Mitochondrial haplogroups associated with elite Japanese athlete status

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ABSTRACT

Purpose It has been hypothesised that certain mitochondrial haplogroups, which are defined by the presence of a characteristic cluster of tightly linked mitochondrial DNA polymorphisms, would be associated with elite Japanese athlete status. To examine this hypothesis, the frequencies of mitochondrial haplogroups found in elite Japanese athletes were compared with those in the general Japanese population.

Methods Subjects comprised 139 Olympic athletes (79 endurance/middle-power athletes (EMA), 60 sprint/power athletes (SPA)) and 672 controls (CON). Two mitochondrial DNA fragments containing the hypervariable sequence I (m16024–m16383) of the major non-coding region and the polymorphic site at m.5178C>A within the NADH dehydrogenase subunit 2 gene were sequenced, and subjects were classified into 12 major mitochondrial haplogroups (ie, F, B, A, N9a, N9b, M7a, M7b, M*, G2, G1, D5 or D4). The mitochondrial haplogroup frequency differences among EMA, SPA and CON were then examined.

Results EMA showed an excess of haplogroup G1 (OR 2.52, 95% CI 1.05 to 6.02, p=0.032), with 8.9% compared with 3.7% in CON, whereas SPA displayed a greater proportion of haplogroup F (OR 2.79, 95% CI 1.28 to 6.07, p=0.007), with 15.0% compared with 6.0% in CON.

Conclusions The results suggest that mitochondrial haplogroups G1 and F are associated with elite EMA and SPA status in Japanese athletes, respectively.

Elite athletic performance is a complex, multifactorial phenotype. A number of familial studies have assessed the relative contribution of genetic and environmental factors to physical performance-related traits and estimated that there is a significant genetic component to phenotypes such as maximal oxygen uptake ($\dot{V}O_2max$)[1 2] and muscle strength.[3 4] Therefore, it is commonly believed that genetic factors are likely determinants of elite athlete status, which may require a synergy of advantageous physical performance phenotypes. Over 200 genes, in both the nuclear and mitochondrial genomes, have recently been suggested to have an effect on physical performance and health-related fitness.[5]

Mitochondria are essential to all higher organisms for sustaining life, and are extremely important in energy metabolism, providing 36 molecules of ATP per glucose molecule in contrast to the two ATP molecules produced by glycolysis. Although most DNA is packaged in chromosomes within the nucleus, mitochondria also possess their own circular DNA: mitochondrial DNA (mtDNA). The 16 569-bp human mtDNA contains 13 genes for mitochondrial oxidative phosphorylation (OXPHOS), as well as two ribosomal RNA and 22 transfer RNA genes that are necessary for protein synthesis within mitochondria.[6] Unlike nuclear DNA, mtDNA is inherited maternally. Interestingly, in familial studies, aerobic capacity has been found to have stronger maternal inheritance than paternal.[7 8] It has also been reported that mtDNA polymorphisms can influence the interindividual variations in aerobic capacity and its trainability.[9 10] Furthermore, patients with mutations in mtDNA commonly present with exercise intolerance, muscle weakness and increased production of lactate.[11] It is clear, therefore, that mtDNA polymorphisms represent a promising candidate to contain variants influencing physical performance.

The matrilineal inheritance of mtDNA and linear accumulation of polymorphisms has allowed the construction of detailed mtDNA phylogenies.[12] These phylogenies display the variation and diversity of human mtDNA and allow haplogroup identification through the analysis of a small number of haplogroup-specific polymorphisms. This matrilineal pattern of descent means that individual haplotypes share linked complexes of polymorphisms common to all sequences in a haplogroup. We previously reported that elite Kenyan endurance athletes differed in their mitochondrial haplogroup distribution compared with the general Kenyan population,[13] although this was not the case in elite Ethiopian endurance athletes.[14] Mitochondrial haplogroups have previously been associated with the elite endurance athlete status[15 16] and $\dot{V}O_2max$[17] in Europeans. Mitochondrial haplogroup distributions display geographical diversity, and certain haplogroups can be regionally specific. Indeed, almost all mitochondrial haplogroups in Africans and Europeans are not present in Asians, including Japanese.

