Official Journal of the European Paediatric Neurology Society

Original article

The use of muscle biopsy in the diagnosis of undefined ataxia with cerebellar atrophy in children

Alessandra Terracciano[a,1], *Florence Renaldo*[b,1], *Ginevra Zanni*[a], *Adele D'Amico*[a], *Anna Pastore*[a], *Sabina Barresi*[a], *Enza Maria Valente*[c], *Fiorella Piemonte*[a], *Giulia Tozzi*[a], *Rosalba Carrozzo*[a], *Massimiliano Valeriani*[a], *Renata Boldrini*[d], *Eugenio Mercuri*[f], *Filippo Maria Santorelli*[a,e], *Enrico Bertini*[a,*]

[a] *Unit of Neuromuscular and Neurodegenerative Disorders, Lab of Molecular Medicine and Unit of Neurology, Dept of Neuroscience, Bambino Gesù Childrens Hospital, Rome, Italy*
[b] *Service de Neuropédiatrie, Hôpital Armand Trousseau, Paris, France*
[c] *Research Institute Mendel, Laboratory of Neuroscience, Rome, Italy*
[d] *Unit of Pathology, Bambino Gesù Childrens Hospital, Rome, Italy*
[e] *IRCCS-Stella Maris, Calambrone, Pisa, Italy*
[f] *Institute of Neuropsychiatry, Catholic University, Rome, Italy*

ARTICLE INFO

Article history:
Received 4 April 2011
Received in revised form
21 July 2011
Accepted 24 July 2011

Keywords:
Inherited cerebellar ataxias
Marinesco-Sjogren syndrome
Coenzyme Q10 deficiency

ABSTRACT

Childhood cerebellar ataxias, and particularly congenital ataxias, are heterogeneous disorders and several remain undefined. We performed a muscle biopsy in patients with congenital ataxia and children with later onset undefined ataxia having neuroimaging evidence of cerebellar atrophy. Significant reduced levels of Coenzyme Q10 (COQ10) were found in the skeletal muscle of 9 out of 34 patients that were consecutively screened. A mutation in the *ADCK3/Coq8* gene (R347X) was identified in a female patient with ataxia, seizures and markedly reduced COQ10 levels. In a 2.5-years-old male patient with non syndromic congenital ataxia and autophagic vacuoles in the muscle biopsy we identified a homozygous nonsense mutation R111X mutation in *SIL1* gene, leading to early diagnosis of Marinesco-Sjogren syndrome. We think that muscle biopsy is a valuable procedure to improve diagnostic assesement in children with congenital ataxia or other undefined forms of later onset childhood ataxia associated to cerebellar atrophy at MRI.

1. Introduction

Inherited cerebellar ataxias (ICA) in children are an extremely heterogeneous group of disorders. According to inheritance, inherited cerebellar ataxias can be classified into autosomal recessive, autosomal dominant, X-linked and maternally inherited forms.[1,2]

Autosomal recessive (AR) ataxias are the most frequent group of inherited ataxias with onset in childhood, particularly Friedreich ataxia and Ataxia-telangectasia. Different

* *Corresponding author*. Unit of Neuromuscular and Neurodegenerative Disorders, Laboratory of Molecular Medicine, Department of Neurosciences, Bambino Gesù Childrens Research Hospital, P.za S. Onofrio, 4, 00165 Rome, Italy. Tel.: +39 0668502105; fax: +39 0668592024.
[1] These authors equally contributed to this work.
1090-3798
doi:10.1016/j.ejpn.2011.07.016

criteria have been used to classify these forms.[2–4] Palau and Espinos (2006), in a pathogenic and clinically-oriented classification, established five subgroups of autosomal recessive ataxia including childhood and adult onset forms and in this classification they incorporated the metabolic ataxias, a growing group of genetically defined disorders.[5] Clinical criteria based on age at onset can distinguish inherited cerebellar ataxias (ICA) with onset in childhood as following: a) congenital ataxias (CA), characterized by neonatal hypotonia, developmental delay and early-onset ataxia, and b) later onset childhood ataxias (CHA). Moreover all these conditions can be progressive or non progressive forms, and syndromic or non syndromic ataxias. Syndromic ataxias have associated symptoms such as dysmorphia, oculomotor apraxia, peripheral neuropathy, deafness, optic atrophy, congenital cataracts, pigmented retinopathy, Lebers' amaurosis, microcephaly, recurrent infections, immunodeficiency, that besides are helpful key signs to define the various conditions and address diagnosis.

Because ICA are neurological disorders resulting from degeneration or abnormal development of the cerebellum, brain MRI is of pivotal importance for the subclassification of cerebellar abnormalities (dysgenesis, hypoplasia and/or atrophy) adding the potential association with supratentorial abnormalities.[6] In cases of cerebellar atrophy, only few main pathogenetic categories have been defined[7,8]: metabolic, DNA repair defects and neurodegenerative, often responsible for congenital or later onset childhood ataxias.

