Mitochondrial DNA inherited variants are associated with successful aging and longevity in humans

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ABSTRACT **Mitochondrial DNA (mtDNA) is characterized by high variability, maternal inheritance, and absence of recombination. Studies of human populations have revealed ancestral associated polymorphisms whose combination defines groups of mtDNA types (haplogroups) that are currently used to reconstruct human evolution lineages. We used such inherited mtDNA markers to compare mtDNA population pools between a sample of individuals selected for successful aging and longevity (212 subjects older than 100 years and in good clinical condition) and a sample of 275 younger individuals (median age 38 years) carefully matched as to sex and geographic origin (northern and southern Italy). All nine haplogroups that are typical of Europeans were found in both samples, but male centenarians emerged in northern Italy as a particular sample: *1)* mtDNA haplogroup frequency distribution was different between centenarians and younger individuals ($P=0.017$ by permutation tests); and *2)* the frequency of the J haplogroup was notably higher in centenarians than in younger individuals ($P=0.0052$ by Fisher exact test). Since haplogroups are defined on the basis of inherited variants, these data show that mtDNA inherited variability could play a role in successful aging and longevity.—De Benedictis, G., Rose, G., Carrieri, G., De Luca, M., Falcone, E., Passarino, G., Bonafé, M., Monti, D., Baggio, G., Bertolini, S., Mari, D., Mattace, R., Franceschi, C. Mitochondrial DNA inherited variants are associated with successful aging and longevity in humans. *FASEB J.* 13, 1532–1536 (1999)**

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SUCCESSFUL AGING AND LONGEVITY are the result of the interaction between a variety of genes, environmental conditions, and lifestyles. Because of their critical importance for energy production, mitochondria have attracted the attention of scientists interested in unraveling the complex changes associated with aging and age-related diseases (1). Indeed, available data clearly indicate that mutations and deletions accumulate with age in mitochondrial DNA (mtDNA)[2] derived from several tissues, such as brain and muscle (2–5). The hypothesis supporting these investigations predicts that eventually these alterations impair oxidative phosphorylation (OXPHOS) and energy production below a threshold level incompatible with normal cell functions (6). MtDNA somatic mutations in senescence have been investigated extensively, but a possible role of mtDNA inherited variability on successful aging has not been explored despite some observations suggesting that longevity descends chiefly along the maternal line (7), like the mitochondrial genome (8). We could speculate that long-lived individuals have inherited from their mothers mtDNA types that protect mitochondrial functions from damage caused by oxygen free radical production (9), thus giving them a small but continuous advantage that increases the probability of successful aging and longevity. This model is in agreement with the nonadditive variance observed in human longevity inheritance, which presupposes epistatic interaction among loci (10) that clearly recalls nuclear–mitochondrial genome interactions (11).

Recently the hypothesis that mtDNA inherited variability plays a role in longevity has been investigated by sequencing the entire mtDNA in 11 centenarians from Japan (12). Three associated mutations (Mt5178A, Mt8414T, Mt3010A) have been found at higher frequency in centenarians than in 43 controls. One of these mutations (Mt5178A) has also been screened by restriction analysis in an additional

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[2] Abbreviations: mtDNA, mitochondrial DNA; OXPHOS, oxidative phosphorylation;

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TABLE 1. *Identification of mtDNA haplogroups by restriction analysis of the specific target sequence*

	Polymorphic restriction sites[a]									
Haplogroup	1715 *Dde*I	4577 *Nla*III	7025 *Alu*I	8249 *Ava*II	9052 *Hae*II	10394 *Dde*I	12308 *Hin*fI[b]	13366 *Bam*HI	16065 *Hin*fI	16389 *Bam*HI
H			0			0				
I	0		1	1		1				1
J			1			1			0	
K			1		0	1	1			
T			1			0		1		
U			1			0	1			
V		0	1			0				
W			1	1		0				
X	0		1			0				

[a] Sites are numbered from the first nucleotide of the restriction site according to ref. 17. Presence/absence of the site is indicated as 1/0.
[b] Restriction site created by mismatched primers (16).

