ELSEVIER

Seminars in
Pediatric
Neurology

Mitochondrial Dysfunction in Autism

Agustín Legido, MD, PhD, MBA,[*,†] Reena Jethva, MD, MBA,[*,†] and Michael J. Goldenthal, PhD[*,†]

Using data of the current prevalence of autism as 200:10,000 and a 1:2000 incidence of definite mitochondrial (mt) disease, if there was no linkage of autism spectrum disorder (ASD) and mt disease, it would be expected that 1 in 110 subjects with mt disease would have ASD and 1 in 2000 individuals with ASD would have mt disease. The co-occurrence of autism and mt disease is much higher than these figures, suggesting a possible pathogenetic relationship. Such hypothesis was initially suggested by the presence of biochemical markers of abnormal mt metabolic function in patients with ASD, including elevation of lactate, pyruvate, or alanine levels in blood, cerebrospinal fluid, or brain; carnitine level in plasma; and level of organic acids in urine, and by demonstrating impaired mt fatty acid β-oxidation. More recently, mtDNA genetic mutations or deletions or mutations of nuclear genes regulating mt function have been associated with ASD in patients or in neuropathologic studies on the brains of patients with autism. In addition, the presence of dysfunction of the complexes of the mt respiratory chain or electron transport chain, indicating abnormal oxidative phosphorylation, has been reported in patients with ASD and in the autopsy samples of brains. Possible pathogenetic mechanisms linking mt dysfunction and ASD include mt activation of the immune system, abnormal mt Ca^{2+} handling, and mt-induced oxidative stress. Genetic and epigenetic regulation of brain development may also be disrupted by mt dysfunction, including mt-induced oxidative stress. The role of the purinergic system linking mt dysfunction and ASD is currently under investigation. In summary, there is genetic and biochemical evidence for a mitochondria (mt) role in the pathogenesis of ASD in a subset of children. To determine the prevalence and type of genetic and biochemical mt defects in ASD, there is a need for further research using the latest genetic technology such as next-generation sequencing, microarrays, bioinformatics, and biochemical assays. Because of the availability of potential therapeutic options for mt disease, successful research results could translate into better treatment and outcome for patients with mt-associated ASD. This requires a high index of suspicion of mt disease in children with autism who are diagnosed early.

Semin Pediatr Neurol 20:163-175

Introduction

Since Kanner's original description of autism 70 years ago, the definition of autism, initially described as "autistic disturbances of affective contact," has undergone a variety of changes and currently refers to a heterogeneous group of disorders. Autism spectrum disorder (ASD) is the broad term encompassing autistic disorder, Asperger disorder or syndrome, and pervasive developmental disorder, not otherwise specified. These disorders share common features of impaired social relationships, impaired communication and language, and stereotypic mannerisms or a narrow range of interests.[1]

The fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V), published in 2013, has modified the diagnostic criteria.[2] The first criteria of deficits of social interaction and communication from the DSM-IV have now been merged into a single criterion of social communication and interaction. The deficits must be clinically significant and persistent and should include the following: (1) marked impairment of both verbal and nonverbal communication used for social interaction, (2) lack of social reciprocity, and (3) a failure to develop peer relationships at the appropriate developmental level. The second criterion consists of restrictive repetitive interests shown by at least 2 of the following: (1) stereotyped motor or verbal behaviors or unusual sensory

*Section of Neurology, St. Christopher's Hospital for Children, Philadelphia, PA.

†Departments of Pediatrics and Neurology, Drexel University College of Medicine, Philadelphia, PA.

This work was in part supported by grants from the St. Christopher's Foundation (St. Christopher's Hospital for Children), PHEC (Philadelphia Health Education Corporation), and DUCOM (Drexel University College of Medicine), Philadelphia, PA.

Address reprint requests to Agustin Legido, MD, PhD, MBA, Section of Neurology, St. Christopher's Hospital for Children, 3601 A Street, Philadelphia, PA 19134. E-mail: alegido@drexelmed.edu

1071-9091/13/$-see front matter
http://dx.doi.org/10.1016/j.spen.2013.10.008

behaviors, (2) excessive adherence to routines and ritualize patterns of behavior, and (3) restricted, fixated interests. The third criterion states that the symptoms of autism must be present in early childhood (DSM-V).

From a clinical point of view, children who present to a pediatrician should be screened for ASD if they seem excessively shy, are socially awkward, have language impairment, or seem to be obsessed with certain topics of interests. Also, any child who has associated morbidities such as intellectual disability, epilepsy, severe hyperactivity, and obsessive-compulsive behavior should be screened.[3]

Overall, epidemiologic studies have shown that the prevalence of autism has increased over the past decades not only in the United States but also in other countries in the world.[1] In the United States, most studies conducted between 1960 and 1980 reported the prevalence of ASD to be 2-5 per 10,000, whereas studies published in early 2000 reported prevalence ranging from 30-60 per 10,000 individuals.[4] The US Department of Developmental Services reported that, between 1991 and 1997, there was a 556% increase in the prevalence of autism in childhood.[5] From 1997-2008, the rate of prevalence of autism increased 4-fold from a prevalence of 19 per 10,000 (0.19%) to 74 per 10,000 (0.74%) individuals, respectively.[6] A recent study by the US Department of Health and Human Services has reported an astonishing prevalence figure of 200 per 10,000 (2%).[7]

Although several explanations have been offered for the epidemiologic data mentioned previously, including previous poor diagnosis, screening recommendations by the American Academy of Pediatrics, increased awareness, diagnosis at an earlier age, and change in diagnostic criteria, some authors believe that the higher prevalence of the disease is real.[1]

Despite many recent scientific advances in the research of autism, its pathogenesis remains elusive. A variety of factors have been implicated, including controversial environmental factors such as exposure to heavy metals like mercury, lead, and immunizations. No scientific data have proved a causal effect of any of these environmental factors.[8-10]

Genetic: From studies on twins to the analysis of familial, inherited cases, and genetic investigations (eg, nucleotide polymorphisms, gene defects in autistic syndromes, and specific candidate genes), all of them have demonstrated that genetics is an important factor in the pathogenesis of autism.[10-12]

Dysgenetic: Neuroanatomical and neuroimaging studies reveal macrocephaly and abnormalities of cellular configurations in several regions of the brain, including the frontal and temporal lobes and the cerebellum. Very typical is also the presence of cortical minicolumns.[10,13,14]

Immunological: Several studies have demonstrated an activation of the immune system in the cerebrospinal fluid and in the brain of patients with autism, with presence of reactive neuroglia and inflammatory response.[10,13,15,16]

Metabolic: In experimental models of autism syndromes (eg, Rett syndrome) and in patients with ASD, dysfunction of excitatory (glutamate) and inhibitory (gamma-amino butyric acid or GABA) neurotransmitters has been reported. Other neurotransmitters like serotonin and dopamine may also have abnormal physiological functions.[11,17,18] Endocrine dysfunction of growth hormone, oxytocin, vasopressin, apelin, and other endocrine factors may also be related to pathogenetic mechanisms or symptoms in patients with ASD.[18]

Furthermore, some inborn errors of metabolism and genetic metabolic disorders (eg, tuberous sclerosis) have been associated with an ASD-like phenotype.[11,19]

Among the metabolic diseases, mitochondrial (mt) disorders have recently been considered as possibly related to ASD. The mt are intracellular organelles that play a crucial role in adenosine 5′-triphosphate (ATP) production through oxidative phosphorylation (OXPHOS). The latter process is carried out by the electron transport chain (ETC) made up of complex I (nicotinamide adenine dinucleotide [NAD] + hydrogen [H]: ubiquinone oxidoreductase or dehydrogenase), complex II (succinate: ubiquinone oxidoreductase), complex III (coenzyme Q: cytochrome-c reductase or cytochrome bc complex), complex IV (cytochrome-c oxidase), and complex V (ATP synthase). The ETC is situated in the inner membrane of the mt and contains proteins encoded by both nuclear and mtDNA. About 100 proteins coded by nuclear genes are required for assembly of the complexes of the respiratory chain.[20-22]

The epidemiology of mt disease has evolved rapidly over the past 15 years. The first estimates of its prevalence were as low as 1:33,000.[23] More recent figures available are that 1 in 2000 children born each year in the United States would develop definite mt disease in their lifetimes.[24] However, when Elliott et al[25] studied the frequency of 10 mtDNA point mutations in 3168 neonatal cord blood samples, they found that at least 1 in 200 healthy humans harbors a pathogenic mtDNA mutation that potentially causes disease in the offspring of female carriers; the most common was the MELAS A3243G mutation. Approximately, 15% of pediatric mt disease is caused by mtDNA mutations and 85% is caused by nuclear DNA mutations that are inherited in a Mendelian fashion.[26]

A possible relationship between mt disease and autism was initially suggested by findings of elevation in the levels of lactate and pyruvate in the plasma[27-31] or in the brain, which was measured with magnetic resonance spectroscopy,[29] a sign of mt dysfunction in patients with ASD. Furthermore, Sue et al[32] in 1999 and Graf et al[33] in 2000 were the first ones to report 2 patients with ASD and a mtDNA mutation thought to be causing the ASD clinical picture. Since then, other similar cases have been reported and research on mt gene mutations and mt metabolism (OXPHOS) has been developed, the results suggest a possible pathogenetic link between mt dysfunction and autism.[21,26,32,33] Using data of the current prevalence of autism and a 1:2000 incidence of definite mt disease, if there was no linkage of ASD and mt disease, then it would be expected that 1 in 110 subjects with mt disease would have ASD and 1 in 2000 individuals with ASD would have mt disease. The co-occurrence of autism and mt disease appears to be much higher than these figures would suggest, again supporting a possible pathogenetic relationship.[21,30,31] The relative proportions and overlaps between children with autism and mt disease are displayed

