Neurobiology of Disease 54 (2013) 349–361

Contents lists available at SciVerse ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Mitochondrial abnormalities in temporal lobe of autistic brain

Guomei Tang [a], Puri Gutierrez Rios [a], Sheng-Han Kuo [a], Hasan Orhan Akman [a], Gorazd Rosoklija [b], Kurenai Tanji [c], Andrew Dwork [b,c], Eric A. Schon [a,d], Salvatore DiMauro [a], James Goldman [c], David Sulzer [a,b,e,*]

[a] Department of Neurology, Columbia University, New York, USA
[b] Department of Psychiatry, Columbia University, New York, USA
[c] Department of Pathology & Cell Biology, Columbia University, New York, USA
[d] Department of Genetics & Development, Columbia University, New York, USA
[e] Department of Pharmacology, Columbia University, New York, USA

ARTICLE INFO

Article history:
Received 4 September 2012
Revised 25 December 2012
Accepted 10 January 2013
Available online 17 January 2013

Keywords:
Autism
Mitochondria
Temporal cortex
Development

ABSTRACT

Autism spectrum disorder (ASD) consists of a group of complex developmental disabilities characterized by impaired social interactions, deficits in communication and repetitive behavior. Multiple lines of evidence implicate mitochondrial dysfunction in ASD. In postmortem BA21 temporal cortex, a region that exhibits synaptic pathology in ASD, we found that compared to controls, ASD patients exhibited altered protein levels of mitochondria respiratory chain protein complexes, decreased Complex I and IV activities, decreased mitochondrial antioxidant enzyme SOD2, and greater oxidative DNA damage. Mitochondrial membrane mass was higher in ASD brain, as indicated by higher protein levels of mitochondrial membrane proteins Tom20, Tim23 and porin. No differences were observed in either mitochondrial DNA or levels of the mitochondrial gene transcription factor TFAM or cofactor PGC1α, indicating that a mechanism other than alterations in mitochondrial genome or mitochondrial biogenesis underlies these mitochondrial abnormalities. We further identified higher levels of the mitochondrial fission proteins (Fis1 and Drp1) and decreased levels of the fusion proteins (Mfn1, Mfn2 and Opa1) in ASD patients, indicating altered mitochondrial dynamics in ASD brain. Many of these changes were evident in cortical pyramidal neurons, and were observed in ASD children but were less pronounced or absent in adult patients. Together, these findings provide evidence that mitochondrial function and intracellular redox status are compromised in pyramidal neurons in ASD brain and that mitochondrial dysfunction occurs during early childhood when ASD symptoms appear.

Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental condition that encompasses a range of cognitive and behavioral phenotypes including autism, Asperger's syndrome, and pervasive developmental disorder characterized by impaired social interaction, communication, and language, and repetitive and stereotyped behaviors (American Psychiatric Association, 1994). Pathological findings suggest that altered neurodevelopment during early postnatal life is crucial to ASD pathogenesis, and may result in excessive neurons and/or overgrowth of axons (Courchesne et al., 2007) and dendritic spines (Hutsler and Zhang, 2010). The etiology and biological basis of ASD pathology are unknown in most cases and both genetic predisposition and environmental factors are suggested to participate (Rossignol and Frye, 2012b).

Impaired mitochondrial function has been implicated in normal aging and neuropsychiatric disorders, including Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD), mood disorder and schizophrenia (Lin and Beal, 2006; Manji et al., 2012; Schon and Manfredi, 2003; Trushina and McMurray, 2007). Mitochondria produce energy via oxidative phosphorylation (OXPHOS) through the respiratory chain, which generates more than 95% of neuronal ATP under normal physiological conditions (Benard et al., 2007). The respiratory chain involves electron transport chain (ETC.) proteins NADH-ubiquinone oxidoreductase (Complex I), succinate ubiquinone oxidoreductase (Complex II), ubiquinone cytochrome *c* oxidoreductase (Complex III), cytochrome *c* oxidase (Complex IV), and the ATP synthase (Complex V). Inefficient electron transfer through ETC. complexes causes brain pathology due to loss of energy, while defects of these enzymes, particularly Complexes I, II and III, cause the respiratory chain to leak electrons that react with oxygen to form toxic reactive radical species.

