# Glaucoma as a Neurodegenerative Disease: Why We Must 'Look for the Protein'

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## ABSTRACT

For years, clinicians and scientists interested in glaucoma have focused on the anterior segment of the eye and lowering of the intraocular pressure with respect to glaucoma causes and therapies. Yet glaucoma progresses in many individuals despite lowering the intraocular pressure. Herein, the concept of glaucoma as a neurodegenerative disease is presented.


**KEYWORDS**: actin, Alzheimer disease, cytoskeletal protein, cortactin, glaucoma, human genome, Huntington disease, intraocular pressure, neurodegenerative disease, optic neuropathy, DNA trinucleotide repeat.

## INTRODUCTION

Glaucoma is a leading cause of blindness in the U.S. and worldwide (1). Glaucoma is a group of diseases that features a progressive optic neuropathy accompanied by characteristic visual field changes, with or without increased intraocular pressure. Glaucoma has been aptly described as an "*optic vasobaropathy*" indicating that both vascular and mechanical pressure processes contribute to the optic nerve damage and visual loss (2). Risk factors associated with the development of glaucoma include elevated intraocular pressure (> 21mmHg), increasing age, race (blacks more than whites), and a family history of glaucoma. Genes associated with glaucoma have been identified (3,4). Other potential risk factors include diabetes mellitus, hypertension, myopia, cigarette smoking, and alcohol consumption.

## GLAUCOMA AND INTRAOCULAR PRESSURE

A primary contributor of glaucoma development is a relative increase in intraocular pressure (IOP). I use the term 'relative' because although a pressure greater than 21 mmHg is considered high, IOPs less than 21 mmHg could contribute to glaucoma (5,6). High IOP results from a decrease in the outflow of aqueous fluid. The aqueous fluid is normally produced by the ciliary body in the eye and exits through the trabecular meshwork and Schlemm's canal. The build-up of pressure in the eye is associated with retinal ganglion cell/optic nerve fiber loss which is exemplified by enlargement of the optic nerve cup-disc ratio and subsequent vision loss (**Figure**). The Ocular Hypertension Treatment Study (OHTS) documented individuals *without* glaucoma, but who have high IOP, have a cumulative increased risk of developing glaucoma at 9.5% incidence in 5 years, or approximately 1–2% cumulative incidence per year (7,8). Older individuals

**Figure A.** Optic nerve appearance of a patient with glaucoma with optic nerve cup-disc ratio of 0.6.

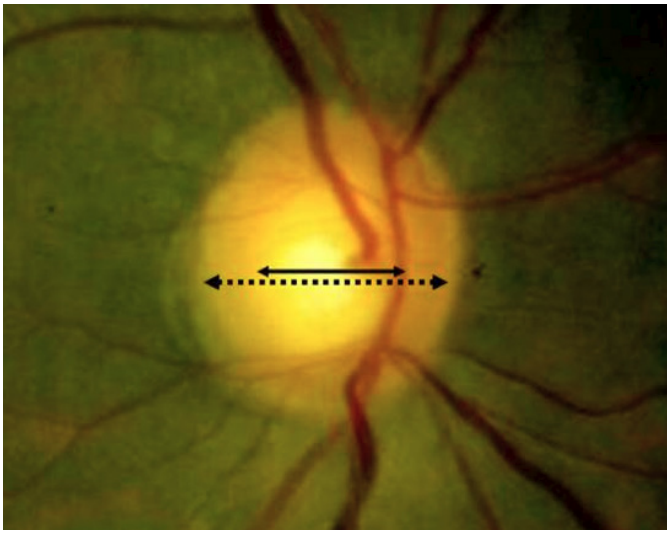
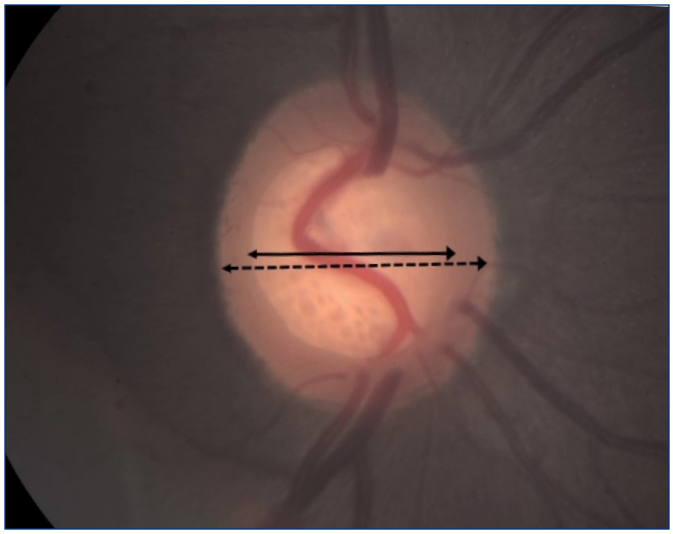


**Figure B.** Increased glaucomatous cupping from another patient with optic nerve cup-disc ratio of 0.8.



and those with thin (less than 555 microns) central corneal thickness measurements and large vertical cup-disc ratios (greater than 0.3) are more likely to develop glaucoma as the IOP increases.

