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MELAS: clinical phenotype and morphological brain abnormalities

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Abstract We describe the clinical and neuropathological findings of three unrelated autopsy cases of MELAS harboring the A3243G transition in the mitochondrial DNA (mtDNA). Using immunohistochemical techniques, we studied the expression of several subunits of the respiratory chain in various brain regions from the same cases. In all three cases there was a reduced immunocytochemical staining for mtDNA-encoded subunits of the respiratory chain, confirming the presence of a defective mitochondrial protein synthesis in this disease. Mitochondrial abnormalities were mostly confined to multiple areas of different size and shape, in agreement with the focal character of the brain pathology in MELAS, and were most prominent in the cerebral cortex, providing a morphological contribution to the explanation of the cognitive regression of the patients. Immunoreactivity for mtDNA-encoded subunits was reduced in the walls of many pial and intracerebral arterioles of different brain regions but there was no clear correlation between territories of affected vessels and distribution of the histological and immunohistochemical lesions. Cerebral focal lesions in MELAS might have a metabolic nature and several pathogenetic mechanisms might be involved in the genesis of stroke-like episodes when there is a local increased ATP demand.

Keywords Mitochondrial encephalomyopathy · MELAS syndrome · Mitochondrial DNA · Stroke · Neuropathology

Introduction

Along with Kearns-Sayre syndrome (KSS) and myoclonic epilepsy with ragged-red fibers (MERRF), mitochondrial encephalopathy, lactic acidosis and stroke-like episodes (MELAS) is one of the three most distinctive mitochondrial encephalomyopathies [35, 41]. An A3243G transition in the transfer RNA (tRNA) leucine (UUR) gene of the mitochondrial DNA (mtDNA) is the most common cause of the disease, being responsible by itself for about 80–86% of the cases [13, 18, 23, 40]. The same mutation has been associated with other distinct maternally inherited phenotypes including progressive external ophthalmoplegia (PEO) [37], diabetes with or without deafness [11] and hypertrophic cardiomyopathy [46].

Affected patients typically have: stroke before 40 years of age, lactic acidosis, ragged-red fibers (RRFs), and encephalopathy with seizures, dementia or both [18]. The severity of the clinical phenotype in unrelated patients as well as among members of the same maternal lineage is usually variable, and it is thought that this might reflect various degrees of mtDNA heteroplasmy (i.e., coexistence of mutant and wild-type mtDNAs) in individuals carrying the A3243G mutation [27].

Pathologically the disease is characterized by multiple softenings, varying in size and age, predominantly involving the cerebral cortex bilaterally and not conforming to the distribution of large vessels [48]. Basal ganglia calcification, spongiosis and neuronal loss, mostly in the cerebral and cerebellar cortex, are other common neuropathological findings [48].

Even if the central nervous system (CNS) is usually affected in MELAS, only few data are available about the regional variability of mitochondrial abnormalities in the CNS, so that the morphological basis for the variable neurological manifestations of the disease have not been fully understood.

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In the present work, using immunohistochemical techniques, we studied the expression of several subunits of the mitochondrial respiratory chain in various brain regions from three unrelated autopsy cases of MELAS that harbored the A3243G mutation. We also correlated clinical and neuropathological features with immunohistochemical data and we evaluated the significance of the distribution of mitochondrial abnormalities in determining clinical manifestations.

Case reports

Case 1

This patient was the first daughter of a non-consanguineous couple. She had one healthy brother. The mother and one of five maternal uncles had suffered from bilateral sensorineural deafness. The patient was born after a normal pregnancy. Delivery was by cesarean section for umbilical cord prolapse. Motor milestones were attained normally only during the first 6 months of life.

At 14 months the child appeared small and microcephalic. She could not sit or stand even with support. On neurological examination she was hypotonic and deep tendon reflexes were increased. Occasional dystonic postures were seen. On fundoscopy, the retinal-pigmented epithelium had a granular appearance. Laboratory testing showed normal CSF, except for a lactate level of 38.3 mg/100 ml (normal range 3.5–18.1 mg/100 ml). Blood lactate was 35.1 mg/100 ml (normal range 3.5–18.1 mg/100 ml) and pyruvate 1.8 mg/100 ml (normal range 0.3–1.1 mg/100 ml). An EMG demonstrated fibrillations, polyphasic potentials, and myotonia; nerve conduction velocities were normal. CT scan re-

vealed moderately enlarged ventricles and a small cerebellar vermis. An MRI study showed mild cerebral atrophy. Skin, conjunctiva, muscle, and rectal biopsy specimens were all normal. Sural nerve had minor ultrastructural abnormalities suggestive of a disorder of the myelin.

At 19 months, she developed flexor spasms, and at 21 months, generalized seizures. At 3.5 years, she had frequent myoclonic and generalized seizures. On neurological examination the patient was somnolent; athetoid movements were occasionally seen. The optic discs were pale, and salt and pepper changes were seen on fundoscopy. An ERG showed signs of diffuse retinal degeneration, classified as a retinitis pigmentosa variant. By 5.5 years, she was hypotonic with decorticate posture and rare seizures. The patient died at home at 7 years of age.

