Phosphorylated α-synuclein-immunoreactive retinal neuronal elements in Parkinson's disease subjects

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Abstract

Visual symptoms are relatively common in Parkinson's disease (PD) and optical coherence tomography has indicated possible retinal thinning. Accumulation of aggregated α-synuclein is thought to be a central pathogenic event in the PD brain but there have not as yet been reports of retinal synucleinopathy. Retinal wholemounts were prepared from subjects with a primary clinicopathological diagnosis of PD ($N = 9$), dementia with Lewy bodies (DLB; $N = 3$), Alzheimer's disease ($N = 3$), progressive supranuclear palsy ($N = 2$) as well as elderly normal control subjects ($N = 4$). These were immunohistochemically stained with an antibody against α-synuclein phosphorylated at serine 129, which is a specific molecular marker of synucleinopathy. Phosphorylated α-synuclein-immunoreactive (p-syn IR) nerve fibers were present in 7/9 PD subjects and in 1/3 DLB subjects; these were sparsely distributed and superficially located near or at the inner retinal surface. The fibers were either long and straight or branching, often with multiple en-passant varicosities along their length. The straight fibers most often had an orientation that was radial with respect to the optic disk. Together, these features are suggestive of either retinopetal/centrifugal fibers or of ganglion cell axons. In one PD subject there were sparse p-syn IR neuronal cell bodies with dendritic morphology suggestive of G19 retinal ganglion cells or intrinsically photosensitive ganglion cells. There were no stained nerve fibers or other specific staining in any of the non-PD or non-DLB subjects. It is possible that at least some of the observed visual function impairments in PD subjects might be due to α-synucleinopathy.

Keywords

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1. Introduction

Visual symptoms are relatively common in Parkinson's disease (PD), including reading difficulties and perceptual distortions. Detailed examinations suggest that visual acuity, contrast sensitivity and color vision are impaired [1–4]. A number of ocular structural and functional abnormalities have been reported in PD subjects and much attention has been paid to the possibility of an ophthalmological biomarker or diagnostic test. Perhaps the most activity has come from the field of optical coherence tomography (OCT), which has enabled high resolution structural imaging of the retina. Several groups have used OCT to measure the thickness of retinal layers and generally concur that the retina is thinner in PD subjects as compared to healthy controls [5]. It is unclear, however, whether the differences are sufficient and consistent enough to serve as a diagnostic marker and whether they are specific to PD or are a change held in common with other neurodegenerative conditions. Retinal thinning has also been reported, for example, in Alzheimer's disease [6], which often co-exists with PD [7].

The cellular basis of retinal thinning has been ascribed by some to loss of dopaminergic amacrine neurons and/or retinal ganglion cells. In some animal models of Parkinson's disease there is both loss of dopaminergic retinal cells and development of parkinsonian motor disabilities [8,9]. In monkeys treated with MPTP there is a decrease in the number of retinal dopaminergic cell bodies, dendritic processes and branching complexity; amacrine cells located postsynaptically are also affected [8]. However, the status of intrinsic retinal neurons in human subjects with PD is still unknown.

The protein that accumulates to form the characteristic Lewy bodies of PD, α-synuclein, is normally present in several types of retinal cells in a wide range of vertebrate species including humans [10] and therefore there is a possibility that this may accumulate in PD retinas as it does in the brain. One group has reported that perikaryal α-synuclein cytoplasmic staining increases with age in neurologically normal elderly humans but PD cases were not studied [11]. There is a case report of unusual retinal inclusions in a subject with DLB but these were not immunoreactive for α-synuclein and their composition is still unknown [12]. A single previous study reported no pathological α-synuclein, tau or Aβ deposits in the retinas of 6 PD subjects, 11 AD subjects and 6 age-similar control subjects [13]; we sought to extend the search for retinal synucleinopathy with alternative methodologies, using retinal wholemounts and an immunohistochemical method for phosphorylated α-synuclein, reasoning that these changes might offer greater sensitivity.

