MITOCHONDRIAL DNA ABNORMALITIES AND AUTISTIC SPECTRUM DISORDERS

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Objectives **To further characterize mtDNA defects associated with autistic features, especially the A3243G mtDNA mutation and mtDNA depletion.**

Study design **Five patients with autistic spectrum disorders and family histories of mitochondrial DNA diseases were studied. We performed mtDNA analysis in all patients and magnetic resonance spectroscopy in three.**

Results **Three patients manifested isolated autistic spectrum features and two had additional neurologic symptoms. Two patients harbored the A3243G mutation. In two others, the A3243G mutation was not found in accessible tissues but was present in tissues from their mothers. The fifth patient had 72% mtDNA depletion in skeletal muscle.**

Conclusions **Autistic spectrum disorders with or without additional neurologic features can be early presentations of the A3243G mtDNA mutation and can be a prominent clinical manifestation of mtDNA depletion. Mitochondrial dysfunction should be considered in patients who have autistic features and associated neurologic findings or who have evidence of maternal inheritance.** ***(J Pediatr 2004;144:81-5)***

There is strong evidence that genetic factors are important in autistic spectrum disorders. However, the mode of inheritance remains unclear. Multiple interacting genes have been proposed, and linkage to a number of loci has been reported.[1] Autism also has been associated with different metabolic disorders,[2,3] and there are biochemical data suggesting that impairment of mitochondrial energy metabolism may play a role in the pathophysiology.[4-6]

Mitochondrial function is under the dual genetic control of nuclear DNA and mitochondrial DNA (mtDNA).[7] MtDNA-related disorders can be due to primary defects of mtDNA (eg, point mutations in tRNA) or to defects of intergenomic communication (eg, multiple deletions or depletion of mtDNA). The genetics of primary mtDNA disorders differ from classic mendelian genetics. Because every cell contains hundreds or thousands of mtDNA molecules, when mutations in the mtDNA arise, they usually result in a mixture of wild-type and mutant mtDNA, a state known as heteroplasmy. When heteroplasmic cells divide, mutant and wild-type mtDNAs are randomly distributed to daughter cells (mitotic segregation), such that over time the mutational load in a given tissue may change. Finally, as mitochondria are transmitted through the oocyte, the diseases caused by primary defects of mtDNA are maternally inherited.[7]

Clinical phenotypes of mtDNA disorders generally correlate with specific mutations, but it is not uncommon that one syndrome may be caused by different mutations and one mutation may cause different syndromes.[7] For example, mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS) is typically caused by the A3243G mutation in the mitochondrial $tRNA^{Leu(UUR)}$ gene, but this same mutation is also

dGK	Deoxyguanosine kinase	MDS	Mitochondrial DNA depletion syndrome
mtDNA	Mitochondrial DNA	MRS	Magnetic resonance spectroscopy
MELAS	Mitochondrial encephalopathy lactic acidosis and stroke-like episodes	TK2	Thymidine kinase 2

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associated with maternally inherited progressive external ophthalmoplegia[8] and with developmental delay and seizures in early childhood.[9]

MtDNA depletion syndrome (MDS) is an autosomal recessive disorder of infancy or early childhood characterized by decreased mtDNA copy number in affected tissues. Recently, mutations were identified in two genes involved in nucleotide metabolism.[10,11] In this report, we describe the association of the A3243G mtDNA mutation and mtDNA depletion with neurodevelopmental behavior disturbances within the autistic spectrum.

PATIENTS

Patient 1

This 6-year-old boy was born at term by vaginal delivery, after induction of labor. Motor and language development were normal. At age 3 years, he showed behavioral difficulties: He was stubborn, overactive, impulsive, and had temper tantrums when frustrated. Neuropsychologic evaluation at 5 years of age revealed an IQ of 94. He showed minimal eye contact and had difficulty engaging in cooperative play. He displayed a perseverative quality in many responses and showed hand stereotypies. He was diagnosed with pervasive developmental disorder (PDD).

His family history was remarkable for four affected maternal uncles. One had MELAS, the second had Alpers syndrome, the third had diabetes mellitus and deafness, and the fourth had obesity and depression. One maternal aunt had hypothyroidism and another had attention deficit hyperactivity disorder.

At 5 years of age, he was evaluated at our institution. He was hyperactive, inattentive, distractible, had a low threshold for frustration, and had outbursts of temper. His head circumference was at the third percentile, and the rest of his neurologic examination was normal.

Patient 2

This 13-year-old boy was born at term after a normal pregnancy. His gross motor milestones were normal. However, he showed delays in fine motor acquisitions and language development. He had behavioral difficulties since early life and was diagnosed with Asperger syndrome. At 12 years of age, he started showing unusual clumsiness shortly after walking up stairs and became very tired even with moderate physical activities.