Clinical, laboratory, and neuroradiological features of the patient are summarized in Table 1.

Case 2

This male was borne after a normal pregnancy and delivery. There was no family history of neurological diseases and the parents were not related. Motor and intellectual milestones were normally attained during infancy.

Since the age of 30 years, he began to suffer from bilateral hearing loss. At 47, the patient had generalized seizures and was first admitted to hospital. On examination he was short (160 cm), deaf and mentally euphoric. He also had slight right hemiparesis, alexia, right hemianopsia and tactile agnosia. Laboratory investigations were normal except for a moderate hyperuricemia. EEG showed slowing of background activity and focal epileptiform abnormalities (paroxysmal high-voltage discharges in a pseudoperiodic pattern) in temporal and posterior regions of the left hemisphere. CT scan revealed a left temporo-parietal ischemic area (Fig. 1). Cerebral angiography was normal.

At 49 years, the patient appeared weak and aboulc. SPECT revealed reduced tracer uptake in the left temporal and occipital areas. EMG and short-latency somatosensory evoked potentials were normal. Over the next few years he had recurrent seizures, asthenia and slight intellectual deterioration. Laboratory abnormalities were confined to hyperuricemia and raised serum lactate (24.5 mg/100 ml). ECG demonstrated a lateral subepicardial ischemia. Sporadic slow abnormalities in temporal regions of the left hemisphere were found on EEG. CT scan showed minimal basal ganglia calcification, cerebral and cerebellar atrophy. Muscle biopsy revealed RRFs.

At 53 years, he developed left hemiplegia with aphasia. CT scan demonstrated a large right capsular infarct involving the homolateral postrolandic cortex (Fig. 1). The patient died of heart failure few days after the hospitalization (Table 1).

Table 1 Summary of clinical, biochemical and neuroradiological features of the patients

	Case 1	Case 2	Case 3
Age at onset (years)	1.2	30	15
Onset symptom	Hypotonia	Hearing loss	Seizures
Seizures	+	+	+
Cognitive regression	+	+	+
Hemiparesis	-	+	+
Hemianopsia	-	+	+
Pigmentary retinopathy	+	-	-
Hearing loss	+	-	+
Dystonia and athetosis	+	-	-
Muscle weakness	-	-	+
Cerebellar signs	+	-	+
Lactic acidosis	+	+	+
RRF	-	+	+
CT-MRI abnormalities			
Focal lesions	-	+	+
Cortical atrophy	+	-	+
Basal ganglia calcification	-	+	+
Age at death (years)	7	53	23