There are highly diverse mtDNA sequences in Africans because of a time depth for mtDNA lineages of approximately 200 000 years.[18] Although mtDNA lineages in east Asians are shorter than in Africans (approximately 30 000–55 000 years), they also have a relatively long history. Japanese people are descendents of immigrants from the northern route, who had adapted to cold climates and/or famine, and those from the southern route.[19] Therefore, acquired mtDNA variations may have permitted the founding populations of modern Japanese individuals to adapt to extreme environmental conditions such as cold climates

and/or famine, and consequently influencing modern human phenotypes related to altered bioenergetics or mitochondrial function.[20] Indeed, we previously reported that certain mitochondrial haplogroups were associated with metabolic disorders closely related to mitochondrial function, such as obesity,[21] type 2 diabetes mellitus,[22–24] metabolic syndrome[22 25] and myocardial infarction[26] in Japanese individuals. Therefore, here we hypothesise that mitochondrial genetic variation in Japanese people may also associate with exercise capacity, because certain mitochondrial haplogroups are related not only to mitochondrial dysfunction, but also to enhanced mitochondrial function. Given elite athletes are a select population with greater exercise capacity, it is of interest to study the genetic determinants of their superior exercise performance. In the present study, we assessed the mitochondrial haplogroup frequencies of elite Japanese athletes compared with the general Japanese population in order to determine the influence of mitochondrial haplogroups on elite Japanese athlete status.

METHODS

Subjects

The subjects consisted of 141 elite Japanese athletes (110 men and 31 women). All athletes had represented Japan at the Olympic Games. A total of 141 athletes was classified as endurance/middle-power athletes (EMA, 81) and sprint/power athletes (SPA, 60) based on the criteria of Yang *et al*.[27] The EMA group included 13 endurance runners competing in events of 800 m or more, 10 sailing athletes, seven swimmers competing in events of 200 m or more, seven rowers, five long-distance cyclists, seven canoeists, nine volleyball players, six basketball players, six hockey players, four soccer players, four water polo players, two boxers and one modern pentathlete. The SPA comprised 18 track and field athletes (seven sprinters competing in events of ≤400 m, six jumpers and five throwers), nine swimmers competing in events of 100 m or less, seven gymnasts, seven competitive fencers, six divers, five wrestlers, four weightlifters, two short-distance track cyclists and two judo athletes. Mitochondrial haplogroup frequencies determined in 672 general Japanese populations (387 men and 285 women) from our Human Mitochondrial Genome Single Nucleotide Polymorphism Database (http://mtsnp.tmig.or.jp/mtsnp/index_e.shtml) represented the control group (CON).[19] Written consent was obtained from each subject, and the study was approved by the Ethics Committees of the Japan Institute of Sports Sciences and Tokyo Metropolitan Institute of Gerontology.

Data collection and analysis

Total DNA was isolated from venous blood by the use of QIAamp DNA Blood Maxi Kit (QIAGEN, Hilden, Germany). Two fragments of mtDNA including the hypervariable sequence I (m.16024–m.16383) and nucleotide position m.5178 were amplified by PCR as detailed in table 1. The forward primer was a 38-mer oligonucleotide, consisting of an 18-base sequence of a universal forward sequencing primer (–21M13: 5′–TGTAAAACGACGGCCAGT–3′) connected on its 5′ side to the 3′ side of a 20-base light-strand-specific sequence (table 1). PCR amplifications were carried out in a final reaction volume of 10 µl, containing 20 ng of total DNA, 0.25 units of *TaKaRa Ex TaqHS* (Takara, Shiga, Japan), 1 µl of 10×*Ex Taq* buffer, 0.2 mM of each dNTP and 0.5 µM of each primer. The PCR conditions used were an initial denaturation step at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 15 s, annealing at 60°C for 15 s, and extension at 72°C for 1 min, with a final extension of 10 min at 72°C. Following this, sequence reactions were performed by use of the PCR template, –21M13 forward primer, and a BigDye Terminator Cycle Sequencing Kit version 3.1 (Applied Biosystems, Foster City, California, USA). Sequences were analysed with an automated DNA sequencer Applied Biosystems 3130*xl* Genetic Analyzer (Applied Biosystems). In some cases, the light-strand sequences could not be determined on stretches of C due to the m.16189T>C transition. In such cases, the heavy-strand (reverse) sequences were also determined. For this purpose, the primer H81 (table 1) was used. Each of the mtDNA sequences was compared with the original[6] and the revised[28] Cambridge reference sequences, and polymorphisms were confirmed visually by the use of DNA Sequencing Software Sequencher version 4.2.2 (Gene Codes Corporation, Ann Arbor, Michigan, USA).