2. Materials and methods

2.1. Source of human subjects

This study was conducted as part of the Arizona Study of Aging and Neurodegenerative Disorders (AZSAND), by the Arizona Parkinson Disease Consortium and the Banner Sun Health Research Institute Brain and Body Donation Program (BBDP; www.brainandbodydonationprogram.org). Brain necropsies and neuropathological examinations were performed on elderly subjects who had volunteered for the BBDP [14]. The BBDP has been approved by the BSHRI Institutional Review Board and research

conducted on BBDP subjects is carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

2.2. Clinical and neuropathological characterization of human subjects

Subjects are clinically characterized with annual standardized test batteries that include general neurological, cognitive, and movement disorder components [14]. Additionally, private medical records are requisitioned, reviewed and abstracted for each subject. Deceased subjects received standardized neuropathological examinations as described previously [14]. Specific neuropathological diagnostic criteria were used for PD [15], dementia with Lewy bodies (DLB) [16], Alzheimer's disease (AD) [17] and progressive supranuclear palsy (PSP) [18,19]. Alzheimer's disease cases with synucleinopathy but not meeting clinicopathological diagnostic criteria for PD or DLB were designated as AD with Lewy bodies (ADLB), versus AD without Lewy bodies (ADNLB).

2.3. Tissue processing

Eyes were fixed in neutral-buffered 10% formalin and retinal wholemounts prepared [20]. To increase antibody penetration, retinal wholemounts were cryoprotected with 15% sucrose in 0.1 M phosphate buffer and subjected to three freeze–thaw cycles by placing and removing from a slab of dry ice [21]. Wedge-shaped pieces of retina were cut with the optic disk at the apex; these were immunohistochemically stained as free-floating preparations with an antibody against α-synuclein phosphorylated at serine 129 [22–25]. Positive and negative control sections were processed with each batch of stained sections. The stained wholemounts were dehydrated in an ethanol series and then cleared in two changes of dibenzyl ether (Sigma–Aldrich, St. Louis, MO).

3. Results

3.1. Human subject characteristics

There were nine subjects with a primary neuropathological diagnosis of PD, three with DLB (two also met neuropathological diagnostic criteria for AD), three with AD (excluding the two cases that also had DLB), of which one was ADLB and two were ADNLB), two with PSP (one also met neuropathological diagnostic criteria for PD) and four normal elderly control subjects. One additional subject had mild cognitive impairment and parkinsonism with non-specific neuropathology. Subject age ranges and means were: PD 63–90, 82.3; DLB 78–82, 80.1; AD 74–87, 82.6; PSP (both subjects were 83); control 86–92, 89.5. For PD subjects, symptom duration ranged from 2 to 13 years with a mean of 9.7 years; final motor scores on the Unified Parkinson's Disease Rating Scale (UPDRS; except one case with no scores available) ranging from 15 to 73, with a mean score of 42.4.

Nine subjects had a medical history of either cataract (five subjects), glaucoma (five subjects) or macular degeneration (two subjects), with three subjects having more than one eye condition. Four of these nine had a non-PD, non-DLB diagnosis while five had PD or DLB.

3.2. Microscopic observations

Phosphorylated α-synuclein-immunoreactive (p-syn IR) nerve fibers were present in 7/9 PD subjects and in 1/3 DLB subjects. There were no stained nerve fibers or other specific staining in any of the other subjects. Stained nerve fibers (Fig. 1) were sparsely distributed and superficially located on the inner retinal surface (the surface facing the vitreous). Focusing on the fibers, as well as the top and bottom surfaces of the retinal wholemount, with a 100× oil-immersion objective indicated that they were either right at the inner retinal surface or were located within the inner 25% of the retina, with an average depth equal to 14% of the entire retinal thickness. This is consistent with locations within the nerve fiber layer, the ganglion cell layer and the inner strata of the inner plexiform layer. The fibers were either long and straight (Fig. 1A and B) or branching (Fig. 1C–E); both types often had multiple en-passant varicosities along their length. The straight fibers most often had an orientation that was radial with respect to the optic disk and thus parallel to the radially-oriented dissection margin (Fig. 1A). Neuronal inclusions were not present and neuronal perikarya, glia or other features were not stained.

In a single PD subject, a very few p-syn IR neuronal perikarya, complete with extensive, planar, beaded dendritic trees, were strongly stained (Fig. 2). These had large cell bodies (22 × 25 μm diameter) and their dendritic trees were the largest of any ganglion cell types at more than 1 mm dendritic spread (Fig. 2). Surface focusing indicated their cell bodies are likely to be located in the ganglion cell layer and their dendritic trees in the lower inner plexiform layer (IPL). By morphology and location, these cells are very likely a type of retinal ganglion cell.

