Mitochondrion xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Mitochondrion

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

Optic nerve histopathology in a case of Wolfram Syndrome: A mitochondrial pattern of axonal loss

Fred N. Ross-Cisneros [a], Billy X. Pan [a], Ruwan A. Silva [b], Neil R. Miller [c], Thomas A. Albini [b], Lisbeth Tranebjaerg [d,e], Nanna D. Rendtorff [e], Marianne Lodahl [e], Milton N. Moraes-Filho [f], Milton N. Moraes [f], Solange R. Salomao [g], Adriana Berezovsky [g], Rubens Belfort Jr. [g], Valerio Carelli [h,i], Alfredo A. Sadun [a,*]

[a] Doheny Eye Institute and Department of Ophthalmology, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA
[b] Bascom Palmer Eye Institute and Department of Ophthalmology, Miller School of Medicine, University of Miami, Miami, FL, USA
[c] Wilmer Eye Institute and Department of Ophthalmology, Johns Hopkins School of Medicine, Baltimore, MD, USA
[d] Departmet of Audiology, Bispebjerg Hospital, Copenhagen, Denmark
[e] Wilhelm Johannsen Center for Functional Genome Research, Department of Cellular and Molecular Medicine, The Panum Institute, University of Copenhagen, Copenhagen, Denmark
[f] Instituto de Olhos de Colatina, Colatina, Brazil
[g] Department of Ophthalmology, Federal University of Sao Paulo, Sao Paulo, Brazil
[h] Department of Biomedical Neuromotor Sciences (DIBINEM), University of Bologna, Bologna, Italy
[i] IRCCS Istituto delle Scienze Neurologiche, Bologna, Italy

ARTICLE INFO

Article history:
Received 11 March 2013
Received in revised form 29 May 2013
Accepted 30 May 2013
Available online xxxx

Keywords:
Wolfram Syndrome
Leber hereditary optic neuropathy
Optic nerve
Retinal ganglion cell
Axonal degeneration
Myelin basic protein

ABSTRACT

Mitochondrial dysfunction in Wolfram Syndrome (WS) is controversial and optic neuropathy, a cardinal clinical manifestation, is poorly characterized. We here describe the histopathological features in postmortem retinas and optic nerves (ONs) from one patient with WS, testing the hypothesis that mitochondrial dysfunction underlies the pathology. Eyes and retrobulbar ONs were obtained at autopsy from a WS patient, and compared with those of a Leber hereditary optic neuropathy (LHON) patient and one healthy control. Retinas were stained with hematoxylin & eosin for general morphology and ONs were immunostained for myelin basic protein (MBP). Immunostained ONs were examined in four "quadrants": superior, inferior, nasal, and temporal. The WS retinas displayed a severe loss of retinal ganglion cells in the macular region similar to the LHON retina, but not in the control. The WS ONs, immunostained for MBP, revealed a zone of degeneration in the inferior and temporal quadrants. This pattern was similar to that seen in the LHON ONs but not in the control. Thus, the WS patient displayed a distinct pattern of optic atrophy observed bilaterally in the temporal and inferior quadrants of the ONs. This arrangement of axonal degeneration, involving primarily the papillomacular bundle, closely resembled LHON and other mitochondrial optic neuropathies, supporting that mitochondrial dysfunction underlies its pathogenesis.

1. Introduction

Wolfram Syndrome (WS) was initially described in 1938 as a disease associated with juvenile diabetes and optic atrophy, which currently remains as the minimal criteria for diagnosis (Wolfram and Wagener, 1938). WS is also known as DIDMOAD, the acronym based on the four primary clinical features of Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy and Deafness (Barrett and Bundey, 1997). WS is a progressive, multisystem autosomal-recessive disorder characterized by neurodegeneration that typically is diagnosed during childhood. Besides these cardinal features of the disease, a recent study of the largest cohort of WS patients reported to date revealed a large variety of other neurological complications. These included dysfunction of the brainstem and cerebellum, peripheral neuropathy, cognitive decline, and epilepsy. Additionally, renal tract abnormalities, psychiatric symptoms, and gastrointestinal anomalies were also present in some individuals (Chaussenot et al., 2011).