Recent evidence suggests that mitochondrial dysfunction could participate in the development and clinical features of ASD (Rossignol and Frye, 2012a). Studies have identified features associated with the biochemical endophenotype of mitochondrial energy deficiency, including abnormal plasma biomarkers that relate to mitochondrial dysfunction, such

* Corresponding author at: Departments of Neurology, Psychiatry, and Pharmacology, Black Building, Room 309, 650 W 168th St, New York, NY 10032, USA. Fax: +1 212 342 3664.
E-mail address: ds43@columbia.edu (D. Sulzer).
Available online on ScienceDirect (www.sciencedirect.com).

0969-9961/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nbd.2013.01.006

as plasma lactic acid, pyruvate, carnitine and amino acids (Weissman et al., 2008), and depressed ETC. complex function (Giulivi et al., 2010) with reduced mitochondrial membrane potential (James et al., 2009) in ASD lymphoblastoid cell lines. Complex I deficiency is the most common mitochondrial defect identified in ASD and has been found in association with Complex III and Complex IV deficiencies (Haas, 2010). Evidence for low pyruvate dehydrogenase complex (PDHC) activities, a higher rate of mitochondrial hydrogen peroxide production and mitochondrial DNA (mtDNA) overreplication and/or deletions, has been identified in a subset of ASD children (Giulivi et al., 2010).

In postmortem human brain, Chauhan et al. (2011) reported decreased expression of mitochondrial respiratory chain complexes in cerebellum, temporal lobe, and frontal lobe of ASD children. ASD patients showed 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 cortex. Anitha et al. (2012) identified downregulation of the expression of mitochondrial ETC. genes in anterior cingulate gyrus, motor cortex and thalamus of autism patients, compared to matched controls. More recently, oxidative damage to DNA (Rose et al., 2012) and proteins (Sajdel-Sulkowska et al., 2011) and inflammation have been found to be associated with low glutathione redox status (Rose et al., 2012) in cerebellum and temporal cortex of autism brain.

Here, we confirm findings of altered respiratory chain proteins in ASD brain, and identify novel features that further characterize abnormal mitochondrial function in ASD. To do so, we measured mitochondrial proteins in ASD brain in a larger cohort of postmortem brain tissue samples, analyzing BA21 in the lateral temporal lobe, a site involved in auditory processing, language and social perception implicated in ASD-associated behaviors (Bigler et al., 2007; Jou et al., 2010). In addition to confirming a decrease in protein expression and Complex I and IV enzyme activities in the temporal cortex from ASD cases, we identified decreased protein levels of the mitochondrial antioxidant enzyme SOD2 and increased oxidative mtDNA damage in ASD patients aged 2–9 years. We also identified increased mitochondrial mass in ASD brain, as indicated by increased protein levels of mitochondrial membrane proteins Tom20, Tim23 and porin. Altered mitochondrial dynamics were evidenced by increased mitochondrial fission proteins (Fis1 and Drp1) and decreased fusion proteins (Mfn1, Mfn2 and Opa1) in ASD patients. We did not identify any significant changes in mtDNA sequence, mtDNA copy number or levels of the mitochondrial gene transcription factor TFAM and cofactor PGC1α, suggesting that a mechanism other than an altered mitochondrial genome or gene expression underlies the mitochondrial abnormalities observed in ASD. Our findings provide further evidence for compromised mitochondrial function and intracellular redox status in ASD brain.

Methods and materials

Brain tissue

Frozen and fixed temporal lobe sections of ASD (n = 20, age range 3–60 years) and control subjects (n = 25, age range 2–65 years) were obtained from the Autism Tissue Portal, the Harvard Brain Bank and the NICHD Brain and Tissue Bank for developmental disorders at the University of Maryland. All frozen brain samples were kept at −80 °C and fixed brain sections in 10% formalin. Brain samples were divided into three age groups: 2–9 y, 13–20 y and 46–67 y. The case information and sample size within each age group in patients and controls were summarized in Table 1. Donors with autism fit the diagnostic criteria of DSM-IV and were confirmed by the Autism Diagnostic Interview—Revised. All controls are age-, PMI- and gender-matched subjects without known neurologic disease. The postmortem intervals did not exceed 32 h (ASD patients, range 3–32 h; controls, range 5–30 h). This study was approved by the Columbia University Medical School Institutional review board.

