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MELAS syndrome, cardiomyopathy, rhabdomyolysis, and autism associated with the A3260G mitochondrial DNA mutation

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ABSTRACT

The A to G transition mutation at position 3260 of the mitochondrial genome is usually associated with cardiomyopathy and myopathy. One Japanese kindred reported the phenotype of mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS syndrome) in association with the A3260G mtDNA mutation. We describe the first Caucasian cases of MELAS syndrome associated with the A3260G mutation. Furthermore, this mutation was associated with exercise-induced rhabdomyolysis, hearing loss, seizures, cardiomyopathy, and autism in the large kindred. We conclude that the A3260G mtDNA mutation is associated with wide phenotypic heterogeneity with MELAS and other "classical" mitochondrial phenotypes being manifestations.

1. Introduction

Over 200 pathologic point mutations in the mitochondrial DNA (mtDNA) have been documented in association with mitochondrial disease [1,2]. Several "classical" acronym-based phenotypes have been described; including mitochondrial encephalopathy, lactic acidosis and stroke-like episodes (MELAS), myoclonic epilepsy with ragged red fibres (MERRF), Leber's hereditary optic neuropathy (LHON), chronic progressive external ophthalmolplegia (CPEO), and neuropathy, ataxia, and retinitis pigmentosa (NARP). Phenotypic and genotypic heterogeneity are very common with mtDNA disorders. For example, at least 30 mtDNA mutations have been associated with MELAS syndrome [2], and conversely, mutations at the A3243G position have been associated with a wide clinical phenotype including; MELAS, type 2 diabetes, hearing loss, CPEO, Leigh disease, and mitochondrial myopathy. The most commonly reported confirmed pathogenic mtDNA mutation is the A3243G transition in leucine tRNA [3], with a prevalence as high as 60/100,000 in the general population [4]. Other reported mutations in the tRNA for leucine include; G3244A [5], A3252G [6], C3256T [7], T3258C [8], T3271C [9,10], T3291C [11], and A3260G [12].

The A3260G transition mutation is listed on Mitomap (http://www.mitomap.org/MITOMAP/MutationsRNA) as a confirmed mutation associated with Maternal Myopathy and Cardiomyopathy (MMC). One Japanese family has been reported to have the MELAS syndrome in association with the A3260G transition. The proband of the Japanese family had headaches, complex partial seizures and myoclonic epilepsy, several stroke-like episodes and lactic acidosis [12]. Two reports of families with the A3260G mutation from Italy [13], and the United Kingdom [14], have described adult onset MMC with no evidence of central nervous system dysfunction, suggesting that the MELAS phenotype with the A3260G mutation is rare and possibly restricted to the Japanese. Here we report the first family outside of Japan to have multiple family members affected with MELAS-like syndrome due to the A3260G mtDNA point mutation. We also expand the A3260G phenotype to include exercise-associated rhabdomyolysis, hearing loss, and possibly, autism.

2. Materials and methods

2.1. Case histories, examination, and pedigree

The family (Fig. 1) is a large, non-consanguineous family of Italian origin now living in Canada. Patients III-3, IV-1,2,4,5,6,7,9,10,

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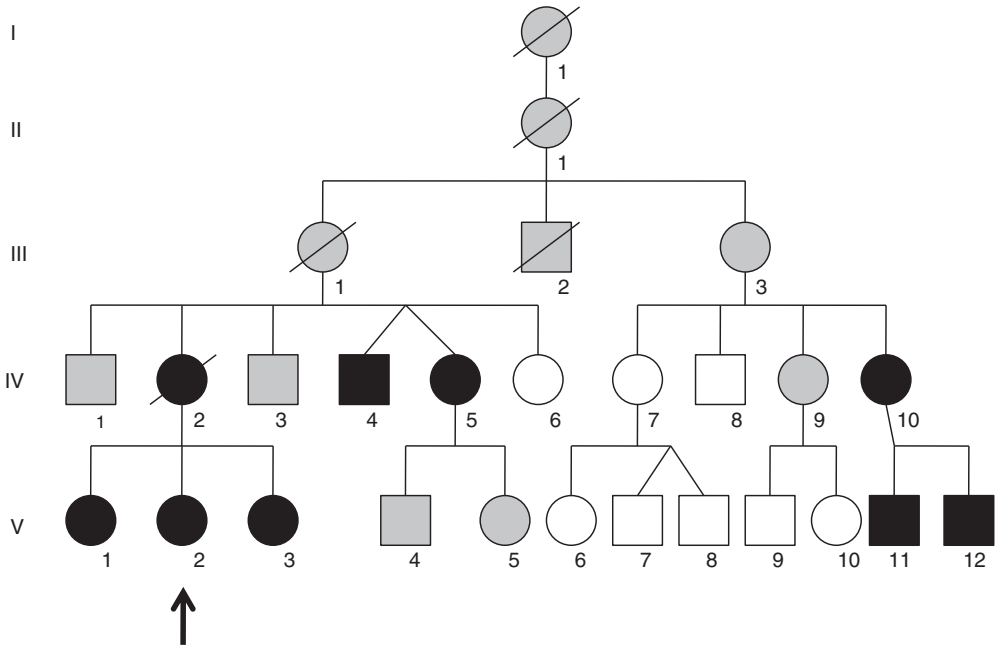

