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Authors

Filipek, Pauline A
Juranek, Jenifer
Smith, Moyra
[et al.](#)

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Mitochondrial Dysfunction in Autistic Patients with 15q Inverted Duplication

Pauline A. Filipek, MD,^{1,2} Jenifer Juranek, PhD,¹ Moyra Smith, MD, PhD,^{1,3} Lee Z. Mays, BS,^{1,3} Erica R. Ramos, BS,^{1,3} Maureen Bocian, MD,^{1,3} Diane Masser-Frye, MS,^{1,3} Tracy M. Laulhere, MA,⁴ Charlotte Modahl, PhD,¹ M. Anne Spence, PhD,^{1,3} and J. Jay Gargus, MD, PhD^{1,3,5}

Two autistic children with a chromosome 15q11-q13 inverted duplication are presented. Both had uneventful perinatal courses, normal electroencephalogram and magnetic resonance imaging scans, moderate motor delay, lethargy, severe hypotonia, and modest lactic acidosis. Both had muscle mitochondrial enzyme assays that showed a pronounced mitochondrial hyperproliferation and a partial respiratory chain block most parsimoniously placed at the level of complex III, suggesting candidate gene loci for autism within the critical region may affect pathways influencing mitochondrial function.

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Several articles have reported that duplications of the proximal long arm of chromosome 15 are associated with autistic features, mental retardation (often profound), seizures, and minor dysmorphic features.^{1,2} The duplications span the imprinted Angelman/Prader-Willi region, 15q11-q13,³ and appear to be of maternal origin.^{4,5} The duplication may be interstitial, resulting in a trisomic genetic load, or may produce a marker chromosome with either a trisomic or tetrasomic (ie, four copies) genetic load.^{4,6–8} One report has suggested that this abnormality occurs in up to 5% of autistic individuals.⁵ We report two children with this characteristic marker chromosome and phenotype who also show biochemical evidence of modest mitochondrial dysfunction, suggesting an additional dimension to this phenotype.

From the ¹Department of Pediatrics, ²Division of Child Neurology, ³Division of Human Genetics, College of Medicine; ⁴Department of Psychology and Social Behavior, School of Social Ecology; and ⁵Department of Physiology and Biophysics, College of Medicine, University of California, Irvine, CA.

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Address correspondence to Dr Filipek, UCI Medical Center, Route 81-4482, 101 City Drive South, Orange, CA 92868-4482. E-mail: filipek@uci.edu

Subjects and Methods

Two children met criteria for autistic disorder by the Diagnostic and Statistical Manual, fourth edition,⁹ the Autism Diagnostic Observation Schedule–Generic,¹⁰ and the Autism Diagnostic Interview.¹¹ Each were identified as having a marker chromosome by routine karyotype at diagnosis, and the marker was defined as an inverted duplication of chromosome 15q (15q inv dup) using fluorescence in situ hybridization (Fig). Both had uneventful perinatal courses, moderate motor delay, and severe hypotonia with periods of lethargy (particularly when ill). Serum total and free carnitine levels were drawn in anticipation of potential valproate therapy for seizures and were low in both children on repeated studies. Routine clinical magnetic resonance imaging and electroencephalograms were interpreted as normal.

Case 1

Case 1 was a girl who first presented to a neurologist with hypotonia and global developmental delay in her first year of life. As part of an evaluation at another medical center, an increased anion gap, low plasma carnitine, and elevated creatine phosphokinase were documented and a diagnostic muscle biopsy was performed at 19 months of age. Histopathology showed nonspecific type I predominance and focal myofiber type disproportion. At 4 years of age, autistic disorder was diagnosed. She had no dysmorphic features; however, routine karyotype showed 47, XX, +mar. The flash-frozen, preserved muscle biopsy subsequently was assayed for mitochondrial enzyme activity and showed pronounced mitochondrial proliferation and a respiratory chain block most parsimoniously placed at the level of complex III (Table). These findings were supported by the results of mitochondrial enzyme assays on her cultured skin fibroblasts, showing a trend toward mitochondrial proliferation and relative inactivity of complex III (see Table). Although plasma lactate, pyruvate, and ammonia levels were in the normal range, plasma carnitine was low, plasma amino acids had a twofold elevation of alanine, and organic acid analysis showed elevated lactate, pyruvate, and fumarate. In view of these findings, she was given carnitine and mitochondrial cofactor supplementation.

