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Mitochondrial respiratory chain disease discrimination by retrospective cohort analysis of blood metabolites

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ABSTRACT

Diagnosing primary mitochondrial respiratory chain (RC) dysfunction has long relied on invasive tissue biopsies, since no blood-based biomarker has been shown to have sufficiently high sensitivity and specificity across the myriad of individual clinical presentations. We sought to determine whether cohort-level evaluation of commonly obtained blood analytes might reveal consistent patterns to discriminate a heterogenous group of primary mitochondrial RC disease subjects both from control individuals and from subjects with pyruvate dehydrogenase deficiency.

Methods: Following IRB approval, 62 biochemical analyte concentrations or ratios were retrospectively analyzed in three well-defined and intentionally heterogeneous subject cohorts reflective of clinical practice: [1] Primary mitochondrial disease (n = 19); [2] pyruvate dehydrogenase deficiency (n = 4); and [3] controls (n = 27). Blood analyte categories included comprehensive chemistry profile, creatine kinase, lipoprotein profile, lactate, pyruvate, and plasma amino acid profile. Non-parametric analyses were used to compare the median of each analyte level between cohorts.

Results: Disease cohorts differed significantly in their median levels of triglycerides, lactate, pyruvate, and multiple individual plasma amino acids. Primary mitochondrial disease was significantly discriminated at the cohort level from pyruvate dehydrogenase deficiency by greater pyruvate and alanine elevation in pyruvate dehydrogenase deficiency, as well as significantly increased branched chain amino acid (BCAA) levels and increased ratios of individual BCAAs to glutamate in mitochondrial disease. In addition, significant elevation of median blood triglyceride level was seen in the primary mitochondrial disease cohort.

Conclusions: Blood metabolite profile analysis can discriminate a heterogeneous cohort of primary mitochondrial disease both from controls and from pyruvate dehydrogenase deficiency. Elevated BCAA levels, either absolutely or when considered relative to the level of glutamate, are common metabolic sequelae of primary mitochondrial RC disease. Prospective study is needed to validate observed plasma metabolite alterations as a potential biomarker of disease both in larger cohorts and at the individual subject level.

1. Introduction

Dysfunction of mitochondria, which support a myriad of cellular processes, causes diffuse cellular impairment or death. The minimal prevalence of mitochondrial respiratory chain (RC) disease is estimated at 1 in 5000 worldwide [1,2]. Primary mitochondrial diseases result either from mitochondrial DNA (mtDNA) mutations that conform to a maternal inheritance pattern or from nuclear gene mutations that can reflect any of the classic Mendelian inheritance patterns [3]. Mitochondrial DNA has 37 genes, including 13 that encode subunits of the energy-generating RC, while the remaining estimated 1500 proteins

Abbreviations: RC, respiratory chain; PDH, pyruvate dehydrogenase; BCAAs, branched chain amino acids; CK, creatine kinase; PC, pyruvate carboxylase; PDHc, pyruvate dehydrogenase complex.

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necessary for mitochondrial activities are encoded by the nuclear genome [4,5]. Clinical manifestations of mitochondrial disease are highly variable, both within a family, and often, in the same person over time. Multiple organ systems commonly are involved [1,6], such that affected individuals may present at any age with a progressive range of neurologic problems including migraines, stroke, epilepsy, encephalopathy, and dementia, as well as vision loss, deafness, gastrointestinal dysmotility, muscle weakness, heart failure, arrhythmias, diabetes mellitus, and kidney or liver problems [1]. This inherent variation in the constellation of clinical presentations that may be seen in mitochondrial disease poses a substantial diagnostic challenge [4].

Definitive diagnosis of primary mitochondrial disease increasingly obliges identification of a pathogenic mutation in either the mitochondrial or nuclear genomes [7]. This can be a formidable undertaking, since more than 100 different genes have been associated with primary mitochondrial disease [4,8] and upward of 1000 other likely candidates remain based on current knowledge of mitochondria-localized proteins [9,10]. Biopsy samples can be obtained from skin, muscle, or liver not only to determine the sequence and content of the mtDNA genome within each tissue, but also to assess for alterations in mitochondrial morphology and RC enzyme activity [2]. Such testing can differentiate biochemical-based mitochondrial dysfunction from other disorders [4], but approximately one third to one-half of patients with biochemically-confirmed mitochondrial RC disease have no readily identifiable pathogenic mutation in known mitochondrial disease genes even when newer massively-parallel genomic sequencing is performed of either focused panels of candidate nuclear genes or of the whole exome [4,11,12]. Further, it is possible to have biochemical findings of altered RC function in other classes of genetic or environmental disease in which the causative defect is not a mutation in a gene linked to mitochondrial function [12].

