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Original Contribution

Degree of heteroplasmy reflects oxidant damage in a large family with the mitochondrial DNA A8344G mutation

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Abstract

Mitochondria are the source of most oxygen-derived free radicals. Mutations in mitochondrial DNA can impair mitochondrial electron transport resulting in decreased ATP production and increased free radical-induced oxidant injury. The specific mitochondrial DNA mutation A8344G alters the TΨC loop or the mitochondrial tRNA for lysine. We investigated a large five-generational family harboring this mutation to determine whether the degree of heteroplasmy (proportion of mutated mitochondrial genomes) for the mtA8344G mutation correlated with a marker of oxidant damage. We measured F_2-isoprostanes because they are specific and reliable markers of oxidant injury formed when free radicals attack esterified arachidonate in cell membranes. Family members with high heteroplasmy (>40%) had significantly higher F_2-isoprostane levels (62 ± 39 pg/ml) than those with lower heteroplasmy (33 ± 13 pg/ml, $P < 0.001$). The degree of heteroplasmy for the mtA8344G mutation in this family correlated positively with F_2-isoprostane levels ($P = 0.03$). This study highlights the underappreciated role free radicals play in the complex pathophysiology of inherited mitochondrial DNA disorders. The most important novel finding from this family is that some currently asymptomatic individuals with moderate heteroplasmy have evidence of ongoing free-radical mediated oxidant injury.

Keywords: Heteroplasmy; Oxidant injury; F_2-isoprostanes; Mitochondrial DNA mutation; mtA8344G

Introduction

Mitochondrial DNA (mtDNA) encodes for a distinct set of ribosomal RNAs and tRNAs as well as for 13 subunits of the electron transport chain [1]. We report a family that harbors an A–G base transition at nucleotide position 8344 in the mtDNA that alters the TΨC loop of the mitochondrial tRNA for lysine (MTTK gene) [2]. Specific deficiencies in muscle energetics and mitochondrial respiratory complexes I and IV were characterized in a family with maternally inherited myoclonic epilepsy and ragged red fibers (MERRF) even before this phenotype's association with this specific mutation [3]. Since the initial reports of the classic MERRF phenotype, other less clearly defined phenotypes have been associated with the mtA8344G mutation [4–6]. The index case of this family had years of medical evaluations before his progressive muscle wasting and unsteadiness were explained by his high heteroplasmy (proportion of mutated mitochondrial genomes) for the mtA8344G mutation. Mutations in mtDNA are transmitted almost exclusively through the maternal line. Upon learning that the index case's maternal grandmother was one of six sisters, we realized that further investigation of this large family could test the hypothesis that oxidant damage correlates with the degree of heteroplasmy for the mtA8344G mutation.

Under normal physiological conditions, as many as 2% of electrons leak from the mitochondrial electron transport chain and reduce oxygen to superoxide anion [7–9]. Thus, mitochondria are a major source of oxygen-derived free

Abbreviations: MERRF, inherited myoclonic epilepsy and ragged red fibers.

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radicals in the normal physiologic state. When electron transport is impaired, more electrons leak and form superoxide radicals, triggering a cascade of free radicals that indiscriminately damage biological macromolecules [10–12]. Oxidant damage has been implicated in a host of degenerative diseases and is increased in cigarette smokers [13,14]. In this family with a mutation altering the mitochondrial electron transport chain, F_2-isoprostanes were measured to determine the extent of oxidant damage. F_2-isoprostanes are a specific group of prostaglandin F_2-like compounds formed when free radicals attack esterified arachidonate in cell membranes [15]. Measurement of these specific products of lipid peroxidation is emerging as one of the most reliable indices of in vivo oxidant injury [16,17]. Increasing heteroplasmy for the mtA8344G mutation in members of this large, five-generational family correlated positively with plasma F_2-isoprostane levels and therefore with oxidant injury.

Materials and methods

Ascertainment/phenotyping

All participating family members provided informed consent prior to enrollment in this study. This study was limited to adults over the age of 18. Demographic data, including age, sex, height, and weight, were collected. Each participant supplied a medical history including a list of ongoing medical problems, current medications, prior hospitalizations, and past surgeries. A specific symptom survey inquired about muscle weakness, unsteadiness, spontaneous muscle contractions, hearing loss, visual difficulties, seizures, and diabetes. Smoking status, a known potential confounder of F_2-isoprostane levels, was reported [13].

Genotyping

Genomic DNA was isolated from 3 ml of whole blood and from buccal epithelium using the Wizard kit (Promega; Madison,WI). PCR in the region of the MTTK gene was carried out using the forward primer CTACCCCCTCT-AGAGCCCAC and the reverse primer GTAGTATT-TAGTTGGGGCATTTCACTGTAAAGCCGTGTTGG as described by Zevani et al. [18]. The reverse primer creates a new *Bgl*I site in the presence of a PCR-amplified A8344G mutation. Products of *Bgl*I (New England Biolabs, Beverly MA) digestion were resolved on 3% agarose gel. The intensities of the wild-type (108 bp) and the mutant (73 bp) were measured using a Hitachi FMBioII (Tokyo, Japan) gel scanner. The raw intensity value for each band was divided by the size of the fragment to obtain a corrected value. Percentage heteroplasmy is presented as the corrected mutant value divided by the sum of the corrected wild-type and mutant values.

Plasma sample acquisition/mass spectroscopic analysis of F_2-isoprostanes

Individual blood specimens were centrifuged promptly for 10 min at 14,000 rpm. The separated plasma was then immediately frozen on dry ice to prevent auto-oxidation. No specimens were shipped. All specimens were stored at −80°C. No antioxidants were added to the plasma samples prior to storage. Specimens were stored no more than 2 weeks before F_2-isoprostane determination. Plasma F_2-isoprostanes do not vary diurnally or with meals, so plasma specimens were obtained throughout the day depending on the availability of the subjects. A highly accurate gas chromatographic/negative-ion chemical ionization mass spectroscopic assay was utilized to measure F_2-isoprostanes [19].

Statistical analysis

Means of continuous variables measured in two groups were compared using Student's t test taking into account unequal variances when necessary. The Pearson product-moment correlation coefficient was used to measure the strength of association between two continuous variables. The level of significance was set at 0.05. All statistical analyses were done utilizing STATA 8.0 (College Station, TX).

Results

Ascertainment/demographics

Twenty-five of 32 (78%) of adults in this family provided DNA samples for analysis. Of these adults, 23 (92%) had F_2-isoprostane levels measured.

Fig. 1 presents the five-generational family pedigree. The mean age of the adult family members was 48 years (range 22–80). Forty-four percent were male. Six (24%) were cigarette smokers. Ten members of Generation III (50%) are deceased. The median age at the time of death was 40 years (range 4 to 71).

Symptom survey/selected case reports

No family members, other than the index case, were previously diagnosed with a mitochondrial disorder. No family member was known to have both myoclonic epilepsy and progressive muscle wasting as would be expected with the classic MERRF phenotype. One deceased member of Generation III (III-7) had a progressive muscle disorder and died at age 54. This person's sister (III-9) had 15% heteroplasmy for mtA8344G detected and transmitted the mutation to all three of her sons (IV-8, 9, 10). Another deceased member of Generation III (III-17) developed seizures at age 31. Systematic survey of family members for symptoms that are related to mitochondrial disorders