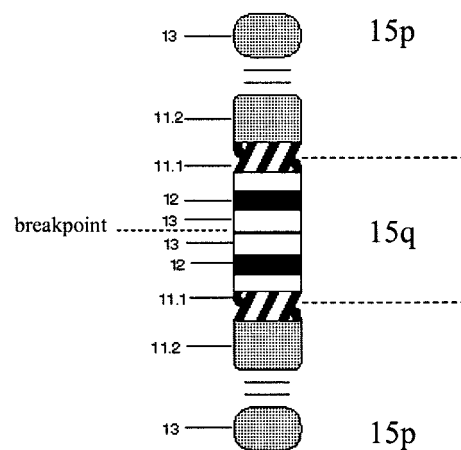


Fig. Ideogram of marker chromosome 15q11-q13.

Table. Mitochondrial Dysfunction in Autism

Mitochondrial ETC Assays	ETC Complex	Case 1	Case 2	Reference Range
Muscle^a				
NADH dehydrogenase	I	13.45	22.03	>14.22
NADH-cytochrome <i>c</i> reductase	I, III	0.40	0.64	>0.52
Succinate-cytochrome <i>c</i> reductase	II, III	0.25	1.01	>0.35
Cytochrome <i>c</i> oxidase	IV	1.88	2.18	>1.41
Citrate synthetase	Mitochondrial abundance	22.01	22.45	7.33–12.43
Skin fibroblast^b				
Succinate-cytochrome <i>c</i> reductase	II, III	6.8	15.2	6.8 ± 3.7
Decylubiquinol-cytochrome <i>c</i> reductase	III	87.2	85.6	173.0 ± 76.7
Cytochrome <i>c</i> oxidase	IV	3.0	3.1	1.1 ± 0.4
Citrate synthetase	Mitochondrial abundance	50.4	84.4	42.9 ± 10.4

^aAssays performed by Athena Diagnostics, Worcester, MA.

^bAssays performed by CIDEM, Cleveland, OH.

ETC = Electron transport chain.

Case 2

Case 2 was a boy who presented first to a neurologist at 1 year of age with global developmental delay, but autistic disorder was not diagnosed until 3 years of age. A genetics evaluation noted mild dysmorphic features including posteriorly rotated ears, mildly downslanting eyes, a right epicanthal fold, a short anteverted nose, and a short philtrum. A karyotype was obtained, showing 47,XY,+mar. Beginning at age 2 years, he began to have episodes of lethargy and profound hypotonia during minor illnesses, such as otitis media. We ordered metabolic studies to be drawn during subsequent illnesses, which showed modest hyperammonemia and lactic acidosis, low carnitine, and urine organic acids, with elevated pyruvic and glutaric acid. Because of the low carnitine levels observed in this clinical setting, he was started on carnitine supplementation. At 5 years of age, a muscle biopsy was performed to evaluate mitochondrial function. The histology was normal, but mitochondrial enzyme assays showed pronounced mitochondrial hyperproliferation and a respiratory chain block most parsimoniously placed at the level of complex III (see Table). Mitochondrial cofactor supplements were added to his carnitine supplements. Mitochondrial enzyme assays obtained on a cultured skin biopsy (obtained off all supplements) confirmed the marked mitochondrial hyperproliferation and showed a trend toward reduced relative activity of respiratory complex III (see Table). Since supplementation was initiated, he has had no further episodes of lethargy or vomiting.