Optic atrophy is a constant and prominent pathological manifestation of WS. A few case reports and a major study on a large cohort of WS patients focusing on ophthalmologic features of this syndrome describe progressive loss of visual acuity, color vision deficits, and cecocentral scotomas on visual field examination, with median age of onset at 11 years (Barrett et al., 1997; Mtanda et al., 1986; Niemeyer and Marquardt, 1972; Seynaeve et al., 1994). Neurophysiological investigations indicate that the retinal ganglion cells (RGCs) appear to be the site of pathology (Barrett et al., 1997; Niemeyer and Marquardt, 1972). Earlier postmortem studies have shown optic atrophy with demyelination and gliosis of the optic nerve, chiasm, and tracts (Barrett et al., 1997; Carson et al., 1977; Genís et al., 1997; Jackson et al., 1994; Shannon et al., 1999).

* Corresponding author at: Departments of Ophthalmology and Neurological Surgery, Doheny Eye Institute, 1450 San Pablo Street, Los Angeles, CA 90033, USA. Tel: +1 323 442 6417.
E-mail address: Asadun@usc.edu (A.A. Sadun).

1567-7249/$ – see front matter © 2013 Elsevier B.V. and Mitochondria Research Society. All rights reserved.
http://dx.doi.org/10.1016/j.mito.2013.05.013

The syndromic association of many clinical features in WS that are common in mitochondrial disorders has raised the question of whether or not the pathophysiology of WS is related to mitochondrial dysfunction and mitochondrial DNA (mtDNA) defects (Barrett et al., 1995, 2000; Barrientos et al., 1996a, 1996b; Bu and Rotter, 1993; Bundey et al., 1992; Jackson et al., 1994; Pilz et al., 1994; Rötig et al., 1993).

The genetic basis of WS was clarified by identifying a locus for WS on chromosome 4p16.1 in 1994 (Polymeropoulos et al., 1994) and subsequently the causative mutations in a gene (*WFS1*) encoding for an endoplasmic reticulum (ER) transmembrane protein named Wolframin (Inoue et al., 1998). *WFS1* mutations may behave as a dominant (Eiberg et al., 2006) or a recessive trait (Rendtorff et al., 2011; Tranebjaerg et al., 2009). The exact function of Wolframin is still under investigation, but its role in the ER-stress response (Fonseca et al., 2005; Yamada et al., 2006), including Na^+/K^+ ATPase dysfunction mediated by the wolframin-interacting protein β1 subunit (Zatyka et al., 2008), as well as defective calcium handling (Osman et al., 2003; Takei et al., 2006), have all been documented.

A homozygous single missense mutation in a second gene, *CISD2*, identified at chromosome 4q22-24, has been designated Wolfram Syndrome 2 (WFS2), and accounts for some Wolframin-negative cases (Amr et al., 2007). The *CISD2* gene product has been shown to localize on the outer mitochondrial membrane, and a knockout mouse model has a neurodegenerative phenotype resembling WS associated with biochemical evidence of mitochondrial dysfunction (Chen et al., 2009a, 2009b), thus partly reconciling the argument that mitochondria may play a role in the pathogenic mechanism of WS.

In this study, we compared retinas and the pattern of axonal loss in post-mortem optic nerves from a 25-year-old male patient diagnosed with WS carrying Wolframin mutations with a molecularly confirmed case of Leber hereditary optic neuropathy (LHON), in consideration of a final common mitochondrial pathogenic mechanism.

2. Materials and methods

2.1. Case report

The proband is a male, who died in 1972 at 25 years of age of cardiopulmonary arrest after developing severe pneumonia. In early infancy, he was noted to have excessive water intake and visual impairment. At 6 years of age he developed visual field defects and was diagnosed with optic atrophy. At 11 years of age he was hospitalized for glycosuria and abdominal pain, leading to the diagnosis of diabetes mellitus. Despite a well-controlled glycemia by diet, the patient continued to have a urinary output of 5 to 6 l, leading to the added diagnosis of diabetes insipidus. At 19 years of age, the patient had an episode of generalized seizures, and at hospital admission high blood sugar levels compelled insulin administration. Three years later, an ophthalmological examination revealed pale and excavated optic discs. He had poor pupillary light responses, with intermittent nystagmus in both eyes and visual function of light perception.