Haplogroup classification

Subjects were classified into 12 major mitochondrial haplogroups (ie, F, B, A, N9a, N9b, M7a, M7b, M*, G2, G1, D5 or D4) on the basis of the presence of hypervariable sequence I polymorphisms and several protein-coding-region polymorphisms (table 2).[19] Data from two of 141 individuals were deemed to be unusable because of incomplete haplogroup classification.

The mitochondrial haplogroups were determined by the combination of both the main polymorphism and one of the secondary polymorphisms in the mitochondrial DNA. Four protein-coding-region polymorphisms (m.15851A>G [Cyt*b*], m.15860A>G [Cyt*b*], m.15874A>G [Cyt*b*] and m.5178C>A [ND2]) were used to determine haplogroups B, G1, D4 and D5, respectively (Cyt*b*, Cytochrome *b*; ND2, NADH dehydrogenase subunit 2).

Statistical analysis

Mitochondrial haplogroup frequencies between EMA and CON, and between SPA and CON were compared by a χ^2 test by the use of JMP version 8 (SAS Institute Japan, Tokyo, Japan). For haplogroup analysis, we compared each haplogroup versus the sum of all other haplogroups. The p value, OR and 95% CI were calculated. A p value of less than 0.05 was considered statistically significant.

RESULTS

The distribution of mitochondrial haplogroups in EMA and SPA compared with CON is shown in figure 1. When the frequency of each haplogroup versus the sum of all others was

Table 1 Primers used for analysis

Primer				
Forward		Reverse		
Name	Sequence (5′–3′)	Name	Sequence (5′–3′)	Product (bp)
FL4827	CAAGGCACCCCTCTGACATC	H5528	TTGAAGGCTCTTGGTCTGTA	739
FL15696	TTCGCCCACTAAGCCAATCA	H81	CAGCGTCTCGCAATGCTATC	992

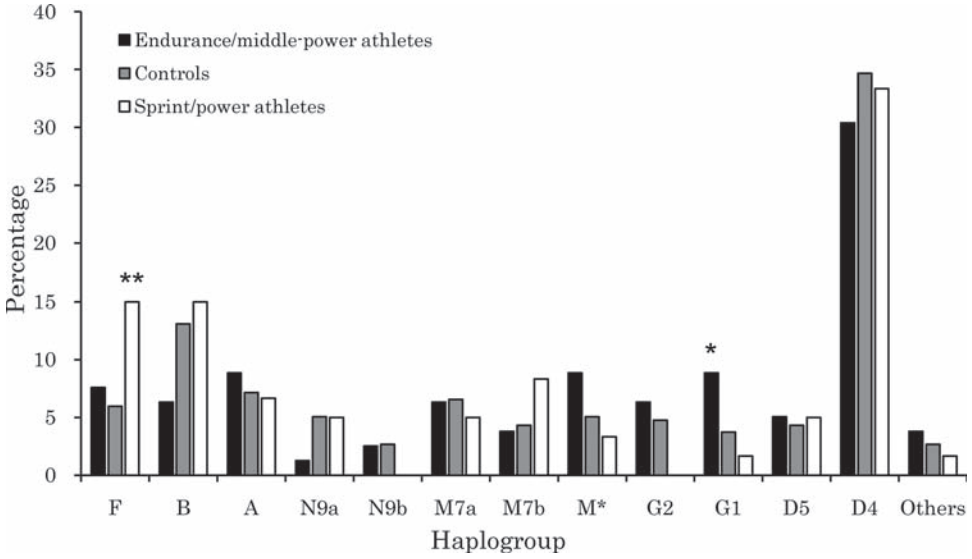

Figure 1 Mitochondrial haplogroup distribution in endurance athletes, sprint/power athletes and controls. Significant differences from controls (grey bars) are highlighted by asterisks (*p<0.05, **p<0.01). Endurance athletes displayed an excess of the haplogroup G1 compared with controls. Sprint/power athletes displayed an excess of haplogroup F compared with controls.