Molecular Evaluation of the Marker Chromosomes

Routine cytogenetic studies showed that both of these patients had an extra dicentric marker chromosome that hybridized to a chromosome 15-specific centromeric probe, D15Z. Extensive analyses using fluorescent in situ hybridization and analysis of microsatellite repeat polymorphisms showed that the marker chromosomes 15q11-q13 arose de novo, were at least 22,810kb in length, were maternally derived, and contained two copies of each of the following genes: GABA receptor A5, GABA receptor B3, UBE3A,

SNRPN, HERC2, Necdin, and FMRP interacting protein 1. These patients therefore have a tetrasomic load of genes that map in this region.

We conclude that these patients are also duplicated for the ATP10C gene and for a series of partially characterized genes that map in the duplicated region. These genes include several CDK activated kinases, transcription factors, and ribosomal proteins. We demonstrated that our patients have four copies of the gene that encodes FMRP interacting protein 1 (KIAA0068). This protein interacts with and modulates the activity of the fragile X mental retardation protein,¹² which is of interest given the fact that deficiency of Fragile X protein is associated with autism.¹³

Discussion

These two children with 15q11-q13 inv dup and a tetrasomic genetic load for a similar 23 megabase region of chromosome 15 commonly share the phenotype of autism and a modest functional defect in oxidative metabolism most consistently revealed as mitochondrial hyperproliferation. This observation presents a novel aspect of this phenotype, because no association of 15q inverted duplications with mitochondrial dysfunction has been reported previously to our knowledge. Mitochondrial biosynthesis and proliferation occur via a coordinated activation of nuclear and mitochondrial transcription in a homeostatic response to deficient oxidative phosphorylation, possibly via redox-sensitive transcription factors.¹⁴ It has become clear that mitochondrial hyperproliferation is a sensitive indicator of deficient mitochondrial function either because a normal system is excessively stressed¹⁵ or because a molecular mitochondrial lesion has rendered a normal mitochondrial number insufficient to support a normal metabolic demand.¹⁶ Other subtle phenotypes resulting from mitochondrial dysfunction, such as cyclic vomiting syndrome, have mitochondrial hyperprolifera-

tion as their only consistent biochemical feature, with inconsistent demonstration of defective respiratory complex function.¹⁷

In our two patients, it is most parsimonious to suggest that the mitochondrial hyperproliferation occurs secondary to a relative functional defect in respiratory complex III of the electron transport system. The apparent relative excess activity of complex IV seen in both fibroblast assays likely reflects the *normal* activity of this complex and the hyperproliferation, because these assays are normalized to total protein content (a correlate of cell number) rather than citrate synthetase activity itself (a correlate of mitochondrial number and the normalization used on the muscle samples). The complex III activity *per mitochondrion* is reduced, and the hyperproliferation tends to normalize the activity *per cell*. The precise nature of the primary defect in these children is not yet known but likely represents a perturbation in mitochondrial structure, function, or biogenesis caused by the genes impacted by the chromosomal anomaly they share. It remains to be determined if the primary defect is loss of one gene's function in this region, a gene dosage effect from one or more genes in this well-studied imprinted region, or some more subtle lesion. There are, however, several candidate genes in the 15q11-13 critical region with the potential to alter mitochondrial function.

Because both marker chromosomes contain duplicated segments of maternally derived 15q11-q13, it is of interest to consider genes in this region expressed from the maternal chromosome UBE3A¹⁸ and ATP10C.¹⁹ The ATP10C gene encodes a haloacid dehalogenase hydrolase that is apparently involved in ion transport.¹⁹

Because both of our cases had a very similar marker chromosome 15, it is uncertain that all autistic children with similar marker chromosomes, or those with *interstitial* 15q inv dup, are similarly affected. It will require a larger study to determine the critical region involved in this phenotype and whether the gene dosage is related to the degree of metabolic or clinical deficit.