4. Discussion

As α-synuclein phosphorylated at serine 129 is only immunohistochemically detectable in the nervous systems of human subjects with α-synucleinopathies [7,25–27], its presence in the retina in these seven PD subjects and one DLB subject is consistent with retinal synucleinopathy. This is in contrast to a recent report [13] that did not find pathological α-synuclein, tau or Aβ deposits in the retinas of six PD subjects, 11 AD subjects and six age-similar control subjects. As the p-syn IR nerve fibers detected in the present report were sparsely distributed, it is possible that the much greater sample volume obtained with retinal whole-mounts (versus paraffin-embedded retinal cross-sections in the previous study) allowed greater sensitivity. Other methodological differences were the usage of an antibody against unmodified α-synuclein in the earlier study while the present study employed an antibody against phosphorylated α-synuclein. The presence of phosphorylated α-synuclein is restricted to the brains of subjects with synucleinopathy while unmodified α-synuclein is extremely abundant in normal brains and therefore precautions must be used to ensure differential staining of normal and pathological α-synuclein. Two prior publications have noted the presence of immunohistochemical staining for unmodified α-synuclein within retinal neuronal perikarya [10,13].

From the great length and radial orientation of the straight fibers, and the probable location of fibers within the optic nerve fiber layer, ganglion cell layer and inner plexiform layer, together with the absence of stained retinal neuronal perikarya in all subjects except one, it

is possible that these are centrifugal/retinopetal fibers originating from the brain [28]. Such fibers are generally sparsely distributed in the retina, and most arise from either histaminergic neurons in the hypothalamus or dorsal raphe serotonergic neurons, although tyrosine hydroxylase-immunoreactive fibers arising from the hypothalamus have also been reported in the mouse [29]. Neurons in all of these brain locations are selectively vulnerable to the development of synucleinopathy in PD [30]. Alternatively, it is possible that these represent centripetal/retinofugal fibers arising from retinal ganglion cells, although retinal ganglion cell bodies were not stained in 6/7 of these subjects. The superficial inner retinal location and the dendritic morphology of the p-syn IR neurons seen in one PD subject are suggestive of both a G19 retinal ganglion cell, as described by Kolb and co-workers [31] and a melanopsin-positive intrinsically photosensitive retinal ganglion cell (ipRGC), as described by Schmidt and co-workers [32]. Co-localization studies will be needed to conclusively identify the observed fibers and neuronal cell bodies.

There were p-syn IR fibers in the retina of 1/3 subjects with DLB. These were similar to those seen in the PD subjects. As with PD, retinal thinning has been reported with OCT in DLB [33]. To our knowledge, there has been only a single prior report of retinal histology in DLB subjects; cytological inclusions were reported but these were not immunoreactive for α-synuclein and did not resemble Lewy bodies ultrastructurally [12].

The numbers of subjects included in this study is small and therefore while we have shown that pathological α-synuclein deposits are present in some Lewy body disease subjects, it is not yet possible to conclude that these are restricted to such subjects. Additionally, the association is not universal as only one of three DLB subjects, and seven of nine PD subjects, had retinal p-syn IR. Previous investigations [26] have noted that synucleinopathy does not extend quite as widely outside of the brain in DLB as in PD. It may be that extension of synucleinopathy to the retina is not an early event, as for both of the p-syn IR-negative PD subjects, the symptom durations were less than the median for the group (3 and 7 years versus 9.7 years). Only one p-syn IR-negative PD subject had a UPDRS motor score available, and, at 15, it was well below the mean PD group score of 42. Again, however, with the small subject numbers it is difficult to conclude much with certainty from this.

Histaminergic and serotonergic retinal inputs modulate the maintained activity and light responses of retinal ganglion cells [28] while ipRGCs mediate aspects of circadian rhythm, pupillary light reflex and sleep, as well as contributing to image formation [32]. At least some of the reported visual function impairments in PD subjects might be due to α-synucleinopathy. It is possible that the psyn-IR fibers might allow an ophthalmological diagnostic test for PD should appropriate technology be developed for this.

Acknowledgments

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HIGHLIGHTS

- Structural and functional retinal changes are present in Parkinson's disease (PD).

- Phosphorylated α-synuclein (psyn) is a specific biomarker of PD.

- Psyn-immunoreactive nerve fibers were present in the retina in 7/9 subjects with PD.

- Psyn-immunoreactive neuronal perikarya were present in 1 PD subject.

