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MELAS SYNDROME

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CONTENTS

ABSTRACT

MELAS syndrome, an acronym for Mitochondrial myopathy, Encephalopathy, Lactic Acidosis, and Strokelike episodes, is a very severe form of mitochondrial encephalopathy. It has several genetic causes, the most frequent of which is the m.3243A>G mutation in the MT-TL1 gene on the mitochondrial DNA (mtDNA), often called the "MELAS mutation". This mutation is the most recurrent deleterious mtDNA point mutation. It is always heteroplasmic and responsible for very diverse clinical presentations in addition to MELAS syndrome. Diseases associated with the m.3243A>G mutation are thought to be due to oxidative phosphorylation defects. They display complex genotype/phenotype relationships and pathophysiological mechanisms, as well as difficulties for the set up of controlled therapeutic trials.

MELAS SYNDROME AND MELAS MUTATION

The acronym MELAS (Mitochondrial myopathy, Encephalopathy, Lactic Acidosis, and Strokelike episodes) was first coined by Pavlakis and colleagues (1) to describe a disease that presented in childhood with a combination of acute episodes resembling strokes and chronic symptoms including short stature, seizures, mitochondrial myopathy and chronic hyperlactatemia. This maternally transmitted syndrome was associated with a very poor outcome, with mental deterioration and premature death.

The histological hallmarks of mitochondrial myopathy are the presence of scattered muscle fibers with defective cytochrome *c* oxidase (COX) activity and significant mitochondrial proliferation (responsible for the ragged-red appearance of the muscle fibers stained with Gomori trichrome or for their dark staining with succinate dehydrogenase [SDH] histochemical activity) (Fig. 1). These mitochondrial alterations are intrinsic to the definition of MELAS syndrome (1). They are, however, also seen in numerous mitochondrial diseases unrelated to MELAS syndrome (2). The relative preservation of COX activity in the muscle fibers with striking mitochondrial proliferation (the so-called COX-positive ragged-red fibers; Fig. 1) differs, however, from the usually complete COX defect of the ragged-red fibers observed in other types of mitochondrial myopathy. Small muscle vessels with increased mitochondrial mass are also frequently reported in MELAS syndrome and may have relevance for its physiopathology (3, 4).

The m.3243A>G point mutation in one of the two mitochondrial DNA (mtDNA) leucine transfer RNA genes (*MT-TL1*) was identified shortly thereafter as the cause of MELAS syndrome (5, 6). Although this mutation is by far the most frequent cause of MELAS syndrome

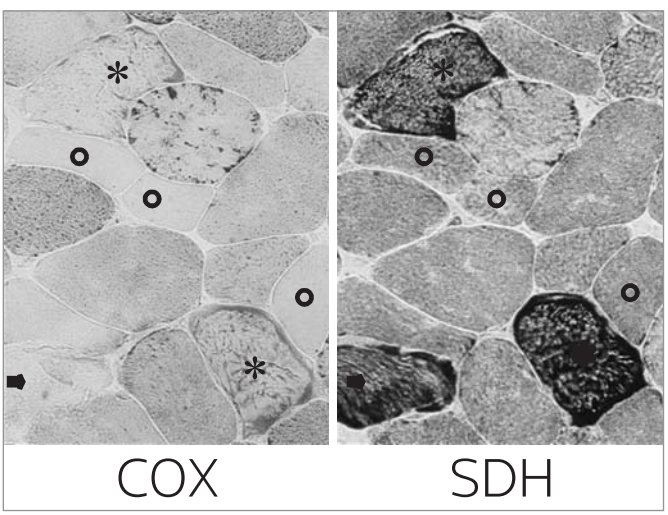

Figure 1. Muscle histological anomalies observed in association with the m.3243A>G mtDNA mutation. Cytochrome *c* oxidase (COX), or respiratory chain complex IV, activity depends on mtDNA-encoded subunits and is therefore defective when mtDNA translation is altered. Succinate dehydrogenase (SDH), the proximal part of respiratory chain complex II, activity only depends on nuclear DNA-encoded subunits. Its level therefore reflects the muscle mitochondrial population. Fibers with a complete COX defect (empty circles) have a normal mitochondrial population whereas fibers with striking mitochondrial proliferation often present with residual COX (asterisk). However, some fibers with mitochondrial proliferation may be completely COX-defective (arrow).

