Clinical/Scientific Notes

Carlos E. Mendoza-Santiesteban, MD*
Jose-Alberto Palma, MD, PhD*
Isabel Ortuño-Lizarán
Nicolás Cuenca, PhD
Horacio Kaufmann, MD

PATHOLOGIC CONFIRMATION OF RETINAL GANGLION CELL LOSS IN MULTIPLE SYSTEM ATROPHY

Multiple system atrophy (MSA) is a rare adult-onset rapidly progressive fatal neurodegenerative disorder characterized by the abnormal aggregation of misfolded α-synuclein primarily in oligodendrocytes.[1]

In vivo studies of retinal structure using optical coherence tomography (OCT) have shown a remarkable reduction in the thickness of the retinal ganglion cell layer (GCL) and their axons in the retinal nerve fiber layer (RNFL) in patients with MSA.[2] These retinal abnormalities worsen over time and are associated with motor and overall clinical worsening.[2]

Here we report a detailed neuropathologic assessment of the retina in 3 patients (6 eyes) with autopsy-proven MSA, well-characterized clinically while alive. All 3 patients were women with the cerebellar predominant phenotype. Patient 1 died at age 69; she had been bedridden for 15 months and died during her sleep 10 years after disease onset. Patient 2 died at age 59; she had been bedridden for 4 months and died of pulmonary embolism and respiratory failure 7 years after disease onset. Patient 3 was a 61-year-old woman who became bedridden 7 months prior to death and died during sleep 8 years after disease onset.

In patients 1 and 2, comprehensive neuro-ophthalmologic evaluations including OCT (e-Methods at Neurology.org) had been performed 18 and 5 months before death, respectively. Four eyes from 4 age-matched normal subjects with no ophthalmologic or neurologic conditions were also studied as controls. The reported cause of death in the 4 controls was cardiorespiratory arrest.

Eyes were fixed in neutral-buffered 10% formalin and retinal whole mounts prepared. To increase antibody penetration, retinal whole mounts were cryoprotected with 30% sucrose in 0.1 M phosphate buffer and subjected to a freeze–thaw cycle. Wedge-shaped pieces of retina were immunohistochemically stained as free-floating preparations with blue fluorescent Hoechst 33258 pentahydrate (bisbenzimide) (Molecular Probes, Eugene, OR). Analogous samples were stained using a mouse monoclonal antibody against α-synuclein phosphorylated at serine 129 (1:1,000; Wako Chemicals, Richmond, VA; clone no. pSyn#64). Retinal whole mounts were mounted in Citifluor (Citifluor Ltd., London, UK) and observed in a TCS SP2 confocal laser scanning microscope or in a Leica DMR microscope (Leica Microsystems, Wetzlar, Germany). Cells in the GCL were manually counted in 7 1-mm concentrically defined annular areas centered in the fovea, using a previously reported method.[3] A total of 7 retinal whole mounts for blue fluorescent Hoechst (3 MSA and 4 control) and 5 whole mounts for anti-α-synuclein phosphorylated staining (3 MSA and 2 control) were prepared.

Clinical neuro-ophthalmologic examination, visual acuity, color discrimination, and funduscopy were normal in patient 1 (69-year-old woman), patient 2 (59-year-old woman), and patient 3 (61-year-old woman). OCT in patients 1 and 2 showed generalized thinning of the RNFL, more evident in the inferior quadrant of the RNFL, where the axons of the peripheral ganglion cells are located. The thickness of the ganglion cell complex at the macular area was also reduced (figure).

