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# Y Chromosome Binary Markers to Study the High Prevalence of Males in Sardinian Centenarians and the Genetic Structure of the Sardinian Population

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**Key Words**
Y chromosome · Centenarians · Longevity · Sardinia · Isolated population

**Abstract**
We have analyzed a sample of 40 centenarians and 116 young controls from Sardinia, with a set of new Y chromosome binary markers, to evaluate if Y chromosome genes are involved in the high prevalence of males among centenarian Sardinians (1/2 vs. 1/4 in other populations studied). The results indicate that none of the seven lineages that account for >97% of the Y chromosome diversity in Sardinia provide an advantage with respect to the extreme longevity. However, our results, although based on the male-specific Y chromosome polymorphisms, give a clear profile of the pattern of genetic variability in Sardinia. Indeed they indicate that the Sardinian population had two main founder populations that have evolved in isolation for at least the last 5,000 years. These findings set the stage for future studies on longevity and other complex traits in Sardinia.



## Introduction

Centenarians are recognized as the best model to study the determinants of physiological aging and longevity in humans [1]. Indeed, a still unexplained gender difference exists in centenarians, where females greatly outnumber males. A recent survey reported that Sardinian centenarians display a male/female ratio of 1/2 [2] instead of the most common 1/4 ratio [3], a result which has been recently validated by an ad hoc European Committee [M. Poulain, pers. commun.]. In addition, the overall prevalence of centenarians in Sardinia turned out to be higher with respect to continental Italy [2]. Environmental and genetic factors may contribute to such a high prevalence of males among Sardinian centenarians.

As to the possible genetic causes, the Y chromosome attracted our attention for three main reasons. First, the Y chromosome (the AZF a–c regions), strongly influences the development of testes and fertility in mammalian males [4]. In fact, a trade-off between fertility and longevity has been predicted by evolutionary theories on aging, and it has recently been confirmed by data on both humans and model organisms [5, 6].

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Second, the Y chromosome is transmitted from father to son without recombination. Thus, any mutation or chromosomal rearrangement affecting longevity (or fertility) present on a Y chromosome will be transmitted as an intact haplotype. For instance, it was shown that a Y chromosome haplotype common among Japanese conferred a disadvantage for fathering children, being in linkage disequilibrium with some mutations affecting spermatogenic ability, although no candidate mechanism was proposed [7].

Third, after many years when the Y chromosome was thought to harbor few polymorphisms, a large set of new Y chromosome binary markers has recently become available as a result of the application of the denaturing high performance liquid chromatography technique [8, 9]. These markers were shown to be capable of distinguishing shared ancestry and geographic affiliation, and provide new opportunities to assess epidemiological phenomena. Indeed, because of their often characteristic regional specificity, they are probably the best tools to give a summary of the components which are present in a population [10]. Intriguingly, in a study on European populations, a lineage almost completely absent elsewhere was found at a frequency of 35% in Sardinia [11].

In this paper a subset of new Y chromosome binary markers was used to study a sample of male centenarians and a control sample of younger males from Sardinia to verify if any of the Sardinian Y chromosome lineages were advantageous as far as the longevity phenotype was concerned. Besides contributing to studies on the genetics of human longevity, this approach allowed us to further characterize the genetic structure of the population of this Mediterranean Island which is different from all the surrounding geographic areas [12]. In fact, the peculiar frequencies of most studied polymorphisms that have resulted from its long isolation have prevented a clear assessment of the origin and the number of the founder populations, which is critical in epidemiological studies on complex genetic traits [13].

## Materials and Methods

The centenarian sample was composed of 40 subjects, i.e. all the centenarian males who were traced and interviewed on the occasion of a representative study performed on the whole of Sardinia (about 1,600,000 inhabitants) in 1997 [2]. This sample represents more than 50% of the centenarians living on the island at the time of the study [2]. The controls were 116 males collected throughout Sardinia whose ages were between 20 and 60. The polymorphisms studied were: M9, M13, M26, M35, M70, M89, M124, M170, M172, M173, M201, YAP [14], RPS4YC711T [15]. With the exception of YAP, which is due to an *Alu* sequence insertion, all the polymorphisms are single-base substitutions. The phylogenetic relationships of these Y chromosome polymorphisms with other 155 world wide Y polymorphisms, as well as marker details are described by Semino et al. [11] and Underhill et al. [16]. The statistical comparison between cases and controls was performed with the $\chi^2$ test and the AMOVA program, which is encompassed in the Arlequin package (http://lgb.unige.ch/arlequin/index.php3). In the AMOVA analysis, molecular distances are not counted.

## Results and Discussion

We have analyzed 13 Y chromosome binary markers in a sample of Sardinian centenarians and geographically matched younger controls. On the basis of the polymorphisms surveyed, 11 haplotypes were constructed. The phylogenetic relationships among the haplotypes we found are shown in figure 1. They represent a subset of the haplotypes found across Europe [11].

Table 1 reports the frequency of the Y chromosome haplotypes found in our survey. With the exception of four control samples that exhibited lineages found at low frequency across Europe, seven Y chromosome haplotypes accounted for the totality of both samples. These lineages also characterize, in different proportions most of the Y chromosome variation in Western European areas, namely in Spain, France, Italy [11]. Haplotype Eu18 is also very frequent in North West Europe (The Netherlands and Germany), while haplotypes Eu4, Eu9, Eu10 and Eu11 are common throughout the Mediterranean. Neither the $\chi^2$ test nor the AMOVA showed any differences between the two samples. No differences were observed even after dividing the samples according to geographic origin (North and South Sardinia). Thus, our data indicate that none of the major Western European Y chromosome types confer any advantage with respect to longevity. It cannot be excluded, however, that other lineages (especially those which have been associated with low fertility [7]) may play a role in the fertility-longevity trade-off. If true, then this result should help in better understanding some results regarding the heritability estimates for life span in European families [17, 18], although, it is worth noting that three other distinct lineages account for most of the Y chromosome variation spread in Eastern Europe (namely Tat, M17, and a different lineage of M170 [11]).