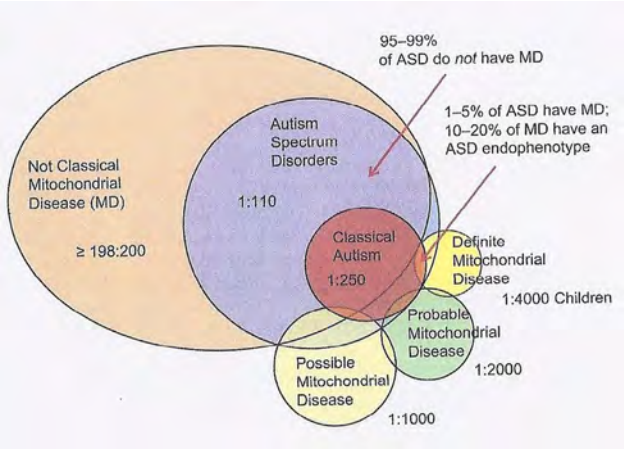

Figure 1 Most children with autism do not have classic forms of mitochondrial disease. Epidemiologic studies show that fewer than 5% of children with autism spectrum disorders have classic mitochondrial disease. Other forms of mitochondrial dysfunction, such as persistent danger response, or segmental overfunction, or certain mitochondrial functions in innate immunity, may be more common and impair cellular communication by effects of metabolism. These nonoxidant-phosphorylation functions of mitochondria are not routinely measured in the evaluation of children for classic forms of mitochondrial disease. (Adapted with permission from Naviaux.[26])

in Fig. 1.[26,30] In this article, we review the current evidence linking mt dysfunction and ASD.

Genetic Evidence of a Link Between Mitochondrial Dysfunction and Autism

Peripheral Studies

During the past decade and a half, several cases and series of children with autism and genetic abnormalities have been reported. Specifically, mtDNA mutations or chromosome anomalies involving nuclear genes that regulate mt OXPHOS function have been described.[32-47]

Mitochondrial DNA Gene Defects

As indicated before, the first evidence of a mtDNA mutation that could cause ASD was published in 1999 and 2000.[32,33] Sue et al[32] reported 3 children with infantile encephalopathy with the mtDNA MELAS mutation A3243G, one of whom was noted to have autistic features on follow-up. Graf et al[33] published the case of a 6-year-old girl, who had a heteroplasmic point mutation in the mt transfer RNA for lysine (G8363A) that was the cause of Leigh syndrome. Her psychomotor development was normal during the first year of life, and she developed ataxia and myoclonus afterward. T2 magnetic resonance imaging findings in the basal ganglia and brain stem were typical for Leigh disease. Her younger brother, who presented the same mtDNA mutation, but at a lower level of heteroplasmy (61% vs 86%), was diagnosed with ASD after developmental regression at 1.5-2 years of age. By 3.5 years of age, he had no functional speech or language, was hyperactive, and displayed bouts of self-injurious behavior.

In 2002, Filiano et al[34] reported a group of 12 children clinically presenting with hypotonia, intractable epilepsy, autism, and developmental delay, who did not fall in the previously described categories of mt disorders. The authors presented the acronym HEADD to facilitate the pursuit of mt defects in patients with this clinical constellation. Five cases exhibited increased levels of large-scale mtDNA deletions.

In 2004, Pons et al[35] studied 5 children with ASD and family histories of mtDNA diseases, one of whom had been previously reported.[29] Three patients manifested isolated ASD features and 2 had additional neurologic symptoms. Two patients had the A3243G mutation. In 2 others, the A3243G mutation was not found in accessible tissues, but it was present in tissue samples from their mothers. The fifth patient had 72% mtDNA depletion in skeletal muscle. The mtDNA depletion syndrome is an autosomal recessive disorder characterized by decreased mtDNA copy number in affected tissues.

In 2008, Weissman et al[36] reviewed the medical records of 25 patients with a primary diagnosis of ASD, 21 of them with definite mt disease. Of the 16 (44%) patients tested, 7 had mtDNA mutations—1 transfer RNA mutation; 2 probable pathogenic mtDNA point mutation: 3397 A > G and 4295 A > G; and 4 possible pathogenic mtDNA point mutations: 3394 A > C, 10394C > T, 11809 T > C, and 11984 T > C.

In 2010, Shoffner et al[37] retrospectively identified 28 patients who met the diagnostic criteria for ASD and mt disease. Autistic regression occurred in 17 (61%), a statistically significant increase over the general population with ASD (25%). Of the 17, 12 (71%) regressed with fever and 5 (29%) without fever. Of the 20 patients tested, a single patient had depletion of skeletal muscle mtDNA.

In 2010, Giulivi et al[38] evaluated mt function and mtDNA abnormalities in leukocytes in 10 children, between the ages of 2 and 5 years, with ASD. They were a subset of patients participating in the Childhood Autism Risk from Genes and Environment study in California. A statistically significant higher mean mtDNA copy number was obtained in 5 children with autism (239) vs controls (179). Of the 10 children, 2 (20%) with autism had deletions at the segment of cytochrome b, which was accompanied by over replication.

In 2013, Napoli et al[39] analyzed mtDNA deletions in 67 patients with autism enrolled in the Childhood Autism Risk from Genes and Environment study and 46 controls. The authors focused on the *ND4* gene, which encodes for the ND4 subunit in complex I, and the *CYTB* gene, which encodes for cytochrome b. A total of 10 (15%) patients with ASD had *ND4* deletions and 14 (21%) had *CYTB* deletions, these figures were statistically significantly higher compared with the controls (7% and 9%, respectively, $P < 0.001$).

A summary of the data described earlier is presented in Table 1.

Nuclear DNA Gene Defects

Replication and maintenance of mtDNA is regulated by enzymes that are proteins encoded by the genes of the nuclear

Table 1 Mitochondrial (mt) DNA Abnormalities in Children With Autistic Spectrum Disorders

Reference	Number of Patients Tested	mtDNA Abnormality	
		Number	Type/Number
Sue et al[32]	1	1	A3243G mutation
Graf et al[33]	1	1	G8363A mutation
Filiano et al[34]	12	5	mtDNA deletions
Pons et al[35]	3	3	A3243G mutation/2 mtDNA depletion/1
Weissman et al[36]	25	7	mtDNA mutations tRNA mutation/1 mtDNA point mutations Probable pathogenic/2 Possible pathogenic/4
Shoffner et al[37]	20	1	mtDNA depletion
Giulivi et al[38]	10	7	Increased mtDNA copy number/5
Napoli et al[39]	67	24	*CYTB* deletions/14 *ND4* deletions/10

Modified with permission from Haas.[30]

DNA.[22,40] As indicated before, 85% of all the pediatric mt diseases are caused by mutations in the nuclear DNA.[26]

In 2003, Filipek et al[41] published 2 cases of children with autism and a chromosome 15q11-q13 inverted duplication, who had decreased activity of ETC complex III in mt isolated from muscle biopsy. It can be hypothesized that a nuclear gene located on this region of chromosome 15 may code for the protein involved in the activity of complex III.

In 2004, Ramoz et al[42] investigated candidate genes for autism in the susceptibility region 2q24q33. They studied 411 patients, and in 197 (48%) of them, they identified 2 single-nucleotide polymorphisms (rs2056202 and rs2292813) in the *SLC25A12* gene. This gene encodes the mt aspartate-glutamate carrier, which is functionally important in neurons with high metabolic activity. *SLC25A12* is associated with neurite outgrowth and has been found to be upregulated in the prefrontal cortex of patients with autism.[43] It would be interesting to find out if biochemical markers of mt dysfunction are present in the population where association with *SLC25A12* has been found. Findings by Ramoz et al[42] were confirmed by some other authors,[44,45] but not all, trying to replicate their results[46,47]; a discrepancy that may reflect the expected genetic heterogeneity, particularly in different ethnic populations.[46,47]

In 2010, Marui et al[48] investigated candidate genes for autism in the susceptibility region 7q32. They studied 235 Japanese patients. The authors identified 2 single-nucleotide polymorphisms (rs12666974 and rs23779262) in the *NDUFA5* gene, which were associated with autism. This gene encodes the product of NADH-ubiquinone oxidoreductase 1 alpha subcomplex 5, which is included in the mt ETC.

In 2010, Ezugha et al[49] reported the case of a 12-year-old boy with ASD and mt disease, cognitive deficit, and dysmorphic features. Chromosomal microarray studies, using array-based comparative genomic hybridization, revealed a 1-Mb deletion in the 5q14.3 region. The patient had a significantly lower activity of ETC complexes I and IV, which was measured in buccal cells. The authors suggested that the features seen in this child were consistent with mt dysfunction and a nuclear gene located on chromosome 5, which codes for a protein involved in the activity of these mt respiratory chain complexes.

In 2010, Guevara-Campos et al[50] reported another case of a 3-year-old girl with autism who showed deficiency of complexes III and IV in mt isolated from muscle biopsy.

