be simplistic to assume that all children with severe feeding disorders have an identical disorder and will respond to a single dietary change. Other mechanisms, such as visceral hypersensitivity and centrally mediated responses to feeding, surely play a role.[16,17]

An obvious limitation of the present study is the small number of patients involved, but this is not surprising because this reflects a very selective cohort of the most severely impaired and medically refractory children.

Since these children are so difficult to manage, it is important that we have the ability to offer practical diagnostic tests and clinical solutions to our patients. Although we do not know how common this subset of patients is, a therapeutic trial involving fat reduction is simple and relatively easy to implement. The benefits in quality of life in patients who respond must be weighed against the need for weight gain and growth in these children. Further studies in a larger cohort of patients with symptoms unresponsive to present treatment and interventions might help resolve both the clinical challenge and the underlying mechanism of this disability and improve the quality of life and growth of these children with severe feeding disorders. In conclusion, we suggest that a therapeutic trial with a low-fat diet should be tested in children refractory to medical and other nutritional interventions.

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## Developmental Regression and Mitochondrial Dysfunction in a Child With Autism

### ABSTRACT

Autistic spectrum disorders can be associated with mitochondrial dysfunction. We present a singleton case of developmental regression and oxidative phosphorylation disorder in a 19-month-old girl. Subtle abnormalities in the serum creatine kinase level, aspartate aminotransferase, and serum bicarbonate led us to perform a muscle biopsy, which showed type I myofiber atrophy, increased lipid content, and reduced cytochrome *c* oxidase activity. There were marked reductions in enzymatic activities for complex I and III. Complex IV (cytochrome *c* oxidase) activity was near the 5% confidence level. To determine the frequency of routine laboratory abnormalities in similar patients, we performed a retro-

spective study including 159 patients with autism (*Diagnostic and Statistical Manual of Mental Disorders-IV* and Childhood Autism Rating Scale) not previously diagnosed with metabolic disorders and 94 age-matched controls with other neurologic disorders. Aspartate aminotransferase was elevated in 38% of patients with autism compared with 15% of controls ($P < .0001$). The serum creatine kinase level also was abnormally elevated in 22 (47%) of 47 patients with autism. These data suggest that further metabolic evaluation is indicated in autistic patients and that defects of oxidative phosphorylation might be prevalent. (*J Child Neurol* 2006;21:170–172; DOI 10.2310/7010.2006.00032).

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The autism spectrum disorders comprise a heterogeneous group of patients who exhibit similar behavioral phenotypes. The etiologies of autism remain idiopathic in most cases despite comprehensive investigations. Others have reported functional mitochondrial abnormalities in patients with autism and epilepsy,[1] mitochondrial DNA G8363A transfer ribonucleic acid (Lys) mutation,[2] chromosome 15q inverted duplication,[3] and A3243G mitochondrial DNA mutation or mitochondrial DNA depletion.[4] Patients with Rett syndrome are known to have mitochondrial ultrastructural abnormalities,[5] as well as abnormalities in oxidative phosphorylation enzymes.[6] We describe a female patient in whom developmental regression and autism followed normal development and subtle laboratory abnormalities suggesting mitochondrial dysfunction led to a diagnostic muscle biopsy.

### *Case Report*

A 19-month-old girl was born after a normal full-term pregnancy. There was no family history of autism or affective, neuromuscular, or hearing disorders. Her development was progressing well, with normal receptive and expressive language and use of prelinguistic gestures, such as pointing for joint attention. Imaginary play and social reciprocity were typical for age. She used at least 20 words and could point to five body parts on command. Several immunizations were delayed owing to frequent bouts of otitis media with fever. Within 48 hours after immunizations to diphtheria, tetanus, and pertussis; *Haemophilus influenzae* B; measles, mumps, and rubella; polio; and varicella (Varivax), the patient developed a fever to 38.9°C, inconsolable crying, irritability, and lethargy and refused to walk. Four days later, the patient was waking up multiple times in the night, having episodes of opisthotonus, and could no longer normally climb stairs. Instead, she crawled up and down the stairs. Low-grade intermittent fever was noted for the next 12 days. Ten days following immunization, the patient developed a generalized erythematous macular rash beginning in the abdomen. The patient's pediatrician diagnosed this as due to varicella vaccination. For 3 months, the patient was irritable and increasingly less responsive verbally, after which the patient's family noted clear autistic behaviors, such as spinning, gaze avoidance, disrupted sleep/wake cycle, and perseveration on specific television programs. All expressive language was lost by 22 months. The patient continued to have chronic yellow watery diarrhea intermittently for 6 months, which was evaluated with negative testing for *Clostridium difficile*, ova/parasites, and culture. Four months later, an evaluation with the Infant and Toddlers Early Intervention program for possible autism was initiated. Along with the regression, her appetite remained poor for 6 months and her body weight did not increase. This resulted in a decline on a standard growth chart for weight from the 97th to the 75th percentile.

