.337 for Biella) and the lower heterozigosity value (0.284±0.006, vs 0.349±0.007 for Cavaglià and 0.447±0.001 for Biella). The F_{ST} value was 0.036. Insertion frequency and heterozigosity values calculated for all the subjects were 0.245 and 0.360 respectively. These values are lower than those observed in the German population (0.54 and 0.50 respectively), the only European population for which data are available in literature, and similar to some African populations. These results could explain the position of the studied populations in the Neighbor-Joining tree obtained for some worldwide populations.

Introduction

Recently, whole-genome computational approaches have revealed thousands of pseudogenes in the genomes of the human and other eukaryotes (Zhang and Gerstein, 2004). Pseudogenes are non-functional copies of coding sequences present in the genomes. Insertion of some pseudogenes could have a deleterious effect on normal function of other genes and could be rapidly selected against and lost. Many other pseudogenes persist and evolve with time. Zischler et coll. (1995) discovered a human specific mtDNA segment of 540 bp (mtDNA pseudogene or Numt) that had been inserted into the human nuclear genome in the past and that shows a presence-absence dimorphism. The low frequency of the insertion in African populations and the pattern of increasing frequency away from Africa (Thomas et al. 1996) most closely resembles the ancestral state of the dimorphism (absence of insertion) and suggests that the insertion arose in Africa, as has been found for other insertion polymorphisms. The increased frequency in other areas may have occurred through genetic drift, as humans migrated out of Africa, or perhaps through selection involving a closely linked gene.

There are not studies in literature about Italian populations using this pseudogene insertion dimorphisms. In the present study, we analyze the frequency of the Numt insertion dimorphisms in the nuclear genome of three North-West Italian populations. The aim of our study was to gather data about frequency of this pseudogene in the Italian population.

Material and methods

In this study, a sample of 236 individuals was analysed: 102 came from Postua, a small village (500 inhabitants) characterised by partial isolation due to its geographic position; 83 from Biella, a small manufacturing town (47,000 inhabitants) and 51 from Cavaglià (3,680 inhabitants), an agricultural village near Postua. The Postua group consisted of individuals whose parents and the four grandparents were born in Postua. The samples of Biella and Cavaglià came from blood donors born in these places. All individuals under study had given informed consent. Peripheral blood samples (5-10 ml venipuncture) were collected and stored heparinized at –20°C. To extract DNA we used Chelex· protocol as described by Walsh et al. (1991). PCR reactions were carried out according to Thomas et al. (1996). The NEIGHBOR program was used to construct a neighbor-joining tree (Saitou and Nei, 1987) from a matrix distance.

Result and discussion

The three possible genotypes of the mtDNA insertion dimorphism, are shown in figure 1.

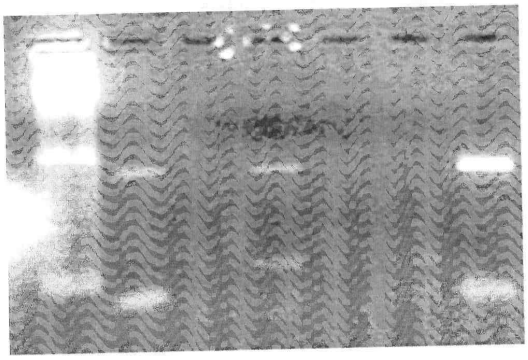

Fig. 1 – Agarose gel electrophoresis illustrating the mtDNA insertion dimorphism.

Alleles containing the insertion yield a 684-bp product, while alleles lacking the insertion produced a 150-bp product, respectively. Homozygotes for the insertion (+/+) show a single band at 684-bp; homozygotes for lack of the insertion (-/-) show a single band at 150-bp; heterozygotes (+/-) show a band at 684-bp and a band at 150-bp. Observed genotypes and allele frequencies for each population are given in Table 1.

		Genotype (N)			Insertion	
Population	**N**	**-/-**	**+/-**	**+/+**	**Frequency**	**Heterozigosity ± SE**
Postua	102	79	11	12	0.172	0.284±0.006
Cavaglià	51	36	7	8	0.225	0.349±0.007
Biella	83	40	30	13	0.337	0.447±0.001
TOTAL	236	155	48	33	0.245	0.360±0.004

F_{ST} = 0.036
SE = Standard Error

Tab. 1- Distribution of the Mitochondrial Insertion Polymorphism

The insertion frequency and the heterozigosity values vary significantly among populations, with Postua showing the lower insertion frequency value (0.172, vs 0.225 of Cavaglià and 0.337 of Biella) and the lower heterozigosity value (0.284±0.006, vs 0.349±0.007 of Cavaglià and 0.447±0.001 of Biella). Insertion frequency and heterozigosity values calculated for all the subjects were 0.245 and 0.360, respectively. These values are lower than those observed in the German population (0.54 and 0.50 respectively; Thomas et al., 1996), the only European population for which data are available in literature, and similar to some African populations (Thomas et al., 1996). These results could explain the position of the studied populations in the Neighbor-Joining tree obtained from the insertion frequencies of this mtDNA pseudogene for some worldwide populations (Thomas et al., 1996) (Figure 2).

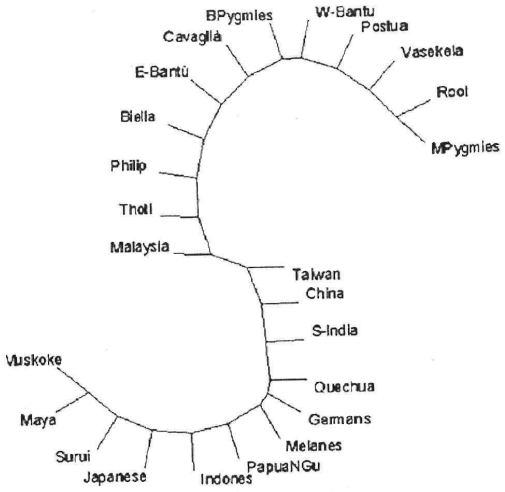

Fig. 2 - Neighbor-Joining tree of population relationships.

The F_{ST} value was 0.036, which could mean that 3.6% of the total variance in allelic frequency differences at this locus was due to differences between populations and 96.4% was due to differences within populations. χ^2 test showed that Postua and Cavaglià deviated significantly from the expected Hardy-Weinberg equilibrium, with an excess of homozygous for the insertion.
In conclusion, with this work we provide data about frequencies of Numt insertion dimorphism in some Italian populations. This marker, along with other insertion polymorphisms, could represent an useful tool for elucidating human populations origins. It expands the growing list of molecular markers that allow to take full advantage for studying human genomic variation.

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