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HETEROPLASMIC MUTATION OF M.3243A>G MITOCHONDRIAL DNA IN A YAKUT FAMILY WITH MELAS SYNDROME: ASSOCIATION WITH PHENOTYPIC MANIFESTATIONS

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For the first time, the diagnosis of MELAS syndrome in a Yakut family was genetically verified using mitochondrial genome sequencing. The substitution of adenine for guanine at position 3243 (m.3243A>G) in the *tRNA*$^{Leu(UUR)}$ gene (MT-TL1) was confirmed. The level of the mutant allele (heteroplasmia) in the patient was 38.5%, while in the mother only 9.8%, which is explained by the selection of rapidly dividing blood cells with a low level of mutant alleles during life. It has been shown that the phenomenon of mtDNA heteroplasmy forms a significant clinical heterogeneity in the manifestation of the disease and demonstrates the complexity of diagnosing subclinical forms of MELAS.

Keywords: mitochondrial diseases, MELAS syndrome, m.3243A>G mutation.

Introduction. MELAS syndrome is an orphan disease caused by a mutation in mitochondrial DNA [2, 3, 11]. The mitochondrial genome, inherited through the maternal line, controls the processes of oxidative phosphorylation and ATP production in the cell, playing a key role in the energy balance of the body. Diseases caused by mutations in the mitochondrial genome are characterized by wide clinical heterogeneity and the multisystem nature of damage to organs and tissues [12]. The most characteristic signs of MELAS syndrome are a combination of encephalopathy with seizures and lactic acidosis [2, 3].

An important role in the phenotypic manifestations of mitochondrial diseases is played by the phenomenon of heteroplasmy - the ratio of the levels of normal and mutant variants of mitochondrial DNA (mtDNA). It has been shown that a small increase in the level of heteroplasmy leads to moderate defects in oxidative phosphorylation, changes in nuclear gene expression and cell phenotype [15, 16].

Objects and methods. The family of a 27-year-old female patient with clinical manifestations of MELAS syndrome was studied. Whole genome sequencing of mtDNA was carried out in the Research Institute of Medical Genetics of the Tomsk Scientific Research Center of Siberian Branch of Russian Academy of Science.

Results. Patient N, born in 1995 (27 years old), yakut, a doctor by training, unemployed, not previously registered with a neurologist, without chronic diseases. The onset of the disease occurred at the age of 23 (January 2019) in the form of generalized tonic-clonic status epilepticus during a trip to Japan.

The patient was hospitalized in the intensive care unit. MRI of the brain, lumbar puncture, and general clinical tests were performed. Signs of inflammation and structural changes in the brain were not found. Status epilepticus was treated with fosphenytoin. The patient was discharged the next day after the attacks subsided; it was decided to undergo further examination in Yakutsk due to the lack of medical insurance.

Upon arrival to home in the city of Yakutsk, an epileptologist was consulted; video-EEG monitoring revealed no pathological changes.

In March 2019, the patient began to again experience weakness, headaches, began to forget the names of objects and words, and also began to experience episodes of visual hallucinations (for example, “a huge cockroach ran across her face”). On March 30, 2019, headache, low-grade fever, and right-sided hemianopia appeared, then a series of four generalized tonic-clonic epileptic seizures developed. A CT scan of the brain and a lumbar puncture were performed; the examination did not reveal any evidence of cerebrovascular accident or inflammatory process.

General clinical and biochemical blood tests did not reveal pathological deviations from the norm. The patient was under drug sedation for 3 days.

After the restoration of consciousness, pronounced cognitive impairments were noted in the form of sensorimotor aphasia, apraxia of dressing. Patient did not recognize relatives, and was disoriented in place and time. Right-sided homonymous hemianopsia persisted, hyporeflexia in the hands, and Babinski's sign on the left were detected.

MRI of the brain revealed signs of cerebral hyperperfusion with damage to the left occipital, parietal lobes, and the thalamic cushion on the left (Fig. 1).

Based on the examination, a diagnosis of autoimmune meningoencephalitis with cognitive impairment, elements of sensorimotor aphasia, visual impairment (homonymous hemianopsia), symptomatic simple focal epileptic seizures, with evolution into bilateral synchronous, and a tendency to a serial course, was established.