The Collaborative Initial Glaucoma Treatment Study (CIGTS), Collaborative Normal-Tension Glaucoma Study (CNTGS), Early Manifest Glaucoma Trial (EMGT), Advanced Glaucoma Intervention Study (AGIS), and Glaucoma Laser Trial (GLT) have documented that reducing the baseline IOP by at least 20%, but preferably 30% or more, generally will prevent progression of visual field loss, even in patients with glaucoma who have an initial baseline IOP within the normal range (7–15). Accordingly, methods of lowering the IOP below the aforementioned 20% to 30% target pressure have been the mainstay of treatment for glaucoma, either by topical and/or oral medications, laser surgery (e.g., argon laser trabeculoplasty [ALT], selective laser trabeculoplasty [SLT]), conventional surgery or device placement (e.g., trabeculectomy, drainage implant device placement), or a combination of these therapies. However, despite lowering the IOP by these methods alone or in combination, there is still a cumulative 1–3% per year failure rate among individuals who received these treatments.

## IMPORTANCE OF LOOKING FOR THE PROTEIN

Only 4.4% of participants without glaucoma in the OHTS who received treatment to lower the IOP developed glaucoma within 5 years in comparison with 9.5% of untreated participants. The OHTS clinical trial documented participants with high IOP *without* glaucoma at baseline had an approximate 50% reduction in glaucoma conversion at 5 years when IOP was lowered. But this meant the conversion to glaucoma continued in the other 50% of participants despite some of them having had significantly lowered IOP, indicating that IOP is not the sole major factor causing glaucoma. We must look elsewhere. It is my impression that, "*We must look for the protein*."

Most clinicians and scientists interested in glaucoma have focused on the anterior segment of the eye for glaucoma causes and therapies, in particular, evaluation of the trabecular meshwork and aqueous outflow mechanisms. The recognition that glaucoma is a neurodegenerative disease similar to Creutzfeldt-Jakob disease (CJD), Huntington's disease, Alzheimer's disease, Parkinson's disease, myotonic dystrophy, amyotrophic lateral sclerosis (ALS, Lou Gehrig's disease), chronic traumatic encephalopathy (CTE), and spinocerebellar ataxia, is now beginning to gain traction. There is a great need for further investigation of the retinal ganglion cells and their connecting neurons. It is my impression that glaucoma will be identified as a "protein-folding" neurodegenerative disease involving either a trinucleotide repeat (TNR) genetic disorder such as *Poly-GAG* (also called Poly-E) that could occur in actin or its fellow structural cell membrane protein component (e.g., actinin, vinculin, or cadherin), or a variable number tandem repeat (VNTR) genetic disorder as may occur in the cytoskeletal protein cortactin. Why might I suggest this?

## REDUNDANCIES IN THE HUMAN GENOME

The haploid human genome consists of 23 chromosomes and 3 billion DNA nucleotide base pairs. The human genome contains approximately 20,000 to 25,000 protein-coding genes, with each gene comprised of approximately 4 exons, and each exon comprised of approximately 250 DNA nucleotide base pairs (16-18). Given that there are 3 billion DNA base pairs of the haploid human genome, then the protein-coding genes comprise only about 1.5% of the total genome. The remainder of the human genome, the 98.5% which previously had been called "junk DNA," is very important as it can be likened to a road map containing the following: regulatory sequences (enhancers, silencers, and locus control regions); promoter sites which bind RNA polymerases; introns; RNA genes (transfer RNA [tRNA], ribosomal RNA [rRNA], microRNA [miRNA], short nuclear RNA [snRNA], short nucleolar RNA [snoRNA], small interfering RNA [siRNA], and other non-coding RNA); and repetitive non-coding DNA which accounts for 50% of the genome.

Genetic defects producing disease may arise from single nucleotide polymorphisms (SNPs) due to DNA nucleotide base pair substitutions, insertions, or deletions. These SNPs cause missense mutations by incorporating the wrong amino acid in the protein sequence (e.g., sickle cell anemia), nonsense mutations by incorporating an early stop signal (coded as TAG, TAA or TGA) causing a shortening of the produced protein, and frameshift mutation by causing a misreading of the 3-letter word DNA sequences due to the insertion or deletion of a nucleotide. Sometimes there could be large-scale rearrangements of the genome resulting in copy number variations (CNVs) which arise from insertion (translocation), deletion, duplication, or inversion of large segments of DNA at the time of meiosis resulting in haploid excess or haploid insufficiency. These CNVs cause genetic effects by increasing or decreasing the gene dose, or by influencing gene transcription and translation through position effects. The SNPs and CNVs alter the gene structure, function, or regulation, causing phenotypic changes or diseases (16-18).