- This pathology may contribute to structural and functional retinal changes in PD.
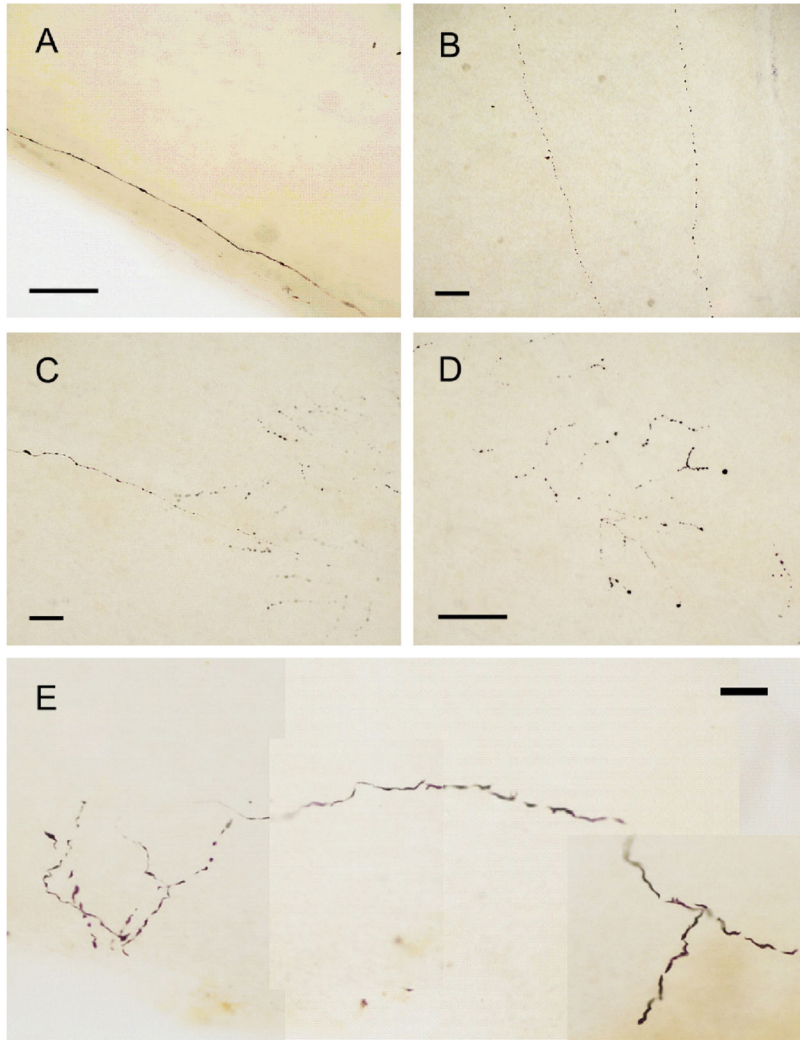
Fig. 1.
Photomicrographs of p-syn IR nerve fibers, seen in the retinas of 7/9 PD subjects. Stained fibers were either long and straight, without branches (a, b) or had multiple branches (c–e). Both types of fibers often had multiple varicosities. All calibration bars equal 100 μm.
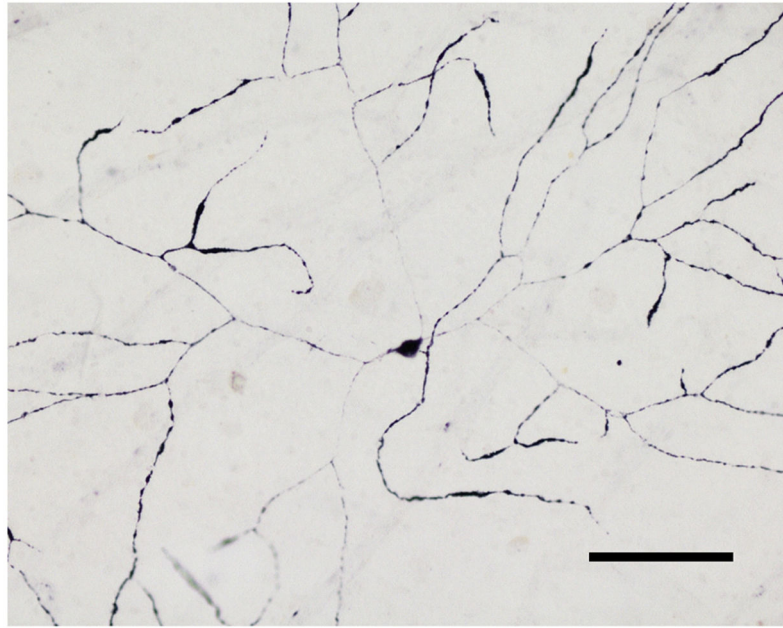
Fig. 2.
Photomicrograph of a neuronal type found in the retina of a single PD subject. These neurons were located near the inner retinal surface. Their dendritic trees were large, planar and beaded. Their size and morphology are consistent with a type of retinal ganglion cell. Calibration bar = 500 μm.