Brain Studies

Most of the previous genetic studies of mt dysfunction in autism have been based on mutations in the mtDNA, as previously described. However, most of the proteins essential for mt replication and function are encoded by the genomic DNA; so far, there have been very few of those genes studied.[51]

Anitha et al[51] used postmortem brains of patients with autism and healthy controls and compared the expression of 84 genes involved in diverse functions of the mt in 3 brain regions: anterior cingulate gyrus, motor cortex, and thalamus. (Table 2) The authors observed brain region–specific alterations in the expression of several genes in the autism group compared with the control group. A total of 22 genes in the anterior cingulate gyrus, 15 genes in the motor cortex, and 12 genes in the thalamus showed aberrant expression in patients with autism compared with the controls.[51] These genes belong to the following functional groups: (1) membrane polarization and potential, (2) mt transport, (3) small molecule transport, (4) targeting proteins to mt, (5) mitochondrion protein import, (6) outer membrane translocation, (7) inner membrane translocation, (8) mt fission and fusion, (9) mt localization, and (10) apoptosis.[51] The genes *MTX2*, *NEFL*, and *SLC25A27* showed consistently reduced expression in all the 3 brain regions studied. The expression of *DNAJC19*, *DNM1L*, *LRPPRC*, *SLC25A12*, *SLC25A14*, *SLC25A24*, and *TOMM20* was consistently reduced in at least 2 brain regions of patients with autism compared with controls.[51] All of these genes participate in different brain functions controlled by those anatomical areas that may be affected in autism (eg, emotion formation and processing, learning and memory, cognitive control, social orientation and target detection, and response monitoring). The gene *SLC25A12* was previously discussed in the studies by Ramoz et al[42] in the peripheral blood and by Lepagnol-Bestel et al[43] in the brain.

In addition, the same group of authors studied the expression of mt respiratory chain complex (or ETC) in the

Table 2 Mitochondria-Related Genes with Altered Expression in the Brains of Patients With Autism

Gene	Anterior Cingulate Gyrus	Motor Cortex	Thalamus	Functional Group
Decreased				
AIP		X		b, d, e
DNAJC19		X	X	b, d, e
DNM1L	X	X		i, j
LRPPRC	X	X		i
MFN2	X			b, d, h, j
MIPEP		X		b, d, e
MTX2	X	X	XX	b
NEFL	X	X	X	i
RHOT2	X			i
SLC25A12	X	XX		c
SLC25A14	X	XX		c
SLC25A15	X			c
SLC25A22	XX			c
SLC25A24		XX	X	c
SLC25A27	X	X	X	c
SLC25A3		X		c
SLC25A4	X			c
SLC25A5		X		c
TIMM17A	X			g
TIMM17B	XX			g
TIMM23		X		g
TIMM50	X			g
TIMM8A	X			g
TIMM9			XX	g
TOMM20	X	X		f
TOMM22	X			f
TOMM34			XX	f
TOMM70A	X			f
Increased				
BCL2	X			a, b, j
HSP90AA1			X	b
SLC25A25			X	c
SLC25A37			X	c
SOD2			X	j
TIMM44			X	g
TP53	X			a, b, j
TSPO	X			b, d

X = $P < 0.05$; XX = $P < 0.01$, for *t* test vs controls, under brain region where altered expression (decreased or increased) was found; a = membrane polarization and potential; b = mitochondrial transport; c = small molecule transport; d = targeting proteins to mitochondria; e = mitochondrion protein import; f = outer membrane translocation; g = inner membrane translocation; h = mitochondrial fission and fusion; i = mitochondrial localization; j = apoptotic genes. (Modified with permission from Anitha et al.[51])

Table 3 Downregulation of Mitochondrial Electron Transport Complex Genes in the Brains of Patients With Autism

Gene	Anterior Cingulate Gyrus	Motor Cortex	Thalamus
Complex I			
NDUFA2		XX	
NDUFA4	X		X
NDUFA5	X	XX	X
NDUFA8	X	XX	
NDUFAB1	X		X
NDUFB3			X
NDUFB4		X	
NDUFB5		X	X
NDUFB6			X
NDUFS2			X
NDUFS4			X
Complex III			
CYC1			X
UQCRC1		X	
UQCRC2			X
UQCRFS1		X	X
UQCRH	X		X
Complex IV			
COX5A	X		
COX6A1	X	X	
COX7A2			X
COX7B			X
COX8A	X		
Complex V			
ATP5A1	X	X	X
ATP5B			X
ATP5F1		X	X
ATP5G3	X	X	XX
ATP5H	X		
ATP5J			X
ATP6V0A2		XX	

X = $P < 0.05$; XX = $P < 0.01$, for *t* test vs controls, under brain region where downregulation was found. (Modified with permission from Anitha et al.[52])

brains of patients with autism. Genes in the following 3 anatomical areas were selected: anterior cingulate gyrus, motor cortex, and thalamus[20-22,52] (Table 3). The authors found reduced expression of ETC genes in the brains of patients with autism compared with controls. A total of 11 genes of complex I, 5 genes of complex III and complex IV, and 7 genes of complex V showed brain region–specific reduced expression in autism. *ATP5A1* (complex V), *ATP5G3* (complex V), and *NDUFA5* (complex I) showed consistently reduced expression in all the brain regions of patients with autism.[52] The gene *NDUFA5* was previously discussed in the study by Marui et al[48] in the peripheral blood.

Gu et al[53] analyzed the presence of deletion in 3 mtDNA genes, *ND1*, *ND4*, and *Cyt B*, that encode the subunits of complexes I and III in the frontal lobe of the brains of patients with autism and demonstrated that 44% of the autistic group had a *ND4* deletion and 33% had a *Cyt B* deletion present. Furthermore, they calculated the ratio of those genes to nuclear genes and found that it was significantly elevated, demonstrating a higher mtDNA copy number in autism.

Overall, the results of the studies summarized previously show that mtDNA and nuclear DNA gene abnormalities are frequently seen in patients with ASD, suggesting that mt disease may be one of the most common conditions associated with autism.[21,20,31] As a consequence, patients with ASD would have mt dysfunction, which might augment and disseminate several brain abnormalities related to autism.[51]

Metabolic Evidence of a Link Between Mitochondrial Dysfunction and Autism

Multiple metabolic abnormalities, indicating dysfunction of mt, have been described in children with ASD. They include elevation in levels of lactate, pyruvate, or alanine in blood, cerebrospinal, or brain[21,27-30,32-39,41,54,55]; decreased levels of plasma carnitine[21,41]; abnormal levels of urine organic acids[36,55]; and impaired mt fatty acid β-oxidation.[56,57]

As indicated previously, the most important metabolic role of mt, though, is the production of energy, facilitating the synthesis of ATP through the process of OXPHOS, regulated by the respiratory chain or ETC.[20-22] Therefore, we now concentrate on summarizing the information, gathered from multiple investigations during the past decade and a half, about demonstrated ETC abnormalities in peripheral tissues (blood, fibroblasts, muscle, and buccal cells) or in the brain of patients with ASD.

Peripheral Studies

Some of the studies previously summarized while describing the mtDNA and nuclear mtDNA abnormalities in patients with autism also measured the activity of the mt ETC complexes (Table 1).

In 2000, Graf et al[33] found an increased activity of complex I in mt isolated from muscle biopsy of the patient with ASD who they reported. In 2002, Filiano et al[34] evaluated muscle mt ETC complexes in 8 of the 12 patients with ASD as part of the symptom constellation of the HEADD syndrome they described; 7 of them had decreased ETC activity, including defects of complex I in 1, III in 6, IV in 1, and V in 3, including combinations of various complexes. In 2003, Filipek et al[41] published 2 cases of children with autism and a chromosome 15q11-q13 inverted duplication, and both had a deficit of complex III activity in muscle mt. In 2004, Pons et al[35] measured mt ETC activity in the muscle biopsy of 1 patient of the 5 case series that they had published and found it to be decreased for complexes I, II, II + III, and IV. In 2008, Weissman et al[36] performed muscle biopsies in 25 patients with ASD and mt disease and analyzed the activity of ETC complexes in isolated mt; the authors demonstrated it to be significantly decreased in 20 of them, including complex I in 16, II in 2, III in 5, and IV in 1; some patients had more than 1 deficit. In 2010, Shoffner et al[37] studied 28 children with ASD and mt disease and measured ETC complexes' activity in isolated muscle mt and found a deficit of complex I in 14 cases, complex I+III in 5, complex I + III + IV in 5, and complex V in 4. In 2010, Giulivi et al[38] studied in leukocytes of 10 children with ASD the activity of mt complexes, which was decreased for complex I in 6 cases, for III in 3, for IV in 3, and for V in 4; these abnormalities, in different combinations, were found in 8 patients. In 2010, Ezugha et al[49] reported a case of a patient with autism and mt disease, who had a defect in complexes I and IV that were evaluated in buccal cells.

The following are publications regarding patients with ASD who had mt function evaluated by measuring ETC complexes, but who did not have genetic studies performed and, therefore, they were not initially discussed in the previous sections.

In 2005, Oliveira et al[54] published a population-based study of mt dysfunction in ASD. A subgroup of 11 children had the ETC studied in mt isolated from muscle. In 6 cases, the results showed abnormalities, including complex I in 2, complex IV in 2, and complex V in 3.

In 2007, Tsao et al[58] published 2 cases of children with ASD and mt disease demonstrating a decreased activity of mt ETC in muscle biopsy, including a partial defect of complex I in 1 patient (associated with a coenzyme Q10 deficiency) and a combined defect of complexes II + III in the other.