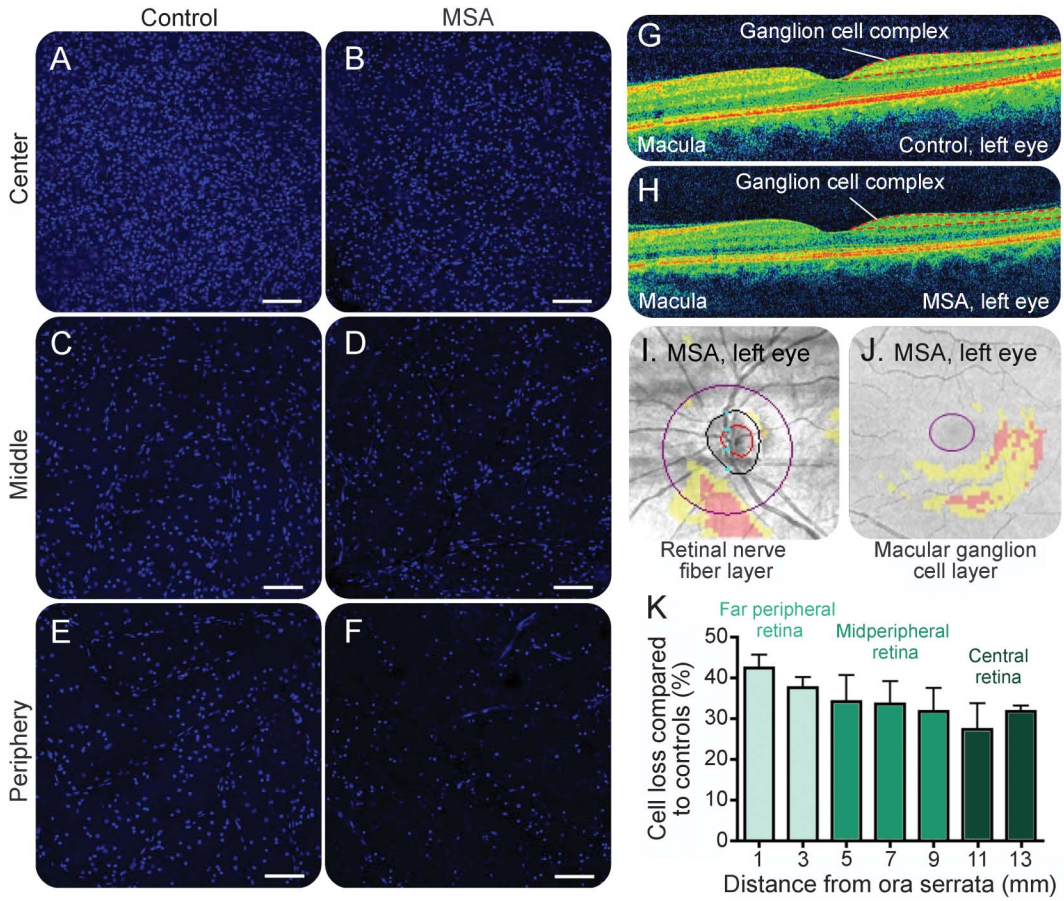
Histopathologic analysis of the Hoechst-stained retinae showed diffuse retinal ganglion cell loss in the eyes of patients with MSA compared to controls (figure). This difference was more pronounced in the peripheral retina rather than in the central retina. In patients with MSA, reduced density of ganglion cells was associated with longer distances from the central retina (figure). α-Synuclein staining failed to show aggregates of phosphorylated α-synuclein in the GCL of any of the 3 patients with MSA or the control retinae.

These histopathology findings in the retinae of patients with MSA confirm our previous in vivo observations using OCT indicating that the retinal ganglion cells and their axons in the RNFL were reduced in these patients.[2] A more severe retinal ganglion cell loss in the peripheral retina, rather than in the macular area (central retina), had also been observed with OCT. The partial sparing of the central retinal ganglion cells with the brunt of the loss in the peripheral retina may explain why patients with MSA are visually asymptomatic and do not exhibit significant loss of visual acuity or color discrimination, as both are functions of the central macular ganglion cells.

Abnormal α-synuclein aggregates have been reported in the retinal ganglion cells of patients with

Supplemental data at Neurology.org

Figure Immunohistochemical analysis of retinal ganglion cells in multiple system atrophy (MSA) and its in vivo correlation with optical coherence tomography (OCT)



Confocal images of representative areas of whole-mounted retinae (superior–temporal area) labeled with the blue fluorescent Hoechst marker of a patient with MSA (A, C, E) and a normal control (B, D, F). Distance from the peripheral retina (ora serrata): center: 11–13 mm; middle: 5–9 mm; periphery: 1–3 mm. Scale bar is 100 μm. The number of ganglion cells is markedly reduced in MSA compared to controls. These pathologic findings are similar to those found with in vivo OCT. (G, H) OCT macular sections where the different layers of the retina can be appreciated. The ganglion cell layer (GCL) complex (delimited with a dashed red line) is thinner in a patient with MSA (H) compared to an age-matched normal control (G). In the same patient with MSA, the thickness of the inferior quadrant of the retinal nerve fiber layer (I) and the macular ganglion cell complex layer (J), measured by OCT, are substantially reduced (reduced thickness identified in yellow and red). These findings were confirmed with ganglion cell quantification, where retinal ganglion cell loss was consistent in the entire retina, particularly in the far peripheral areas (K). Briefly, images of the whole retinal sample (with the focus on the GCL) were taken and sampling regions of 1 mm^2 were established every 2 mm from the ora serrata to the central retina. Cells in the GCL stained with Hoechst were manually counted and the mean density of cells in each quadrant (number of cells per mm^2) was calculated for MSA and controls. The percentage of ganglion cell loss at each sampling region in patients with MSA (with the ganglion cell count of controls as reference) is shown (K).