Western blot analysis

Frozen brain tissue samples were homogenized in 1× RIPA buffer supplemented with protease inhibitors (Roche) and phosphatase inhibitors (Sigma). The suspensions were centrifuged at 13,000 *g* at 4 °C for 30 min. Supernatants were collected and assayed for total protein using the Bradford method (BioRad). Samples were aliquoted into labeled vials and stored at −80 °C until use. Fifty microgram of total protein of each sample was mixed with NuPAGE sample buffer and separated in 4–12% NuPAGE Bis–Tris gel (Invitrogen) and transferred to Whatman PROTRAN Nitrocellulose Transfer Membranes (0.25 μm). For immunoblotting, membranes were washed with 1× Tris-buffered saline with 0.1% Tween 20 (TBS-T), blocked with 5% dry milk in 1× TBS-T at room temperature (RT) for 1 h. The membranes were incubated overnight with the primary antibodies for respiratory chain proteins Complex I NDUFS3 subunit (Mitoscience, MS110), Complex II 30KD subunit (Mitoscience, MS203), Complex III UQCR2 subunit (Mitoscience, MS304), Complex IV subunit COX1 (Mitoscience, MS404), COX2 (Mitoscience, MS405) and COX4 (Mitoscience, MS408), Complex V subunit α ATP5A (Mitoscience, MS507), fusion proteins Mfn1 (Santa Crutz, sc50330), Mfn2 (Abcam ab56889) and Opa1(BD Bioscience, BP612606), fission proteins Fis1 (Proteintech, 10586-1-AP) and Drp1(Santa Crutz, sc32898), mitochondria outer membrane proteins porin (Abcam, ab15895) and Tom20 (Proteintech, 11802-1-AP), inner membrane protein Tim23 (Proteintech, 11123-AP), matrix protein cytochrome *c* (Abcam, ab13575), mitochondrial gene transcription factors PGC1alpha (Abcam, ab54481) and TFAM (Abcam, ab119684), actin (Sigma, A5441), and catalase (Mitoscience, MS721). Following the primary antibody exposure, the membranes were washed with 1× TBS-T buffer three times at 10 min intervals, then incubated for 1 h at RT with appropriate secondary antibodies, followed by three additional washes at 10 min intervals. Protein bands were visualized using ECL or Supersignal Chemiluminescent reagents (Pierce). The densities of immunoreactive bands were quantified using ImageJ and presented as mean ± SEM. A reference standard sample was used on each gel to normalize every other band on the same blot, and then compare across multiple blots because every band in the dataset is normalized to the same standard.

MitoChip assay

Samples were prepared for MitoChip analysis as previously described (van Eijsden et al., 2006). Briefly, long-range PCR was performed using three PCR primer sets that can amplify the entire mtDNA, using 100 ng of input DNA for each reaction. The cycling conditions for all reactions were: 1) 95 °C for 2 min; 2) 95 °C for 15 s; 3) 68 °C for 7 min; 4) repeat step 2 for 29 times; 5) final extension for 12 min. As a control for PCR amplification and subsequent hybridization, a 7.5-kb plasmid DNA (Tag IQ-EX template) was amplified concomitantly with the test samples, using forward and reverse primers included in the CustomSeq™ kit (Affymetrix). The procedures for sample pooling, DNA fragmentation, and labeling were identical for both first- and second-generation MitoChip assays. Pre-hybridization, hybridization, washing, and scanning of the MitoChip were performed as described in the Affymetrix CustomSeq™ resequencing protocol. The analysis of microarray data was performed using resequencing analysis (RA) Software (Genechip Sequencing Analysis Software Version 4.1.0, Affymetrix Inc., 2005).

mtDNA quantification

mtDNA content was assessed with the Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems) in 100 ng total DNA extracted from autopsy samples. We measured mtDNA using the 12S ribosomal TaqMan mitochondrial assay labeled with 6FAM fluorochrome and the primers 5′-CCA CGG GAA ACA GCA GTG ATT-3′ and 5′-CTA TTG ACT TGG GTT AAT CGT GTG A-3′. The nuclear single copy gene RNAseP was measured with the kit PDARs RNAseP (Applied