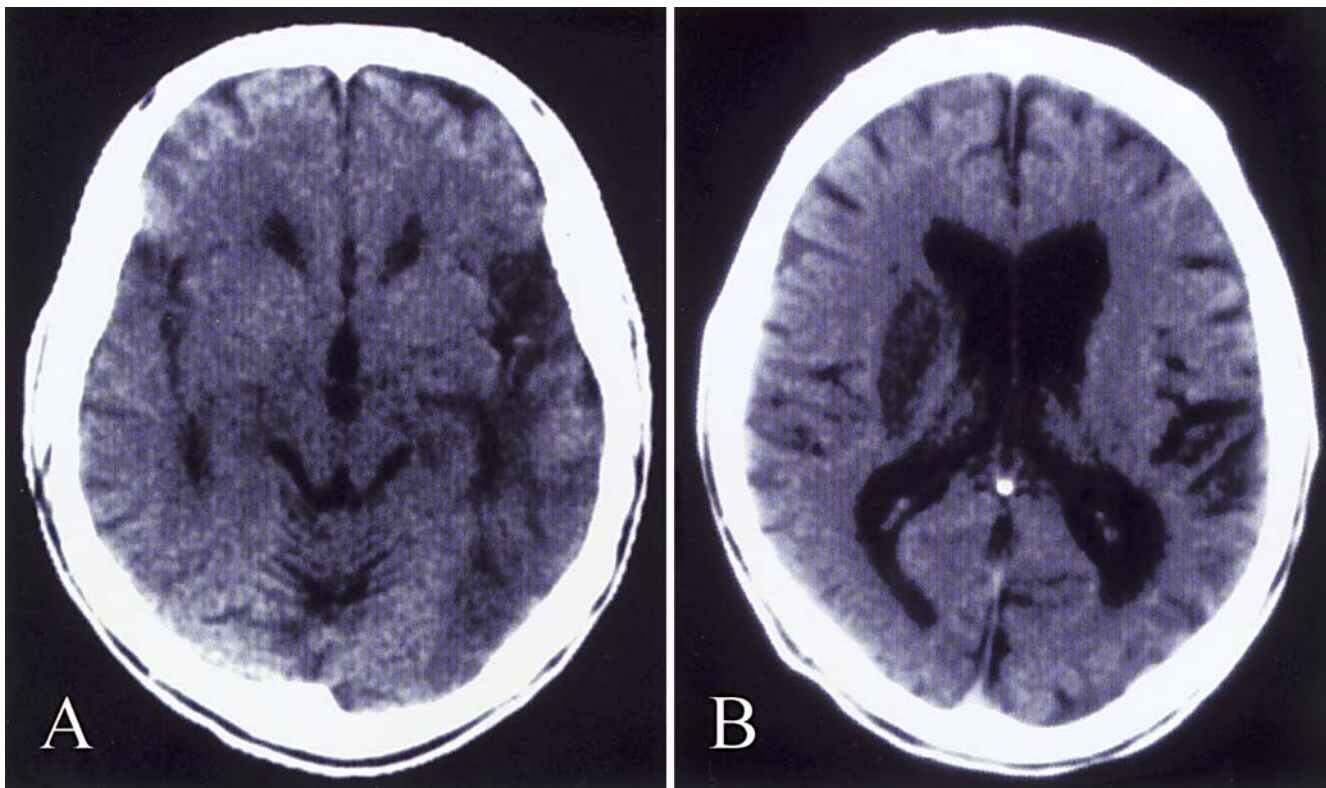


Fig. 1 Non-contrast CT scan of case 2 performed at 47 (A) and 53 (B) years of age, showing a left temporo-parietal ischemic area (A) and a right capsular infarct (B)

Case 3

The clinical findings of this case have been described in greater detail elsewhere (patient III-4 of family B in [29]) (Table 1). This male patient was born after a normal pregnancy, by vacuum extraction. Family history was positive for mitochondrial diseases and the parents were not related. Motor and intellectual development was delayed. At 15 years, he had a left motor seizure secondarily generalized and was first hospitalized. He was short (156 cm), thin, and partially deaf. He also had kyphoscoliosis and pectus excavatum. Neurological examination showed unsteady gait, head titubation, dysmetria, decreased tendon reflexes, and slight intellectual delay. Biochemical abnormalities were confined to a moderate hyperuricemia and raised serum lactate at rest (38.3 mg/100 ml). ECG revealed the Wolf-Parkinson-White abnormality, and echocardiography showed a left ventricular apex hypertrophy. EEG demonstrated slowing of background activity with paroxysmal high-voltage waves and spike-and-wave complexes in a pseudoperiodic pattern. CT scan and MRI showed cerebellar atrophy and bilateral basal ganglia calcification. There were myopathic changes in the EMG and RRFs in the muscle biopsy. The first wave in a brain stem auditory evoked response (BAERs) test was not identifiable.

At 16 years, he had one episode of headache, left hemianopsia and hemiparesis, which completely resolved in a

month. CT scan and MRI at that time revealed an ischemic area in the right occipital cortex. Over the next few years the patient experienced two more stroke-like episodes and had a progressive motor and intellectual deterioration. He became incontinent and had aphasia, visual agnosia, oro-buccal apraxia, and focal myoclonic jerks.

Since the age of 19 he started to suffer of recurrent generalized seizures. He died of heart failure at 21 years of age.

Materials and methods

Human samples

Tissues from patients and controls were obtained at autopsy 2–10 h after death. As controls we used tissues from ten individuals (aged 2–61 years) with no history of neurological diseases and no neuropathological lesions at post-mortem examination. The samples from patients and controls were fixed in 10% neutral formalin for several weeks, sliced, and embedded in paraffin.

Monoclonal and polyclonal antibodies

Immunological probes used in this work included a monoclonal antibody against subunit IV of cytochrome c oxidase (COX) (COX-IV), encoded by nuclear DNA (nDNA), and polyclonal antibodies against the following subunits of the respiratory chain: (1) mtDNA-encoded subunit II of COX (COX-II) (complex IV); (2) nDNA-encoded iron sulfur protein (FeS) of complex III (kindly provided by Dr. Diego Gonzalez-Halphen, Department of Bioenergetics, Universidad Nacional Autonoma de Mexico); (3) mtDNA-encoded subunit 8 of ATPase of complex V (ATPase8). The immunological and immunohistochemical characteristics of the antibodies have been published elsewhere [12, 26, 28, 49, 52].

Immunohistochemistry and analysis of immunostaining

Immunohistochemical studies were performed on paraffin-embedded transverse sections from frontal, parietal and occipital cortex, corpus striatum, cerebellum, midbrain, and medulla of the patients and the controls. Transverse sections, 6 μ m thick, were deparaffinized with xylene and immunostaining was carried out according to the instructions of the Vectastain ABC Kit (Vectors Labs, Burlingame, CA) using 3,3'-diaminobenzidine as a chromogen [3, 49]. Antibodies against FeS, COX-IV, COX-II and ATPase8, were used at dilution 1:250–1:3,500, with overnight incubation at 4°C. Negative control sections were incubated with PBS alone, without the first antibody, overnight at 4°C.

To assess the intensity of the immunostaining in different anatomical regions, sections were observed under high magnification and immunostaining was scored arbitrarily on a scale from 0% to 100% with five categories: +++++, very intense staining (100%); +++++, normal staining (75%); ++, weak staining (50%); +, very weak staining (25%); –, no staining (0%). The Purkinje cells in the cerebellum and the Betz cells in the frontal motor cortex were the most intensely stained cells in sections from normal controls and were used as reference for the grading and were assigned a value of +++++ (100%). Sections from normal controls and from patients, incubated with PBS alone without the first antibody were assigned a value of – (0%). To avoid observer bias, the intensity of staining was assessed independently by two investigators (M.S. and L.B.) on several micrographs from each area in both patients and controls.