Evaluation at 23 months showed atopic dermatitis, slow hair growth, generalized mild hypotonia, toe walking, and normal tendon reflexes. The Childhood Autism Rating Scale (CARS) score was 33 (mild autism range), and she also met *Diagnostic and Statistical Manual for Mental Disorders-IV* criteria for autism. Laboratory findings included repeated measurements of aspartate aminotransferase 40 IU/L (normal < 31 IU/L), serum bicarbonate 20 mmol/L (normal 21–31 mmol/L), serum creatine kinase level 203 IU/L (normal < 170 IU/L), and fasting lactic acid 3.3 mmol/L (normal 0.5–2.2 mmol/L). Quantitative urinary organic acid analyses showed trace amounts of dicarboxylic acids (adipic, suberic, octenedioic acids) and small amounts of ethylmalonic and methylsuccinic acids, consistent with a fatty acid oxidation dysfunction. Quantitative plasma amino acids were all within the normal range; however, the alanine to lysine ratio (a surrogate marker for pyruvate; Dr Richard Kelley, personal communication, 2001) was elevated at 3.2 (normal 1.5–2.5). Cranial magnetic resonance imaging, otoacoustic emission testing, overnight electroencephalography with slow-wave sleep, serum lead, chromosomes, and fragile X by DNA testing were all normal.

The patient was referred for muscle biopsy (J.S.) because of persistent mild lactic acidosis, elevated serum creatine kinase level, and increased aspartate aminotransferase. A fresh vastus lateralis biopsy was performed and examined as described previously.[7,8] The biopsy showed abnormal histology with type I myofiber atrophy, increased myofiber lipid content, and reduced cytochrome *c* oxidase activity. Oxidative phosphorylation enzymology showed markedly reduced complex I, I + III, and III activity. Complex IV activity was near the 5% confidence limit of the control group (Table 1). Mitochondrial DNA sequencing of the skeletal muscle was normal.

Now 6 years old, our patient has been treated with vitamin supplements since 2½ years of age. Even before starting supplementation, the patient began speaking again at 23 months old and had a four-word vocabulary of "bubbles," "ball," "drink," and "cracker." Levocarnitine 250 mg and thiamine 50 mg three times per day were initiated when the patient was 29 months old. Coenzyme $Q_{10}$ was added at age 33 months. Although she still exhibits mild autistic behaviors, our patient has continued to improve in language functions and sociability such that she now attends a regular kindergarten with an aide. There have been slow yet steady improvements in muscle tone, motor coordination, and gastrointestinal symptoms with occupational therapy, applied behavioral analysis interventions, and mitochondrial enzyme cofactor supplements. After the age of 2 years, growth trajectory has continued along the 75th percentile for both height and weight. Laboratory tests were repeated at ages 2 years and 10 months (aspartate aminotransferase 47 IU/L, normal < 38 IU/L; alanine transferase 20 IU/L, normal < 40 IU/L; serum creatine kinase level 105 IU/L, normal < 194 IU/L), 4 years old (aspartate aminotransferase 36 IU/L; alanine transferase 19 IU/L; serum creatine kinase level 169 IU/L), and 6 years old (aspartate aminotransferase 36 IU/L; alanine transferase 21 IU/L; alanine to lysine ratio 1.58, normal < 1.5 to 2.5). During an acute illness owing to *C difficile*, the aspartate aminotransferase was on one occasion elevated to 50 IU/L; however, the serum creatine kinase level remained normal at 169 IU/L. Urine organic acids and serum amino acids have been normal at ages 3 and 6 years. Childhood Autism Rating Scale scores since beginning kindergarten have been under 30.