Fig. 1. Pedigree of the family. Circle = female, square male, oblique line deceased. Black symbols: affected with abnormal muscle biopsy or lactic acidosis; grey symbols: suspicious symptoms or signs, normal muscle biopsy, or not biopsied. Proband indicated by arrow.

and V-1 to V-6, 11,12 had neurological examinations and history completed by one of the authors (MAT). The histories of patients I-1, II-1, III-1, and III-2, were obtained through interviews with the patients in generation IV. Patient III-1 died at McMaster University Medical Center and her file was reviewed by the authors (MAT and ASF).

2.2. Muscle histology and ultrastructure

Needle muscle biopsies were obtained from the vastus lateralis of five patients using a 5 mm suction-modified Bergstom needle-biopsy technique [15]. As previously described by our group, all muscle biopsies were evaluated using a standard set of histological stains and light microscopy, and a further piece of approximately 50 fibres was obtained and immediately submerged in ice cold 2% glutaraldehyde, embedded, sectioned, and stained for ultrastructural analysis [15]. The muscle biopsy of the proband was performed and analyzed at the Hospital for Sick Children in Toronto, Canada, using the same standard stains and electron microscopic methods.

2.3. Molecular and biochemical analysis of blood and muscle

Mitochondrial DNA from whole blood (patients IV-2,4,5,6,7,9,10, V-2,3,4,5,11,12) and muscle (patients IV-2,4,5,6, V-2) was analyzed using the following technique: the mitochondrial DNA region 3130–3310, encompassing $tRNA^{Leu}$, was amplified by polymerase chain reaction, using forward primer AGG ACA AGA GAA ATA AGG CC and reverse primer GTA TGT TGT TAA GAA GAG GAA TTG AAC CTC TGA CTG TAA AGG AAT AAG TT containing three mismatched residues, which together with the mutation A3260G form a single recognition site for restriction endonuclease Xmn 1. Digestion of the 180 bp PCR product generates two fragments of 134 plus 46 nucleotides, respectively, in amounts proportional to the mutant DNA. The heteroplasmy of DNA from different patients and tissues was estimated using densitometric measurement of the undigested vs. digested bands visualized on agarose gels with ImageJ 1.35p (National Institutes of Health). The maximal enzyme activity of complex I + III, II + III, cytocrome *c* oxidase, and citrate synthase was completed on frozen muscle homogenates, as described by our group [16].

3. Results

3.1. Case histories, examination, and pedigree

The clinical features and lactate values (where available) of family members are summarized in Table 1. The proband (patient V-2) presented at 8 years of age with symptoms of dyspnea and exercise intolerance. She was noted to have mild ptosis but no other myopathic signs, short stature, elevated serum creatine kinase (CK) (708 IU/L) and borderline high venous lactate (2.4 mmol/L) ($N < 2.2$ mmol/L) but normal urine organic acids. Serum mtDNA analysis did not include the A3260G mutation and was negative. Echocardiography and endomyocardial biopsy revealed hypertrophic cardiomyopathy with some increased microvesicular lipid and vacuolar degeneration but no mitochondrial ultrastructural changes. She was stable on medications for 2 years but then progressively deteriorated and presented with severe dilated cardiomyopathy and heart failure, and went on to receive an orthotopic heart transplantation in 2001. Ultrastructural analysis of the heart at that time showed accumulation of pleomorphic mitochondria. Histochemical stains were normal. Initial muscle mtDNA sequencing was negative using temporal temperature gradient gel electrophoresis (TTGE). In the post-operative period she became hypertensive (180/130 mmHg) and developed seizures. MRI showed focal subcortical changes consistent with hypertensive encephalopathy. Persistent plasma lactate elevations up to 14 mmol/L were noted, venous pH was 6.9, bicarbonate was 5 mmol/L, CSF lactate was 4.7 mmol/L, and CK, 1239 U/L. She received dichloroacetate (DCA) acutely and stabilized. She continued on long-term DCA (and thiamine) therapy titrated against her lactate levels and clinical symptoms. She was also treated with a cocktail of supplements consisting of coenzyme Q10, creatine monohydrate, alpha-lipoic acid, riboflavin, vitamin E, and vitamin C. Her cranial MRI imaging abnormalities resolved after 4 months.