and is therefore often referred to in the literature as the "MELAS mutation", numerous mutations of mtDNA have subsequently been found to be responsible for almost identical clinical presentations (see Mitomap database at http://www.mitomap.org/). These mutations modified other nucleotide positions in the *MT-TL1* gene, other tRNA genes, the 16S ribosomal gene or genes encoding structural subunits, most often subunits of the respiratory chain complex I. Alterations of the nuclear gene encoding the mtDNA polymerase (*POLG*) have also recently been found to be responsible for MELAS syndrome (7). The m.3243A>G mutation, as well as all the mtDNA mutations subsequently associated with MELAS syndrome, were shown to be heteroplasmic, i.e., to coexist with a residual amount of wild-type mtDNA molecules within each cell, a feature common to severely deleterious mtDNA mutations.

CLINICAL SPECTRUM OF DISEASES ASSOCIATED WITH THE M.3243A>G MUTATION

In surveys of patients with mitochondrial diseases, the m.3243A>G mutation is the most recurrent mtDNA point mutation (8, 9). Recent population-based surveys have shown an incidence of up to 0.25% for the mutation, thus implying that particularly great attention should be applied to its genotype/phenotype relationship (10, 11).

MELAS syndrome due to the m.3243A>G mutation usually presents in childhood or young adulthood, with acute episodes resembling strokes associated with severe headaches, vision disorders with hemianopsia and, less frequently, hemiparesis, seizures and altered consciousness. Chronic symptoms such as failure to thrive, intolerance to exercise and deafness are often associated with the acute episodes.

Clinical presentations associated with the m.3243A>G mutation are, however, very diverse, including presentations that differ from MELAS syndrome in the age at onset, the rate of progression and the nature of symptoms (4, 12, 13). Hypertrophic cardiomyopathy and cardiac insufficiency, progressive renal insufficiency (14) or gastrointestinal disease with chronic pseudo-obstruction (15) may be prominent in an otherwise multisystemic disease. Numerous patients have also been reported with relatively paucisymptomatic forms, the most frequent being the association of diabetes and deafness leading to "mitochondrial inherited diabetes and deafness" (MIDD) (16), which represents up to 2% of adult forms of diabetes (17). Several patients present with only muscle symptoms first involving ocular muscles and later on skeletal muscles (18, 19). A number of cases without any symptoms until late in life have also been observed either as sporadic patients or as relatives diagnosed during familial investigations of severely affected patients (11-20). In our diagnostic center, the latest onset of symptoms associated with the m.3243A>G mutation was observed in a woman who had been healthy until the age of 71 years, when she experienced a subacute drop of the head due to axial muscle weakness.

PATHOPHYSIOLOGY OF THE M.3243A>G MUTATION

Lactate accumulation reflects a defect in NADH oxidation by the respiratory chain flux and its effects on pyruvate utilization and cellular redox ratios. Abnormal accumulation of lactate in body fluids is an almost constant trait of patients affected with MELAS syndrome, but is much less frequently observed in patients presenting with paucisymptomatic clinical forms (4). It may therefore be considered an indicator of the severity of metabolic impairment (21).

Defective oxidative phosphorylation in muscle

Biochemical analyses of the oxidative phosphorylation pathway have mostly been performed in patients' muscle biopsies. As expected from a tRNA mutation with a global impact on mtDNA translation, the studies disclosed combined defects of respiratory chain activities, only sparing respiratory chain complex II, which is the sole respiratory complex with only nuclear DNA-encoded subunits (22, 23). Respiratory chain complex I often appeared to have the most severely defective activity and some patients even presented with an apparently isolated complex I defect (19).

