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Cardiometabolic phenotypes and mitochondrial DNA copy number in two cohorts of UK women

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ABSTRACT

The mitochondrial genome is present at variable copy number between individuals. Mitochondria are vulnerable to oxidative stress, and their dysfunction may be associated with cardiovascular disease.

The association of mitochondrial DNA copy number with cardiometabolic risk factors (lipids, glycaemic traits, inflammatory markers, anthropometry and blood pressure) was assessed in two independent cohorts of European origin women, one in whom outcomes were measured at mean (SD) age 30 (4.3) years (N = 2278) and the second at 69.4 (5.5) years (N = 2872). Mitochondrial DNA copy number was assayed by quantitative polymerase chain reaction. Associations were adjusted for smoking, sociodemographic status, laboratory factors and white cell traits.

Out of a total of 12 outcomes assessed in both cohorts, mitochondrial DNA copy number showed little or no association with the majority (point estimates were close to zero and nearly all p-values were > 0.01). The strongest evidence was for an inverse association in the older cohort with insulin (standardised beta [95%CI]: − 0.06, [− 0.098, − 0.022], p = 0.002), but this association did not replicate in the younger cohort.

Our findings do not provide support for variation in mitochondrial DNA copy number having an important impact on cardio-metabolic risk factors in European origin women.

Author summary

Mitochondria are organelles that liberate adenosine triphosphate in order to provide energy for a cell's requirements. Mitochondria contain their own circular genome, which is present at variable copy number between individuals. A number of predominantly small studies have examined the relationship between mitochondrial DNA copy number (mtDNA CN) and cardiometabolic traits. In one of the largest studies of its kind, we have studied mtDNA CN in relation to a variety of cardiometabolic risk factors in two large cohorts of European women. We were able to make use of these rich data resources in order to control for a range of confounding variables, including cellular heterogeneity. We found no consistent evidence to suggest that mtDNA CN was related to the cardiometabolic traits studied, although after considering multiple testing, we did find weak evidence of a positive association with cholesterol in the younger cohort, and an inverse association with insulin in the older cohort. This latter association has been reported more consistently in previous literature. Whilst we cannot rule out associations between mtDNA CN and some cardiometabolic traits, our results do not suggest that variation in mtDNA CN has a major impact on cardio-metabolic risk factors in European origin women.

1. Introduction

Mitochondria are organelles responsible for the liberation of energy in the form of adenosine triphosphate (ATP), which is hydrolysed to meet a cell's energy requirements. Mitochondria contain a double-stranded genome of 16.6 kb that encodes 37 genes, including the complexes of the electron transport chain (Dimauro and Davidzon, 2005). The electron transport chain represents the end-point of cellular respiration that facilitates ATP synthesis (Jonckheere et al., 2012).

Each mitochondrion contains a relatively constant (Jonckheere et al., 2012; Ziegler et al., 2015) number of mitochondrial DNA (mtDNA) (Wiesner et al., 1992), yet mitochondria number varies

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enormously according to cell lineage and function (Robin and Wong, 1988). This translates into considerable interindividual differences in mtDNA content, and overall mtDNA copy number (mtDNA CN) has been observed to decline with age (Short et al., 2005).

Hereditary mutations in nuclear DNA (nDNA) in genes controlling mitochondrial deoxyribonucleoside triphosphate (dNTP) synthesis or replication (reviewed elsewhere (El-Hattab and Scaglia, 2013)) may lead to the autosomal recessive mitochondrial depletion syndromes (MDS). Diseases resulting from mitochondrial depletion may be multisystem, with organs relying heavily on aerobic metabolism (e.g. skeletal/cardiac muscle, liver, brain, kidney) (El-Hattab and Scaglia, 2013) affected most (Chinnery, 2014). Age of onset varies, although symptoms and signs are often most severe in childhood disease (Mattman et al., n.d.).

Mitochondrial pathologies manifest clinically as the consequences of respiratory chain dysfunction (Chinnery, 2014). Mitochondria are the principal producers of reactive oxygen species (ROS), which may damage lipids, proteins and nucleic acids, and mtDNA is exquisitely vulnerable to ROS-induced damage (St John et al., 2007), which may lead to inefficiency of the electron transport chain, and further ROS production (Ziegler et al., 2015).

MtDNA CN has been associated with cardiovascular disease (Moslehi et al., 2012; Chen et al., 2014; Huang et al., 2016) and its risk factors, chronological age (Mengel-From et al., 2014), as well as markers and diseases associated with age, e.g. telomere length (Kim et al., 2012a; Kim et al., 2013; Qiu et al., 2015; Sahin et al., 2011), and frailty (Ashar et al., 2015). Cognitive phenotypes associated with decreased mtDNA CN include cognitive impairment (Lee et al., 2010), dementia (Coskun et al., 2004; Coskun et al., 2012; Gatt et al., 2013; Podlesniy et al., 2013; Rice et al., 2014), and psychiatric morbidities (depression (Kim et al., 2011; Chang et al., 2015) bipolar disorder (Chang et al., 2014; De Sousa et al., 2014), and post-traumatic stress disorder [PTSD]) (Bersani et al., 2016). For detail, see Online Resource 1, Online Resource 2, and Online Resource 3.