In 2011, our group retrospectively evaluated the incidence of mt respiratory chain function in a group of 39 consecutive children with ASD and 63 age-matched controls.[59] We used cells obtained from buccal swabs to determine the activity of ETC complexes I and IV by immunocapture and the referential citrate synthase activity using microspectrophotometry. This is a technique that has a high correlation with the study of the mt respiratory chain complexes in the muscle.[60] Of the 39 children, 21 (54%) had deficiency of mt ETC complexes, including complex I in 4, complex IV in 12, and both complexes in 5.

In 2012, Frye[61] reported the case of a patient with 22q13 duplication syndrome, with mt disease and autism. Studies of mt ETC in fibroblasts showed a marked deficit of complexes II and II + III and a deficit of complexes I + III and II + III in the muscle.

In 2012, Craig et al[62] published data on 2 patients with Dravet syndrome and ETC abnormalities. One of them had autism and the function of complex IV was decreased in the mt isolated from the muscle.

Overall, except the patient reported by Graf et al,[33] who was found to have an increased complex I activity, all the remaining patients presented a deficit of the activity of ETC complexes. Of the 130 patients with ASD studied, 99 (76%) demonstrated decreased functional OXPHOS activity of the mt respiratory chain. Complex I was the most frequently involved (40%), followed by complex IV (26%), complex III (14%), complex V (12%), complex II (4%), and several complex combinations (4%) (Table 4).

Brain Studies

In 2011, Chauhan et al[63] studied the ETC complex levels in the brains of patients with autism, and in matched controls, in the cerebellum and in the frontal, temporal, parietal, and occipital cortical regions. The patients were divided according to age into group A (children between the ages of 4 and 10 years) and B (adults between the ages of 14 and 39 years). In children with autism, the authors found significantly lower levels of complexes III and V in the cerebellum; of complex I in the frontal cortex; and of complexes II, III, and V in the temporal lobe, whereas none of the 5 ETC complexes were

Table 4 Mitochondrial ETC Abnormalities in Children With Autistic Spectrum Disorders

Reference	Number of Patients Tested	ETC Deficiency	
		Number	Type/Number
Graf et al[33]	1	1	↑ Complex I
Filiano et al[34]	8	7	↓ Complex I/1 ↓ Complex III/6 ↓ Complex IV/1 ↓ Complex V/3
Filipek et al[41]	2	2	↓ Complex III/2
Pons et al[35]	1	1	↓ Complexes I, II, II + III, IV
Oliveira et al[54]	11	6	↓ Complex I/2 ↓ Complex IV/2 ↓ Complex V/3
Tsao et al[58]	2	2	↓ Complex I, CoQ10/1 ↓ Complexes II, III, IV/1
Weissman et al[36]	25	20	↓ Complex I/16 ↓ Complex II/2 ↓ Complex III/3 ↓ Complex IV/1
Shoffner et al[37]	28	28	↓ Complex I/14 ↓ Complex I + III/5 ↓ Complex I + III + IV/5 ↓ Complex V/4
Giulivi et al[38]	10	8	↓ Complex I/6 ↓ Complex III/3 ↓ Complex IV/3 ↓ Complex V/4
Ezugha et al[49]	1	1	↓ Complexes I, IV
Guevara-Campos et al[50]	1	1	↓ Complexes III, IV
Goldenthal et al[59]	39	21	↓ Complex I/4 ↓ Complex IV/12 ↓ Complexes I, IV/5
Frye[61]	1	1	↓ Complexes II, II + III, I + III
Craig et al[62]	1	1	↓ Complex IV

Modified with permission from Haas.[30]

affected in the cortical parietal and occipital lobes. A striking observation was that the levels of ETC complexes were similar in adults with autism and control subjects.

In 2013, Gu et al[53] analyzed the ETC complexes exclusively in the frontal lobe of the brains of patients with autism. The activities of complexes I and V were most often affected, being significantly reduced by 31% and 36%, respectively, in the subjects. The authors also measured the activity of pyruvate dehydrogenase, which was found to be reduced by 35% in autism.

In 2013, Tang et al[64] studied mt function data in the temporal lobe of the brains of patients with ASD. They exhibited decreased complex I and IV activities compared with controls. Furthermore, the authors demonstrated higher levels of the mt fission proteins, Fis1 and Drp1, and decreased levels of the fusion proteins, Mfn1, Mfn2, and Opa1, in patients with ASD, indicating altered mt dynamics in the brain of children with ASD. These changes were evident in cortical pyramidal neurons and were observed in children with ASD, but the changes were less pronounced or absent in adult patients, in agreement with the findings reported by Chauhan et al.[63] The mt membrane mass was higher in the brains of patients with ASD, as indicated by higher protein levels of mt membrane proteins, Tom20, Tim23, and porin. No differences were observed in either the mtDNA or levels of the mt gene transcription factor TFAM or cofactor PGC1α, indicating that a mechanism other than alterations in mt genome or mt biogenesis underlies these mt abnormalities.[63]

Pathogenetic Mechanisms Underlying the Relationship of Mitochondria and Autism

In spite of the genetic and biochemical findings presented previously, suggesting a relationship between ASD and mt, it is not clear whether mt dysfunction contributes to the development or pathogenesis of ASD or whether it is merely an epiphenomenon of ASD.[21] Several metabolic abnormalities or exposures to environmental toxic substances could result in secondary mt dysfunction in children with ASD. Alternatively, these factors could worsen mild mt dysfunction in some children, transforming it into more severe dysfunction.[21] As previously indicated, some children demonstrated regression into ASD and mt disease following fever, suggesting children who develop mt disease may be vulnerable to external factors causing regression into ASD in children who may already have an existing, but unidentified, mt disease.[21,37]

In the following sections, we discuss mechanisms triggered by mt dysfunction, which could explain the existence of unified pathogenetic theories explaining the causative relationship between mt dysfunction and ASD.

Mitochondrial Activation of the Immune System

One of the most ancient functions of mt is in cell defense. When a cell is attacked by a virus, a cascade of events is initiated to protect the cell from injury, limit viral replication, and warn neighboring cells of the intrusion.[26] Healthy cells can increase or decrease their response to a given infection or inflammatory stimulus by a process of priming, so that cells differentiate to respond rapidly to an attack.[26] If a cell is injured in the attack, then a large number of molecules are released into the extracellular space as "danger" signals. Many of these are present in high concentrations within the mt, and they are called DAMPs or damage-associated molecular patterns. ATP is a DAMP.[65]

Another reason that a number of mt molecules act as DAMP is the evolutionary origin of mt as the ancestors of ancient,

free-living, Gram-negative bacteria.[66] The mtDNA itself contains unmethylated CpG dinucleotides that resemble bacterial DNA and activate toll-like receptor 9. Proteins synthesized in mt start with a bacteria-like formylmethionine. *N*-formylmethionine–containing peptides from mt bind to the formyl peptide receptors, FPR1 and FPRL1, and activate innate immunity.[67]

There is evidence of abnormal immune responses to several putative antigens in genetically vulnerable children with ASD.[30] Multiple immune dysfunctions have been described in patients with ASD, including abnormal accumulation of T lymphocytes in tissues such as the gastrointestinal tract, reduced number of circulating $CD4^+$ T cells, abnormal lymphocyte responsiveness, and abnormal production of Th1 and Th2 cytokines.[68,69] Particularly, 2 Th1 or Th2 cytokines that have been implicated in ASD are tumor necrosis factor-α (TNF-α) and interferon-γ.[69]

These immunologic abnormalities found in ASD could lead to an abnormal brain growth and development by disrupting the delicate balance between the immune system, neural growth factors, neural stem cells, and neurotransmitters.[30]

The hypothesis of dysfunction of the immune system and abnormal brain development and function is supported by the study of Smith et al,[70] who created a mouse model of autism by injection of a double-stranded RNA poly(I:C) into pregnant mice, to cause maternal immune activation (MIA), and identified IL-6 as a key mediator of the effects of MIA on fetal brain development. In humans, Pardo et al[15] studied the neuropathology of the brains of patients with autism and demonstrated activated microglia and astroglia and increased levels of cytokines (eg, IL-6 and transforming growth factor-β1) in the prefrontal and cerebellar gray and white matter. Glia play essential roles during brain development. Stimuli, such as infection, injury, or immune activation, can persistently cause glial activation, a finding common to many neurodegenerative diseases, including Alzheimer disease, multiple sclerosis, and stroke.[30] In addition, the authors also identified high levels of several growth factors, including vascular endothelial growth factor, fibroblast growth factor-9, and insulin-like growth factor-binding protein-1, among others.[15] A number of studies have implicated growth factors in the pathogenesis of ASD in some patients, suggesting that they have an abnormal robust immunologic response.[30,71]

Furthermore, mt play an important role in the regulation of the immune system. Interferons can induce the expression of over 300 genes, some of which are likely to be mt genes. They may be the actual mediators of the antiviral effects and antitumor effects of interferons, but they may also promote proapoptotic actions that could lead to neurodegeneration.[30]

Corticosteroids released by the autonomic nervous system and hypothalamic-pituitary-adrenocortical neuraxis are important modulators of the immune response.[30] Glucocorticoids inhibit production of proinflammatory cytokines, chemokines, and cytokine receptors, including TNF-α, IL-2, IL-6, IL-1β, and IL-8. They also upregulate production of anti-inflammatory cytokines, including IL-10, IL-4, and transforming growth factor-β.[72] Both nuclear- and mt-encoded OXPHOS subunits are regulated by glucocorticoids, and their

