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Molecular and bioenergetic differences between cells with African versus European inherited mitochondrial DNA haplogroups: Implications for population susceptibility to diseases

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ABSTRACT

The geographic origins of populations can be identified by their maternally inherited mitochondrial DNA (mtDNA) haplogroups. This study compared human cybrids (cytoplasmic hybrids), which are cell lines with identical nuclei but mitochondria from different individuals with mtDNA from either the H haplogroup or L haplogroup backgrounds. The most common European haplogroup is H while individuals of maternal African origin are of the L haplogroup. Despite lower mtDNA copy numbers, L cybrids had higher expression levels for nine mtDNA-encoded respiratory complex genes, decreased ATP (adenosine triphosphate) turnover rates and lower levels of reactive oxygen species production, parameters which are consistent with more efficient oxidative phosphorylation. Surprisingly, GeneChip arrays showed that the L and H cybrids had major differences in expression of genes of the canonical complement system (5 genes), dermatan/chondroitin sulfate biosynthesis (5 genes) and CCR3 (chemokine, CC motif, receptor 3) signaling (9 genes). Quantitative nuclear gene expression studies confirmed that L cybrids had (a) lower expression levels of complement pathway and innate immunity genes and (b) increased levels of inflammation-related signaling genes, which are critical in human diseases. Our data support the hypothesis that mtDNA haplogroups representing populations from different geographic origins may play a role in differential susceptibilities to diseases.

1. Introduction

Mitochondria (mt) have their own unique, circular DNA that can be categorized into haplogroups defined by single nucleotide polymorphism (SNP) variants, and these haplogroups represent populations of different ancestral origin. Recent studies have shown that mtDNA haplogroups can be associated with human aging and diseases [1]. The coding region of mtDNA encodes for 37 genes including 13 protein subunits that are essential for oxidative phosphorylation (OXPHOS), 2 ribosomal RNAs and 22 transfer RNAs [2–4]. The non-coding mtDNA D-loop (also called the Control Region) contains 1121 nucleotides and is important for replication and transcription. It is known that mtDNA is critical for OXPHOS but recent studies have provided evidence that mtDNA haplogroups can also influence expression of genes related to oxidative stress [5,6] and the clinical severity of diseases [7,8]. Most

Abbreviations: ABI, Applied Biosystems; ARPE-19, Retinal pigmented epithelium cell line; ATP, Adenosine triphosphate; CFH, Complement factor H; C1s, Complement component 1, s subcomponent; C3, Complement component 3; C4B, Complement component 4B; DMEM, Dulbecco's modified Eagle's medium; DNA, Deoxyribonucleic acid; ECAR, Extracellular acidification rate; EDTA, Ethylenediaminetetracetic acid; ETC, Electron transport chain; FCCP, Carbonyl Cyanide 4-trifluoromethoxy-phenylhydrazone; μM, MicroMolar; MT-CYB, Mitochondria encoded cytochrome B; MT-ND1, Mitochondria encoded NADH dehydrogenase 1; MT-ND3, Mitochondria encoded NADH dehydrogenase 3; MT-ND5, Mitochondria encoded NADH dehydrogenase 5; MT-CO1, Mitochondria encoded cytochrome oxidase 1; MT-CO2, Mitochondria encoded cytochrome oxidase 2; MT-CO3, Mitochondria encoded cytochrome oxidase 3; OCR, Oxygen consumption rate; OXPHOS, Oxidative phosphorylation; Q-PCR, Quantitative polymerase chain reaction; SEM, Standard error mean; SNPs, Single nucleotide polymorphisms; UCLA, University of California, Los Angeles; $VO2_{max}$, Maximal oxygen uptake

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importantly mtDNA haplogroups have been associated with various diseases, including Alzheimer's disease (AD) [9], Parkinson's disease [10–12], osteoarthritis [13], type 2 diabetes (T2D) [14] and various cancers [15].

In medicine, it has long been recognized that certain diseases are more prevalent in specific racial/ethnic populations [16–21]. For example, there are differences in the prevalence of Alzheimer's disease (AD) depending upon the ethnic/racial groups. Proportionately to the size of their population, older African-Americans are ~2-times more likely and Hispanics are approximately 1.5-times more likely to have AD or dementia compared to older non-Hispanic whites [22–24]. Another example of racial/ethnic differences can be found in the incidence of diabetes. Compared to non-Hispanic white adults, Asian-Americans have an 18% rate, Hispanic/Latinos have 66%, and non-Hispanic blacks a 77% higher risk of diabetes (http://www.diabetes.niddk.nih.gov/dm/pubs/statistics). With systemic lupus erythematosus (SLE), there is higher incidence within the African-American community compared to white subjects [20,21]. While some have suggested that epigenetic changes may contribute to these ethnic/racial differences [25], we suspect that non-synonymous mtDNA variants (causing amino acid changes) associated with the different haplogroups may contribute to altered functions and disease susceptibilities. In addition, the SNP variants within the mt-D-loop region can cause changes in replication and transcription rates, leading to lower levels of mtDNA and mtRNA [26].