Polymerase chain reaction screening for the MELAS-3243 mutation

Paraffin-embedded sections, 10- μ m thick, of frontal cortex from patients and controls were placed in 1.5-ml Eppendorf tubes and deparaffinized with xylene. Total DNA was extracted according to established protocols [46, 58]. PCR analysis was performed using primers corresponding to the light-strand positions 3,116–3,134 and to the heavy strand positions 3,353–3,333 (nucleotide positions are according [2]) for 30 cycles of 1 min. at 94°C, 1 min. at 55°C, and 1 min. at 72°C. The concentrations of Taq polymerase, deoxynucleotides, and primers were as suggested by the manufacturer (Perkin Elmer Cetus). The amplified DNA was ethanol-precipitated, resuspended in restriction enzyme buffer and digested with *Hae*III (New England Biolab) at 37°C for 1 h. The A3243G transition creates a new *Hae*III site, which is diagnostic for the MELAS mutation [7]. The digestion products were electrophoresed through a 12% non-denaturing polyacrylamide gel, stained with ethidium bromide, and viewed by UV transillumination.

Neuropathological examination

The brain of the first two cases showed evidence of a mild cerebral and cerebellar atrophy, while only the cerebellum appeared atrophic in case 3. A large necrotic-hemorrhagic area was found in the globus pallidus and striatum of the right hemisphere of case 2. Multiple softenings, varying in size and age, were seen in the cerebral cortex and subcortical white matter of both hemispheres, mostly in occipital and frontal lobes, in all cases. Similar necrotic foci were also seen, to a lesser degree, in the putamen and amygdala of the left hemisphere of case 2 and in caudate nucleus and putamen of both hemispheres of case 1.

Histologically, the right capsular infarct of case 2 showed ischemic cell changes, diapedetic hemorrhages and infiltration by mononuclear phagocytes. More recent softenings consisted of tissue necrosis with spongiosis, infiltration by macrophages and proliferation of capillary endothelial cells. Older lesions were associated with severe neuronal loss and gliosis. Cortical lesions in occipital lobes were often laminar and involved chiefly intermediate layers. The cytoarchitecture of the remaining cerebral cortex was largely normal in cases 1 and 3, whereas appeared not well preserved because of a widespread neuronal loss in case 2. There was mild but

widespread pallor of cerebral white matter, most severe near the cortical lesions, in case 1.

Finely granular siderocalcific deposits, localized in and around blood vessels, were found in all cases throughout the globus pallidus of both hemispheres. There were gliosis and neuronal loss in the inferior olivary nuclei of the first two cases. Mild loss of Purkinje cells, depletion of cerebellar granule cells and mild spongiosis of substantia nigra were findings common to all cases.

Results

Controls

Sections of the frontal, parietal, and occipital cortex, corpus striatum, cerebellum, midbrain and medulla, stained with antibodies against FeS, COX-IV, COX-II and ATPase8, showed no significant differences in the immunostaining pattern. A finely spotted immunoreactivity was uniformly distributed throughout all the layers of the cerebral and cerebellar cortex. Perikarya and dendrites of pyramidal cells, Purkinje cells and neurons of the oculomotor nuclear complex reacted strongly and homogeneously with all antibodies. Cellular immunostaining was preferentially distributed under the cellular membrane in inferior olivary nuclei (Fig. 2) and on the side facing the lumen in ependymal cells covering the cerebral aqueduct and the fourth ventricle.

In the substantia nigra, brown granular reaction products present in the cytoplasm of pigmented cells were distinguished from neuromelanin because of their brighter staining. Intensity and distribution of cellular immunostaining were normal in the remaining nuclei of the corpus striatum, cerebellum and brain stem. White matter reacted normally with all antibodies and oligodendrocytes showed a thin perinuclear immunoreactivity.

MELAS

In all three cases, the general immunostaining pattern of the cerebral and cerebellar cortex was characterized by multiple areas with abnormal immunostaining, varying in size and shape. Weak staining of the neuropil with all antibodies and marked decrease of neuronal staining for COX-II and ATPase8 were the main immunohistochemical findings in these areas. Immunoreactivity for mtDNA-encoded subunits (COX-II and ATPase8), especially for COX-II, was unevenly distributed among the neurons, such that negative or barely stained cells were interspersed with normally or even strongly stained cells (Fig. 3). Slight decrease of neuronal staining for nDNA-encoded subunits (COX-IV and FeS), especially for FeS, and presence of astrocytes normally reacting with all antibodies were other common findings in affected areas. The distribution pattern of these areas with abnormal immunostaining, predominantly involving the cerebral cortex and, to a lesser degree, the subcortical white matter, often but not always matched the distribution of the infarct-like lesions found at conventional histological examination.