37 centenarians and in 252 controls. However, longevity is strongly affected by environmental factors as well as by the entire genetic background. Different populations are therefore expected to have different gene/longevity associations. On the other hand, sequence analysis of the whole mtDNA is very expensive and time-consuming, and it is unfeasible for large size samples from various populations to be analyzed by this approach.

The novelty of our approach is the use of stable, ancestral, and associated polymorphisms, which are currently used in studies of human evolution, as markers of mtDNA inherited variability. Restriction fragment-length polymorphism studies of mtDNA from a wide range of human populations have revealed sets of ancestral mutations that define groups of mtDNA types (haplogroups) that have common ancestry and, because of uniparental inheritance, evolve independently from each other (13–15). Each of these haplogroups, which includes evolutionary related types of mtDNAs, is defined by specific sets of associated mutations, thus allowing for a quick and precise classification of the mtDNA molecules within a certain population. Nine mtDNA haplogroups (H, I, J, K, T, U, V, W, and X) have been identified that are characteristic of Europeans and encompass virtually all mtDNAs in Europe (16). We used these haplogroups as mtDNA stable markers to explore the role of mtDNA inherited variability on successful aging and longevity in Europeans.

MATERIALS AND METHODS

Samples

A total of 487 unrelated subjects gathered in both northern and southern Italy were studied. The origin of each individual in the specific geographic area up to the maternal grandmother was ascertained by interview. Control groups (20–75 years age range, median age 38 years) were made up of 125 individuals (74 females and 51 males) in northern Italy (Lombardia, Liguria, Veneto, Emilia) and by 150 individuals (76 females and 74 males) in southern Italy (Calabria, Campania, Puglia, Sicilia). The groups of centenarians included only subjects over 100 years old on the day of the blood collection (109 years maximum age) and was made up of 109 subjects (83 females and 26 males) in northern Italy and 103 subjects (67 females and 36 males) in southern Italy. Both groups were composed of people from various social-economic extraction. All the subjects included in the study were free of clinically manifest pathologies. In particular, the centenarians were self-sufficient, had quite an active lifestyle, and, on the whole, were in a fairly good health status. All the subjects had been invited to participate in the study by an appropriate campaign and had given their informed consent.

MtDNA analysis

DNA was extracted from blood buffy coats following standard procedures. Haplogroups typing was carried out by restriction analyses of mtDNA according to reference (16). For each individual, mtDNA fragments encompassing the relevant polymorphic site (**Table 1**) were amplified by polymerase chain reaction. Primers and amplification conditions are given in **Table 2**. The amplified fragments were then digested with the appropriate enzyme (Table 1) and separated by a 2% agarose gel electrophoresis. The combination of the results obtained at the different polymorphic sites allowed the mtDNA of the subject to be classified in a certain haplogroup.

Statistical analysis

Permutation tests were used to verify whether the overall distribution of haplogroup frequencies was different between groups (18). For each test, the significance value was computed using 10,000 random permutations of the total set of haplogroups.

Fisher exact tests were used to verify whether the frequency of a specific haplogroup was different between sex-matched centenarians and controls. In each comparison, the level of significance was reduced to $\alpha = 1-0.95^{1/9} = 0.0057$ (nine independent variables).

RESULTS

Screening for mtDNA haplogroups (Table 1) was carried out in 487 individuals, made up of a group of

TABLE 2. *Primers and conditions used for mtDNA amplification*[a]

Primers		Amplification conditions		
Forward	Reverse	Denaturation	Annealing	Polymerization
1561–1581	3717–3701	93°C for 30	51°C for 30	72°C for 60
3951–3970	5917–5890	93°C for 30	61°C for 30	72°C for 60
5720–5738	7608–7588	93°C for 30	57°C for 30	72°C for 60
7392–7410	8628–8608	93°C for 30	57°C for 30	72°C for 60
8232–8305	10107–10088	93°C for 30	57°C for 30	72°C for 60
9911–9932	11873–11851	93°C for 30	69°C for 30	72°C for 60
11711–11727	14208–14190	93°C for 30	49°C for 30	72°C for 60
15553–15569	725–706	93°C for 30	45°C for 30	68°C for 60

[a] The primer coordinates are given according to the Anderson sequence (17). Amplifications were performed in a 9600 Perkin Elmer apparatus.