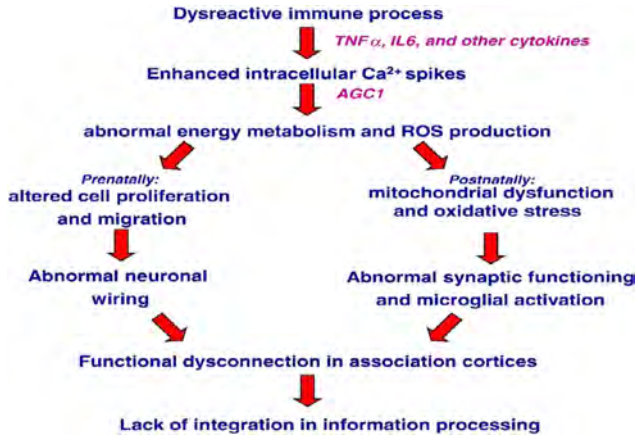

Figure 2 Pathogenetic model proposed for immune-mediated forms of autism spectrum disorders. An abnormal neuroimmune response is a relevant player in elevating intracellular Ca^{2+} levels, deranging neurodevelopment, driving oxidative stress, and ultimately affecting synaptic function and neural connectivity, especially in long-range neuronal pathways that are physiologically responsible for integrted information processing. Adapted with permission from Palmieri and Persico.[78]

receptors have been identified in the mt.[73] There is evidence that mt are important mediators of the stress-induced cytokine-mediated apoptosis, and the efficiency of mt function helps to determine apoptotic response. Indeed, mt-nuclear interactions play a role in cytokine gene expression.[30]

In summary, mt dysregulation of the immune system may play a role in triggering immune-mediated mechanisms, which can damage prenatal brain development or postnatal brain function, thus establishing a causal relationship with the ASD phenotype (Fig. 2).

Abnormal Mitochondrial Calcium Handling

The regulation of intracellular Ca^{2+} release from the endoplasmic reticulum to mt through the inositol trisphosphate receptor and ryanodine receptor channel is a crucial point of regulation of the immune response to infection and stress.[74] Furthermore, an abnormal immune process enhances intracellular penetration of Ca^{2+}. Cytokines, such as TNF-α and IL-6, and cytokine receptors, like CD38, are all known to mobilize Ca^{2+} stores or to facilitate Ca^{2+} entry from extracellular compartment.[15,75,76]

The mt are well known to have a pivotal role in the regulation of plasma membrane Ca^{2+} channels and transporter activities.[77] Therefore, mt are key players in cellular Ca^{2+} handling, and an increase in the intracellular Ca^{2+} levels produces mt dysfunction, thus impairing OXPHOS and generating oxidative stress (OS), as discussed later. This would, in turn, be able to trigger an immune response similar to the one observed in the brains of patients with autism[78-81] (Fig. 2).

Abnormal Ca^{2+} handling by mt could also contribute to abnormalities in neuronal function, reported in ASD, including disruption of neurotransmitters involved in the process of neurodevelopment.[78] Intracellular Ca^{2+} homeostasis regulates

the release of the excitatory neurotransmitter glutamate. An imbalance between glutamate and the inhibitory neurotransmitter GABA has been implicated in the pathogenesis of ASD, with a relative increase in the excitatory-to-inhibitory ratio.[17,31,82] The mt dysfunction can lead to reduced synaptic neurotransmitter release in neurons that have high firing rates, such as inhibitory GABA interneurons.[83] Thus, mt dysfunction can result in a reduction in inhibition and the relative increase in the excitatory-to-inhibitory ratio observed in ASD. Furthermore, Ca^{2+} signaling is essential for the integrity of synaptic function, and abnormal Ca^{2+} signaling has also been implicated in ASD.[84] Recent genetic studies have also supported this concept.[75,76] Ca^{2+} homeostasis in the central nervous system is controlled by multiple genes, which ultimately have an effect on brain development.[84]

The gene *SLC25A12* has been associated with autism[43-45] and has been found to be downregulated in the cingulate gyrus and motor cortex and upregulated in the prefrontal cortex[42] of the brains of patients with autism. This gene controls the synthesis of the mt aspartate-glutamate carrier, isoform 1 (AGC1). The end terminal of its 678 amino acid sequence contains 4 EF hand Ca^{2+}-binding domains, which bind Ca^{2+} *in vitro* and *in vivo*.[76] Other genes, participating in Ca^{2+} homeostasis, have been associated to autism, including *ATP13A4*, *ATP2B2*, *CACNA1C*, *CACNA1F*, *CNCNA1H*, *KCNMA1*, *IL1RAPL1*, *NCS1*, and *CAPS2*.[76] The proteins encoded by these genes play a pivotal role in adjusting mt energy production to functional demands in excitable cells through their responsiveness to cytosolic Ca^{2+} levels. Also, these proteins exert a profound influence on neurodevelopment not only through the regulation of Ca^{2+} homeostasis but also by modulating neuronal migration, dendritic spine formation, synaptogenesis, myelin synthesis, etc.

Palmieri et al[85] studied AGC, isoform 1 and Ca^{2+} levels in the brains of patients with ASD. They found high neocortical Ca^{2+} levels, which were responsible for boosting AGC activity, mt metabolism, and OS in the brains of patients with autism. The authors concluded that AGC and altered Ca^{2+} homeostasis play a key interactive role in the cascade of signaling, leading to autism.

Abnormal Mitochondrial Oxidative Stress

Additional evidence pointing toward abnormal mt function in autism comes from investigation addressing OS.[21,31,75,86] Under normal conditions, a dynamic equilibrium exists between the production of reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical, singlet oxygen, and hydrogen peroxide, and the antioxidant capacity of the cell. The mt represent the major cellular source of ROS. The inner membrane of the mt contains a large amount of free-radical scavengers, including glutathione, vitamin C, and vitamin E, as well as superoxide dismutase. Excess production of free radicals or impaired antioxidant mechanisms may cause OS.[78] This may be a key mechanism by which mt are negatively influenced by factors linked to ASD, such as prooxidant environmental toxic substances and immune abnormalities, as ROS can result in mt dysfunction. Further, dysfunctional mt leads to further OS and a vicious negative cycle ensues[21,31,78,86] (Fig. 2). For example, dysfunction of ETC complex I can produce high levels of ROS and reactive nitrogen species. In turn, ROS and reactive nitrogen species can inhibit complex I, causing further mt dysfunction.[87,88] The decreased function of the ETC also causes cellular ATP depletion that disrupts the mt inner transmembrane potential, which activates the mt permeability transition pore and causes apoptosis.[89]

Glutathione (GSH) is a key antioxidant that protects the mt; its deficit promotes OS and increases vulnerability to prooxidant environmental exposures and oxidative damage.[86] Several studies have found that patients with autism have GSH deficits in the plasma, immune cells, and urine.[86,90-92] In addition, 3-nitrotyrosine, a biomarker of oxidative damage to proteins, and 3-chlorotyrosine, a biomarker of chronic inflammation, have been found to be elevated in the plasma and brain of children with ASD.[92,93] In the brain, a prooxidant balance has been demonstrated by the presence of decreased levels of GSH and oxidized GSH (GSSG),[93] decreased ratio of GSH-GSSG redox-antioxidant capacity,[93] decreased levels of superoxide dismutase 2,[64] and elevated levels of lipid hydroperoxide.[63] Overall, these findings of increased OS in the brains of patients with autism suggest that it may have functional consequence in terms of a long-term inflammatory response, increased mt superoxide production, and oxidative protein and DNA damage.[93] Recently, Napoli et al[39] have demonstrated that ROS mediate damage to mtDNA in children with autism.

A recent study by Frye et al[86] compared the redox or antioxidant status of 2 groups of children with autism, with and without mt disease. As expected, in comparison with controls, both of them showed higher levels of oxidative damage (3-chlorotyrosine) owing to long-term immune activation and decreased levels of GSH, GSSG, and 3-nitrotyrosine and decreased GSH-GSSG ratio, indicating a prooxidant balance. However, the group of children with ASD and mt disease had significantly higher GSH-GSSG ratio and lower levels of GSSG, suggesting a more favorable glutathione redox status. Moreover, only in the patients with ASD and mt disease, there was a significant correlation of 3-nitrotyrosine with cognitive function, development, and behavior, suggesting that these children with a more chronic oxidized pattern have a better development. The authors interpreted the findings as the consequence of compensatory upregulation of dysfunctional mt, which can increase the activity and function at the expense of a more oxidized microenvironment. It is, therefore, important to identify patients with ASD who have mt disease, as they may respond differently to specific treatments because of their specific metabolic profile.[86]

It should be highlighted that other authors believe that ROS and chronic oxidative changes in membrane lipids and proteins found in many chronic diseases are the results of a machinery of "oxidative shielding" that evolved from pathways of innate immunity, designed to protect the cell from attack and limit the spread of infection. Therefore, according to them, ROS are the response to disease, not the cause.[94]

Unified Pathogenetic Theories

Abnormal Neuroimmune Response

This immune-mediated mechanism could characterize a large subgroup of patients with ASD. An abnormal neuroimmune response would be the primary trigger in causing an increase in intracellular Ca^{2+} levels, producing mt dysfunction and, as a consequence, abnormal energy metabolism and stimulation of OS. Prenatally, deranging neurodevelopment would occur, affecting cell proliferation and migration, which ultimately would cause abnormal wiring, functional disconnection in association cortices, and the manifestation of lack of integration in information processing. Postnatally, the same outcome would be the consequence of mt dysfunction and OS, which would also affect synaptic functioning and would stimulate the immune system, causing microglial activation (Fig. 2).[78]