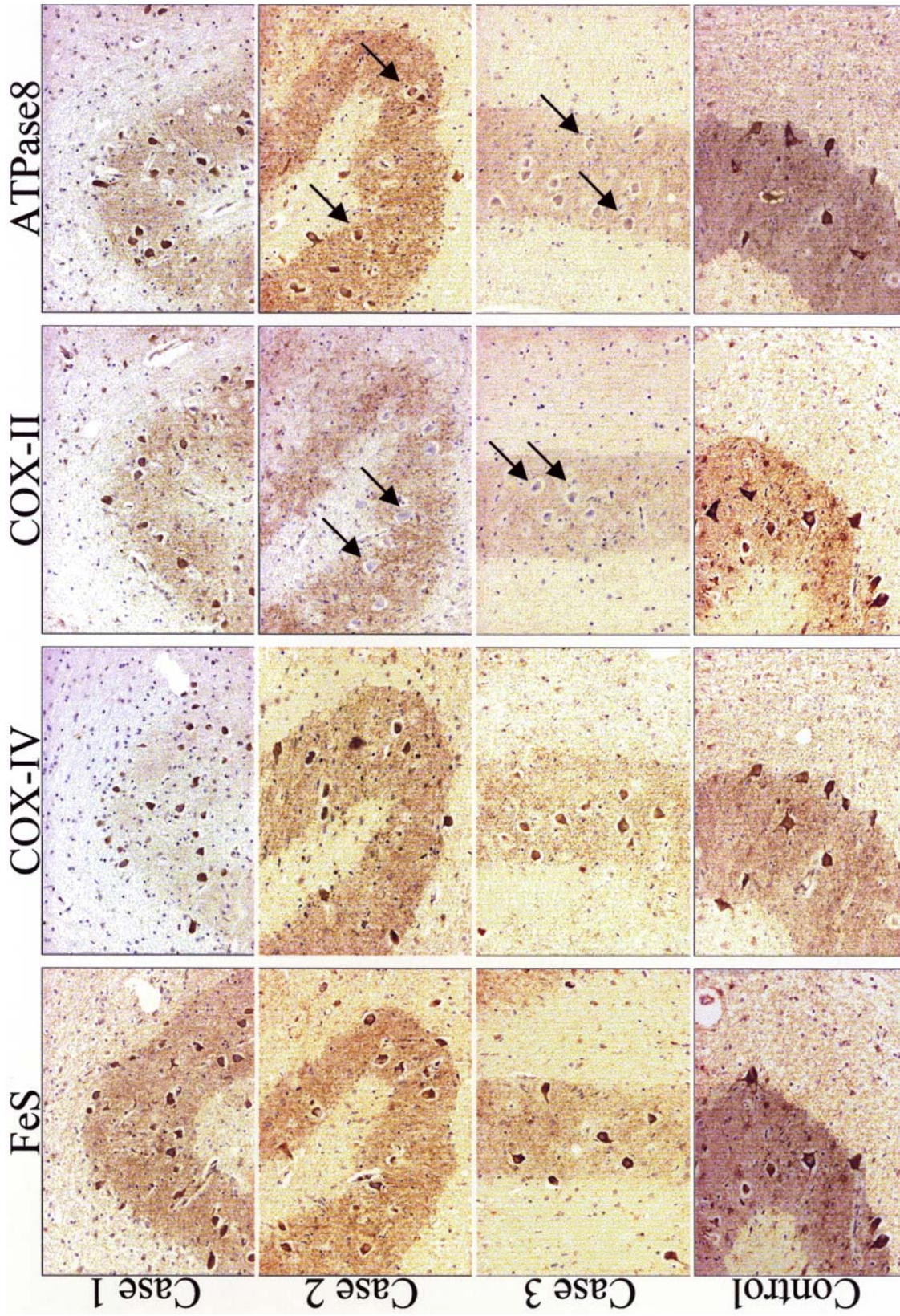


Fig. 2 Immunostaining of sections of olivary nucleus from a control and the patients for the localization of FeS, COX-IV, COX-II, and ATPase8. Case 2 and 3 show decrease or lack of immunostain for mtDNA-encoded subunits (COX-II and ATPase8) (arrows) and normal immunostain for nDNA-encoded subunits (FeS and COX-IV) in the neurons (FeS iron sulfur protein, COX cytochrome c oxidase, mtDNA mitochondrial DNA). $\times 100$