### *Additional Studies*

The subtle laboratory abnormities identified in this case led us to retrospectively evaluate the laboratory records of other patients with autism. Records from the Kennedy Krieger Institute between January 1995 and September 2002 were selected. Available laboratory tests processed by the Johns Hopkins Hospital clinical laboratory were reviewed in 159 patients with autism and 94 patients of a similar age with other neurologic disorders. Patients with autism met both *Diagnostic and Statistical Manual of Mental Disorders-IV* and Childhood Autism Rating Scale criteria. Only a subset of laboratory values could be analyzed owing to sample size limitations. Liver function tests were available in a reasonable number of subjects. Serum levels of aspartate aminotransferase, but not alanine transferase, were significantly higher in autistic patients compared with control patients (autism vs control mean [standard error]: aspartate aminotransferase 36.3 [1.2] vs 29.7 [3.0]; alanine transferase 24.6 [3.0] vs 20.6 [2.5]; *t*-test: aspartate aminotransferase $P = .00005$; alanine transferase $P = .22$). Chi-square analysis also demonstrated that significantly more autistic patients demonstrated abnormal values for aspartate aminotransferase but not alanine trans-

**Table 1. Skeletal Muscle Oxidative Phosphorylation Enzymology Results**

| *Complex Assay* | *Patient Results** | *Mean* | *SD* | *5% Level* |
|---|---|---|---|---|
| Complex I (n-decyl coenzymeQ) | 0 | 106 | 46 | 33 |
| Complex I assay (coenzymeQ$_1$) | 47 | 209 | 59 | 93 |
| Complex I + III | 35 | 262 | 93 | 105 |
| Complex II + III | 283 | 526 | 140 | 276 |
| Complex III | 527 | 1377 | 367 | 735 |
| Complex IV (freeze/thaw) | 794 | 1151 | 324 | 589 |
| Complex IV (sonicated) | 941 | 1583 | 370 | 923 |

*Units of enzyme activity are expressed as nanomoles of substrate/min/mg mitochondrial protein.

ferase (autistic vs control: aspartate aminotransferase 46% vs 22%; alanine transaminase 6% vs 7%; chi-square: aspartate aminotransferase 20.8, $P$ = .000005; alanine transaminase 0.1, $P > .50$). Too few laboratory values for the serume creatine kinase level were available from control subjects to directly compare this laboratory value between autistic and control patients. However, the number of abnormal serum creatine kinase level values was unusually high for the autistic group (22 of 46 [47%]; binomial probability: $P < 1 \times 10^{-14}$).

## *Discussion*

To our knowledge, this is the first description of an autistic child with mitochondrial dysfunction, growth failure, and abnormal muscle histopathology without seizures or a defined chromosomal abnormality. This patient exemplifies important questions about mitochondrial function in autism and developmental regression. It is unclear whether mitochondrial dysfunction results from a primary genetic abnormality, atypical development of essential metabolic pathways, or secondary inhibition of oxidative phosphorylation by other factors. If such dysfunction is present at the time of infections and immunizations in young children, the added oxidative stresses from immune activation on cellular energy metabolism are likely to be especially critical for the central nervous system, which is highly dependent on mitochondrial function. Young children who have dysfunctional cellular energy metabolism therefore might be more prone to undergo autistic regression between 18 and 30 months of age if they also have infections or immunizations at the same time. Although patterns of regression can be genetically and prenatally determined,[9] it is possible that underlying mitochondrial dysfunction can either exacerbate or affect the severity of regression. Abnormalities of oxidative phosphorylation can be developmental and age related and can normalize with time.[10]

Our findings of mildly increased aspartate aminotransferase and serum creatine kinase level in children with autism might reflect abnormal mitochondrial function in skeletal muscle since alanine transaminase and other liver enzymes were normal. Without muscle biopsy data, it is not entirely possible for us to exclude that false-positive laboratory results contribute to our high rate of aspartate aminotransferase and serum creatine kinase level abnormalities. As pointed out by others, children who struggle during venipuncture can skew the results of blood analysis.[11] However, our laboratory uses a hemolysis index that specifically avoids falsely elevated aspartate aminotransferase. Further prospective biochemical studies are needed to evaluate mitochondrial function in children within the autistic spectrum. There is a need for reliable laboratory markers to detect abnormalities of mitochondrial function, which will then facilitate further clinical investigations in this subgroup of children with autism.

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