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Short communication

Cerebrospinal fluid biomarkers of central dopamine deficiency predict Parkinson's disease

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ABSTRACT

Background: Consistent with nigrostriatal dopamine depletion, low cerebrospinal fluid (CSF) concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC), the main neuronal metabolite of dopamine, characterize Parkinson's disease (PD) even in recently diagnosed patients. Whether low CSF levels of DOPAC or DOPA, the precursor of dopamine, identify pre-clinical PD in at-risk healthy individuals has been unknown.

Methods: Participants in the intramural NINDS PDRisk study entered information about family history of PD, olfactory dysfunction, dream enactment behavior, and orthostatic hypotension at a protocol-specific website. After at least 3 risk factors were confirmed by on-site screening, 26 subjects had CSF sampled for levels of catechols and were followed for at least 3 years.

Results: Of 26 PDRisk subjects, 4 were diagnosed with PD (Pre-Clinical PD group); 22 risk-matched (mean 3.2 risk factors) subjects remained disease-free after a median of 3.7 years (No-PD group). The Pre-Clinical PD group had lower initial DOPA and DOPAC levels than did the No-PD group ($p = 0.0302$, $p = 0.0190$). All 3 subjects with both low DOPA (<2.63 pmol/mL) and low DOPAC (<1.22 pmol/mL) levels, based on optimum cut-off points using the minimum distance method, developed PD, whereas none of 14 subjects with both normal DOPA and DOPAC levels did so (75% sensitivity at 100% specificity, $p = 0.0015$ by 2-tailed Fisher's exact test).

Conclusions: In people with multiple PD risk factors, those with low CSF DOPA and low CSF DOPAC levels develop clinical disease during follow-up. We suggest that neurochemical biomarkers of central dopamine deficiency identify the disease in a pre-clinical phase.

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1. Introduction

By the time Parkinson's disease (PD) manifests with motor signs, substantial loss of nigrostriatal dopaminergic neurons has already occurred. There is great interest in identifying cerebrospinal fluid (CSF) biomarkers of the neurodegenerative process, both for early diagnosis and to track effects of putative disease-modifying treatment or prevention strategies.

Consistent with profound striatal dopamine depletion, CSF concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC), the main neuronal metabolite of dopamine, are decreased in PD [1,2], even in patients with a recent diagnosis [3]. CSF levels of endogenous 3,4-dihydroxyphenylalanine (DOPA), the precursor of dopamine, are also decreased in levodopa-untreated patients [2] however, whether low CSF DOPAC or low CSF DOPA provides a neurochemical biomarker of pre-clinical PD in at-risk individuals has been unknown.

Here we report data from the PDRisk prospective cohort study of the National Institute of Neurological Disorders and Stroke (NINDS) that are relevant to this issue. In the PDRisk study, a small group of

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individuals with multiple PD risk factors (at least 3 of: family history, olfactory dysfunction [4], dream enactment behavior [5], orthostatic hypotension [6]) but without motor symptoms suggestive of PD, have been intensively and comprehensively studied and then re-evaluated at about 1.5-year intervals. The PDRisk subjects reported here underwent lumbar puncture for assays of CSF DOPAC and DOPA and were followed for at least 3 years.

2. Methods

Subjects in this study gave written informed consent to participate in intramural protocols approved by the NINDS institutional review board (IRB). Consent for the PDRisk study was in two forms—electronic at a protocol-specific, IRB-approved website and in writing at the time of evaluation at the Clinical Center of the National Institutes of Health (NIH).

2.1. Inclusion criteria

There were 4 categories of risk—genetic, olfactory, symptoms of RBD, and OH. With respect to genetics, a positive family history (one immediate or more than one non-immediate family member with PD) or positive genetic testing (e.g., LRRK2, alpha-synuclein, glucocerebrosidase) satisfied this criterion. Olfactory dysfunction reported at the protocol-specific website satisfied the criterion. To satisfy the RBD risk factor criterion, the individual or member of the individual's household must have reported movements of the body or limbs associated with dreaming and at least one of the following: potentially harmful sleep behavior, dreams that appeared to be acted out, and sleep behavior that disrupted sleep continuity. To satisfy the OH risk factor criterion, the individual must have reported symptoms of OH or having OH as defined by consensus criteria.

