Available online at www.sciencedirect.com
ScienceDirect
Gene 411 (2008) 69–76
GENE
www.elsevier.com/locate/gene

Experimental assessment of bioenergetic differences caused by the common European mitochondrial DNA haplogroups H and T

Taku Amo [a], Nagendra Yadava [b], Richard Oh [b], David G. Nicholls [b], Martin D. Brand [a,*]

[a] *MRC Dunn Human Nutrition Unit, Hills Road, Cambridge CB2 0XY, UK*
[b] *Buck Institute for Age Research, Novato, California, USA*

Received 4 September 2007; received in revised form 15 January 2008; accepted 15 January 2008
Available online 26 January 2008
Received by M. Di Giulio

Abstract

Studies of both survival after sepsis and sperm motility in human populations have shown significant associations with common European mitochondrial DNA haplogroups, and have led to proposals that mitochondria bearing haplogroup H have different bioenergetic capacities than those bearing haplogroup T. However, the validity of such associations assumes that there are no non-random influences of nuclear genes or other factors. Here, we removed the effect of any differences in nuclear genes by constructing transmitochondrial cybrids harbouring mitochondria with either haplogroup H or haplogroup T in cultured A549 human lung carcinoma cells with identical nuclear backgrounds. We compared the bioenergetic capacities and coupling efficiencies of mitochondria isolated from these cells, and of mitochondria retained within the cells, as a critical experimental test of the hypothesis that these haplogroups affect mitochondrial bioenergetics. We found that there were no functionally-important bioenergetic differences between mitochondria bearing these haplogroups, using either isolated mitochondria or mitochondria within cells.

Keywords: Oxidative phosphorylation; Coupling efficiency; Sepsis; Sperm motility; Cybrid

1. Introduction

Analysis of the variation in normal human mitochondrial DNA (mtDNA) has identified many haplogroups–specific patterns of polymorphisms that have arisen over the last 150,000–200,000 years–that have been used to study the origin, radiations and evolution of human populations (Cann et al., 1987; Wallace et al., 1999; Wallace, 2005). These mtDNA haplogroups have been less studied bioenergetically than mtDNA mutations that lead to disease (Dimauro and Davidzon, 2005; Taylor and Turnbull, 2005), but normal variation in mtDNA may affect disease susceptibility (Herrnstadt and Howell, 2004) and longevity (Santoro et al., 2006). These effects could be explained by differences in mitochondrial coupling efficiency (the percentage of oxygen consumption used for ATP synthesis rather than heat generation) or mitochondrial production of ROS (reactive oxygen species). It has been proposed that particular haplogroups cause inefficient oxidative phosphorylation and greater heat production and were therefore selected during the radiations of humans into Arctic environments (Mishmar et al., 2003; Ruiz-Pesini et al., 2004; Wallace, 2005; Montiel-Sosa et al., 2006), but this proposal is controversial (Elson et al., 2004; Kivisild et al., 2006; Ruiz-Pesini and Wallace, 2006) and is not supported by direct measurement of mitochondrial coupling efficiencies in mitochondria carrying representative "arctic" and "tropical" mtDNAs (Amo and Brand, 2007; Elson et al., 2007).

Abbreviations: ρ^0, mtDNA-less; $\Delta\psi$, mitochondrial membrane potential; TPMP, triphenylmethylphosphonium; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

* Corresponding author. Tel.: +44 1223 252800; fax: +44 1223 252805.
E-mail address: martin.brand@mrc-dunn.cam.ac.uk (M.D. Brand).

0378-1119/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.gene.2008.01.007

Two lines of evidence suggest that common European haplogroups may affect bioenergetic function: there are associations of both survival after sepsis and sperm motility with haplogroup H. In a prospective study of intensive care patients in Newcastle-upon-Tyne, U.K., individuals with haplogroup H (about 40% of the population studied) were more than twice as likely to survive 180 days after sepsis than those with non-H haplogroups (primarily the closely-related haplogroups J and T; about 30% of the population) (Baudouin et al., 2005). Mitochondrial dysfunction may be linked to sepsis-induced multiple organ failure (Protti and Singer, 2007), so altered mitochondrial bioenergetics might be the causal link between haplogroup and survival. Two possibilities have been proposed: haplogroup H might protect through greater heat generation (because of higher electron transport rates or looser coupling) (Baudouin et al., 2005), or through greater ROS production (because of tighter coupling and raised protonmotive force), which could reduce bacterial infection (Wallace, 2005). There have been no direct experimental tests of these possibilities.