He was evaluated at our institution at 13 years of age, when a maternal relative was diagnosed with MELAS. He had significant fine-motor difficulties. He was hyperactive and distractible. He had a low frustration threshold, difficulties with impulse control, and frequent temper outbursts. He showed poor self-regulatory skills, poor attention, and organization difficulties. He engaged poorly with the examiner and displayed perseverations. Neuropsychologic evaluation (Wechsler Intelligence Scale for Children) showed a full scale score of 94, a verbal score of 88, and a performance score of 102. His head circumference was at the 90th percentile. He had peripheral retinal hyperpigmentation and a pale optic nerve. He had mild right ptosis and was mildly ataxic. His brain MRI was normal. Serum lactate level was normal.

Patient 3

This patient has been previously reported.[9] Briefly, he presented with global developmental impairments and respiratory difficulties since early infancy. Evaluation showed increased lactic acid levels in blood and CSF and his brain MRI showed bilateral signal abnormalities in the caudate and pallidum. Head circumference was in the 50th percentile. He acquired skills slowly. He had neurologic deterioration during intercurrent illnesses and recovered gradually over several weeks.

At age 5 years, Merrill-Palmer Scale of Mental tests showed Full Scale IQ of 80. He had poor language development, involving expression, comprehension, and pragmatic skills. He showed poor eye contact and limited facial expression. Attention was erratic, and he had difficulties with impulse control. He engaged in limited, repetitive activities. Evaluation was consistent with autism spectrum disorder.

Patient 4

This 2-year-old boy, the younger brother of patient 3, was born at term after a normal pregnancy and delivery. Motor milestones were normal. At age 2 years, he babbled but produced no words. He was not pointing and did not use gestures to indicate his needs. He showed repetitive behavioral and social difficulties. Neurologic examination and audiologic evaluation were normal. Head circumference was in the 50th percentile. Brain MRI was normal.

At 2 years, the Merrill-Palmer Scale of Mental Tests along with other measurements showed Mental Age Score of 3 years 8 months. He had excellent visual-spatial skills, but speech and language were delayed. Eye contact was poor and he engaged in activities only intermittently. He was diagnosed with autism spectrum disorder. Motor skills and language continued to improve gradually.

Patient 5

This 5-year-old girl was born at term after a normal pregnancy. Her older sister was diagnosed with MDS. At age 6 months, she was evaluated for poor oral intake, failure to thrive, irritability, and developmental delay. Lactic acid level in blood was elevated. Brain MRI showed abnormal signal intensities in the cerebellum, brainstem, and thalamus and partial agenesis of the corpus callosum. At 14 months of age, she had a viral illness with high fever, encephalopathy, regression of previous acquired skills, and significant acidosis. She gradually recovered and continued developing slowly. She sat at 2 years and walked at 4 years of age. She did not develop language, had poor eye contact, and displayed stereotypies and self-injurious behavior. She had significant mood changes with episodes of screaming, crying, and biting herself. Her behavior was consistent with PDD.

At 5 years of age, she had episodes of eye deviation and unresponsiveness. An electroencephalogram showed right frontal spikes and left temporal slowing. Antiepileptic medications caused no changes in her behavior. Her head circumference was in the 3rd percentile. She was irritable and had severe compulsive self-injurious behavior. She did not speak or follow commands. She had bilateral ptosis but full extraocular movements, hypotonia, and had brisk deep tendon reflexes with extensor plantar responses and an ataxic gait.

METHODS

Clinical Assessment

In addition to specific neuropsychologic tests described for each patient, the diagnosis of autistic spectrum disorder was based on the criteria for 299.00 autistic disorder from the American Psychiatric Association.[12]

Biochemical Analysis

Respiratory chain enzyme activities in skeletal muscle were measured as described.[13]

DNA Analysis

Patients 1 through 4 and their relatives were screened for the A3243G mtDNA mutation by single-strand conformational polymorphism analysis, as previously described.[14]

Southern blot analysis and quantitative evaluation of mtDNA in skeletal muscle from patient 5 was performed as described.[15] Sequencing analyses of the deoxyguanosine kinase (*dGK*) and thymidine kinase 2 (*TK2*) genes was performed by published methods.[16,17]

Magnetic Resonance Spectroscopy

In patients 2, 3, and 4, three-section brain proton magnetic resonance spectroscopic imaging (1H MRSI) was performed with TE/TR 280/2300. All three patients were sedated. The three 15-mm sections were oriented so that the bottom slice traversed the cerebellum posteriorly and the anterior horn of the lateral ventricle anteriorly; the second slice was oriented to traverse the posterior horn of the lateral ventricle.