Studies have generally found an inverse correlation between mtDNA CN and cardio-metabolic risk factors and outcomes (Kim et al., 2012b; Huang et al., 2011; Antonetti et al., 1995; Chien et al., 2012; Kaaman et al., 2007; Lindinger et al., 2010; Ding et al., 2015), although a number of studies have also found null (Ding et al., 2015; Asmann et al., 2006; Mozhei et al., 2014), tissue-dependent (Hsieh et al., 2011), or results in the opposite direction (Lee et al., 2014; Malik et al., 2009; Weng et al., 2009). Elevated low-density lipoprotein cholesterol (LDLc) has also been associated with decreased mtDNA CN, with the inverse association reported for high-density lipoprotein cholesterol (HDLc) (Lee et al., 2014; Liu et al., 2005), and a recent case-control study found that mtDNA CN was generally lower in coronary heart disease (Chen et al., 2014). However, whilst mtDNA is heritable (Ding et al., 2015), and the majority of studies have conceptualised mtDNA CN as an exposure, reverse causation is possible: recently, an axis proposed between mitochondria, telomeres and p53 has been discussed (Sahin and DePinho, 2012), suggested that telomere dysfunction and attrition may be associated with impaired mitochondrial function (Kim et al., 2013).

The majority of studies relating mtDNA CN to cardiometabolic traits have been of small sample size (in the order of tens to hundreds of patients, with one recent larger study analysing 2077 patients) (Ding et al., 2015). We studied observational associations between mtDNA CN and cardiometabolic risk factors in two large, independent cohorts of European origin: mothers of the Avon Longitudinal Study of Parents and Children (ALSPAC) study, and participants of the British Women's Heart and Health Study (BWHHS) cohort, a population-based cohort of post-menopausal women. Longitudinal associations were studied in ALSPAC, and cross-sectional associations were studied in BWHHS. We used these rich data sources to control for potential confounding variables. Since the relationship between mtDNA CN and leucocyte subtypes is well-documented, and may bias mtDNA CN analyses (Urata et al., 2008; Knez et al., 2016), we also examined the association between mtDNA CN and white cell traits (Pyle et al., 2010).

2. Methods

2.1. Cohort details

2.1.1. ALSPAC

The Avon Longitudinal Study of Parents and Children is a prospective cohort of mothers and children. Between 1991 and 1992, 14,541 women living in the former county of Avon, UK were recruited during pregnancy, of whom 13,761 were enrolled into the study. Antenatal blood samples were assayed for mtDNA CN in this study. Participants have been followed up longitudinally since recruitment. Outcome data are those measured at the Focus on Mothers Research Clinic, which took place between 2008 and 2011. Further details are available in the cohort profile paper (Fraser et al., 2013), and the study website contains details of available data through a fully searchable data dictionary: http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/.

Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local National Health Service (NHS) Research Ethics Committees.

2.1.2. BWHHS

The British Women's Heart and Health Study (BWHHS) recruited 4286 women between the ages of 60–79 from UK general practices [https://www.lshtm.ac.uk/eph/ncde/research/bwhhs/]. Blood was collected at baseline interview (1999–2001) after an overnight or minimum 6-h fast. Details of the sampling strategy and available data, including details of follow-up and data linkage, are described elsewhere (Lawlor et al., 2003). Data for the current analysis are those taken or derived at baseline, unless otherwise stated. Ethical approval for the BWHHS was obtained from NHS Research Ethics Committees of individual centres, in addition to the London Medical Research Ethics Committee.

2.2. Assay of mitochondrial DNA copy number

ALSPAC mothers' DNA was extracted from whole blood or white cell pellet samples (anticoagulated with EDTA and heparin, respectively) using a phenol-chloroform method. For BWHHS a salting-out procedure was used to extract DNA from K-EDTA whole blood samples (Zabaneh et al., 2011).

MtDNA CN was measured using a quantitative PCR (qPCR) assay that relates the relative copy number of a mitochondrial DNA amplicon [bases 317–381 in the D-loop region] to a nuclear reference gene [*B2M*] (Malik et al., 2011). For details of the assay, see Online Resource 4.

MtDNA CN was calculated as the relative magnitude of the signal from the mitochondrial amplicon to the nuclear amplicon. PCR efficiency values were calculated from standard curves to adjust raw values, and 'calibrator' DNAs were amplified on each microplate, in order to generate a 'calibration factor' for each batch. This factor was applied to the previously calculated copy numbers, resulting in efficiency- and calibrator- adjusted value for mtDNA CNs.

2.3. Outcome variables

Unless otherwise stated, outcomes were measured at baseline in BWHHS, and at the Focus on Mothers Research Clinic (2008–2011) in ALSPAC.

2.3.1. Anthropometric variables

After measuring height and weight, BMI was calculated as weight (kg)/height (m^2). Averages of two measures of waist and hip circumference were calculated, and waist-to-hip ratio was derived.