Abnormal Purinergic Signaling

The role of the purinergic system in autism is a novel theory developed by Naviaux.[26] The metabolism of a child adjusts to match the changing environment by the process of metabolic matching: changes in nutrition, infectious agents, environmental toxic substances, and activity. Mitocellular hormesis refers to a long-term adaptation process of mt to past metabolic stresses, by producing long-term changes in cellular reactivity.[95] Mitocellular hormesis to severe stress can produce a long-term and pathologic increase in many components of mt metabolism and an increase in extracellular ATP levels, which is a DAMP, or damage-associated molecular pattern, that binds to purinergic receptors P2X and P2Y. P2X receptors are ATP-gated cation channels that regulate Ca^{2+} conductance and they are known as the ionotropic purinergic receptors. In humans, there are 7 subclasses of them, designated P2X1-7. P2Y receptors are G protein-coupled receptors, collectively called the metabotropic purinergic receptors. In humans, there are 8 subclasses designated P2Y1, 2, 4, 6, 11, 12, 13, and 14. Nucleotide signaling via P2X and P2Y receptors mediates a large number of biological phenomena of relevance to autism.[26] These include normal synaptogenesis and brain development,[96] regulation of the PI3K-AKT pathway,[97] control of immune responses and chronic inflammation,[98] gut permeability,[99,100] taste chemosensory transduction,[101] sensitivity to food allergies,[102] hearing,[103] innate immune signaling, neuroinflammation, antiviral signaling, microglial activation, neutrophil chemotaxis, autophagy, and chronic pain syndromes.[96]

From the perspective of brain development and function, purinergic signaling represents an important new area for study in ASD as it has direct effects on pathways implicated in ASD[26] (Fig. 3).

Abnormal Genetic Regulation

Other ASDs associated to mt dysfunction could be well explained either by rare genetic mutations or by the coexistence of several unfavorable common gene variants in the same individual. In these genetically based cases, mutations or polymorphic variants could affect, among other mtDNA or nuclear loci directly involved in mt function, genes involved in Ca^{2+} signaling or genes critical to downstream synaptic function.[78]

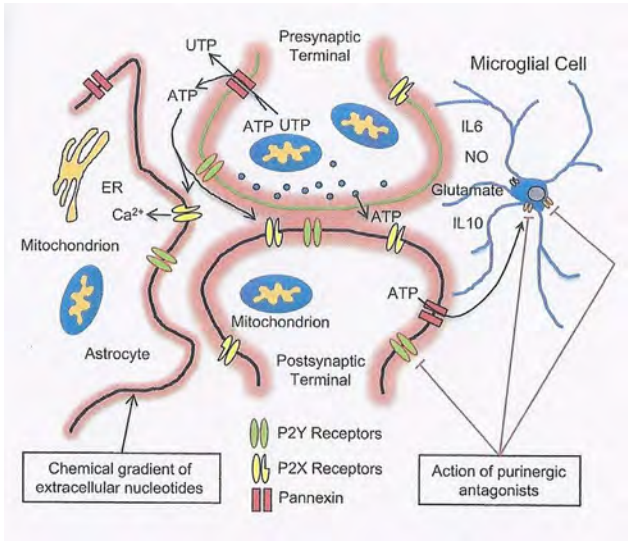

Figure 3 Purinergic regulation of synaptogenesis. ATP is a coneurotransmitter at every synapse studied to date. Mitochondria are the ultimate source of extracellular ATP (eATP). The activity and usage of each synapse regulates the concentration of eATP surrounding the synaptic junction. Microglial cells monitor synaptic activty and respond to eATP to either stabilize or inhibit synapse formation. Excitotoxicity results in excessive eATP that binds to microglial purinergic receptors and stimulates neuroinflammation. ER: endoplasmic reticulum, IL: interleukin, NO: nitric oxide. (Adapted with permission from Naviaux.[26])

It is also important to mention the possible role of epigenetics, which refers to nonpermanent heritable changes that alter expression of genes without altering the DNA sequence itself and considers the role of environment in this modulation of gene expression,[104] which takes place through a process of gene methylation.[22] An epigenetic approach may be very promising in clarifying genetic factors involved in autism, and an increasing number of epigenetic diseases are being associated with mt dysfunction.[104,105]

The epigenetic process of gene methylation is affected by OS. Production of ROS in brain cells because of genetic or environmental causes leads to decreased methionine synthase activity.[106] Methionine synthase action involves 2 processes: (1) dopamine-stimulated phospholipid methylation, which is required for synchronizing normal brain activity during attention and (2) DNA methylation, which silences gene activity. The important interaction between methionine metabolism and methylation with overall reactions of folate and B12 metabolism is regulated by mt reduction or oxidative reaction controlling nuclear gene methylation (epigenetics) and glutathione metabolism. When this process is disturbed because methionine synthase is impaired, children may manifest attentional impairment and other symptoms from defective gene expression, leading to ASD.[107]

Mitochondria as Therapeutic Targets in Autism

The previously mentioned pathogenetic theories about the possible relationship between mt dysfunction and ASD not only explain the mechanisms underlying such interaction but also open the door to the possibility of developing new therapies to improved mt function and, therefore, the associated ASD clinical manifestations.[30,36,51,52,64]

Although treatment with mt cofactor supplementation, including antioxidants, coenzyme Q10, carnitine, and vitamin B, may improve mt function and behavior of some children with ASD, systematic studies documenting the efficacy of these treatments for mt dysfunction in children with ASD are lacking.[21,64]

A therapeutic trial of mt cofactor and antioxidants may be reasonable in children with ASD and mt disease given the fact that (1) OS is associated with impaired mt function, (2) antioxidant supplementation in individuals with mt disease increases GSH levels, (3) mt are dependent on cytosolic GSH levels, and (4) these compounds are generally recognized as safe.

However, according to Naviaux,[94] who believes that ROS are the response to disease, OS should not be the target for therapy, but rather the metabolic conditions that create them should be targeted.

In 2011, Ghanizadeh[89] hypothesized that olesoxime and complement 1q-binding protein, which prevent the opening of the mt permeability transition pore by OS, may improve the condition of some patients with autism. The authors concluded that it is worthwhile conducting the translational appropriate animal model studies to assess mt functioning.[108]

Mt abnormalities have been identified in *MECP2* mutant,[109] *TSC*-mutant ASD mouse,[110] and Drosophila models.[111] Further characterization of the mechanisms underlying mt dysfunction in these model systems may lead to an increased understanding of the pathology of the brain with ASD and provide a therapeutic direction.[64]

In 2013, Naviaux et al[112] used the maternal immune activation (MIA) mouse model of gestational poly(IC) exposure and treatment with the nonselective purinergic antagonist suramin to test the role of purinergic signaling in C57BL/6J mice. The results showed that antipurinergic therapy (APT) corrected 16 multisystem abnormalities that defined the ASD-like phenotype in this model. The authors concluded that antipurinergic therapy provides a new tool for refining current concepts of pathogenesis of ASD and represents a fresh path toward new drug development.

Conclusion

There is genetic and biochemical evidence for a mt role in the pathogenesis of ASD in a subset of children.[21,22,30,36,51-53]

The mt dysfunction can unify the seemingly disparate clinical findings and physiopathologic abnormalities associated with ASD. However, it should be noted that, even if mt dysfunction underlies the abnormalities in multiple organs and physiological systems, mt dysfunction could still be secondary to other biological processes, such as elevated ROS or long-term immune dysfunction, both of which have been implicated in ASD.[31]

The mt role in autism requires further studies with the latest genetic technology such as next-generation sequencing, microarrays, bioinformatics, and biochemical assays, which help to determine the prevalence and type of mt defects in ASD.[22,30]

Because of the availability of potential therapeutic options for mt disease, investigation into the molecular abnormalities underlying the pathogenetic link between mt dysfunction and ASD could translate into better treatment and outcome for patients with mt-associated ASD. This requires a high index of suspicion of mt disease in children with autism who are diagnosed early.[22,36,52]