Oxidative phosphorylation (OXPHOS) activities have also been analyzed in single muscle fibers from patients' muscle biopsies using histoenzymological methods. The activity of the mtDNA-dependent respiratory chain complex IV is analyzed by the COX histochemical stain, while that of respiratory chain complex II is analyzed by the SDH stain (Fig. 1). Defective mtDNA translation was demonstrated in the patients' single muscle fibers by the parallel decrease in the amount of mtDNA-encoded proteins and mtDNA-dependent COX activity in association with increased mtDNA-encoded mRNA levels (24). Very high proportions of mutations were observed in single muscle fibers with defective COX activity whatever the state of mitochondrial proliferation (25), suggesting that the first consequence of a high mutation load was respiratory chain defects. Apoptosis, however, was only observed in muscle fibers with prominent mitochondrial proliferation in addition to abnormal COX activity, thus indicating that the occurrence of mitochondrial proliferation reflected further cellular alteration associated with a very high mutation load (24-26).

Molecular consequences of the m.3243A>G mutation in cellular models

Cybrid (for cytoplasmic hybrid) cells are obtained by a fusion of cells devoid of mtDNA (rho0 cells) and cells from a patient carrying the mtDNA mutation (27). They are essential tools for studying the consequences of an mtDNA alteration independently of its initial nuclear genetic background. Several analyses of cybrids carrying the m.3243A>G mutation have been reported (28-31). They all showed that the mutation induced loss of function of the $tRNA^{leu(UUR)}$-associating global translation defect and respiratory defect, albeit only at very high mutation loads, generally above 90%. However, defective respiration on complex I substrates was observed at lower mutation loads (around 50%), a finding reminiscent of enzymatic observations in patients' muscle (30).

The m.3243A>G mutation was associated with reduced steady state of the $tRNA^{leu(UUR)}$ and disruption of the 5-taurinomethyl modification of the wobble position of the $tRNA^{leu(UUR)}$ anticodon, which further impairs the tRNA decoding function but may also permit amino acid misincorporation (32).

Primary cells derived from patients have also shown the respiratory defect associated with the mutation (33, 34). Primary myoblasts derived from patients' muscle have recently been used to demon-

strate the presence of gain of function of the mutant $tRNA^{Leu(UUR)}$, with probable amino acid misincorporation (34).

GENOTYPE/PHENOTYPE RELATIONSHIP, NATURAL HISTORY AND TREATMENT OF DISEASES ASSOCIATED WITH THE M.3243A>G MUTATION

Evaluation of potential therapeutics cannot be effective in diseases for which the genotype/phenotype relationship and natural history have not been elucidated.

Genotype/phenotype relationship

Heteroplasmy is an important confounding parameter, as it makes it possible for the proportion of mutations to greatly vary in different organs of a patient. This is the case for the m.3243A>G mutation (35, 36). Relevant symptoms in MELAS patients originate from cerebral dysfunction, but the m.3243A>G mutation load cannot be assessed in the brain. The challenge is therefore to correlate symptoms in MELAS patients to mutation load in a tissue easily available for genetic analysis.

Searching for such a relationship does not appear to be an unrealistic project. Previous analyses of autopsied patient samples have shown relatively homogeneous tissue distribution of the mutation load (37). The tissue distribution of the mutation load observed in patients follows a nonrandom pattern, with the proportion of m.3243A>G mutations always being the highest in muscle and cells from the urinary sediment, followed by buccal mucosa and blood (35, 36-38). A decrease in the proportion of m.3243A>G mutations in blood with age has been reported, thus explaining the low mutation load of this tissue (39, 40). These data suggest that the observed heterogeneous tissue distribution of the mutation represents the evolution over time of an initially homogeneous mutation load modified in rapidly dividing tissues by efficient selection against cells with a high mutation load (35).

Indeed, the mutation load in urinary cells was reported to correlate with the global severity of the disease evaluated using a global clinical rating scale (41, 42). In a series of 111 patients recruited from a local patient database and also from literature reports, a significant positive correlation was found between the frequency of recurrent strokes, dementia, seizures and ataxia and the proportion of m.3243A>G mutations in muscle, but not in blood (43). The relationship between the occurrence of symptoms and mutation load was, however, relatively loose as, for example, the frequency of epilepsy was roughly 35% with a proportion of mutations in muscle ranging from 40% to 80%. The relationship between mutation load and cerebral symptoms was further shown by NMR spectroscopy analysis of brain lactate in eight carriers of the m.3243A>G mutation, demonstrating a tight link between the lactate/creatine ratio and the mean proportion of mutations in all available samples (blood, buccal and urine cells) (38).