Table 2 Polymorphisms used to determine mitochondrial haplogroups

Haplogroup	Main polymorphism (s)	Secondary polymorphism (s)
F	16304T>C	16129G>A or 16189T>C or (16203A>G and 16291C>T) or 16207A>G
B	16183A>C, 16189T>C, 16519T>C	16217T>C or (15851A>G [Cyt*b*] and 16140T>C)
A	16223C>T, 16290C>T, 16319G>A	16187C>T or 16519T>C or (16129G>A and 16213G>A) or 16362T>C
N9a	16172T>C, 16223C>T, 16257C>A, 16261C>T	
N9b	16183A>C, 16189T>C, 16223C>T, 16519T>C	
M7a	16209T>C, 16223C>T	16140T>C or 16324T>C
M7b	16129G>A, 16189T>C, 16223C>T, 16297T>C, 16298T>C	
M*	16223C>T	16497A>G or (16184C>T and 16298T>C and 16319G>A) or (16234C>T and 16316A>G and 16362T>C) or (16311T>C and 16519T>C)
G2	16223C>T, 16278C>T, 16362T>C	16269A>G or 16519T>C
G1	16223C>T, 16362T>C, 16519T>C	(15860A>G (Cyt*b*) and 16325T>C) or (16184C>T and 16214C>T)
D5	5178C>A (ND2), 16189T>C, 16223C>T, 16362T>C	16167C>T or 16390G>A or (16092T>C and 16266C>T)
D4	5178C>A (ND2), 16223C>T, 16362T>C	16129G>A or 16291C>T or 16319G>A or 16245C>T or 15874A>G (Cyt*b*) or 16278C>T or 16174C>T or 16294C>T or (16274G>A and 16290C>T and 16319G>A) or (16145G>A and 16368T>C)

compared between EMA or SPA and CON, it was found that EMA displayed an excess of haplogroup G1 (OR 2.52, 95% CI 1.05 to 6.02, p=0.032; table 3), with 8.9% in EMA compared with 3.7% in CON. In addition, the frequency of haplogroup B in EMA tended to be lower than in CON (OR 0.45, 95% CI 0.18 to 1.14, p=0.084; table 3), although this difference was not statistically significant. On the other hand, SPA showed a greater proportion of haplogroup F (OR 2.79, 95% CI 1.28 to 6.07, p=0.007; table 3), with 15.0% in SPA compared with 6.0% in CON. The overall frequency of mitochondrial haplogroups F and G1 in our control samples was 7.0% and 3.5% (394 and 198 of 5651); 6.7% and 3.0% in Gunma Prefecture (95 and 43 of 1418) (unpublished data), 7.6% and 3.8% in Gifu Prefecture (208 and 104 of 2748) (unpublished data) and 6.1% and 3.4% in Tokyo (91 and 51 of 1493) (unpublished data). Performance-associated haplogroups F and G1 thus have a wide geographical distribution in Japan.

DISCUSSION

We found that the frequencies of mitochondrial haplogroups differed significantly between elite Japanese athletes and the general Japanese population. EMA displayed an excess of haplogroup G1, whereas SPA showed an excess of haplogroup F. This is the first study to report that Asian-specific mitochondrial haplogroups G1 and F are associated with elite athlete status. We recently reported that mitochondrial haplogroups were associated with elite Kenyan athlete status,[13] but not with elite Ethiopian[14] and Jamaican/African-American athlete status (Deason et al, 2010, manuscript in preparation). Elite Kenyan athletes displayed an excess of haplogroup L0 and a dearth of haplogroup L3 compared with the general Kenyan population. Niemi *et al*[16] also found differences in mitochondrial haplogroup frequencies between elite Finnish sprint and endurance athletes; no endurance athletes belonged to mitochondrial haplogroup K and subhaplogroup J2. Furthermore, Castro *et al*[15] reported that haplogroup T was negatively associated with elite Spanish endurance athlete status. These associations with elite athlete status may suggest that these haplogroups contain polymorphisms that influence some aspect of exercise performance or its trainability.