2.2. Exclusion criteria

People younger than 18 years old or older than 70 years old were excluded. A candidate subject was excluded if there was a disqualifying condition or clinical considerations required continued treatment with a drug likely to interfere with the scientific results.

2.3. Study design

The primary outcome measure was a diagnosis of PD by a neurologist who was blinded to the results of the catecholaminergic biomarkers testing. Data collection has been ongoing since 2009.

Recruitment was by advertisement using a Google ad referring to a protocol-specific, IRB-approved web page. Candidate participants who were flagged as reporting at least 3 risk factors were prescreened by a Research Nurse, to confirm eligibility criteria.

The screening examination at the NIH Clinical Center was to obtain consent for further participation in the study, verify at least 3 risk factors, and perform clinical and laboratory tests (e.g., autonomic function tests described below). Subjects with at least 3 confirmed risk factors underwent inpatient biomarkers testing.

For follow-up testing subjects were re-evaluated at about 1.5-year intervals, for neurological examinations and neuroimaging reassessments. Participants who started on exclusionary drugs after enrollment were not brought back for follow-up inpatient testing; however, they could be contacted by phone, mail, or secure e-mail, to learn of their clinical status.

PD was diagnosed according to accepted clinical criteria such as bradykinesia, rigidity, resting tremor, and imbalance.

2.4. Identification of OH

Each subject was evaluated in a dedicated patient testing room in the NIH Clinical Center. After at least 15 min with the subject at supine rest, the subject was tilted head-up at 90° for 5 min. OH was defined as a decrease in systolic blood pressure of at least 20 mmHg or in diastolic pressure of at least 10 mmHg between lying supine and head-up tilting for at least 3 min.

2.5. CSF collection

To obtain CSF, subjects underwent lumbar puncture by a neuroradiologist under fluoroscopic guidance. A total of 12 1-mL aliquots of CSF were obtained in cold 1.5-mL plastic sample tubes that were placed immediately in dry ice. We report data about CSF catechols in the 6th aliquot. CSF levels of DOPA and DOPAC were measured in our laboratory by batch alumina extraction followed by liquid chromatography with series electrochemical detection, as described previously [7].

2.6. ^{18}F-DOPA PET scanning

Each PDRisk study participant underwent head ^{18}F-DOPA positron emission tomographic (PET) scanning, as described previously by our group [1]. Briefly, 10 mCi of ^{18}F-DOPA was injected intravenously without carbidopa pre-treatment. The putamen/occipital cortex (PUT/OCC) ratio of ^{18}F-DOPA-derived radioactivity was calculated for the static 15-min image ending at 120 min after tracer injection. The putamen posterior/anterior (P/A) ratio of radioactivity was also measured, since in early PD the posterior putamen is more affected than the anterior putamen [8].

2.7. Study size, data analysis, and statistics

To estimate the required numbers of subjects, we used a log-rank test for the interval between biomarkers testing and diagnosis of PD and predicted that among individuals at risk of PD and who had abnormal catecholaminergic biomarkers, 80% would develop PD by 7.5 years of follow-up; and that among at-risk participants without abnormal biomarkers, 20% would develop PD during follow-up. Considering the possibility of dropouts, at an alpha value of 0.05 and beta value of 0.20, follow-up from a group of 26 subjects with complete baseline biomarkers data would be sufficient. An analysis was done after $\geq$ 3 years of follow-up, for calculation of the positive predictive value of the biomarkers. Calculation of the negative predictive value of absent biomarkers will be done at the end of the study (7.5 years of follow-up).

The individual carrying out the neurochemical assays (C.H.) was blinded as to the clinical risk factors and other biomarkers.

For each of the dependent measures, normality in the distribution of residuals was assessed by the Shapiro-Wilk test. The data for CSF DOPA had to be log-transformed to achieve normality. The data were then subjected to two-sample t-tests, to examine the differences between the PDRisk subjects with pre-clinical PD (Pre-Clinical PD group) and the PDRisk subjects without symptomatic PD at 3 years of follow-up (No-PD group). We also analyzed the data by the non-parametric Wilcoxon rank sum test.

Receiver operating characteristic curve analysis and a method based on the minimum distance between the point (0,1) and points on the curve were used to select the optimal cutoff thresholds for binary predictions. The dichotomized biomarkers data based on the cutoff values were then analyzed by 2-tailed Fisher's exact test.

A p value less than 0.05 defined statistical significance.

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