Parkinson disease[4,5] but we did not find similar aggregates in patients with MSA. In the brain of patients with Parkinson disease, accumulation of α-synuclein occurs in neurons,[6] whereas in MSA brains, pathologic α-synuclein aggregates occur mostly in oligodendroglial cells.[1] Because there are no oligodendroglial cells in the retina, it is conceivable that the abnormal α-synuclein deposits in patients with MSA occur in oligodendroglial cells in the optic nerve. Optic nerve oligodendroglial cells myelinate the axons of magnocellular ganglion cells, which are located in the retinal areas most affected in patients with MSA. Our small sample size precluded statistical correlations between OCT findings and ganglion cell density, which should be addressed in future studies.

We report neuropathologic evidence that retinal ganglion cells are reduced in patients with MSA, an abnormality that had previously been reported in vivo using OCT. These findings provide further support for the use of retinal thickness as measured by OCT as a potential biomarker of efficacy of disease-modifying agents in patients with MSA.

*These authors contributed equally to this work.

From the Dysautonomia Center (C.E.M.-S., J.-A.P., H.K.), New York University School of Medicine, New York University, New York; and University of Alicante (I.O.-L., N.C.), Spain.

Author contributions: Dr. Mendoza-Santiesteban: study concept and design, acquisition of data, analysis and interpretation, critical revision of the manuscript for important intellectual content, study supervision. Dr. Palma: study concept and design, acquisition of data, analysis and interpretation, critical revision of the manuscript for important intellectual content, study supervision. I. Ortuño-Lizaran: acquisition of data, analysis and interpretation, critical revision of the manuscript for important intellectual content. Dr. Cuenca: acquisition of data, analysis and interpretation, critical revision of the manuscript for important intellectual content. Dr. Kaufmann: study concept and design, critical revision of the manuscript for important intellectual content, study supervision.

Acknowledgment: The authors thank the patients with MSA who donated their organs and Dr. Tatiana Bakaeva for her help with the autopsies.

Study funding: NIH (U54-NS065736-01), Multiple System Atrophy Coalition, Michael J. Fox Foundation, Dysautonomia Foundation, Regional Government of Valencia (Prometeo 2016/158), Spanish Health Research Institute Carlos III (ISCIII RETICS-FEDER RD12/0034/0010), Ministry of Economy of Spain (MINECO-FEDER-BFU2015-67139-R), and Ministry of Education of Spain (FPU14/03166).

Disclosure: C. Mendoza-Santiesteban receives research support from The Dysautonomia Foundation and the Michael J. Fox Foundation. J. Palma receives research support from the NIH (U54NS065736), the Food and Drug Administration, the Dysautonomia Foundation, the MSA Coalition, and the Michael J. Fox Foundation. I. Ortuño-Lizaran receives financial support from the Ministry of Education of Spain. N. Cuenca receives research support from the Regional Government of Valencia, Spanish Health Research Institute Carlos III, and the Ministry of Economy of Spain. H. Kaufmann serves as Editor-in-Chief of Clinical Autonomic Research *and receives research support from the NIH, the Food and Drug Administration, the Dysautonomia Foundation, and the Michael J. Fox Foundation. Go to Neurology.org for full disclosures.*

Received December 29, 2016. Accepted in final form March 15, 2017.

Correspondence to Dr. Mendoza-Santiesteban: carlos.mendoza-santiesteban@nyumc.org

1. Ahmed Z, Asi YT, Sailer A, et al. The neuropathology, pathophysiology and genetics of multiple system atrophy. Neuropathol Appl Neurobiol 2012;38:4–24.
2. Mendoza-Santiesteban CE, Palma JA, Martinez J, Norcliffe-Kaufmann L, Hedges TR III, Kaufmann H. Progressive retinal structure abnormalities in multiple system atrophy. Mov Disord 2015;30:1944–1953.
3. Gomez-Vicente V, Lax P, Fernandez-Sanchez L, et al. Neuroprotective effect of tauroursodeoxycholic acid on N-methyl-D-aspartate-induced retinal ganglion cell degeneration. PLoS One 2015;10:e0137826.
4. Beach TG, Carew J, Serrano G, et al. Phosphorylated alpha-synuclein-immunoreactive retinal neuronal elements in Parkinson's disease subjects. Neurosci Lett 2014;571:34–38.
5. Bodis-Wollner I, Kozlowski PB, Glazman S, Miri S. Alpha-synuclein in the inner retina in Parkinson disease. Ann Neurol 2014;75:964–966.
6. Halliday G, Hely M, Reid W, Morris J. The progression of pathology in longitudinally followed patients with Parkinson's disease. Acta Neuropathol 2008;115:409–415.