275 people aged from 20 to 75 years (median age 38 years), and a group of 212 centenarians (100–109 years), all selected for their good clinical health status. As expected, all nine haplogroups characteristic of Europeans were found in both samples. They encompassed 94% and 90% of the mtDNAs in centenarians and younger people, respectively. The remaining mtDNAs were not attributable to any one of the above haplogroups and were grouped as 'others', as also found by previous studies (16). These results confirm and extend, over a large group of Italians, the data on mtDNA haplogroups in Europeans.

MtDNA haplogroups observed in northern Italy are shown in **Table 3**. The overall frequency distribution was significantly different between male centenarians and male controls (P=0.017). The frequencies of each of the nine haplogroups observed in the two groups were then compared. We found that the frequency of the J haplogroup increased from 2% in controls to 23% in centenarians (P=0.0052), whereas that of the U haplogroup decreased from 23,5% in controls to ~4% in centenarians (P=0.050). Since we had a separate interest in each haplogroup, we tested the nine null hypotheses separately: therefore, the correction for multiple tests should not be required in the present case (19). At any rate, the increase of J in centenarians would remain significant even if the level of significance were reduced to $\alpha = 1-0.95^{1/9} = 0.0057$. In females, the overall frequency distribution was not statistically different between centenarians and controls (P=0.670). However, in this case, too, the J haplogroup showed a tendency to increase (and the U haplogroup to decrease) from controls to centenarians (see Table 3).

MtDNA haplogroups observed in southern Italy are shown in **Table 4**. The overall frequency distributions were not significantly different between centenarians and controls either in males (P=0.980) or in females (P=0.273). Furthermore, no association was evident between longevity and specific mtDNA haplogroups.

TABLE 3. *MtDNA haplogroups observed in samples of northern Italians of different ages*[a]

	Females				Males			
	Controls (n = 74)		Centenarians (n = 83)		Controls (n = 51)		Centenarians (n = 26)	
Haplogroups	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.
H	27	365 (56)	28	337 (52)	24	471 (70)	13	500 (98)
I	3	41 (23)	2	24 (17)	—	—	—	—
J	5	68 (29)	11	133 (37)	1	20 (19)	6	231 (83)
K	4	54 (26)	4	48 (24)	2	39 (27)	2	77 (52)
T	9	122 (38)	12	145 (39)	4	78 (38)	—	—
U	15	203 (47)	12	145 (39)	12	235 (58)	1	38 (38)
V	—	—	4	48 (24)	1	20 (19)	1	38 (38)
W	2	27 (19)	2	24 (17)	2	39 (27)	—	—
X	2	27 (19)	2	24 (17)	1	20 (19)	1	38 (38)
Others	7	95 (34)	6	72 (28)	4	78 (38)	2	77 (52)
	P = 0.670				P = 0.017			

[a] Controls: 20- to 75-year-old individuals; median age of males 44 years, median age of females 39 years. Centenarians: individuals over 100 years old. Abs. and Rel. indicate absolute and relative (×1000) haplogroup frequencies with standard error in parentheses. P values refer to heterogeneity tests between controls and centenarians performed by permutations (18).

TABLE 4. *MtDNA haplogroups observed in samples of southern Italians of different ages*[a]

	Females				Males			
	Controls (n = 76)		Centenarians (n = 67)		Controls (n = 74)		Centenarians (n = 36)	
Haplogroups	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.
H	26	342 (54)	29	433 (61)	31	419 (57)	15	417 (82)
I	1	13 (13)	3	45 (25)	3	41 (23)	3	83 (46)
J	2	26 (18)	4	60 (29)	5	68 (29)	2	56 (38)
K	9	118 (37)	5	75 (32)	6	81 (32)	4	111 (52)
T	9	118 (37)	4	60 (29)	7	95 (34)	2	56 (38)
U	8	105 (35)	8	119 (40)	11	149 (41)	5	139 (58)
V	3	39 (22)	5	75 (32)	1	14 (14)	1	28 (27)
W	4	53 (26)	1	15 (15)	2	27 (19)	—	—
X	2	26 (18)	4	60 (29)	3	41 (23)	1	28 (27)
Others	12	158 (42)	4	60 (29)	5	68 (29)	3	83 (46)
	P = 0.273				P = 0.980			