In a series of patients affected by non syndromic congenital or later–onset childhood ataxia with neuroradiological evidence of cerebellar atrophy in whom known forms of childhood onset ataxia were ruled out by extensive metabolic, neurophysiological and laboratory examinations, we performed systematically a muscle biopsy in a selected cohort of 34 consecutive patients in order to analyze muscle morphology, and the mitochondrial respiratory chain enzyme activities together with the determination of CoQ10 levels in muscle. Our studies led to a definitive diagnosis in two patients: Marinesco-Sjogren syndrome in one sporadic patient with apparently non syndromic congenital ataxia, and another sporadic patient with a childhood onset non syndromic ataxia with a primary defect of CoQ10 biogenesis.

2. Materials and methods

2.1. Patients

We have evaluated 68 consecutive children with ataxic syndromes that were referred to our centre from 1998 to 2008 and we selected a cohort of 34 unrelated patients with undetermined cause who showed MRI evidence of cerebellar atrophy. A group of patients (14 patients, 41%) were classified as affected by a congenital non syndromic ataxia (CA) whereas most of them (20 patients, 59%) had undetermined ataxia with later onset in childhood (CHA). In all patients, family history was unremarkable for ataxia or other relevant genetic disorders. The brain MRI showed isolated cerebellar atrophy and extensive laboratory investigations including metabolic screening tests (isoelectric focusing of serum transferrin, serum and urine aminoacid chromatography, urinary organic acid chromatography MS, serum lactate, alpha-fetoprotein), and echocardiography were negative. Neurophysiologic examinations excluded a peripheral sensory motor neuropathy and other cranial nerve involvement. Patients were followed up for at least 5 years. All patients were submitted to a skeletal muscle biopsy for measuring mitochondrial respiratory chain enzymes and coenzyme Q10 levels. The procedure of the muscle biopsy was approved by or local Ethics committee. In addition the neurological examination ruled out other relevant associated symptoms and confirmed that most (85%) patients with a congenital onset had early-onset strabismus. Thirthy three patients of our series have been clinically followed up in a range of 4–12 years and have not shown any substantial progression of the disease. Only one patient followed for 3 years and diagnosed with a Marinesco-Sjogren syndrome has a slowly progressive ataxia.

2.2. Muscle biopsy

After obtaining an informed written consent, open muscle biopsies were performed in all patients.

Frozen muscle sections were stained using standard histochemical and histoenzymatic methods, and when appropriate were selected for ultrastructural examination.

Mitochondrial respiratory chain enzymes were analyzed spectrophotometrically in all muscle biopsies. Spectrophotometric measurements of mitochondrial respiratory chain enzyme activities were carried out as reported with modifications.[9] Briefly, approximately 50 mg of muscle biopsy were homogenized in Tris HCl/KCl (pH 7.4), centrifuged at 800× *g* for 10 min and the enzyme activities assayed on the supernatants in a UV double-beam spectrophotometer. The rotenone–sensitive Complex I activity was measured by following the rate of NADH oxidation at 340 nm for 1 min. Complex III specific activity was measured by monitoring the reduction of cytochrome c at 550 nm and the reaction started by adding reduced DB. Complex IV was assayed by following the oxidation of reduced cytochrome c at 550 nm. SDH was assessed by following the reduction of 2,6-dichlorophenolindophenol at 600 nm for 1 min in the presence of succinate. Complex II activity was measured in the same reaction mixture by adding 50 μM DB and monitoring the enzyme kinetic for 3 min. The coupled Complex II + III assay was also determined by starting the reaction with succinate and measuring the reduction of cytochrome c at 550 nm. Complex II + III was performed only in patients who received a muscle biopsy after 2001.In all muscle extracts the levels of CoQ10 were measured using the method developed in our laboratory.[10] Summarizing approximately 2 mg of −80 °C frozen fragments of muscle biopsy specimens were homogenized with 250 μL methanol and 500 μL hexane (containing 50 μL of 100 nmol/L CoQ_9 as internal standard) in a Potter-Elvehjem type homogenizer and a 5 μL aliquot of hexane extract was immediately injected into a 150 × 4.6 mm Hypersil-ODS column. Reduced and oxidized CoQ_{10} was isocratic eluted at a flow rate of 1 mL/min and the retention times for each analyte was calculated using external standards at five different concentrations.

CoQ10 levels were measured with a HPLC-system by an Agilent 1100 Series Liquid Chromatograph with a coulometric electrochemical detector (Coulochem® II) equipped with a Model 5020 Guard Cell (−600 mV) and a Model 5011