PARKINSON'S PROGRESSION MARKERS INITIATIVE

Play a Part in Parkinson's Research

Blood Biomarker for Parkinson's Disease

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Scientific Abstract

Please describe the analysis performed (Should not exceed 200 words)

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 H_2SO_4. 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). We will first analyze the peptoid data from a test group of 41 subjects (from Italy), to be certain that our results are as predicted. Once we know that the results are as expected, we will run the remaining 159 samples.

References

Reddy MM, Wilson R, Wilson J, Connell S, Gocke A, Hynan L, **German D**, Kodadek T. (2011) Identification of candidate IgG biomarkers for Alzheimer's disease via combinatorial library screening. *Cell*, 144: 132-142. One of the 5 most cited Cell article for 2011.

Zaman S, Yazdani U, Deng Y, Li W, Gadad B, Hynan, L, Karp D, Roatch N, Schutte C, Marti CN, Hewitson L, **German DC** (2016). A search for blood biomarkers for autism: peptoids. *Sci Rep*, Jan 14;6:19164. doi: 10.1038/srep19164.

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