The second line of evidence comes from studies of sperm motility, which is correlated with mitochondrial enzymatic activities and depends on the activity of the mitochondrial electron transport chain (Ruiz-Pesini et al., 1998; Ruiz-Pesini et al., 2000). Haplogroup T was over-represented in men with asthenozoospermia (reduced sperm motility), whereas haplogroup H was over-represented in men with other fertility problems. Sperm with haplogroup H performed better in a test of motility and had higher cytochrome oxidase activity than those with haplogroup T (Ruiz-Pesini et al., 2000), suggesting that mitochondria carrying haplogroup H make more ATP than those with haplogroup T. This conclusion has been extended to sublineages of haplogroup U (Montiel-Sosa et al., 2006), but associations between haplogroups and reduced male fertility and sperm motility have been disputed by others (Pereira et al., 2005; Pereira et al., 2007). Less well-coupled mitochondria make less ROS (Korshunov et al., 1997; Liu, 1997; Lambert and Brand, 2004), so people having such mitochondria might suffer less from neurodegenerative diseases caused by ROS (Wallace, 2005). Some epidemiological studies support this prediction; e.g. haplogroup T is under-represented in Alzheimer's disease patients (Chagnon et al., 1999). However, other studies have not replicated this finding (Elson et al., 2006), and correlations between mitochondrial haplogroups and neurodegenerative diseases are controversial (Raule et al., 2007). Again, there have been no direct experimental tests of possible differences in the bioenergetic properties of mitochondria that might underlie the reported associations of haplogroup and phenotype.

In the present study, we analyse bioenergetic capacity and coupling efficiency in mitochondria isolated from cytoplasmic hybrids (cybrids) carrying haplogroups H and T with identical nuclear DNA. Furthermore, to investigate the effects of haplogroups H and T on the bioenergetic status of mitochondria at the cellular level, we analyse the respiratory capacities and mitochondrial coupling efficiencies of intact cybrids.

2. Materials and methods

2.1. Subjects

Healthy volunteers were recruited by advertisement and written informed consent was obtained. Ethical approval was obtained from the Cambridge Research Ethics Committee. DNA was extracted from buccal swab samples using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Mitochondrial haplogroups were determined by PCR-RFLP analysis according to published criteria (Wallace et al., 1999). Entire mtDNAs were amplified in several overlapping fragments by PCR (Torroni et al., 1997). Each fragment was digested by restriction enzymes and resolved on agarose gels. 15 ml of blood were taken from each of three volunteers who had haplogroup H, and three who had haplogroup T, for platelet preparation and cybrid construction. Haplogroup H (Achilli et al., 2004) is subdivided into at least 15 sub-haplogroups (H1-H15). In the Newcastle sepsis study, there were no significant differences in survival of sub-haplogroups H1 (−3008 TaqI), H2 (−4769 AluI), H3 (+6773 NlaIII) and the remaining H sub-haplogroups combined (Baudouin et al., 2005). Our haplogroup H volunteers were H1, H3 and (H, not H1, H2 or H3). Haplogroup T (Macaulay et al., 1999) is subdivided into at least 5 sub-haplogroups (T1–T5) (Pike 2006). Our haplogroup T volunteers were one T (sub-haplogroup not checked), one T1 (−12629 AvaII) and one (T, not T1). No major differences in cybrid phenotypes between sub-haplogroups were observed.

2.2. Generation of cybrid cell lines

Cybrid cell lines are constructed by repopulation of mtDNA-less (ρ^0) cells with exogenous mitochondria (King and Attardi, 1989). A549.B2 ρ^0 (mtDNA-less) cells derived from human lung carcinoma A549 (originally carrying mitochondrial DNA of haplogroup H) were cultivated in Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g/l glucose, 110 μg/ml sodium pyruvate, 10% (v/v) fetal bovine serum (FBS) and 50 μg/ml uridine. Platelets (which have no nuclei) were isolated from the volunteers' blood samples and fused with A549.B2 ρ^0 cells as described elsewhere (Chomyn, 1996). The resultant cybrids had mtDNA from the different donors, but their nuclear DNA was identical. After many generations of cybrid growth (diluting platelet-derived nuclear-encoded subunits), all nuclear-encoded mitochondrial protein subunits will be specified by the host cell, but all mitochondrial-encoded subunits will be specified by the donor mtDNA. The cybrid cell lines were constructed and routinely maintained in DMEM with 10% dialysed FBS. Stocks of cell lines were frozen and kept at −80 °C until required. DNA was extracted from cultured cybrid cells as described previously (Laird et al., 1991) to confirm mitochondrial haplogroups by PCR-RFLP analysis.

2.3. Mitochondrial respiration and membrane potential

Human A549 cell mitochondria were prepared from cultured cybrids as previously described (Amo and Brand, 2007).