In order to clarify the diagnosis, the patient was examined at the Federal State Budgetary Institution "Scientific Center of Neurology" in Moscow, where the blood was additionally tested for: L-lactate – 9.40 mmol/l, pyruvate – 0.11 mmol/l, lactate/pyruvate ratio – 84, 0, in dynamics L-lactate – 9.21 mmol/l (normally up to 3.0 mmol/l), pyruvate – 0.11 mmol/l (normally from 0.03 to 0.09 mmol/l), lactate/pyruvate ratio – 82.4.

MR spectroscopy of the brain revealed a lactate peak in both cerebral hemispheres, predominantly on the left in the affected area (Fig. 2).

Based on the identified data, the diagnosis was clarified as mitochondrial encephalopathy.

Patient N. was consulted by a neuropsychologist; acoustic-mnestic aphasia, acalculia, visual-spatial disorders, and changes in dynamic processes were identified.

In 2019-2022, she was annually admitted to the intensive care unit with the development of status epilepticus, which was associated with an increase in lactate levels to 8 mmol/l (normally 0.5-1.6 mmol/l). She underwent video-EEG monitoring several times, the results of which revealed focal in the frontotemporal regions and/or generalized epileptic activity in the form of a peak-slow wave (Fig. 3).

Further brain MRIs from 2019 (Fig. 4A) and 2022 (Fig. 4B) revealed hyperintense FLAIR signals from the left temporal lobe with increasing expansion of the posterior horn of the left lateral ventricle (Fig. 4A, B)

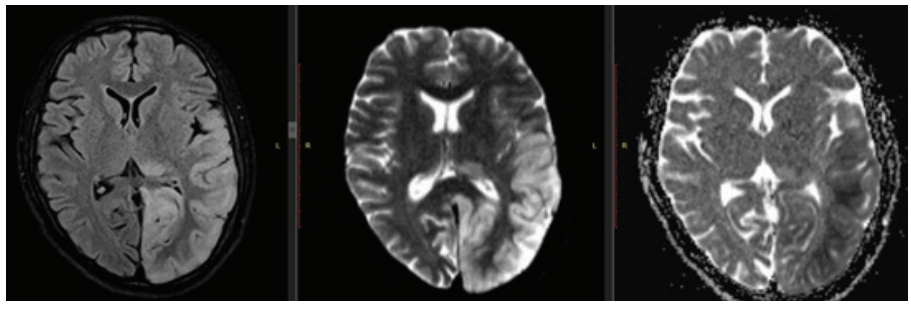

Fig. 1. MRI of the brain of patient N. dated 04/05/2019 with signs of cerebral hyperperfusion with damage to the left occipital, parietal lobe, and thalamic cushion on the left

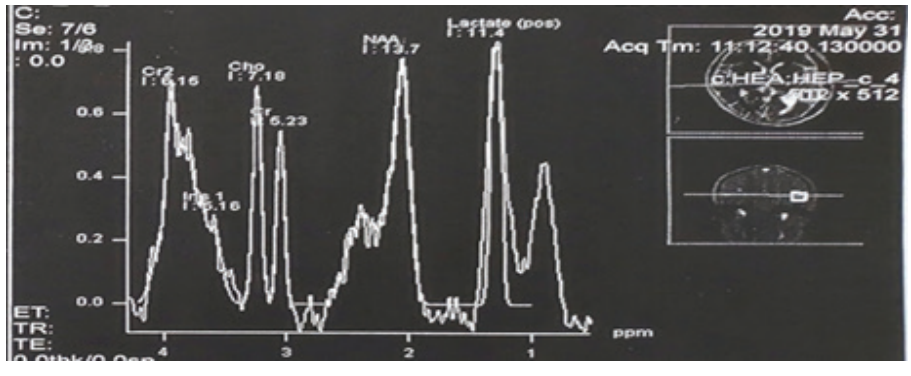

Fig. 2. MR spectroscopy of the brain of patient N. A marked increase in the lactate peak in the area of the altered MR signal in the left temporal lobe and a slight increase in the lactate peak in the unchanged white matter of the right hemisphere of the brain

