PROGRESS REPORT for CURE GLAUCOMA FOUNDATION

TITLE: Neurodegenerative biomarkers in primary open angle glaucoma

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STUDY TEAM: Daniel Vu, M.D. Rand Allingham, M.D. (recently deceased), Brahma Mulugu, Ph.D., Mohamed Abou-Donia, Ph.D.

ABSTRACT:

Primary open angle glaucoma (POAG) is a neurodegenerative disease of the visual pathway in which irreversible blindness result from progressive loss of retinal ganglion cells (RGCs). Intraocular pressure or visual field testing are unreliable methods to screen for POAG. As such, there is currently no effective clinical method for diagnosing POAG at an early stage. Our goal is to identify and develop POAG biomarkers which can be utilized for early diagnosis. We hypothesize that the degeneration of RGCs result in leakage of specific neuronal proteins into the blood, and that autoantibodies developed against these neuronal proteins can be utilized as a POAG biomarkers. These autoantibodies will be present in the serum or tear film in small quantity. Therefore, the goal of this proposal is to develop sensitive proteomic assays to these autoantibodies, and test them in POAG patients. Previous studies by other investigators have examined autoantibodies to inflammatory markers. However, to our knowledge, little is known about autoantibodies to neuronal proteins emanating from dying neurons. In preliminary work, we have already developed an assay to detect low levels of autoantibodies against these neuron-specific proteins, such as tau, MAP, and neuron enolase. Furthermore, our preliminary data have demonstrated elevated autoantibody levels of specific neuronal proteins in POAG patients recruited at the Duke Eye Center. Here, we propose to expand our preliminary work into a larger clinical study, and validate these autoantibodies as serum and tear film biomarkers.

OBJECTIVES:

Our goal is to test the hypothesis that these protein markers can be utilized as neurodegenerative biomarkers for primary open angle glaucoma (POAG), and be used to develop a serum diagnostic assay. These were the two aims proposed:

Aim 1: To establish the expression profile of neurodegenerative markers associated with recent, new POAG diagnosis

Aim 2: To quantitate neurodegenerative markers associated with established POAG patients that exhibit disease progression.

RESULTS:

Substantial progress were made during the one-year funding period, but was significantly limited by major challenges. However, we have developed solutions to partially overcome these hurdles. These are discussed below as part of the progress made during this past year.

First, the 50% decrease in the actual amount of award given to us ($25k out of proposed $50k) made it impossible to support the salary of a clinical study coordinator/technician as originally proposed. This person would have facilitated the research by maintaining institutional research board (IRB) compliance, recruiting patients, setting up time for patients for a research visit, consenting patients, drawing blood, purifying serum, maintaining the samples in the freezer, etc. This work would be typically accomplished outside of normal clinic hours since the blood needs to be immediately processed in the laboratory following phlebotomy in the clinic.

Instead, the PI (myself) and one of the ophthalmology resident physician (Dr. Daniel Vu) performed this work while seeing 40-50 patients during regular clinic hours -- running back and forth between the clinic and the basic science laboratory few buildings away -- to immediately process the blood. Often Dr. Vu typically see patients in another clinic and building, he is often not available in my clinic when appropriate study subjects are available. Subsequently, patient recruitment to fulfill our aims proceeded slower than anticipated.

Secondly, one of our major team member (Dr. Rand Allingham), became ill and rapidly deteriorated last year. He passed away 5 months ago, and thus was not able to assist with research work during the funding period last year. His other research resources (outside of this CGF grant) also became unavailable after his death. As such, we have been working hard to obtain additional support.

Scientifically, we have made significant progress. Our original assay described in the original proposal was based on 2-dimensional protein gel electrophoresis, which is extremely labor intensive. Without the ability to

hire a study coordinator/technician to assist with the assay (discussed above), we first spent the first six months developing and testing an alternative assay that is less labor intensive and quicker using an ELISA with a fluorescent plate reader approach. We have also improved our statistical methodology to compared different serum neurodegeneration biomarkers.

With regards to patient recruitment for serum samples, we have made progress as well. At the time we submitted our proposal, our preliminary data consisted of 4 high-pressure glaucoma, 2 normal tension, and 4 normal control patients. In the last year, we have now successfully obtained and processed serum from 9 high pressure POAG patients, 4 normal tension glaucoma patients, and 13 normal control patients. These were age-matched, and patients conformed to our strict inclusion and exclusion criteria following an extensive review of their electronic medical records. We found that many glaucoma patients also have non-glaucomatous conditions in the retina and optic nerve that may confound our study, and thus were not appropriate study subjects.

With the approved no-cost extension into the upcoming year, we will continue our research effort. Our goal is to obtain at least 20-30 patients for each cohort by the end of this year. We are confident this goal is feasible and look forward to describing the results in a publication. The new alternative assay approach that we have developed this past year will also help accelerate our serum biomarker detection and data analysis. If we are successful with alternative research funding, these additional funds will help support a clinical coordinator/technician to facilitate the completion of this study.

PUBLICATIONS

There were no publications during this past year that are funded by this grant.