References

1. Ducham E, Patel DR: Epidemiology of autism spectrum disorders. Pediatr Clin North Am 59:27-43, 2012
2. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 5th ed. Washington, DC: American Psychiatric Publishing, 2013
3. Nazeer A, Ghaziuddin M: Autism spectrum disorders: Clinical features and diagnosis. Pediatr Clin North Am 59:19-25, 2012
4. Kogan D, Blumberg S, Schieve L, et al: Prevalence of parent-reported diagnosis of autism spectrum disorder among children in the US, 2007. Pediatrics 124:1395-1403, 2009
5. Stokstad E: Development. New hints into the biological basis of autism. Science 36:1561-1569, 1997
6. Boyle C, Boulet S, Schieve L, et al: Trends in the prevalence of developmental disabilities in US children, 1997-2008. Pediatrics 127:1034-1042, 2011
7. Blumberg SJ, Bramlett MD, Kogan MD, et al: Change in the prevalence of parent-reported autism spectrum disorder in school-aged children: 2007 to 2011-2012. Natl Health Stat Rep 65:1-12, 2013
8. Lakshmi Priya MD, Geetha A: Level of trace elements (copper, zinc, magnesium and selenium) and toxic elements (lead and mercury) in the hair and nail of children with autism. Biol Trace Elem Res 142:148-158, 2011
9. Clements CJ, McIntyre PB: When science is not enough-a risk/benefit profile of thiomersal-containing vaccines. Expert Opin Drug Saf 5:17-29, 2006
10. Lai MC, Lombardo MV, Baron-Cohen S: Autism. Lancet 2013; [Epub ahead of print] S0140-6736:61539-1
11. Toriello HV: Approach to the genetic evaluation of the child with autism. Pediatr Clin North Am 59:113-128, 2012
12. Persico AM, Napolioni V: Autism genetics. Behav Brain Res 251:95-112, 2013
13. Silver WG, Rapin I: Neurobiological basis of autism. Pediatr Clin North Am 59:45-61, 2012
14. Courchesne E, Redcay E, Kennedy DP: The autistic brain: Birth through adulthood. Curr Opin Neurol 17:489-496, 2004
15. Pardo CA, Vargas DL, Zimmerman AW: Immunity, neuroglia and neuroinflammation in autism. Int Rev Psychiatry 17:485-495, 2005
16. Zimmerman AW, Jyonouchi H, Comi AM, et al: Cerebrospinal fluid and serum markers of inflammation in autism. Pediatr Neurol 33:195-201, 2005
17. Chugani DC: Neuroimaging and neurochemistry of autism. Pediatr Clin North Am 59:63-74, 2012
18. Tareen RS, Kamboj MK: Role of endocrine factors in autism spectrum disorders. Pediatr Clin North Am 59:75-88, 2012
19. Ghaziuddin M, Al-Owain M: Autism spectrum disorders and inborn errors of metabolism: An update. Pediatr Neurol 49:232-236, 2013
20. Taanman JW, Williams SL: Structure and function of mitochondrial oxidative phosphorylation system. In: AH Schapira, DiMauro S (eds): Mitochondrial Disorders in Neurology 2. Boston, Butterworth Heinemann, 1-34, 2002

21. Rossignol DA, Frye RE: Mitochondrial dysfunction in autism spectrum disorders: A systematic review and meta-analysis. Mol Psychiatr 17: 290-314, 2012
22. Dhillon S, Hellings JA, Butler MG: Genetic and mitochondrial abnormalities in autism spectrum disorders: A review. Curr Genomics 12:322-332, 2011
23. Applegarth DA, Toone JR, Lowry RB: Incidence of inborn errors of metabolism in British Columbia, 1969-1976. Pediatrics 105:e10, 2000
24. Naviaux RK: Developing a systematic approach to the diagnosis and classification of mitochondrial disease. Mitochondrion 4:351-361, 2004
25. Elliott HR, Samuels DC, Eden JA, et al: Pathogenic mitochondrial DNA mutations are common in the general population. Am J Hum Genet 83:254-260, 2008
26. Naviaux RK: Mitochondria and autism spectrum disorders. In: JD Buxbaum, Hof PR (eds): The Neuroscience of Autism Spectrum Disorders. Amsterdam, Academic Press, 179-193, 2013
27. Coleman M, Blass JP: Autism and lactic acidosis. J Autism Dev Disord 15:1-8, 1985
28. László A, Horváth E, Eck E, et al: Serum serotonin, lactate and pyruvate in infantile autistic children. Clin Chim Acta 229:205-207, 1994
29. Chugani DC, Sundram BS, Behen M, et al: Evidence of altered energy metabolism in autistic children. Prog Neuropsychopharmacol Biol Psychiatry 23:635-641, 1999
30. Haas RH: Autism and mitochondrial disease. Dev Disabil Res Rev 16:144-153, 2010
31. Frye RE, Rossignol DA: Mitochondrial dysfunction can connect the diverse medical symptoms associated with autism spectrum disorders. Pediatr Res 69:41R-47R, 2011
32. Sue CM, Bruno C, Andreu AL, et al: Infantile encephalopathy associated with the MELAS A3243G mutation. J Pediatr 134:696-700, 1999
33. Graf WD, Marin-Garcia J, Gao HG, et al: Autism associated with the mitochondrial DNA G8363A transfer RNA (Lys) mutation. J Child Neurol 15:357-361, 2000
34. Filiano JJ, Goldenthal MJ, Rhodes CH, et al: Mitochondrial dysfunction in patients with hypotonia, epilepsy, autism, and developmental delay: HEADD syndrome. J Child Neurol 17:435-439, 2002
35. Pons R, Andreu AL, Checcarelli N, et al: Mitochondrial DNA abnormalities and autistic spectrum disorders. J Pediatr 144:81-85, 2004
36. Weissman JR, Kelley IR, Bauman ML, et al: Mitochondrial disease in autism spectrum disorder patients analysis. PLoS One 3:e3815, 2008
37. Shoffner J, Hyams L, Niedziela GN, et al: Fever plus mitochondrial disease could be risk factors for autistic regression. J Child Neurol 25: 429-434, 2010
38. Giulivi C, Zhang YF, Omanska-Klusek A, et al: Mitochondrial dysfunction in autism. J Am Med Assoc 304:2389-2396, 2010
39. Napoli E, Wong S, Giulivi C: Evidence of reactive oxygen species-mediated damage to mitochondrial DNA in children with typical autism. Mol Autism 4:2, 2013
40. Smith M, Spence MA, Flodman P: Nuclear and mitochondrial genome defects in autisms. Ann N Y Acad Sci 1151:102-132, 2009
41. Filipek PA, Juranek J, Smith M, et al: Mitochondrial dysfunction in autistic patients with 15q inverted duplication. Ann Neurol 53:801-804, 2003
42. Ramoz N, Reichert JG, Smith CJ, et al: Linkage and association of the mitochondrial aspartate glutamate carrier SLC25A12 gene with autism. Am J Psychiatry 161:662-669, 2004
43. Lepagnol-Bestel AM, Maussion G, Boda B, et al: SLC25A12 expression is associated with neurite outgrowth and is upregulated in the prefrontal cortex of autistic subjects. Mol Psychiatry 13:385-397, 2008
44. Segurado R, Conroy J, Meally E, et al: Confirmation of association between autism and the mitochondrial aspartate/glutamate carrier SLC25A12 gene on chromosome 2q31. Am J Psychiatry 162: 2182-2184, 2005
45. Silverman JM, Buxbaum JD, Ramoz N, et al: Autism-related routines and rituals associated with a mitochondrial aspartate/glutamate carrier SLC25A12. Am J Med Genet B Neuropsychiatr Genet 147:408-410, 2008
46. Correia C, Coutinho AM, Diogo L, et al: Brief report: High frequency of biochemical markers for mitochondrial dysfunction in autism: No association with the mitochondrial aspartate/glutamate carrier SLC25A12 gene. J Autism Dev Disord 36:1137-1140, 2006
47. Chien WH, Wu YY, Gau SS, et al: Association study of SLC25A12 gene and autism in Ham Chinese in Taiwan. Prog Neuropsychopharmacol Biol Psychiatry 34:189-192, 2010
48. Marui T, Funatogawa I, Koishi S, et al: The NADH-ubiquinone oxidoreductase 1 alpha subcomplex 5 (NDUFA5) gene variants are associated with autism. Acta Psychiatr Scand 123:118-124, 2011
49. Ezugha H, Goldenthal M, Valencia I, et al: 5q14.3 deletion manifesting as mitochondrial disease and autism: Case report. J Child Neurol 25: 1232-1235, 2010
50. Guevara-Campos J, González-Guevara L, Briones L, et al: Autism associated to a deficiency of complexes III and IV of the mitochondrial respiratory chain. Invest Clin 51:423-431, 2010
51. Anitha A, Nakamura K, Thanseem I, et al: Brain region-specific altered expression and association of mitochondria-related genes in autism. Mol Autism 3:1-12, 2012
52. Anitha A, Nakamura K, Thanseem I, et al: Downregulation of the expression of mitochondrial electron transport complex genes in autism brains. Brain Pathol 23:294-302, 2013
53. Gu F, Chauhan V, Kaur K, et al: Alterations in mitochondrial DNA copy number and the activities of electron transport chain complexes and pyruvate dehydrogenase in the frontal cortex from subjects with autism. Transl Psychiatry 3:e299, 2013
54. Oliveira G, Diogo L, Grazina M, et al: Mitochondrial dysfunction in autism spectrum disorders: A population-based study. Dev Med Child Neurol 47:185-189, 2005
55. Poling JS, Frye RE, Shoffner J, et al: Developmental regression and mitochondrial dysfunction in a child with autism. J Child Neurol 21:170-172, 2006
56. Clark-Taylor T, Clark-Taylor BE: Is autism a disorder of fatty acid metabolism? Possible dysfunction of mitochondrial β-oxidation by long chain acyl-CoA dehydrogenase. Med Hypotheses 62:970-975, 2004
57. Pastural E, Ritchie S, Lu Y, et al: Novel plasma phospholipid biomarkers of autism: Mitochondrial dysfunction as a putative causative mechanism. Prostaglandins Lekot Essent Fatty Acids 81:253-264, 2009
58. Tsao CY, Mendell JR: Autistic disorder in 2 children with mitochondrial disorders. J Child Neurol 22:1121-1123, 2007
59. Goldenthal MJ, Shah N, Sheth S, et al: Buccal swab analysis reveals a high prevalence of mitochondria respiratory chain defects in children and adolescents with autism spectrum disorders. 40th Annual Meeting of the Child Neurology Society. Ann Neurol 70:(Suppl 15)127, 2011; [abstract]
60. Goldenthal MJ, Kuruvilla T, Damle S, et al: Non-invasive evaluation of buccal respiratory chain enzyme dysfunction in mitochondrial disease: Comparison with studies in muscle biopsy. Mol Genet Metab 105: 457-462, 2012
61. Frye EE: Mitochondrial disease in 22q13 duplication syndrome. J Child Neurol 27:942-949, 2012
62. Craig AK, Sotero de Menezes M, Sanetro RP: Dravet syndrome: Patients with co-morbid *SCN1A* gene mutations and mitochondrial electron transport chain defects. Seizure 21:17-20, 2012
63. Chauhan A, Gu F, Wegiel EJ, et al: Brain region-specific deficit in mitochondrial electron transport chain complexes in children with autism. J Neurochem 117:209-220, 2011
64. Tang G, Gutierrez Rios P, Kuo SH, et al: Mitochondrial abnormalities in temporal lobe of autistic brain. Neurobiol Dis 54:349-361, 2013
65. Zhang Q, Raoof M, Chen Y, et al: Circulating mitochondrial DAMPS cause inflammatory responses to injury. Nature 464:104-107, 2010
66. Cavalier-Smith T: Origin of mitochondria by intracellular enslavement of a photosynthetic purple bacterium. Proc Biol Sci 273:1943-1952, 2006
67. West AP, Shadel GS, Ghosh S: Mitochondria in innate immune responses. Nat Rev Immunol 11:389-402, 2011
68. Ashwood P, Anthony A, Torrente F, et al: Spontaneous mucosal lymphocyte cytokine profiles in children with autism and gastrointestinal symptoms: Mucosal immune activation and reduced counter regulatory interleukin-10. J Clin Immunol 24:664-673, 2004
69. Ashwood P, Wills S, Van de Water J: The immune response in autism: A new frontier for autism research. J Leukoc Biol 80:1-15, 2006