In the past it has been difficult to evaluate the contribution that mtDNA variants might have to molecular processes or cellular behavior. However, with the development and use of the novel cybrid (cytoplasmic hybrid) model, many questions related to the functional importance of the mtDNA haplogroup variants and mitochondrial–nuclear interactions can be addressed. These cybrid cell lines are created by fusing mitochondrial-free (Rho^0) cells with mitochondria-rich platelets from different individuals so the resultant cells have identical nuclei but vary in their mtDNA haplogroups.

Our initial interests have been related to age-related macular degeneration and other retinal diseases, so we elected to use a well characterized retinal pigment epithelial cell line, ARPE-19, for our cybrids. Our findings with this novel cybrid model show that surprisingly the L haplogroup mtDNA variants (African origins) can differentially mediate the expression of nuclear genes involved in the complement, inflammatory and innate immunity pathways, which are critical in human diseases. These data also support the hypothesis that the differential susceptibilities to diseases found in ethnic/racial populations may be related in part to their mtDNA haplogroup backgrounds, which can influence nuclear gene expression, cellular functions and induce variable phenotypic severity of diseases.

2. Material and methods

2.1. Transmitochondrial cybrids and culture conditions

Institutional review board approval was obtained from the University of California, Irvine (#2003-3131). For DNA analyses, 10 ml of peripheral blood was collected via venipuncture in tubes containing 3.2% sodium citrate from normal volunteers (H haplogroup, n = 3, average age 35.3 ±7.3 years; L haplogroup, n = 3, average age 44.6 ± 4.8 years, p =0.5). DNA was isolated with a DNA extraction kit (PUREGENE, Qiagen, Valencia, CA). Platelets were isolated by a series of centrifugation steps and final pellets were suspended in Tris-buffered saline. The ARPE-19 cells deficient in mtDNA (Rho^0) were created by serial passage in low dose ethidium bromide [27]. Cybrids were produced by polyethylene glycol fusion of platelets with Rho^0 ARPE-19 cells according to modified procedures of Chomyn [28]. Verification of transfer of the mitochondria into the Rho^0 ARPE-19 cells was accomplished by polymerase chain reaction (PCR), restriction enzyme digestion, and sequencing of the mtDNA to identify the mitochondrial haplogroup of each cybrid [29].

2.2. Identification of cybrid haplogroups

Cybrid DNA was extracted from cell pellets using a spin column kit (DNeasy Blood and Tissue Kit, Qiagen) and quantified using the Nanodrop 1000 (Thermo Scientific, Wilmington, DE). PCR and restriction enzyme digests [29] allelic discrimination and sequencing of the mt-Dloop were performed to determine mitochondrial haplogroups. The H defining SNPs were T7028C, G73A, G2706A, A11719G and T14766C. The samples were further sequenced and identified to be H, H and H5a for the three cybrids (Fig. 1a). The L cybrids were further sequenced using primers to L9611-H12111 and to the mt-Dloop. The L samples were identified to be L0a1′4, L1b, and L2b as defined by the SNP variants (Fig. 1b).

Allelic discrimination was also performed to confirm the haplogroups. The primers for allelic discrimination were synthesized by ABI Assay-by-Design. The samples were run at GenoSeq, the UCLA Genotyping and Sequencing Core, on an ABI 7900HT. Data were analyzed with Sequence

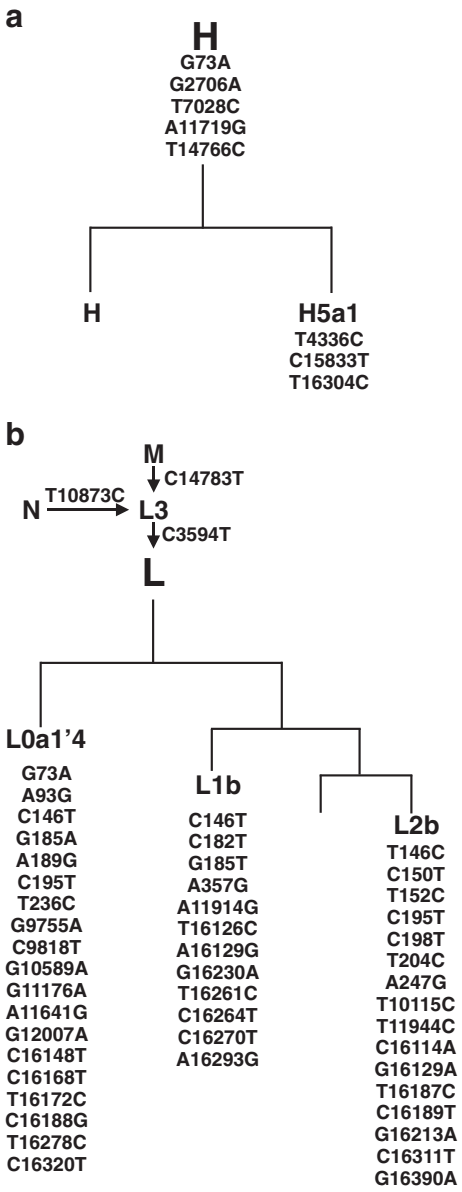

Fig. 1. a and b. Diagrams of haplogroups trees showing the subsets of L cybrids and H cybrids used in this study. The mtDNA from the individual L cybrids were analyzed and the SNPs which define the Loa1′4, L1b, and L2b haplogroup subsets are listed. The SNPs listed were all verified by sequencing and comparison to the H haplogroup represents the Cambridge reference standard used to define the mtDNA sequences.