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Cerebral hyperperfusion and decreased cerebrovascular reactivity correlate with neurologic disease severity in MELAS☆,☆☆

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ABSTRACT

Objective: To study the mechanisms underlying stroke-like episodes (SLEs) in MELAS syndrome.
Methods: We performed a case control study in 3 siblings with MELAS syndrome (m.3243A>G $tRNA^{Leu(UUR)}$) with variable % mutant mtDNA in blood (35 to 59%) to evaluate regional cerebral blood flow (CBF) and arterial cerebrovascular reactivity (CVR) compared to age- and sex-matched healthy study controls and a healthy control population. Subjects were studied at 3 T MRI using arterial spin labeling (ASL) to measure CBF; CVR was measured as a change in % Blood Oxygen Level Dependent signal (as a surrogate of CBF) to repeated 10 mm Hg step increase in arterial partial pressure of CO_2 ($PaCO_2$).
Results: MELAS siblings had decreased CVR ($p \leq 0.002$) and increased CBF ($p < 0.0026$) compared to controls; changes correlated with disease severity and % mutant mtDNA (inversely for CVR: $r = -0.82$ frontal, $r = -0.91$ occipital cortex; directly for CBF: $r = +0.85$ frontal, not for occipital infarct penumbra). Mean CVR was reduced more in frontal ($p < 0.001$) versus occipital cortex ($p = 0.002$); mean CBF was increased more in occipital ($p = 0.001$) than frontal ($p = 0.0026$) cortices compared to controls. CBF correlated inversely with CVR ($r = -0.99$ in frontal; not in occipital infarct penumbra) suggesting that increased frontal resting flows are at the expense of flow reserve.
Interpretation: MELAS disease severity and mutation load were inversely correlated with Interictal CVR and directly correlated with frontal CBF. These metrics offer further insight into the cerebrovascular hemodynamics in MELAS syndrome and may serve as noninvasive prognostic markers to stratify risk for SLEs.
Classification of evidence: Class III.

☆ *Author contributions and disclosures:* Lance H. Rodan MD, contributed to the analysis and interpretation of the data and to the drafting of the manuscript for intellectual content. Dr. Rodan has nothing to disclose.Julien Poublanc MSc contributed to the analysis and interpretation of the data. Mr. Poublanc has nothing to disclose.Joseph A. Fisher MD contributed to the analysis and interpretation of the data and to the revision of the manuscript for intellectual content. Dr. Fisher reports personal fees from Thornhill Research Inc. (TRI) during the conduct of the study. TRI is a spin off from the University Health Network and has designed and constructed the RespirACT used for this study. In addition, Dr. Fisher has a patent (issued) for the underlying principles of the RespirACT™ used in this work. All patents are owned by the University Health Network and licensed to TRI. Dr. Fisher is one of the inventors of the device. TRI has not otherwise commissioned or supported this study.Olivia Sobczyk MSc contributed to the analysis and interpretation of the data. Ms. Sobczyk reports personal fees from Thornhill Research Inc., outside the submitted work. She is a consultant of TRI whose parent company owns IP on the RespirACT device used in the study.Tien Wong BSc contributed to the analysis of the data. Mr. Wong has nothing to disclose.Eugen Hlasny contributed to the analysis of the data. Mr. Hlasny has nothing to disclose.David Mikulis MD contributed to the design of the study, analysis and interpretation of the data and to the revision of the manuscript for intellectual content. Dr. Mikulis reports other from Thornhill Research Inc. outside the submitted work. Dr. Mikulis has a patent pending for a new method and apparatus (RespirACT) used in this work.Ingrid Tein MD, contributed to the design and conceptualization of the study, analysis and interpretation of the data and to the writing and revision of the manuscript for intellectual content. Dr. Tein reports an operating grant from the United Mitochondrial Disease Foundation supporting in part this study. No personal fees were obtained. The UMDF had no role in the study design, analysis of data or writing of the manuscript.

☆☆ This is a prospective non-randomized pilot case–control study of cerebral perfusion and cerebrovascular reactivity in MELAS siblings compared to healthy controls. Statistical analysis was performed by Dr. Ingrid Tein (corresponding and senior author).

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1. Introduction

Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome, the most common disease resulting from a mutation in mitochondrial DNA, most often results from an A to G substitution in bp 3243 of the $tRNA^{Leu(UUR)}$ of MT-TL1 gene (OMIM # 590050), resulting in dysfunctional assembly of mtDNA encoded respiratory chain complexes, particularly complex 1 (DiMauro and Hirano). MELAS syndrome is associated with epilepsy, migraine-like headaches, deafness, neuromyopathy, failure to thrive, short stature, lactic acidosis and stroke-like episodes (SLEs), which are recurrent neurological deficits resembling vaso-occlusive strokes (Sproule and Kaufmann, 2008); however, unlike true vaso-occlusive strokes, MELAS SLEs are not restricted to vascular territories (Ito et al., 2011), often evolve subacutely over hours to days (sometimes weeks) (Iizuka et al., 2003), have greater potential for reversibility (Iizuka et al., 2002), and have complex hemodynamic alterations beyond simple hypoperfusion (Miyamoto et al., 1997; Nishioka et al., 2008; Ooiwa et al., 1993). SLEs have a predilection for the occipital and posterior parietal and temporal cortices (Ito et al., 2011). Pathophysiology of SLEs is incompletely understood, although current literature implicates a combination of neuronal and/or glial injury as the direct consequence of mitochondrial energy failure and cerebrovascular angiopathy resulting in dysregulated cerebral perfusion (Koga et al., 2012). Evidence for cerebral angiopathy in MELAS has largely derived from studies demonstrating an increase in size and number of mitochondria in cerebral vascular endothelial and smooth muscle cells (Gilchrist et al., 1996; Mizukami et al., 1992; Zhang et al., 2010). Five studies have attempted to functionally assess cerebral blood vessels in patients with MELAS using different techniques for the measurement of cerebrovascular reactivity (CVR) with contradictory results (Gropen et al., 1994; Iizuka et al., 2007; Kodaka et al., 1996; Molnár et al., 2000; Nariai et al., 2000). Decreased CVR has been reported in three of these studies (Gropen et al., 1994; Iizuka et al., 2007; Kodaka et al., 1996). There have also been contradictory results in different studies of cerebral perfusion ranging from decreased to normal to increased cerebral blood flow in MELAS (Gropen et al., 1994; Iizuka et al., 2007; Nariai et al., 2000; Watahiki et al., 1988) with documentation of cerebral hyperemia in three studies during either subacute SLEs or chronic interictal periods (Gropen et al., 1994; Iizuka et al., 2007; Watahiki et al., 1988). Recent work has demonstrated a beneficial effect of L-arginine therapy in MELAS for the treatment and preventions of SLEs (Koga et al., 2010).