These studies were approved by the Columbia University Institutional Review Board.

RESULTS

Biochemical Analysis

Respiratory chain analysis in skeletal muscle from patient 3 was normal. In patient 5, complex I, complex III, and complex IV activities were decreased to 34%, 23%, and 25% of control values, respectively. Citrate synthase activity was increased, indicating mitochondrial proliferation (Table I).

Table I. Biochemical studies in skeletal muscle

Respiratory chain enzyme (mmol/min per gram)	Patient 5	Control subjects
Cytochrome *c* oxidase (complex IV)	0.69	2.80 ± 0.52
Succinate cytochrome *c* reductase (complexes II + III)	0.21	0.70 ± 0.23
NADH cytochrome *c* reductase (complexes I + III)	0.23	1.02 ± 0.38
NADH dehydrogenase (complex I)	11.91	35.48 ± 7.07
Citrate synthase	15.71	9.88 ± 2.55
Succinate dehydrogenase (complex II)	0.40	1.00 ± 0.53

DNA Analysis

The A3243 mtDNA mutation was detected in skeletal muscle, blood, urinary sediment, oral mucosa, and hair follicles from patient 3 and in hair follicles from patient 4. The mutation was present in oral mucosa, urinary sediment, and fibroblasts from the mother of patient 1; in blood, hair follicles, oral mucosa, urinary sediment and fibroblasts from the mother of patient 2; and in hair follicles from the mother of patients 3 and 4 (Table II).

Southern blot analysis of patient 5 showed 72% depletion of mtDNA in skeletal muscle. Analysis of the *TK2* and *dGK* genes did not disclose any mutation.

Magnetic Resonance Spectroscopy

Spectra from the bodies of the lateral ventricles were normal in patients 1 and 4 and revealed the presence of a moderately increased level of lactic acid in patient 3 (6.6 IU; control subjects, 3.2 IU). Parenchymal proton metabolites were normal in voxels from both cerebellum and cerebrum in all patients.

DISCUSSION

The "autism spectrum" is a clinically and etiologically heterogeneous developmental disorder of the brain.[3] "Primary" or "idiopathic" autistic spectrum is not associated with brain damage, neurologic findings, or biological markers.[1,18] Multiple interacting genes have been proposed, and linkage to a number of loci has been reported.[1] "Secondary" autistic spectrum disorders refer to cases with defined causes.[18] The list of causes is heterogeneous and includes cytogenetic abnormalities, single gene defects, congenital infections, neurocutaneous diseases, toxic exposure, and metabolic disorders.[2,3]

Autistic features have been reported in patients with mitochondrial disorders caused by diverse molecular defects.[19-21] One patient had the G8363A mutation in the mtDNA $tRNA^{Lys}$ gene documented in blood and skeletal muscle[20] and 5 patients had single large-scale mtDNA deletions.[21] In this report, we present 4 children with autistic spectrum disorders associated with the A3243G mutation in the mtDNA $tRNA^{Leu(UUR)}$ gene. Typically, this mutation causes MELAS, which is characterized by normal early development and by childhood or young-adult onset of recurrent stroke-like episodes, seizures, hearing loss, dementia, lactic acidosis, and by the presence of ragged red fibers in skeletal muscle. The A3243G mutation also was found in

Table II. Molecular analysis

Patients and maternal relatives	Skeletal muscle	Blood	Hair follicles	Oral mucosa	Urine sediment	Fibroblast
1	ND	0%	0%	0%	0%	0%
Mother 1	ND	0%	0%	7%	5%	6%
2	ND	0%	0%	0%	0%	0%
Mother 2	ND	4%	11%	14%	37%	Trace
3*	5%	4%	3–12%	14%	10%	ND
4*	ND	0%	27%	0%	0%	0%
Mother 3 and 4	ND	0%	3%	0%	0%	0%
Aunt 3 and 4	ND	10%	22%	0%	0%	0%
Grandmother 3 and 4	ND	0%	0%	0%	0%	0%
5	72% mtDNA depletion	ND	ND	ND	ND	ND

Presence and relative abundance (in percentage of total mtDNA) of A3243G MELAS mutation in accessible tissues from patients 1, 2, 3, 4, and their maternalrelatives. For patient 5, percentage of mtDNA depletion is indicated in skeletal muscle.
ND, Not done.
*Brothers.

3 cases of infantile encephalopathy,[9] and autistic features were noted on follow-up in one of these patients (patient 3). This prompted us to analyze a possible association of autistic spectrum disorders with the A3243G mutation.