The family history was remarkable for three individuals who were known to have diabetes mellitus: a paternal uncle, a paternal cousin, and a maternal second cousin. Glucose tolerance tests for both parents revealed pre-diabetes. The proband's sister, the oldest of three siblings, was said to be healthy and had normal offspring. The family history was negative for blindness or diabetes insipidus.

2.2. Genetic analysis of the Wolframin gene

In 1972, when the patient died, there was no diagnostic molecular analysis available and, hence, there was no impetus to draw a blood sample from this proband. However, more recently, blood samples were collected, after informed consent, from the parents to pursue the molecular diagnosis of WS by direct sequence analysis of *WFS1*. Primers were as described in Eiberg et al. (2006) or designed to PCR amplify exons and 20–50 bp of surrounding intronic regions of *WFS1* (RefSeq NM_006005.2). Primer sequences and PCR conditions are available upon request. PCR products were sequenced using BigDye Terminator chemistry (Applied Biosystems) and separated on an ABI 3130XL genetic analyzer (Applied Biosystems) according to the manufacturer's instructions.

2.3. Tissue processing and light microscopy

Right and left postmortem eyes and retrobulbar optic nerves were obtained at autopsy from the WS proband. For comparison with a mitochondrial-induced optic atrophy, the optic nerves from a 59-year-old male with LHON from a large Brazilian pedigree (Sadun et al., 2003) harboring the 11778/ND4 mutation were also studied. Control tissues were obtained from a healthy 24-year-old male who died in a motorcycle accident. Tissues were fixed in 10% neutral buffered formalin. The proximal portion of each nerve immediately behind the eye was dissected into cross-sectional profiles. The eyes were cut through the nasal–temporal horizontal meridian. Retinal images were captured approximately 3.5 mm from the center of the optic nerve. All tissues were then embedded into paraffin. Sections were cut at 5 μm. Mayer's hematoxylin was used as a counterstain. Hematoxylin and eosin (H&E) was used to stain the retinas for general morphology and for the identification of RGCs. Optic nerves were immunostained for myelin basic protein (MBP) to evidence the myelinated RGC axons using an indirect method with horseradish peroxidase. The chromogen used was 3,3′-diaminobenzadine (DAB). The eyes were examined throughout the entire extent of the nasal and temporal retina as far as the ora serrata to evaluate the RGCs qualitatively. The optic nerves were examined in four "quadrants": superior, inferior, nasal, and temporal.

2.4. Morphometry of optic nerves

Optic nerve photomicrographs were captured via a Zeiss Axioskop bright-field light microscope (Carl Zeiss, Inc., USA) at 25× magnification. Calibration was performed using a micron ruler, and optic nerve measurements for area and shortest diameter were made using the SPOT Advanced software package (SPOT Imaging Solutions, USA).

3. Results

3.1. Mutations in the Wolframin gene

Two heterozygous mutations were found at position c.1549delC (p.R517AfsX5) in the father and at position c.2648_2651delTCTT (p.F883SfsX68) in the mother, strongly suggesting that the proband was a compound heterozygote for these two pathogenic changes, thus confirming the diagnosis of WS on molecular grounds (Fig. 1). Both mutations have been reported pathogenic before (p.R517AfsX5 in families D and W, and p.F883SfsX68 in families T, W, 3328, 1945 and 5, respectively, at the *WFS1* Gene Mutation and Polymorphism Database at: http://www.khri.med.umich.edu/research/lesperance_lab/wfs_delete.php).

3.2. Hematoxylin and eosin staining of retina

H&E staining of the control retina revealed normal architecture (Fig. 2A). Both the WS and LHON retinas demonstrated severe loss of RGCs in the temporal region, especially in the macula (Fig. 2B and C), with relative sparing of RGCs on the nasal side (data not shown). The remaining structures and cellular components of the WS and LHON retinas appeared to stain within normal limits compared with the staining pattern of the control retina. However, the WS retina showed some inner retinal thinning in the parafoveal region in comparison with retinas from the control and LHON patients.

Please cite this article as: Ross-Cisneros, F.N., et al., Optic nerve histopathology in a case of Wolfram Syndrome: A mitochondrial pattern of axonal loss, Mitochondrion (2013), http://dx.doi.org/10.1016/j.mito.2013.05.013