[a] Controls: 20- to 75-year-old individuals; median age of males 34 years, median age of females 33 years. Centenarians: individuals over 100 years old. Abs. and Rel. indicate absolute and relative (×1000) haplogroup frequencies with standard error in parentheses. P values refer to heterogeneity tests between controls and centenarians performed by permutations (18).

DISCUSSION

We wanted to test whether the analysis of mtDNA haplogroups is able to reveal any association between mtDNA inherited variability and longevity. The approach was a comparative analysis between the mtDNA pool of healthy centenarians and that of younger people matched for sex and geographic area.

Let us consider the results obtained in northern Italian males. Not only was the overall frequency distribution different between cases and controls, but the frequency of a specific haplogroup (J) was notably higher in centenarians than in controls, although both groups had been sampled from the same population. Since case/control matching had been carefully checked (the ancestry of both cases and controls in the specific geographic area had been verified as far as maternal grandmothers), bias caused by long-range mobility occurring in Italy in the last century could be excluded. Likewise, since both centenarians and controls had been collected in very large geographic areas of northern Italy, effects caused by genetic drift could be excluded too. The existence of a true positive association between inherited mtDNA variants and long life expectancy is therefore likely in males of this geographic area.

This finding raised two questions: *1)* Why were these phenomena not observed in females? *2)* Why were these phenomena not observed in southern Italy?

As to the first question, different findings in males and females have already been observed in gene/longevity association studies (20, 21). This is not unexpected if we consider that longevity is a multifactorial trait where the phenotypic effect of a certain gene depends on the physiological background in which the gene is expressed. Since the age-related physiological scenario changes in males and females differently, the effect of mtDNA variability on successful aging and longevity could vary between sexes. On the other hand, life expectancy is significantly higher in females than in males (gender effect). It may be that in order to attain longevity, males more than females need particularly protective mtDNA types.

As for the second question, consider that haplogroups are categories defined on the basis of ancient mutations shared among different haplotypes. Therefore, each haplogroup encompasses many haplotypes, and its composition is defined by the history of the population (22). The different results obtained in northern and southern Italy are therefore not unexpected if we consider that northern and southern Italian populations have different origins, as well documented by both historical and genetic studies (23). In any case, longevity could be attained through various strategies, according to various gene pools and environments. The latter consideration emphasizes the importance of having rapid and cheap genetic markers, such as haplogroups, to test the role of mtDNA on longevity in various populations from various geographic areas. In this regard, it should be noted that the Mt5178A mutation found in Japanese centenarians (12) is included in the M haplogroup, extremely frequent in Asia but virtually absent in Europe (22).

These data show that, at least in males from a well-defined geographic area, not only the haplogroup frequency distribution is different between centenarians and younger people, but also a specific haplogroup (J) is significantly more frequent in

individuals selected for longevity. We could speculate that the J haplogroup includes a mtDNA type (present in northern Italy only) carrying mutations that improve the starting OXPHOS levels, thus delaying age-related mitochondrial decline or lowering the frequency of age-related diseases, such as dementia, Parkinson, and cancer, where reactive oxygen species and mitochondria dysfunction may play a role (6). Indeed, the centenarians tested in our study (as well as the controls) were clinically healthy, had quite an active lifestyle, and had escaped the abovementioned age-associated diseases. The finding that J accounts for only a small proportion of the centenarian sample is not in disagreement with a possible role played by mtDNA in longevity. Since longevity is a complex trait, a number of loci other than mtDNA, with epistatic and pleiotropic effects, are likely to be responsible for this phenotype (24).

In conclusion, the data reported here confirm in a European population the mtDNA/longevity association observed in the Japanese (12). The implication of these results can be far-reaching, taking into account the important role of the mitochondria in the aging process and in the pathogenesis of age-related diseases. FJ

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