



Blood Biomarker for Parkinson's Disease

Dwight C. German, Ph.D.
UT Southwestern Medical Center

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Scientific Abstract

Using a novel high-throughput peptoid screening method, we have recently found a peptoid (PD2) that binds serum IgG3 antibody from PD patients significantly higher ($p=0.001$) than in normal controls. We used 104 normal control serum samples, 75 samples from PD patients, and 25 samples from *de novo* PD cases. The predictive accuracy of the peptoid biomarker is 68% for PD vs. control, but we found that for *de novo* PD cases the predictive accuracy is 84%. *The goal of this project is to validate our antibody biomarker for PD using de novo and age-matched control samples from PPMI (n=100/group).*

Method

PD2 Peptoid Synthesis

The PD2 peptoid will be synthesized on Polystyrene AM RAM resin (Rapp Polymer, Tübingen, Germany) with the methionine linker, used in the screening library, replaced by a cysteine linker so that the compounds can be immobilized using sulfhydryl-reactive chemistry. The peptoid is made with the following 4 amines: diaminobutane, ethanolamine, isobutylamine, and pyrrolidinone. The peptoid will be cleaved off the resin by incubating in a 95% trifluoroacetic acid, 2.5% triethylsilane, 2.5% water solution for 2 hrs. at room temp (RT). The peptoid will be purified using high-performance liquid chromatography and verified by MS analysis.

Peptoid ELISA Measurements

PD2 will be immobilized onto maleimide-activated 96-well plates (Pierce Biotechnology, Rockford, IL) by dissolving to 0.03 – 0.05 mM in a 0.1 M sodium phosphate, 0.15 M sodium chloride, 10 mM EDTA solution adjusted to pH 7.2 and incubating with shaking for 3 hrs. at RT. Plates will be washed with PBST and blocked with a 5% goat serum (Thermo Scientific, Rockford, IL) in PBST solution for 1 hr. at RT. Plates are washed again and incubated with target (serum) samples diluted in blocking buffer (1:1 PBST-1% BSA and SuperBlock) for 2 hrs. at RT. After washing, plates are incubated with mouse anti-human IgG3 (hinge)-HRP conjugate (SouthernBiotech, Birmingham, Alabama) diluted 1:1000 in PBST-1% BSA for 30 min at RT. After another wash, plates are incubated in TMB substrate for 16 min. at RT and stopped with 2M H₂SO₄. Plates are read at 450 nm. All samples are run in duplicate, and every assay contains





PD and Control serum pool samples to serve as internal controls. Results for individual samples are assessed as ratios to the Control serum pool so as to control for plate-to-plate variation. These methods have been used previously in our lab (e.g., Reddy et al., 2011; Zaman et al., 2016).

The serum samples were run twice: one time in the lab of Dr. Tom Kodadek and the second time in Dr. German's lab. Data from the first assay was collected such that the peptoid binding did not closely match with samples run previously in the German lab. Therefore a second analysis of the samples was done in Dr. German's lab during the months of October, November and December 2017. The results from this second assay included pooled control and pooled PD samples on each ELISA plate to be certain that the binding was consistently higher for the pooled PD sample vs. control. In addition, we also measured levels of IgG in each sample and the IgG3 level. In our previous publication (Yazdani et al., 2016) we found higher levels of IgG3 in PD vs. control samples and here we will see if we can replicate this finding with the PPMI samples.

References

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About the Authors

This document was prepared by Dr. Dwight German, UT Southwestern Medical Center, Department of Psychiatry. For more information please contact Dr. German at 214-648-7385, or dwight.german@utsouthwestern.edu.

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