The greater diversity of mtDNA sequences among African population is due to the fact that the most recent ancestor of modern humans (so called 'mitochondrial Eve') originated from Africa.[29 30] As such, each of the African haplogroups

Table 3 Mitochondrial haplogroup distribution of subject groups

	Controls	Endurance/middle-power athletes			Sprint/power athletes		
Haplogroup	**n (%)**	**n (%)**	**p Value**	**OR (95% CI)**	**n (%)**	**p Value**	**OR (95% CI)**
F	**40 (6.0)**	6 (7.6)	0.565	1.3 (0.53 to 3.17)	**9 (15.0)**	**0.007**	**2.79 (1.28 to 6.07)**
B	88 (13.1)	5 (6.3)	0.084	0.45 (0.18 to 1.14)	9 (15.0)	0.677	1.17 (0.56 to 2.46)
A	48 (7.1)	7 (8.9)	0.589	1.26 (0.55 to 2.90)	4 (6.7)	0.891	0.93 (0.32 to 2.67)
N9a	34 (5.1)	1 (1.3)	0.130	0.24 (0.03 to 1.78)	3 (5.0)	0.984	0.99 (0.29 to 3.32)
N9b	18 (2.7)	2 (2.5)	0.939	0.94 (0.21 to 4.15)	0 (0.0)	0.199	–
M7a	44 (6.5)	5 (6.3)	0.941	0.96 (0.37 to 2.51)	3 (5.0)	0.639	0.75 (0.23 to 2.50)
M7b	29 (4.3)	3 (3.8)	0.829	0.88 (0.26 to 2.94)	5 (8.3)	0.157	2.02 (0.75 to 5.42)
M*	34 (5.1)	7 (8.9)	0.160	1.82 (0.78 to 4.26)	2 (3.3)	0.554	0.65 (0.15 to 2.76)
G2	32 (4.8)	5 (6.3)	0.543	1.35 (0.51 to 3.57)	0 (0.0)	0.084	–
G1	**25 (3.7)**	**7 (8.9)**	**0.032**	**2.52 (1.05 to 6.02)**	1 (1.7)	0.410	0.44 (0.06 to 3.30)
D5	29 (4.3)	4 (5.1)	0.759	1.18 (0.40 to 3.46)	3 (5.0)	0.804	1.17 (0.34 to 3.95)
D4	233 (34.7)	24 (30.4)	0.447	0.82 (0.50 to 1.36)	20 (33.3)	0.834	0.94 (0.54 to 1.65)
Others	18 (2.7)	3 (3.8)	0.568	1.43 (0.41 to 4.98)	1 (1.7)	0.637	0.62 (0.08 to 4.69)
Total	672	79			60		

Numbers in bold indicate haplogroups that showed frequency differences between athletes and controls.

(L0–L3) has deep genetic roots. Haplogroup L3 is proposed to be the ancestor of all non-African populations. European haplogroups (H, I, J, K, S, T, U, V, W, etc) belong to macrohaplogroup N,[31] whereas Asian haplogroups belong to both macrohaplogroups N and M (haplogroups A, B, F and N9 to macrohaploup N; and haplogroups M7a, M7b, M8, D and G to macrohaploup M).[19] Both macrohaplogroups N and M have a common root with haplogroup L3.[32] Africans, Europeans and Asians thus have different phylogenetic structures. Therefore, the mtDNA polymorphisms of these ethnic groups and their effects on exercise performance must be analysed according to the haplogroup frequencies in each population.

Mitochondrial haplogroup G1 is characterised by three polymorphisms: m.8200T>C, m.15323G>A and m.15497G>A, in the protein-coding region of the mtDNA.[19 32] Two of these, namely, m.15323G>A and m.15497G>A, are non-synonymous substitutions. These polymorphisms cause the Ala193Thr and Gly251Ser replacements in Cyt*b*, which is a subunit of the complex III. Previously, the m.15498G>A mutation causing the Gly251Asp replacement in the Cyt*b* was reported in a patient with mitochondrial myopathy and exercise intolerance.[33] Interestingly, in middle-aged individuals, we reported an association of obesity-related phenotypes with m.15497G>A transition (Cyt*b*: Gly251Ser), which is a polymorphism characterising haplogroup G1.[34] It is reasonable to speculate that this Gly251Ser replacement is accompanied by functional alterations of Cyt*b*.