Blood oxygen level dependent (BOLD) MRI is a non-invasive means of measuring cerebral blood flow based on differences in the magnetic properties of oxygenated and deoxygenated hemoglobin (Iannetti and Wise, 2007; Nair, 2005). Assuming a constant rate of O_2 extraction, BOLD signal, over a limited physiologic range, varies inversely with the concentration of deoxyhemoglobin and therefore directly with tissue perfusion (Fan et al., 2014). BOLD CVR mapping has not been previously utilized in the setting of MELAS syndrome. Cerebrovascular reactivity (CVR) reflects the capacity of blood vessels to dilate leading to a change in cerebral blood flow in response to a change in a vasoactive stimulus, and is an important marker for brain vascular reserve (Schwertfeger et al., 2006).

The purpose of the present study was to evaluate baseline CVR in patients with MELAS syndrome under conditions of controlled changes in the partial pressure of CO_2 in arterial blood ($PaCO_2$) as the provocative vasoactive stimulus and brain BOLD as a surrogate for regional brain blood flow. We also quantified cerebral blood flow (CBF) in MELAS and controls using arterial spin labeling (ASL) technique. Our goal was to investigate the limitations of vasoactivation and perhaps its role in SLEs.

2. Methods

2.1. Study methodology

We employed a prospective case control methodology for evaluation of baseline CVR and CBF in MELAS patients compared to age and sex matched controls. This case–control study was registered on the ClinicalTrials.gov (NIH) website under identifier: NCT01603446. Research ethics board approval was obtained from the two study sites, namely the Hospital for Sick Children (HSC) and Toronto Western Hospital (TWH). A data safety monitoring committee was set in place. Written informed consent was obtained from all participants (and guardians of participants where indicated). All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki.

2.2. Subjects

Three siblings (two female) aged 17, 21 and 22 years with genetically confirmed MELAS syndrome (m.3243A>G $tRNA^{Leu(UUR)}$) were recruited from the Neurometabolic clinic at HSC, Toronto, as were four healthy age and sex-matched controls (three female). Female patients were also matched to controls for timing of menstrual cycle, and estradiol levels were measured, as estrogen levels have been shown to affect CVR. Control subjects had no ongoing medical conditions that could affect CVR (e.g. migraine, neurological disease, genetic metabolic disorder, cardiac or pulmonary disease, hypertension, prothrombotic disorder, anemia) and were taking no medications. Healthy controls were also screened prior to study entry for a normal baseline physical examination, blood pressure, serum hemoglobin, lactate, creatine phosphokinase, quantitative amino acids, carnitine, and urine organic acids. The patients and healthy subjects did not smoke. All subjects were asked to refrain from caffeine.

2.3. Study design

A complete neurological examination to ensure clinical stability, serum quantitative amino acids (AAs), and CVR and CBF studies were performed on both MELAS patients and controls. MELAS patients also underwent baseline CBC, electrolytes, renal and liver functions, serum glucose, calcium, phosphate, PT, INR, carnitine, CK, lactate, and urine organic acids. Female subjects had serum estradiol measured. Serum AAs were measured at 4 time-points over the day, both pre- and post-prandially, to better assess average AA levels. Serum arginine levels of MELAS and control subjects were compared to our well-established normal control data ($n = 500$) from the HSC Metabolic Diseases Laboratory using the Student's t-test.

2.4. CVR studies

For CVR studies, subjects were fitted with an air-tight mask on the face attached to a sequential gas delivery breathing circuit. Gas delivery to the breathing circuit was controlled by an automated gas blender (RepirAct™ Thornhill Research, Inc., ON, Canada). In our previous study of the RespirAct™ sequential gas delivery circuit in five healthy male adults comparing their $PETCO_2$ values with their arterial PCO_2, repeated measures of ANOVAs revealed no significant differences between the end-tidal PCO_2 ($PETCO_2$) (between 35 to 50 mm Hg), and arterial PCO_2 (Pa, CO_2) over the ranges of PO_2 (between 70 to 300 mm Hg) (Ito et al., 2008). This has been confirmed in animals (Brogan et al., 2004; Fierstra et al., 2011, 2013) and in humans (Ito et al., 2008; Willie et al., 2012). In the current study, the patterns of end-tidal (end-expiratory) partial pressure of CO_2 ($PETCO_2$) and O_2 ($PETO_2$) were programmed into the automated gas blender, which directed mixtures of O_2, CO_2 and N_2 into the breathing circuit according to prospective targeting algorithms developed by Slessarev et al. (2007). Tidal gas was sampled and analyzed for $PETCO_2$ and $PETO_2$ and recorded at 20 Hz.