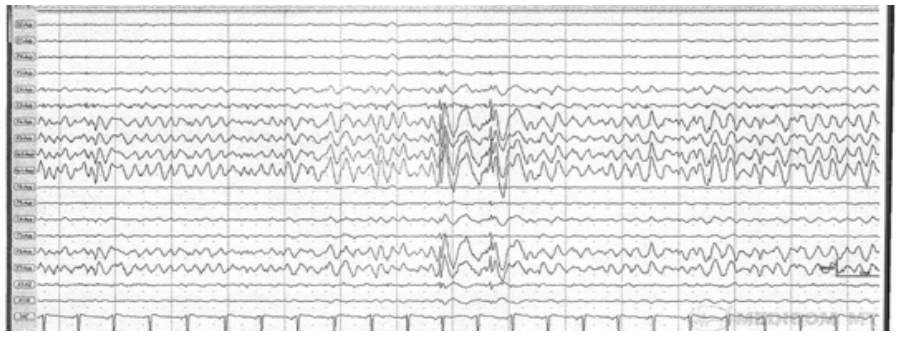

Fig. 2. EEG of patient N. at rest...EEG at rest and activity – focal interictile activity in the form of “peak - slow” waves in the frontotemporal regions, without generation (10/18/2023)

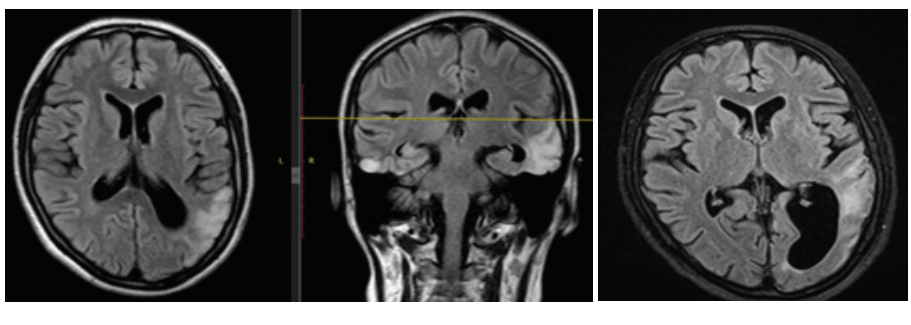

Fig. 4. MRI of the brain: a) from October 2019; b) from October 2022

The patient was examined in May 2023. In neurological status: consciousness is clear, behavior is orderly, makes contact well, sense of smell is normal. Vision is reduced due to myopia, corrected with glasses (–11D), pupils D=S, photoreaction is live. There is no hemianopsia. Sensitivity on the face is preserved, the trigeminal points are painless. Corneal reflexes are alive. The face is symmetrical, the function of facial muscles is preserved. Taste sensitivity in the tongue is preserved. Oral automatism reflexes are not detected. Tongue in the midline, without fibrillations. The strength in the limbs is sufficient, there are no paresis. Diffuse muscle hypotonia. There are no atrophies or hypotrophies in the muscles. Reflexes from the biceps and triceps are low, carporadial are not evoked; knee and Achilles reflexes are not evoked. There are no pathological reflexes. Plantar and abdominal reflexes are not evoked. Performs coordination tests well. There are no sensory disorders. There are no meningeal signs.

Assessment of cognitive functions on the MoCA scale reveals a decrease to 26/30 points: could not repeat two sentences, decreased speech activity (3 words starting with the letter “L” within 1 minute), remembered 4 out of 5 words.

Signs of acoustic-mnestic and optical-mnestic aphasia are determined (for example, the patient cannot remember the names of objects while retaining recognition of their purpose; poorly understands the speech of the interlocutor, especially at a fast pace); phonemic paraphasias are revealed in speech. The patient is unable to read (alexia) and write correctly, both spontaneously and under dictation (agraphia). At the time of examination, no signs of motor, ideational, or constructive apraxia were identified.

During observation, the patient underwent a electrocardiogram (ECG) to exclude non-coronary myocardial infarction type 2. Numerous ECGs revealed sinus tachycardia up to 100 beats/min, early ventricular repolarization, then myocardial changes appeared in the anteroseptal region according to the type of damage (ST elevation V1-V3, negative T wave V4-V6, I-II, (+/-) T IIIAVF0. Consultation with a cardiologist: Dysmetabolic cardiomyopathy associated with the energetic failure of mitochondria (Mitochondrial cardiomyopathy). Chronic heart failure with ejection fraction (EF)=61%, stages I-IIA, functional class (FC) is not differentiated.