The relationship between muscle symptoms and mutation load appears much less straightforward. A significant but inverse correlation was found between muscle mutation load and the frequency of ophthalmoplegia and myopathy in the series of 111 patients with a positive correlation between cerebral symptoms and muscle mutation load (43). When muscle oxidative metabolism was assessed with NMR spectroscopy in a series of eight mutation carriers, it did not correlate with muscle mutation load (44). In contrast, when assessed by maximal oxygen uptake and workload in a series of 51 carriers of the mutation, it showed a tight inverse correlation with muscle mutation load (22). The significant influence of exercise training on muscle aerobic metabolism may in part underlie these discrepancies.

Natural history

Retrospective reports on diseases associated with the m.3243A>G mutation have shown them to be progressive. Recurrent cerebral insults and also chronic progressive cerebral deterioration occurred in the most severe cases of MELAS syndrome. Less severe clinical presentations were also progressive with respect to muscle, heart, glucose metabolism or hearing involvement (4, 5, 12, 13, 19, 38, 45, 48). A prospective study of 3 years' duration in 33 adult carriers of the m.3243A>G mutation has recently shown a high rate of sudden unexplained death, progression of glucose intolerance and hearing impairment, an increase in left ventricular wall thickness and deterioration of quantitative EEG (49). Evaluation of any potential therapy must therefore take into account the wide diversity of clinical presentations, as well as the natural history of the different clinical presentations associated with the m.3243A>G mutation.

Treatment

Beneficial effects of drugs have been reported in MELAS patients, including dichloroacetate, a lactate-lowering agent that acts by activating the pyruvate dehydrogenase complex, lipoic acid, a cofactor of the pyruvate dehydrogenase complex, L-arginine, a nitric oxide precursor, creatine, a phosphocreatine precursor, and ubiquinone, a cofactor in oxidative phosphorylation. These trials either gave contradictory results or were not optimal, thus preventing a firm conclusion from being drawn (50). The most frequent drawbacks were the absence of randomization or placebo control, the small number of patients, the diversity of the causes or clinical presentations of the mitochondrial diseases analyzed and the lack of direct relevance to clinical severity of the proposed outcome measures.

More recently, a controlled clinical trial in MELAS syndrome reported on the use of dichloroacetate in a series of 30 patients with both MELAS syndrome and the MELAS mutation (51). The primary outcome measure was the global assessment of the patients' neurological, neuropsychological and general well-being. It was therefore clearly relevant to the disease natural history. Significant peripheral nerve toxicity led to premature termination of the trial. Within the 2 years' frame of that trial no beneficial effect could be detected on the recurrence of strokes, seizures or the neurological and general well-being of the patients. Despite these negative results, the report demonstrated the feasibility of performing therapeutic trials in mitochondrial diseases.

Another recent double-blind, placebo-controlled study (52) reported positive results for the combination of creatine, ubiquinone and lipoic acid. The outcome measures were numerous, but most had disputable relevance to the pathology. In addition, this study was performed on a small number of patients with very diverse mitochondrial diseases with respect to cause and symptoms.

CONCLUSIONS

MELAS syndrome and other clinical presentations associated with the heteroplasmic m.3243A>G mutation are thought to be due to oxidative phosphorylation defects. They display complex genotype/phenotype relationships and pathophysiological mechanisms, as well as difficulties for the set up of controlled therapeutic trials. Prospective analyses of the natural history of numerous patients, with special emphasis on the tissue distribution of the mutation load, as well as pathophysiological studies on patient tissues instead of immortal cell lines, are needed to help choose relevant criteria for the outcome evaluation, as well as therapeutic strategies. The recurrent nature of the MELAS mutation should then allow the set up of proper double-blind, placebo-controlled studies in sufficient numbers of patients.

DISCLOSURE

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