## CHARACTERISTICS OF NEURODEGENERATIVE DISORDERS

A relatively common CNV is the sequential repetition of several nucleotide base pairs. When 3 nucleotides are repeated, such as CAG being repeated several times, this is called a triplet nucleotide repeat or trinucleotide repeat (TNR). When 6 or more nucleotide base pairs are repeated, this is called a variable number tandem repeat (VNTR). Everyone has CNVs as a result of us normally having TNRs

or VNTRs. In general, it is when the number of these TNR or VNTR repeats exceeds a certain threshold that a disease is triggered (16-18). Creutzfeldt-Jakob disease (CJD), the prototype of neurodegenerative diseases, is characterized by the presence of a transmissible particle, an abnormal prion PrP protein. Huntington's disease (HD), another classic neurodegenerative disease, is inherited as an autosomal dominant trait and becomes manifest when, on chromosome 4, the normally occurring TNR 'CAG' (which codes for glutamine) is repeated more than 39 times. The normally occurring huntingtin protein now has an abnormal excess of glutamine (polyglutamine, poly-Q) resulting in the pathological hallmark of degeneration and atrophy of the neurons in the striatum and their connecting neurons in the cerebral cortex and other subcortical structures in HD (16-18).

## FUNKY PROTEIN CELLULAR DEPOSITS

Like CJD and HD, neurodegenerative disorders have selective destruction of neurons within their associated neuronal network, and deposition of a "funky" or abnormal protein in the affected cell nucleus, cytoplasm, or both. This protein deposition can take the shape of plaques as with Alzheimer's disease, neurofibrillary tangles as with chronic traumatic encephalopathy and Alzheimer's disease, or inclusion bodies (Lewy bodies) in cytoplasm or nucleus as with Parkinson's disease. The "funky" or abnormal protein that is translated from the TNR or VNTR gets cleaved by enzymes in the cytoplasm (16-18). Some of the abnormal protein fragments will enter the nucleus and co-opt the nuclear machinery to make more of itself. The funky protein accumulates within the nucleus, cytoplasm, or both, thereby interfering with the cytoskeleton structure, cell signaling, or mitochondria energy generation machinery, thus killing the cell (18). In the process, the transmissible protein is then passed on to other transsynaptic connecting cells causing selective neuronal destruction. In Parkinson's disease, there is selective destruction of dopaminergic neurons in the substantia nigra and the neurons with which they synapse in the striatum of the basal ganglia and other brain regions. This results in the deposition of proteinaceous inclusion bodies (Lewy bodies) composed of alpha-synuclein protein. In fronto-temporal dementia (FTD) and chronic traumatic encephalopathy (CTE), there is selective destruction of neurons with deposition of tau protein. Alzheimer's disease is characterized by accumulation of amyloid β protein forming senile plaques and tau protein forming neurofibrillary tangles in the nerve cell bodies, resulting in destruction of the cytoskeleton and eventual neuronal death.

There is an increasing body of work documenting glaucoma as a neurodegenerative disease with selective destruction of retinal ganglion cells, the connecting transsynaptic cells in the lateral geniculate nucleus, and occipital cortex (19). Analysis of the vitreous in glaucoma, has shown an excess of glutamate (20). The excess glutamate could be the result of a byproduct of cell death or could be part of the excess "funky" protein that is generated by having TNR or VNTR coding for excess glutamate which then causes cell death. I believe the latter. It is my belief that a potential TNR suspect is actin, a multifunctional protein involved in the cytoskeleton structure, and which normally has a TNR Poly-E (aka Poly-GAG) segment at the beginning of the gene, which could code for the translation of multiple glutamate molecules and abnormal protein folding (21-23). Additionally, a potential VNTR suspect is cortactin, another cytoskeletal protein, which normally has a central 6.5 tandem repeat VNTR consisting of 30 amino acid sequence which also has the potential for coding for the translation of multiple glutamate molecules (21-23). Indeed, recent studies have shown that mechanical pressure on a cell will cause abnormal actin cytoskeletal structural changes, potentially incapacitating the cell (21). In conclusion, it is my impression that glaucoma will be identified as a "protein-folding" neurodegenerative disease. Like other neurodegenerative diseases, *we should look for the protein* in the retinal ganglion cells and their connecting neurons as this hopefully will expand our treatment armamentarium for this group of blinding diseases called glaucoma.

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