Family description and molecular genetic testing. The patient was born from second pregnancy. The mother's first and third pregnancies ended in premature birth. The boys born died due to prematurity after 5-6 hours.

The patient's mother (57 years old) was examined by a rheumatologist, neurologist and geneticist. When examining the mother, a severe deficiency in body weight was revealed (BMI – 15.7 kg/m^2), disorders of phosphorus-calcium metabolism (numerous low-energy bone fractures), osteoporosis, mixed form (surgical menopause in 2000, taking glucocorticosteroids) with multiple clavicle fractures, ribs (2013, 2014), pathological fractures of the distal forearm bones of both upper extremities (2018), proximal left femur (2021). Ischemic heart disease (IHD): Angina pectoris. FC2. Acquired heart disease: aortic valve insufficiency grade 3, mitral valve insufficiency grade 1, tricuspid valve insufficiency grade 1.

Patient’s mother has history of papillary adenocarcinoma of the left lobe of the thyroid gland, condition after the strumectomy operation dated August 25. 2010 and hypothyroidism. Since 2003, she has been suffering from Sjogren's disease, manifested by dry skin, eyes, nephropathy, lymphadenopathy, polyarthralgia, sialadenitis was not excluded, but the diagnosis was not subsequently confirmed by laboratory and instrumental research methods. In addition, the patient’s mother underwent cholecystectomy in 2003, and the lenses of both eyes were replaced with artificial ones in 2014.

No characteristic signs of MELAS syndrome were identified in the patient N.'s mother. The mother and daughter were diagnosed with sensorineural hearing loss.

The mother's brother (patient N.'s uncle, age 60 years) suffers from cerebral palsy, is disabled in group 1, studied in a correctional school, has a neurological deficit in the form of cognitive decline and spastic tetraparesis, in addition, he has structural epilepsy and type 2 diabetes mellitus. Parents of the patient's mother: the mother died in 2023 at the age of 84 from lung cancer. Father, 91 years old, alive. MtDNA sequencing was performed on the patient and her mother. As a result of analysis of the mtDNA sequence in patient N., an adenine to guanine substitution at position 3243 (m.3243A>G) was identified, which, according to the literature, is associated with MELAS syndrome [8]. In the blood sample of patient N., the proportion of the mutant allele G at position 3243, i.e., the level of heteroplasmy, is 38.5% (227 out of 590 reads). In a sample of mitochondrial DNA isolated from the blood of the patient's mother, the mutant allele G was detected with a heteroplasmy level of 9.8% (37 out of 379 reads).

The mtDNA lines of mother and daughter belong to haplogroup D4j5a1a.

Discussion. The m.3243A>G mutation in the *tRNA$^{Leu(UUR)}$* (MT-TL1) mtDNA gene is pathogenic and has significant phenotypic variations. The disease can manifest as multiorgan involvement with a wide variety of clinical manifestations and varying degrees of severity. It is known that the onset of mitochondrial diseases varies from 3 to 40 years [3]. Our patient fell ill at the age of 23, the disease manifested itself in the form of status epilepticus with loss of consciousness and the development of tonic-clonic seizures. During the period from 2019 to 2023, episodes of convulsive seizures with an increase in neurological disorders - cognitive impairment, signs of acoustic-mnestic and optical-mnestic aphasia, motor weakness, decreased hearing and vision - were repeated many times.

Among the characteristic clinical manifestations, the patient has severe cerebral symptoms with epilepsy confirmed by instrumental studies (EEG, MR spectroscopy of the brain), lactic acidosis detected in the blood and by MR spectroscopy of the brain (lactate peaks in both hemispheres of the brain). Other manifestations of MELAS syndrome include cardiomyopathy detected in the patient, which indicates the energetic failure of mitochondria.

According to the literature, cardiac dysfunction occurs in approximately one third of patients with mitochondrial myopathy, encephalopathy, or lactic acidosis, which is a stereotypical example of a mitochondrial disorder leading to cardiomyopathy [6]. Our patient also had visual and hearing impairments in the form of high myopia with astigmatism. Angiopathy of retinal vessels is observed.

