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Charlene Hudgins
Executive Director
Benign Essential Blepharospasm Research Foundation
P.O. Box 12468
Beaumont TX 77726

Dear Charlene:

Here find the final progress report for the project your foundation funded in 2013. The project was entitled *Neuropathology of Blepharospasm*. We are very grateful for the opportunity to pursue this project, where we proposed to evaluate 12 human blepharospasm brains and 12 matched controls over a 2 year period. The original aims are below:

- Aim 1. To determine if blepharospasm brains have evidence for Purkinje neuron loss, a finding recently reported for cervical dystonia in two independent studies.*
- Aim 2. To determine if blepharospasm brains have an increase in ectopic Purkinje neurons, an abnormality reported for other neurological disorders such as essential tremor.*
- Aim 3. To determine if blepharospasm brains have an increase in torpedo bodies, pathological neuritic swellings associated with dysfunction of surviving Purkinje neurons.*
- Aim 4. To determine if blepharospasm brains have an increase in hairy baskets, an abnormally tangled mass of basket cell neurites surrounding Purkinje neurons.*

As you may recall, our requested project period and budget were reduced from 2 years to 1 year. As a result, it was necessary to modify the scope of the original project. Although we proposed to evaluate 12 cases and controls, the total number was reduced to 6 cases and 6 controls. We collected brain samples from 6 individuals with blepharospasm, and 6 normal controls matched for age and sex. This material was collected from Emory University pathology resources, the University of Maryland Brain and Tissue Bank, the Mayo Clinic, and Washington University in St. Louis. It was our original intention was to include brains stored at Harvard in the Dystonia Brain Collective. Unfortunately, despite multiple requests to the managers of the Harvard collection over a period of 2 years, we never received any material from them.

All samples available to us were embedded in paraffin blocks, and sections cut on a microtome. At least one representative section from the cerebellum from each brain was subject to Nissl stains as a screen for tissue quality. All samples were reviewed by a board-certified pathologist and judged to be of good quality. Silver stains were completed next, again with results of sufficient quality to quantify all of the abnormalities described in Aims 1-4 above. Two microscopists evaluated each section, and provided digitized results for each of the target abnormalities. There was good inter-rater correlations between them for most of the measures, validating the methods used.

To summarize results briefly, no gross abnormalities were found in the cerebellum for any of the cases. However, quantitative assessments revealed changes that appeared to be similar to those we have reported in the past for cervical dystonia. Specifically, there was a small reduction in the linear density of Purkinje neurons in the cerebellum. We did not see any significant changes in ectopic Purkinje neurons, torpedo bodies, or hairy baskets.

Using simple statistical comparisons via Student's t-tests, the difference between blepharospasm and control brains was borderline ($p=0.051$). However, because of multiple related comparisons, we are now

taking a more sophisticated approach using multiple logistic regression. Whether or not the abnormalities we have quantified will reach statistical significance using this more tailored approach is being evaluated now.

The statistical outcome obviously depends on the number of cases we were able to study. Because of the cut in timeline and budget, this number was half that which we originally proposed. If the current dataset provides a rigorous statistical difference, we can conclude that neuropathological findings are similar to those of cervical dystonia. This conclusion opens the door to further studies to understand why these brain changes may be occurring. If the current dataset does not yield a statistical difference, we may conclude that insufficient specimens were evaluated to yield a conclusive result.

Sincerely,

A handwritten signature in black ink, appearing to be 'H. A. Jinnah', written in a cursive style.

H. A. Jinnah, M.D., Ph.D.