The lack of correlation between Y chromosome and longevity indicates that the high incidence of males among Sardinian centenarians is rather due to genes which are shared with women (autosomal, X chromo-

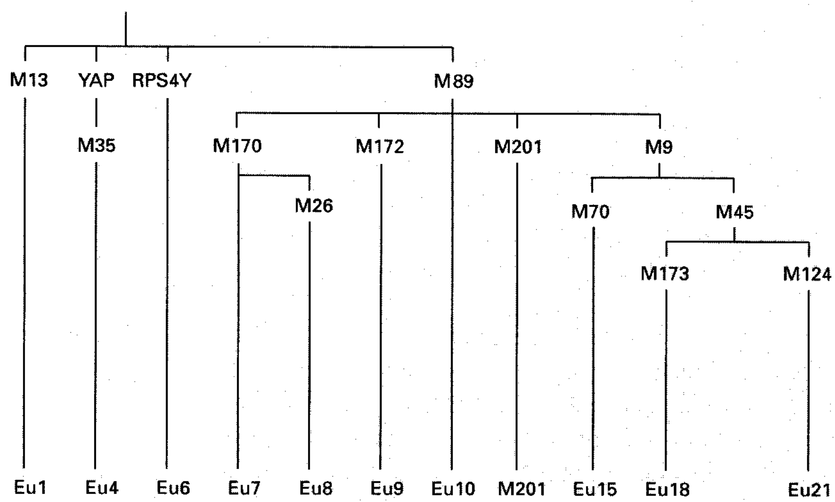


**Fig. 1.** Parsimony tree of the Y chromosome markers studied in the Sardinian samples. The names of the haplotypes are according to Semino et al. [11].

**Table 1.** Y Chromosome haplotypes found in centenarian and younger controls in Sardinia and their relative frequencies

| | Eu1 | Eu4 | Eu6 | Eu7 | Eu8 | Eu9 | Eu10 | Eu11 | Eu15 | Eu18 | Eu21 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Centenarians (n = 40) | | 7.5 | | 5.0 | 32.5 | 5.0 | 10.0 | 15.0 | | 25.0 | |
| Controls (n = 116) | 0.9 | 9.5 | 0.9 | 3.4 | 35.3 | 5.2 | 6.9 | 14.7 | 0.9 | 21.5 | 0.9 |

Haplotypes were numbered according to a large study of Y chromosome variation in Europe [11]. Markers associated with each haplotype are as follows: Eu1: presence of M13. Eu4: Yap+, M35. Eu6: RPS4YC711T. Eu7: M89, M170. Eu8: M89, M170, M26. Eu9: M89, M172. Eu10: M89. Eu 11: M89, M201. Eu15: M89, M9, M70. Eu18: M89, M9, M173. Eu21: M89, M9, M124.

some or mtDNA), but which are probably more effective in males. This is in agreement with previous studies on continental Italy showing that the tyrosine hydroxylase gene (THO) and mitochondrial DNA are associated with longevity in males but not in females [19, 20], suggesting that the two genders likely require different genetic components to attain longevity [3]. Finally, it is possible that a strong environmental influence related to the specific lifestyle of the Sardinian population in the last century could play a major role.

Although the seven haplotypes accounting for most of the Sardinian samples also characterize other areas of Western Europe, their frequencies in Sardinia are very unusual. In particular, while common in Sardinia, haplotype Eu8 is present outside Sardinia only among Basques at a 2% frequency [11]. However, the phylogeographic approach allows to infer the origin of Y chromosome and mitochondrial DNA lineages beyond their frequencies [21]. The data regarding haplotypes Eu7, Eu8 and Eu18 indicate that more than 60% of the present Sardinian Y chromosomes descend from the Paleolithic population of Iberia, where these haplotypes show their maximum antiquity and variability [11]. This population spread all over Europe between 13,000 and 9,000 years ago [11, 12, 22]. The remaining haplotypes, Eu 4, Eu 9, Eu 10 and Eu11, are likely to have been brought from the Middle East by Neolithic migrations which spread these alleles throughout Southern Europe between 9,000 and 5,000 years ago [11, 12]. Thereafter (about 200 generations) isolation and consequent drift shaped the frequencies. On the other hand, Y chromosome polymorphisms have proved to provide a good metaphor of the history of human populations. Indeed the genetic structure of Europe deduced from data on Y chromosome polymorphisms well matched previous data obtained with 95 different genes. In addition they were able to describe components of the European populations not described before [11, 12]. This is extremely important in studies of isolated populations like that of Sardinia where, because of very unusual frequencies of virtually all studied polymorphisms, it is very hard to understand the origin and prevalence of the different components [10, 11]. It is then likely that our results on the genetic structure of the Sardinian population, although based only on the analysis of the paternally transmitted Y chromosome, is actually providing an index of the overall genetic structure of the Sardinian population. It will no doubt contribute to clarify the different founder components that make up the Sardinian gene pool. This perspective can be helpful in future epidemiological studies in Sardinia on complex traits, such as longevity, and linkage disequilibrium [13].

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