Because of the significant sequelae associated with oxidative metabolic defects, including the potential for serious acute clinical decompensations and even death, baseline metabolic studies should be considered on all patients with marker 15q inv dup. However, it is clear that further study is required on a larger series of such patients before any specific recommendations can be made. A point to consider in the evaluation of these children for mitochondrial dysfunction is that blood lactate levels were not consistently elevated and may be observed only during an illness. In addition, their muscle histopathology did not demonstrate ragged red fibers. Clearly, neither

precludes the presence of a mitochondrial disorder in a child.²⁰ Pending further study, we are including an evaluation of urine organic acids and blood for lactate, pyruvate, ammonia, quantitative amino acids, and total and free carnitines in new patients with this chromosomal abnormality, and we intend to utilize cofactor supplementation in children with a documented defect.

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Regular Exercise is Beneficial to a Mouse Model of Amyotrophic Lateral Sclerosis

Ilias G. Kirkinetzos, MSc,¹ Dayami Hernandez, BSc,² Walter G. Bradley, DM, FRCP,² and Carlos T. Moraes, PhD^{1,2}

We tested whether a regular exercise regimen was associated with a change in the life span of G93A-SOD1 transgenic mice, a model of familial ALS. Regular treadmill running for 10 weeks led to a significant increase in the life span of G93A-SOD1 mice. The effect was stronger in male mice, whereas there was only a trend between exercised and sedentary female G93A-SOD1 mice. The data suggest that regular exercise has a beneficial effect on the progression of ALS.

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that mainly affects motor neurons in

From the Departments of ¹Cell Biology and Anatomy and ²Neurology, University of Miami School of Medicine, Miami, FL.

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Address correspondence to Dr Moraes, University of Miami School of Medicine, 1095 NW 14th Terrace, Miami, FL 33136. E-mail: cmoraes@med.miami.edu

cortex, brainstem, and spinal cord. The role of regular exercise and fitness in the pathogenesis and treatment of ALS has been controversial.^{1,2} In the United States, ALS is commonly known as Lou Gehrig's disease, named after a famous professional baseball player who was afflicted by this devastating neurodegenerative disorder during the late 1930s. Other celebrated professional athletes struck by the disease include boxing champion Ezzard Charles, professional football player Glenn Montgomery, and Hall of Fame pitcher Jim "Catfish" Hunter. In addition, three players of the San Francisco 49ers American Football team received diagnoses of ALS during the 1980s.

Recent work suggested that persons with a history of active lifestyle and with reduced body fat have increased risk to develop ALS.² This work comes to support a previously proposed hypothesis that heavy exercise is a suspected risk factor.³ Specifically, the related risk factors include varsity sports, vigorous exercise, fitness, and body mass index under 25.^{2,3} Interestingly, leisure activity was also suspected as a risk factor.³ Several molecular events may underlie the above risk factors. The production of reactive oxygen species increases upon physical exercise, which could mediate damage to macromolecules.⁴

On the other hand, exercise has been associated with increased quality of life^{1,5,6} and has shown neuroprotective properties in several scenarios. It can alleviate motor deficit,⁷ enhance new neuronal formation,⁸ ameliorate neurological impairment in different neurodegenerative process,^{9,10} and hinder age-related neuronal loss.¹⁰ It has been also suggested that exercise mediates its protective effects against various brain insults via the upregulation of the potent neurotrophic hormone IGF-1.¹¹ Also, an "enriched" environment and physical exercise can also enhance neurogenesis,⁸ which suggests that neuronal plasticity can respond to increased functional demands, as well as in injuries.⁹

To help address this controversy, we examined whether regular exercise can be beneficial to mice transgenic for a mutated form of Cu/Zn Superoxide Dismutase (G93A-SOD1). This mutated form of the gene has been identified in patients with familial ALS and has been shown to cause motor neuron degeneration in transgenic mice overexpressing the gene.¹²

Materials and Methods

Mice with a human SOD1 transgene (G93A) were obtained from Jackson Laboratories (Bar Harbor, ME). Transgenic and control mice were placed on a treadmill at 13m/min for 30 minutes, 5 days a week. The treadmill (Columbus Instruments, Columbus, OH) was equipped with a motivation grid at the starting end that discharged a weak electrical current. Performance was measured by the number of times each mouse would fail to stay on the running treadmill, averaged weekly. Treadmill running was performed between 1