70. Smith SE, Li J, Garbett K, et al: Maternal immune activation alters fetal brain development through interleukin-6. J Neurosci 27:10695-10702, 2007
71. Careaga M, Ashwood P: Autism spectrum disorders: From immunity to behavior. Methods Mol Biol 934:219-240, 2012
72. Psarra AM, Solakidi S, Sekeris CE: The mitochondrion as a primary site of action of regulatory agents involved in neuroimmunomodulation. Ann N Y Acad Sci 1088:12-22, 2006
73. Psarra AM, Sekeris CE: Glucocorticoid receptors and other transcription factors in mitochondria and possible functions. Biochim Biophys Acta 1787:431-436, 2009
74. Zecchini E, Siviero R, Giorgi C, et al: Mitochondrial calcium signalling: Message of life and death. Ital J Biochem 56:235-242, 2007
75. Gellerich FN, Gizatullina Z, Trumbeckaite S, et al: The regulation of OXPHOS by extramitochondrial calcium. Biochim Biophys Acta 1797:1018-1027, 2010
76. Napolioni V, Persico AM, Porcelli V, et al: The mitochondrial aspartate/glutamate carrier AGC1 and calcium homeostasis: Physiological links and abnormalities in autism. Mol Neurobiol 44:83-92, 2011
77. Demaurex N, Poburko D, Frieden M: Regulation of plasma membrane calcium fluxes by mitochondria. Biochim Biophys Acta 1787: 1383-1394, 2009
78. Palmieri L, Persico AM: Mitochondrial dysfunction in autism spectrum disorders: Cause or effect? Biochim Biophys Acta 1797:1130-1137, 2010
79. Rizzutto R, Bernardi P, Pozzan T: Mitochondria as all-round players of calcium game. J Physiol 520:Pt 137-47, 2000
80. Tripathy P, Kashyap L, Singh V: The role of nitric oxide in inflammatory reactions. FEMS Immunol Med Microbiol 51:443-452, 2007
81. Larbi A, Kempf J, Pawelec G: Oxidative stress modulation and T cell activation. Exp Gerontol 42:852-858, 2007
82. Rubenstein JL, Merzenich MM: Model of autism: Increased ratio of excitation/inhibition in key neural systems. Genes Brain Behav 2:255-267, 2003
83. Anderson MP, Hooker BS, Herbert MR: Bridging from cells to cognition in autism pathophysiology: Biological pathways to defective brain function and plasticity. Am J Biochem Biotechnol 4:167-176, 2008
84. Krey JF, Dolmetsch RE: Molecular mechanisms of autism: A possible role for Ca^{2+} signaling. Curr Opin Neurobiol 17:112-119, 2007
85. Palmieri L, Vapaleo V, Porcelli V, et al: Altered calcium homeostasis in autism-spectrum disorders: Evidence from biochemical and genetic studies of the mitochondrial aspartate/glutamate carrier AGC1. Mol Psychiatry 15:38-52, 2010
86. Frye RE, Delatorre R, Taylor H, et al: Redox metabolism abnormalities in autistic children associated with mitochondrial disease. Transl Psychiatry 3:e273, 2013
87. Koopman WJ, Nijtmans LG, Dieteren CE, et al: Mammalian mitochondrial complex I: Biogenesis, regulation, and reactive oxygen species generation. Antioxid Redox Signal 12:1431-1470, 2010
88. Chinta SJ, Andersen JK: Nitrosylation and nitration of mitochondrial complex I in Parkinson's disease. Free Radic Res 45:53-58, 2011
89. Ghanizadeh A: Targeting mitochondria by olesoxime or complement 1q binding protein as a novel management for autism: A hypothesis. Mol Syndromol 2:50-52, 2011
90. James SJ, Rose S, Melnyk S, et al: Cellular and mitochondrial glutathione redox imbalance in lymphoblastoid cells from children with autism. FASEB J 23:2374-23383, 2009
91. Damodaran LP, Arumugam G: Urinary oxidative stress markers in children with autism. Redox Rep 16:216-222, 2011
92. Melnyk S, Fuchs GJ, Schulz E, et al: Metabolic imbalance associated with methylation dysregulation and oxidative damage in children with autism. J Autism Dev Disord 42:362-377, 2012
93. Rose S, Melnyk S, Pavliv O, et al: Evidence of oxidative damage and inflammation associated with low glutathione redox status in the autism brain. Transl Psychiatry 2:e134, 2012
94. Naviaux RK: Oxidative shielding or oxidative stress? J Pharmacol Exp Ther 342:608-618, 2012
95. Ristow M, Zarse K: How increased oxidative stress promotes longevity and metabolic health: The concept of mitochondrial hormesis (mitohormesis). Exp Gerontol 45:410-418, 2010
96. Abbracchio MP, Burnstock G, Verkhratsky A, et al: Purinergic signaling in the nervous system: An overview. Trends Neurosci 32:19-29, 2009
97. Franke H, Sauer C, Rudolph C, et al: P2 receptor-mediated stimulation of the PI3-K/Akt-pathway in vivo. Glia 57:1031-1145, 2009
98. Pelegrin P: Targeting interleukin-1 signaling in chronic inflammation: Focus on P2X(7) receptor and pannexin-1. Drug News Perspect 21:424-433, 2008
99. Gallego D, Vanden Berghe P, Farre R, et al: P2Y1 receptors mediate inhibitory neuromuscular transmission and enteric neuronal activation in small intestine. Neurogastroenterol Motil 20:159-168, 2008
100. Matos JE, Sorensen MV, Geyti CS, et al: Distal colonic Na(+) absortion inhibited by luminal P2Y(2) receptors. Pflûgers Arch 454:977-987, 2007
101. Surprenant A, North RA: Signaling at purinergic P2X receptors. Annu Rev Physiol 71:333-359, 2009
102. Leng Y, Yamamoto T, Kadowaki M: Alteration of cholinergic, purinergic and sensory neurotransmission in the mouse colon of food allergy model. Neurosci Lett 445:195-198, 2008
103. Housley GD, Jagger DJ, Greenwood D, et al: Purinergic regulation of sound transduction and auditory neurotransmission. Audiol Neurootol 7:55-61, 2002
104. Hall L, Kelley E: The contribution of epigenetics to understanding genetic factors in autism. Autism 2013; [Epub ahead of print]
105. Wallace DC, Fan W: Energetics, epigenetics, mitochondrial genetics. Mitochondrion 10:12-31, 2010
106. James SJ, Cutler P, Melnyk S, et al: Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. Am J Clin Nutr 80:1611-1617, 2004
107. Naviaux RK: Mitochondrial control of epigenetics. Cancer Biol Ther 7:1191-1193, 2008
108. Patterson PH: Modeling autistic features in animals. Pediatr Res 69: 5 Pt234R-40R, 2011
109. Grosser E, Hirt U, Janc OA, et al: Oxidative burden and mitochondrial dysfunction in a mouse model of Rett syndrome. Neurobiol Dis 48:102-114, 2012
110. Goto J, Talos DM, Klein P, et al: Regulable neural progenitor-specific Tsc1 loss yields giant cells with organellar dysfunction in a model of tuberous sclerosis complex. Proc Natl Acad Sci U S A 108:E1070-E1079, 2011
111. Yao A, Jin S, Li X, et al: Drosophila FMRP regulates microtubule network formation and axonal transport of mitochondria. Hum Mol Genet 20:51-63, 2011
112. Naviaux RK, Zolkipli Z, Wang L, et al: Antipurinergic therapy corrects the autism-like features in the poly(IC) mouse model. PLoS One 8: e57380, 2013