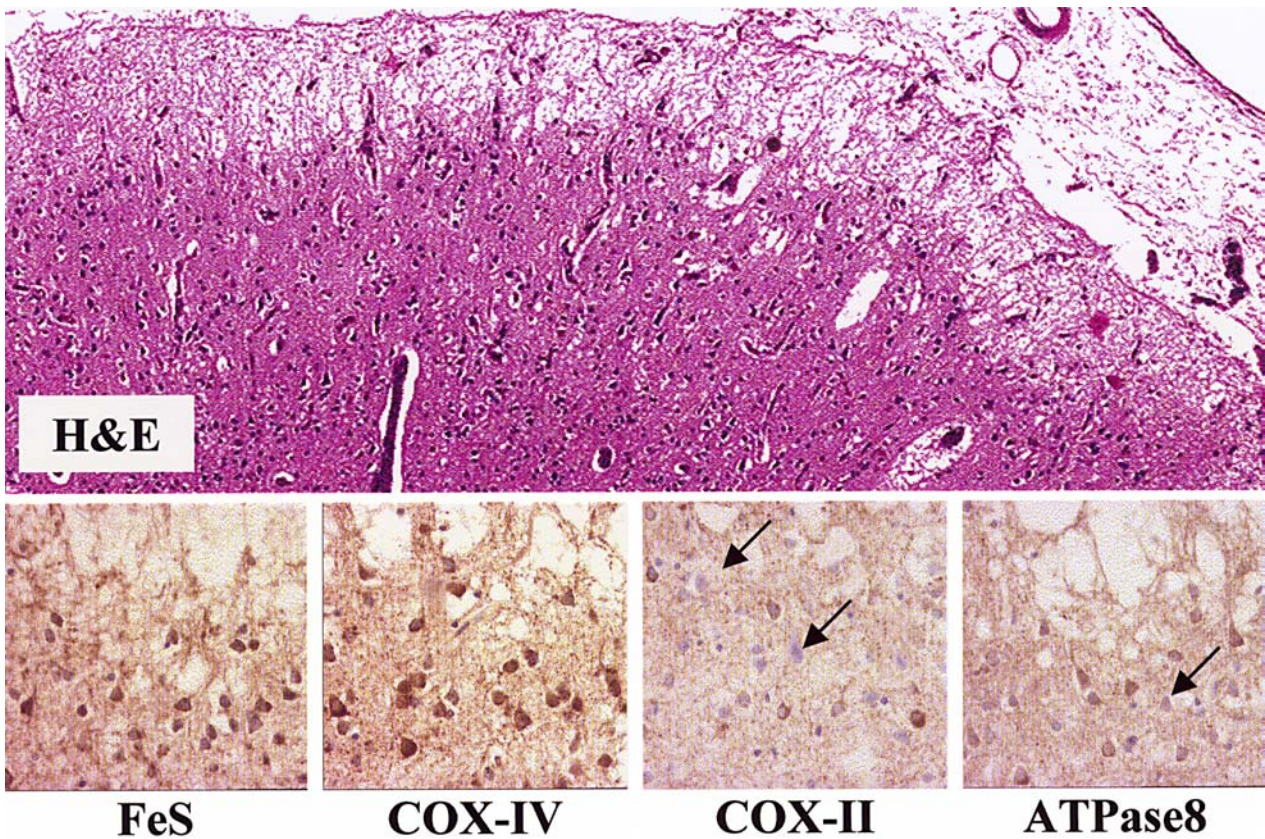


Fig. 3 Spongiosis in the parietal cortex of case 3 stained with hematoxylin-eosin (*H&E*) ($\times 50$) and immunostained for the localization of FeS, COX-IV, COX-II, and ATPase8 ($\times 130$). Neurons under the lesion show decrease and lack of immunostain for COX-II and ATPase8 (arrows), and normal stain for FeS and COX-IV

In all three cases, immunostaining pattern in the cortex between lesions was largely normal or showed to a lesser degree the same immunohistochemical findings described in the areas with abnormal immunostaining.

The large capsular infarct in the right hemisphere of case 2 was characterized by a very weak staining of the neuropil and of the neurons with all antibodies, whereas the decrease in neuronal immunostaining was limited to the mtDNA-encoded proteins in infarct-like lesions present in caudate nucleus and putamen of case 1.

In case 2 and 3 many morphologically normal Purkinje cells were completely COX-II and ATPase 8 negative, not only in areas of abnormal immunostain but also in the remaining cerebellar cortex. Large multipolar neurons of the dentate nucleus were negative for COX-II and hypostained for FeS and ATPase8 in case 2.

Many cells negative or hypostained with COX-II and ATPase8 antibodies were found in several spongiotic areas of the compact part of the substantia nigra, as well as in the red nucleus and in the oculomotor nuclear complex of cases 2 and 3. Immunostaining of sections of medulla was largely normal with all antibodies in case 1, whereas it showed the following changes in the other two cases: decreased immunoreactivity for all subunits, particularly

for COX-II and ATPase8, in many neurons of the spinal nucleus of the trigeminal nerve, gracile and cuneatus nuclei, hypoglossal nucleus, nucleus ambiguus and dorsal motor nucleus of the vagus nerve; and absence or marked reduction of immunostain for mtDNA-encoded subunits in inferior olivary nuclei (Fig. 2).

Immunostaining in the white matter was generally normal with all antibodies. Main immunohistochemical and neuropathological features in the examined brain regions of the three cases are summarized in Table 2.

In all three cases, the walls of many pial and intracerebral arterioles reacted weakly or not at all with antibodies against mtDNA-encoded subunits (Fig. 4). Affected vessels were found in areas with abnormal immunostaining as well as in brain regions showing a normal immunoreactivity with all antibodies.