Although previous studies have identified several individual blood analyte alterations that may raise the likelihood that a given patient has mitochondrial disease, none has sufficiently high sensitivity or specificity to be widely useful as a reliable biomarker. Elevated lactate and/or pyruvate levels commonly occur in mitochondrial disease, but may only be detected during an acute medical crisis or not at all [4]. Furthermore, lactate and pyruvate elevations can result from other diseases, nutritional deficiency, hypoxia, or most commonly, from poor specimen collection and handling techniques [4]. Elevated blood alanine can indicate pyruvate accumulation, a finding observed not only in mitochondrial RC disease, but also in other metabolic disorders such as pyruvate dehydrogenase complex (PDHc) deficiency, pyruvate carboxylase (PC) deficiency, and urea cycle disorders [4]. The ratio of lactate to pyruvate is a well-established indicator of the cytoplasmic $NADH/NAD^+$ redox ratio [4], which is typically, but not exclusively, elevated in mitochondrial RC disease and normal in pyruvate metabolism defects. Other plasma amino acids that have variably been reported to be elevated in primary mitochondrial disease include glycine, proline, sarcosine, and tyrosine [4]. Levels of glutathione and one of its precursor amino acids, citrulline, were recently found to be significantly decreased in the plasma of subjects with mitochondrial disease or organic acidemia, a finding attributed to ongoing oxidative stress [13]. Lipid alterations have also been noted to occur in some patients with mitochondrial disease [14]. In addition, several studies have suggested that altered creatine metabolites may be a hallmark of mitochondrial disease, as the creatine kinase (CK) reaction converts creatine to phosphocreatine, which donates a phosphate group to ADP to generate ATP [15]. Two studies identified elevated blood CK activity in some individuals with mitochondrial disease [14,15]. Low phosphocreatine levels have also been reported to occur in both brain and muscle of RC disease patients [13]. Similarly, analysis of human myotubes treated with a mitochondrial complex I inhibitor, rotenone, identified elevated creatine and reduced phosphocreatine levels in the media [16]. FGF21 concentrations have also been shown to be a potentially reliable biomarker for RC diseases that involve muscle manifestations [17], although this has not yet been widely validated nor have clinical diagnostic assays for FGF21 been widely accessible.

Collectively, such studies are suggestive that alterations in a wide range of blood metabolites can and do occur in primary mitochondrial disease. However, most such alterations have been reported in small sample sizes because of the historic paucity of cases with a clearly-defined genetic etiology. In addition, many prior reports identified biochemical alterations in tissues, cells, or cell derivatives rather than analytes directly assayed in patients' blood. Given recent improvements in the ability to establish definitive genetic diagnoses in the setting of suspected mitochondrial disease, it is now feasible to systematically reevaluate whether any blood-based biochemical parameters are consistently altered across well-defined yet heterogeneous causes of primary mitochondrial RC disease. Such analyses may identify secondary biochemical alterations that commonly result from primary mitochondrial RC dysfunction, contribute to disease manifestations, and thereby, offer potential clinical benefit if therapeutically targeted. Comparison of the metabolic effects of primary RC deficiency and PDHc deficiency may be particularly informative to clarify two categories of mitochondrial dysfunction that impair cellular capacity to generate energy from nutrients, where only RC deficiency directly alters cellular redox balance and electron transport activity. To identify a common blood-based biochemical profile indicative of primary mitochondrial RC dysfunction, we report here results of a retrospective analysis of 62 blood biochemical analyte concentrations or ratios performed in three well-defined cohorts of subjects with primary mitochondrial RC disease, PDHc deficiency, and controls having non-specific medical concerns but no clinical or laboratory evidence suggestive of metabolic disease.

2. Methods

2.1. Human subject enrollment

The study was approved by the Institutional Review Boards of The Children's Hospital of Philadelphia and Arcadia University. Informed consent was obtained for all living subjects. A retrospective chart review was performed among subjects recruited from The Children's Hospital of Philadelphia as part of a larger study to investigate the metabolic consequences of mitochondrial disease (PI, M.J.F.). Only subjects on whom blood metabolite data were available were included in the present study. Data were obtained directly from clinical laboratory reports from all analyses available for each subject.

2.2. Clinical cohort assignment

Subjects were assigned to one of the three clinical cohorts, as follows:

2.2.1. Primary mitochondrial disease

This cohort of 19 subjects (Table 1) included 15 females and 4 males (Table 1). Age at the time of phlebotomy ranged from 1 month to 53 years. Subjects were included in this cohort if their clinical histories were consistent with primary mitochondrial disease and if they had either a known pathogenic mutation in a mtDNA or nuclear-encoded mitochondrial disease gene and/or tissue biochemistry studies consistent with significantly impaired respiratory chain enzyme activity (<30% of control mean) (Supplementary material 1). Overall, 15 had definite genetic etiologies (11 subjects with confirmed pathogenic mtDNA mutations and 4 subjects with confirmed pathogenic nuclear gene mutations in *FBXL4*, *POLG*, *MPV17*, or *RRM2B*), two adult subjects (1041 and 1043) had severe multi-systemic disease, mitochondrial DNA mutations of uncertain pathogenicity, and a deceased first-degree relative who had severe complex I deficiency demonstrated on muscle biopsy, and 2 decedents (1097 and 1157) had severe muscle biopsy biochemical