We detected 3 additional patients who presented with neurodevelopmental difficulties within the autistic spectrum (patients 1, 2, and 4). Given the lack of other associated neurologic abnormalities at the time of diagnosis, they appeared to represent forms of primary autistic spectrum disorders. These patients were brought to our attention because a relative in the maternal lineage was known to have the A3243G mutation. Molecular analysis of five accessible tissues showed the A3243G mutation in hair follicles from one patient but was negative in all tissues from the other two (Table II). However, the mothers of both patients in whom we failed to detect the mutation (patients 1 and 2) harbored the A3243G mutation in several tissues. Given the maternal transmission of mtDNA, it is likely that these women transmitted the mutation to their children. Previous analysis of the relation between maternal mtDNA mutation load and clinical expression in children showed a high frequency of affected offspring irrespective of the degree of heteroplasmy in maternal blood.[22,23] It is therefore possible that the mutation was present in accessible tissues from our two patients at levels below detection. Furthermore, as the result of mitotic segregation, the degree of heteroplasmy varies in different tissues, and lack of detectable mutation in one tissue does not exclude presence of the mutation in other tissues.

We propose that in patients with autistic spectrum disorders, the A3243G mutation may be expressed preferentially in the brain, especially in those areas that have been implicated in the pathology of autism, such as the cerebellum, the prefrontal cortex, the limbic system, and the hippocampus.[3] Because pathologic studies have shown developmental cellular abnormalities in the cerebellar hemispheres,[24,25] we evaluated the concentration of lactic acid by MRS in the brains of 3 patients (patients 2, 3, and 4). We did not find evidence of abnormal accumulation of lactic acid in the cerebellum. The absence of findings on MRS indicates either that—if indeed the mitochondrial dysfunction increases brain lactate level—this technique is not sensitive enough to detect these changes or that the cerebellum is not the anatomic substrate of autism, at least in these patients.

It is known that psychiatric symptoms, including depression, psychosis, and aggressive and paranoid behavior can occur in patients with MELAS[26,27] and that these may precede the onset of neurologic symptoms.[26] We showed that depressive symptoms were frequent in carriers of mtDNA point mutations[28] and that depression was more frequent in asymptomatic A3243G carriers than in control subjects, suggesting that psychiatric symptoms may be early manifestations of the underlying genotype.[28] Similarly, we propose that neurobehavioral disturbances within the autism spectrum may be early clinical presentation in children harboring the A3243G mutation. A prospective study will be needed to elucidate if more characteristic features of the MELAS syndrome will develop in these patients later in life.

Patient 5 presented with features of secondary autistic spectrum disorder and had significant depletion of mtDNA in skeletal muscle. MDS is an autosomal recessive quantitative defect of mtDNA characterized by reduction of mtDNA copy numbers in affected tissues.[7] Recently, mtDNA depletion has been linked to mutations in two nuclear genes coding for dGK and TK2, two enzymes involved in nucleotide metabolism and replication of mtDNA.[10,11] Mutations in the *TK2* gene are associated with myopathy or, more rarely, with lower motor neuron disease,[16] whereas mutations in the *dGK* gene have been identified in patients with multisytemic, "hepatocerebral" disease.[17] Neurologic manifestations of mtDNA depletion are variable and include hypotonia, seizures, myoclonus, and developmental delay.[15] Our patient is the first example of prominent autistic features associated with mtDNA depletion, thus expanding the clinical spectrum of this mitochondrial disorder.

It appears that the neurobehavioral symptoms of our patients are due to the A3243G mtDNA mutation in patients 1 to 4 and to mtDNA depletion in patient 5. A pathogenetic role of these mtDNA defects is reinforced by the report of other patients with mitochondrial disorders and autism.[19-21] The pathogenetic link is that all of these mutations impair overall mitochondrial protein synthesis, leading to combined defects of multiple respiratory chain complexes.[7] The precise mechanism by which impairment of the respiratory chain would lead to autism is unknown, but disruption of energy-dependent processes in the developing brain has been proposed.[20]

In conclusion, autistic features may be the expression of mitochondrial dysfunction in a developing brain. Autism with or without other neurologic findings can be the early presentation of patients with the A3243G mtDNA mutation. Offspring of mothers carrying the A3243G mtDNA mutation can also present with autistic features, even when the mutation is undetectable in easily accessible tissues. Autism can be a prominent clinical feature of mtDNA depletion.

Our report of 5 patients with autism associated with the A3243G mutation and with mtDNA depletion indicates that analysis of mitochondrial metabolism should be considered in autistic patients with associated neurologic findings or evidence of maternal transmission.

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