We previously hypothesised two possibilities to explain the association between the obesity-related phenotype and the m.15497G>A transition characterising mitochondrial haplogroup G1: (1) increased efficiency of mitochondrial energy conservation at the cytochrome bc_1 complex resulting in decreased energy consumption; or (2) inhibiting reduction in ubiquinone at the Qo site (one of the ubiquinone-binding sites of the complex III) resulting in a reduced β-oxidation of fatty acid, which leads to fat accumulation.[34] In the present study, haplogroup G1 was associated with elite Japanese EMA status. The main function of mitochondria is to produce ATP by OXPHOS; and while the uncoupling of mitochondrial OXPHOS generates heat, it concomitantly reduces the production of ATP.[35] Conversely, more tightly coupled OXPHOS would be expected to decrease heat production and result in higher efficiency of ATP production. This improved efficiency of ATP production could explain, at least partly, the association between haplogroup G1 and endurance/middle-power performance reported in the present study. However, this energy conservation by mitochondrial OXPHOS may predispose to obesity in sedentary individuals later on in life; a phenomenon commonly referred to as the 'thrifty' genotype and/or 'thrifty' phenotype.[20] These hypotheses require further investigation.

In the present study, we found that the frequency of mitochondrial haplogroup F was significantly higher in SPA than in CON. This haplogroup is defined by four polymorphisms: m.3970C>T, m.6392T>C, m.10310G>A and m.13928G>C, in the coding region of the mtDNA.[19 32] One of the polymorphisms, namely m.13928G>C, causes Ser531Thr replacement in the ND5, which is a subunit of complex I. Sprint performance relies more on anaerobic glycolysis than OXPHOS. Certain mtDNA polymorphisms and/or mitochondrial haplogroups may influence the regulation of ATP production not only by the OXPHOS system in the mitochondria but also by the glycolytic pathway in the cytosol. We reported that the peak cytosolic calcium levels after histamine stimulation were higher in the cybrids with mitochondrial macrohaplogroup N than in those with mitochondrial macrohaplogroup M.[36] This result suggests that the cytosolic calcium response may be enhanced in the cybrids with macrohaplogroup N. Sprint/power performance-related mitochondrial haplogroup F is a component of macrohaplogroup N. Therefore, haplogroup F may be related to the calcium dynamics in the cell including skeletal muscle. Calcium regulates glycogen breakdown in the skeletal muscle. During activation of contraction in skeletal muscle, calcium is released from the sarcoplasmic reticulum, and also activates phosphorylase kinase. The physiological significance of this calcium activation process is that muscle contraction is triggered by a transient increase in the level of cytosolic calcium through its release from intracellular reservoirs, which may also include mitochondrial calcium, by nerve impulses. The rate of glycogen breakdown is linked to the rate of muscle contraction, an important regulatory link because glycogen breakdown in muscle provides fuel for glycolysis, which, in turn, generates the ATP required for muscle contraction. Mitochondrial haplogroup F, which is associated with SPA status in the present study, may thus influence the rate of glycolytic ATP production and/or the rate of muscle

What is already known on this topic

Mitochondrial haplogroups, which are a set of mitochondrial DNA polymorphisms, appear to influence physical performance. Indeed, previous studies have reported associations between certain mitochondrial haplogroups and elite endurance athlete status or $\dot{V}O_2$max in African and European, but not in Asian individuals.

What this study adds

In the present study, Asian-specific mitochondrial haplogroup G1 was found to associate with elite Japanese EMA status, whereas haplogroup F was related to elite Japanese SPA status. Mitochondria may therefore regulate not only aerobic metabolism but also anaerobic metabolism.

contraction. These hypotheses require further investigation by functional analysis of this haplogroup.

In conclusion, we found significant associations both between mitochondrial haplogroup G1 and elite EMA status and between mitochondrial haplogroup F and elite SPA status in Japanese athletes. These associations may implicate these mitochondrial haplogroups in determining elite athlete status in Japanese individuals (as previously suggested in other populations). It should be noted that the sample size of the present study was relatively small but in line with other similar studies.[15 16] Nevertheless, our previous findings suggest physiological plausibility.[34] Further investigation will require more detailed analysis of mtDNA.

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Competing interests None.

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