Discussion

Using immunohistochemical techniques, we have shown reduced expression of COX-II and ATPase8 in multiple areas of the brain from three unrelated autoptic cases of MELAS harboring the A \rightarrow G transition at mtDNA position 3243. The decrease in immunocytochemical staining was apparently specific to mtDNA-encoded proteins, as there was a normal or slightly reduced expression of nDNA-encoded subunits FeS and COX-IV in all three cases. Our observations are in agreement with the results of previous biochemical and immunostaining studies in skeletal muscle fibers and cytoplasm from patients with the same mu-

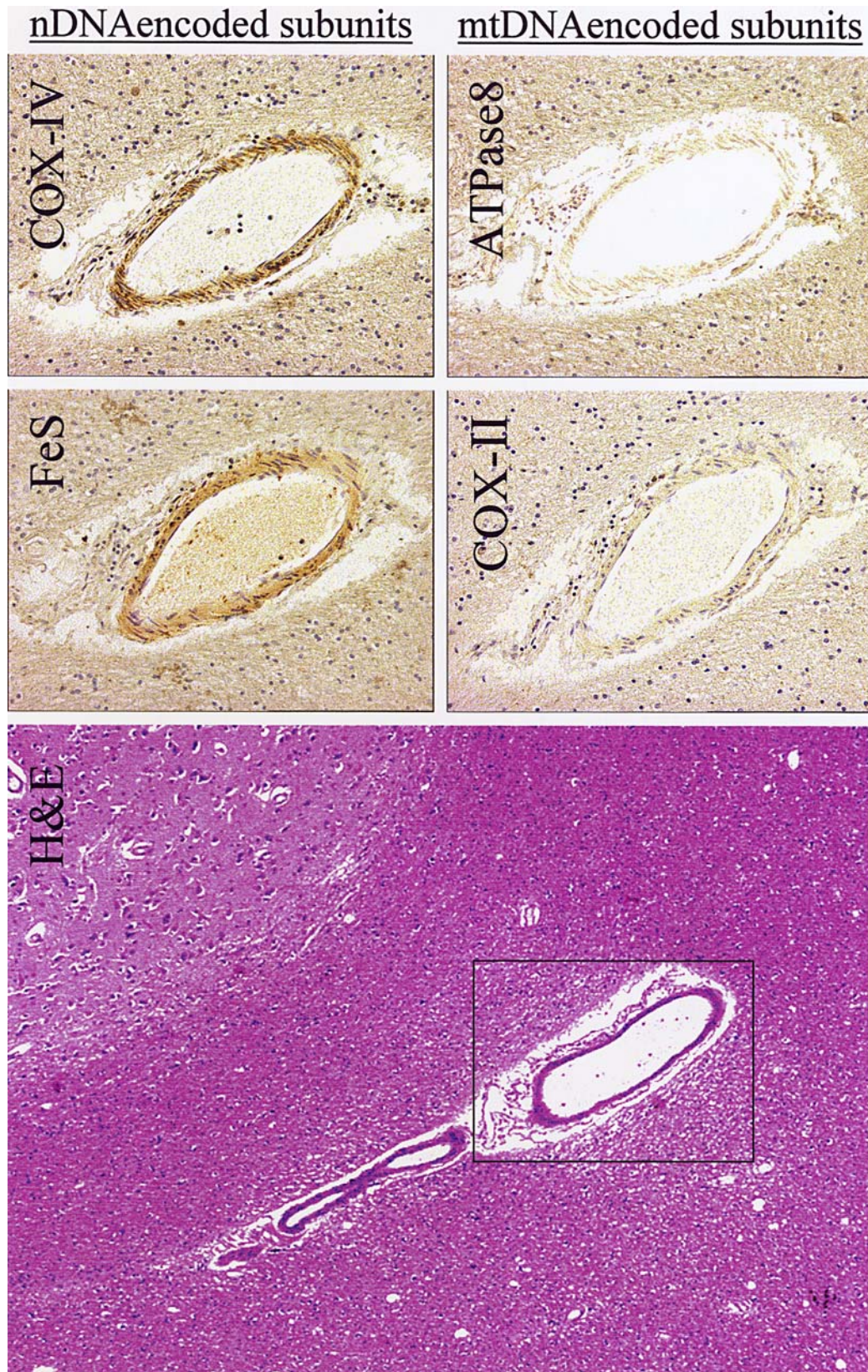


Fig. 4 Sections of parietal cortex from Case 2 stained with H&E ($\times 50$) and immunostained for the localization of FeS, COX-IV, COX-II, and ATPase8 ($\times 70$). The wall of one arteriole in an area free of histological alterations shows reduced immunostain for mtDNA-encoded subunits (COX-II and ATPase8)

tation [22, 36], and seem to be consistent with a reduction in mitochondrial protein synthesis. Several recent lines of evidence indicate that the pathogenetic mechanism of the MELAS A3243G mutation is an aminoacylation defect of the mitochondrial tRNA^{Leu (UUR)} that affects mRNA association with ribosomes and causes a general reduction in mitochondrial protein synthesis [6, 10]. The A3243G transition, in fact, changes the tertiary structure of the tRNA^{Leu (UUR)} so that the mutant tRNA^{Leu (UUR)} becomes metabolically less stable and is charged to a lesser extent by the leucyl-tRNA synthetase [4, 6].

The topographic analysis allowed us to draw several correlations between immunohistochemical and clinical and neuropathological features. The demonstration of a cortical mitochondrial dysfunction in all three cases confirmed the preferential involvement of the cerebral cortex in MELAS [48], and provided a morphological correlate to the explanation of the cognitive regression of our patients (Table 1). In agreement with the results of PET investigations [34] in MELAS, most prominent cerebral immunocytochemical changes were found in occipital regions, particularly in case 3 who had visual agnosia (Table 1).