Thus, based on typical symptoms characteristic of mitochondrial diseases, as well as genetic (detection of the m.3243A>G mutation), general clinical tests (study of blood lactate levels) and instrumental diagnostic methods (EEG, MRI), the patient was diagnosed with the syndrome MELAS. In the Yakut population, this mutation m.3243A>G of the *tRNA$^{Leu(UUR)}$* mtDNA gene was described for the first time.

The identified mutation is often associated with diabetes mellitus, which is found with a frequency of approximately 1.5% to 5% among diabetic patients from different countries and races [10]. Our patient and her mother were not diagnosed with diabetes mellitus. However, it should be noted that the mother’s brother has

type 2 diabetes mellitus, which does not exclude the presence of heteroplasmy of this mutation.

MELAS syndrome may be accompanied by other disorders – hearing loss, short stature/thin build, myopathy or neurological disorders, disorders of the thyroid and parathyroid glands [1, 5, 10]. The patient's mother was found to have low-level heteroplasmy mutation m.3243A>G (9%). The patient's mother did not show any characteristic symptoms of MELAS, but since childhood she has been short stature, underweight, and during her childbearing years there was miscarriage, fetal failure, bleeding during childbirth with amputation of the uterus and ovaries.

The existing numerous low-energy fractures of long bones, ribs, and clavicle in the patient's mother may have been caused not only by early surgical menopause and removal of the thyroid gland, but perhaps by undetected MELAS syndrome. Damage to the thyroid gland and kidneys may indicate the presence of MELAS syndrome in the mother. From the anamnesis it is known that the mother's chronic kidney disease was interpreted in terms of Sjögren's disease, but the diagnosis was not verified by a sufficient amount of laboratory and instrumental research methods.

Studies of familial cases of detection of the m.3243A>G mutation indicate significant clinical heterogeneity in the manifestations of damage to organs and systems, even within the same family [15]. The low level of mutant mtDNA in the mother at present may be explained by the selection of rapidly dividing blood cells with a low level of mutant alleles during life. The results of long-term studies show that such selection is a common feature of mtDNA carrying the m.3243A>G mutation in blood cells [7]. All these facts, including the m.3243 A>G study, demonstrate that even one point mutation in mtDNA, depending on its level in the cell, can have a profound effect on the state of gene expression, shaping the phenotype of complex hormone-dependent metabolic and degenerative diseases.

A hearing test revealed bilateral sensorineural syndromic hearing loss of 1st degree in the patient and her mother. MtDNA sequencing made it possible to identify another mutation in both subjects: m.7445A>C, previously suspected of being associated with sensorineural hearing loss. L.U. Dzhemileva et al. (2009) considered the possible role of the m.7445A>C mutation in the development of sensorineural hearing loss in Kazakhs, but emphasized that its role in the pathogenesis of auditory dysfunction requires clarification [4]. The literature describes a case of sensorineural deafness in 13 family members with mtDNA variant 7445C [17].

At the same time, it was established that m.7445A>C is a polymorphic variant that defines the D4j5a haplogroup [14], which is widespread in Central Asia and Yakutia [9, 13, 14]. Among the indigenous population of Yakutia, haplogroup D4j5a is found among Yakuts with a frequency of 1.2%, Evenks - 1.6%, Evens - 2.9%, Yukaghirs - 4.5% [5]. Clinical and audiological studies have not been conducted in these individuals; therefore, the role of the m.7445A>C mutation in the molecular pathogenesis of auditory dysfunction requires clarification.

Conclusion. MtDNA sequencing revealed a characteristic mutation m.3243A>G with a heteroplasmy level of 38.8% in a patient with MELAS syndrome in a Yakut family. Family history does not exclude the presence of a mutation in the patient's uncle (mother's brother) and a less pronounced subclinical variant of MELAS syndrome in the mother with a low level of the mutant allele m.3243A>G (9.76%). This mitochondrial DNA mutation was described for the first time among the Yakut population.

In the studied Yakut family, based on a typical syndrome complex and the results of laboratory and instrumental diagnostic methods, the patient was diagnosed with MELAS syndrome. It has been shown that the phenomenon of mtDNA heteroplasmy creates significant clinical heterogeneity in the manifestation of the disease and demonstrates the difficulty of diagnosing subclinical forms of MELAS.

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