The presence of multiple infarct-like lesions as well as the reduced expression of COX-II and ATPase8 in caudate nucleus and putamen of case 1 might explain some extrapyramidal features (i.e., dystonia and athetosis) occasionally seen in this patient. Finally, most severe mitochondrial abnormalities found in the cerebellum of case 3 (Table 2) seem to give an explanation for several cerebellar signs of the patient.

We do not believe that a right capsular infarct found in case 2 had a mitochondrial genesis, but was probably due to an embolus from the heart. This patient had a subepicardial ischemia and died of heart failure. Additionally, the finding of a reduced expression of all, mtDNA- and nDNA-encoded, subunits in this area seems to indicate a mitochondrial damage secondary to the ischemia.

Mitochondrial abnormalities in the brains of our cases had a different pattern of distribution; they were present in all regions of cases 2 and 3, while they spared the brain stem in case 1 (Table 2). The heterogeneous phenotypic expression of the A3243G MELAS mutation, which could explain the variable immunohistochemical presentation of our cases, has been initially attributed to different degree of mtDNA heteroplasmy in different tissues of patients with MELAS [7, 8]. Recent genetic studies have provided evidence that the pattern of intra-tissue segregation of mtDNA A3243G mutant genomes could be related to the activity of nuclear genes controlling either the replication of mutant mtDNAs or the regulation of the threshold of wild-type mtDNAs required to complement mutant mtDNAs among different cells or tissues [9, 16, 21, 47, 57]. Because in our study immunohistochemical abnormalities were less diffuse in the youngest patient (case 1), it could be argued that also aging processes might have some influence on the topographic extension of brain lesions in affected individuals. Recent studies, in fact, indicate that mutations of nDNA-encoded factors involved in the posttranscriptional and/or posttranslational regulation of mtDNA ex-

pression might cause the age-dependent reduction of COX activity normally present in human cells [19, 25, 47].

In each region of our cases, immunocytochemical changes were most frequent and severe in nerve cells deemed to be more vulnerable to hypoxia: neurons of intermediate layers in the cerebral cortex, Purkinje cells and cells of inferior olivary nuclei [5] (Table 2). These observations suggest that the degree of cellular dependence on oxidative metabolism might play a role in determining, in various neuronal populations, both the selective vulnerability to oxygen deficiency and the presence of different thresholds for phenotypic expression of a mtDNA mutation. Therefore, neurons most reliant on oxidative metabolism might be more sensitive to a reduction in oxidative phosphorylation capacity and may require a lower percentage of mutant mtDNA to express a respiratory-deficient phenotype. In line with this hypothesis, it has been shown that RRFs are more predominant in oxidative type I than in glycolytic type II muscle fibers [36, 51] of patients with the MELAS A3243G mutation.

A central problem to be solved in MELAS is the nature of the pathogenetic mechanism leading to the stroke-like episodes. So far it has been supposed that infarct-like lesions might result from a focal, transient impairment of oxidative metabolism in the brain parenchyma (metabolic hypothesis) [30, 31, 42] or from large vessels and/or arterioles occlusion (vascular hypothesis) [17, 39, 45, 50].

We have found reduced expression of mtDNA-encoded subunits in the walls of many pial and intracerebral arterioles of different brain regions. However, there was not clear correlation between territories of affected vessels and distribution of the immunohistochemical lesions, such that many vessels reacting weakly or not at all were found not only in areas with abnormal immunostaining but also in normally stained brain regions.

These findings, along with the observation of a prevalent focal expression of mitochondrial abnormalities in the CNS of our cases, suggest that many different mechanisms might be simultaneously involved in the genesis of cerebral focal lesions in MELAS.

The activation or the appearance of different stress conditions in areas characterized by a defective vascularization and by a depressed oxidative metabolism might precipitate an increased metabolic demand, causing anaerobic metabolism activation, parenchyma lactate accumulation and tissue damage. It has been demonstrated that the defect in ATP synthesis in MELAS becomes apparent only when ATP demand is high [20]. Furthermore, MRS, SPECT and PET studies have shown abnormal alteration of lactate and metabolic acidosis in affected brain regions [15, 31, 33, 38, 42].

The impairment of the oxidative phosphorylation and a focal hyperperfusion, as shown before or during the stroke-like episodes [1, 15, 33, 38, 43, 54], might locally enhance the release of reactive oxygen species (ROS), as demonstrated in cybrids carrying the A3243G mitochondrial mutation and exposed to high concentration of oxygen [56].

Finally, a local failure of the oxidative metabolism might cause a rise in intracellular free calcium concentration that

can trigger cell death by means of phospholipase activation, leading to breakdown of cell membranes [48]. The A3243G MELAS mutation decreases both the membrane potential and the driving force for Ca^{2+} uptake in mitochondria [20], causing a rise in intracellular free calcium concentration in basal conditions and after oxidative stress [44, 55]. Furthermore, an excessive uptake of calcium as well as oxidative stress or disease states that inhibit the oxidative phosphorylation increase the propensity of cell to undergo to apoptosis [14, 24, 32, 53].

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