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Extra-nigral pathologies are common in Parkinson disease with freezing of gait: an *in vivo* PET study

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Abstract

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1. Research Project: A. Conception, B. Organization, C. Execution
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BACKGROUND—Cholinergic denervation has been associated with falls and slower gait speed and β -amyloid deposition with greater severity of axial motor impairments in Parkinson disease (PD). However, little is known about the association between the presence of extra-nigral pathologies and freezing of gait (FoG).

METHODS—Patients with PD ($n=143$; age 65.5 ± 7.4 years, Hoehn and Yahr stage 2.4 ± 0.6 , Montreal Cognitive Assessment score 25.9 ± 2.6) underwent [^{11}C]methyl-4-pi-peridiny propionate acetylcholinesterase and [^{11}C]dihydrotetrabenazine dopaminergic PET imaging, and clinical, including FoG, assessment in the dopaminergic “off” state. A subset of subjects ($n=61$) underwent [^{11}C]Pittsburgh compound-B β -amyloid PET imaging. Normative data were used to dichotomize abnormal β -amyloid uptake or cholinergic deficits.

RESULTS—FoG was present in 20 patients (14.0%). Freezers had longer duration of disease ($P=0.009$), more severe motor disease ($P<0.0001$) and lower striatal dopaminergic activity ($P=0.013$) compared to non-freezers. FoG was more common in patients with diminished neocortical cholinergic innervation (23.9%, $\chi^2=5.56$, $P=0.018$) but not in the thalamic cholinergic denervation group (17.4%, $\chi^2=0.26$, $P=0.61$). Subgroup analysis showed higher frequency of FoG with increased neocortical β -amyloid deposition (30.4%, Fisher Exact test: $P=0.032$). FoG frequency was lowest with absence of both pathology (4.8%), intermediate in subjects with single extra-nigral pathology (14.3%) and highest with combined neocortical cholinopathy and amyloidopathy (41.7%; Cochran-Armitage trend test $Z=2.63$, $P=0.015$). Within the group of freezers, 90% had at least one of the two extra-nigral pathologies studied.

CONCLUSIONS—Extra-nigral pathologies, in particular the combined presence of cortical cholinopathy and amyloidopathy, are common in PD with FoG and may contribute to its pathophysiology.

Keywords

acetylcholine; β -amyloid; dopamine; gait freezing; Parkinson’s disease; PET

Introduction

Advancing Parkinson disease (PD) is associated with disabling axial motor complications, such as freezing of gait (FoG), with diminishing responsiveness to dopaminergic medications^{1,2}. Nigrostriatal dopaminergic denervation secondary to α -synuclein related pathology is the defining pathologic feature of the great majority of early stage PD clinical manifestations³. Heterogeneous involvement of other projection systems and other pathologies variably exacerbate the effects of nigrostriatal denervation and is a significant contributor to the varied clinical features of advancing PD. Unlike the uniform presence of striatal dopaminergic denervation in clinically manifest PD, for example, there is significant heterogeneity in the range of cholinergic denervation seen in non-demented PD patients⁴. Neocortical cholinergic denervation is present only in approximately one third of non-demented PD patients when compared to normal control subjects⁴. Comorbid β -amyloid plaques are also variably present in PD⁵.

We previously identified pedunculo pontine nucleus (PPN)-thalamic and forebrain cortical cholinergic projection system degeneration as a prominent factor related to falls and gait

slowing in PD, respectively ^{6, 7}. We recently identified also that cortical β -amyloid deposition is associated with severity of postural instability and gait difficulties features in PD ⁸.

We set out to further refine these observations and test the hypothesis that extra-nigral changes of subcortical or neocortical cholinergic denervation (cholinopathy) and increased β -amyloid deposition (amyloidopathy) are factors associating specifically with FoG in PD.

Subjects and Methods

Subjects and clinical test battery

This cross-sectional study involved 143 subjects with PD (106 males and 37 females), mean age 65.5 ± 7.4 (range 50–84) years. PD subjects met the UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria ⁹. Fifty PD subjects were taking a combination of dopamine agonist and carbidopa-levodopa medications, 67 were using carbidopa-levodopa alone, 15 were taking dopamine agonists alone and 11 were not receiving dopaminergic drugs. No subjects were treated with anti-cholinergic or cholinesterase inhibitor drugs. Most subjects had moderate severity of disease: 4 patients in stage 1, 7 in stage 1.5, 35 in stage 2, 63 in stage 2.5, 30 in stage 3, 3 in stage 4 and 1 in stage 5 of the modified Hoehn and Yahr classification ¹⁰. Mean duration of disease was 6.0 ± 4.3 (SD; range 0.5–20) years. The mean motor examination score on the Movement Disorder Society Revised Unified Parkinson's Disease Rating Scale (MDS-UPDRS) was 32.5 ± 14.2 (range 8–72). Subjects receiving dopaminergic medications were examined in the morning after dopaminergic medications had been withheld overnight. The presence or absence of FoG was based on clinical examination and directly observed by the clinician examiner based on a non-zero score on item 3.11 'Freezing of Gait' of the MDS-UPDRS motor examination ¹¹. For most reliable assessment, FoG classification should be based upon objective confirmation by an experienced observer during clinical assessment rather than on patient recollection ¹². Details of gait speed, UPDRS motor, and cognitive testing from this cohort of patients are reported elsewhere ^{4, 8, 13}. All PD subjects previously reported in the quantitative studies by Petrou *et al.* (n=40) and Muller *et al.* (n=44) are included in the present expanded dataset ^{8, 13}.

Each patient underwent neuropsychological examination as described previously ⁴. Global cognitive composite z-scores were calculated based on normative data. Patients completed also a Montreal Cognitive Assessment with mean score of 25.9 ± 2.6 (range 15–30) ¹⁴.

This study was approved by the Institutional Review Board of the University of Michigan School of Medicine. Written informed consent was obtained from all subjects.

Imaging techniques

All subjects underwent brain MRI and [¹¹C] methyl-4-piperidiny propionate (PMP) (acetylcholinesterase) and [¹¹C]dihydrotetrabenazine (DTBZ) vesicular monoamine transporter type 2 (VMAT2) PET except for one subject where the DTBZ PET scan failed because of technical reasons. All DTBZ PET scans showed evidence of nigrostriatal degeneration. A subset of PD subjects (n=61) were clinically identified as being at increased

risk for development of dementia based on older age, subjective cognitive complaints and more severe motor disease underwent also [^{11}C]Pittsburgh compound B (PIB) β -amyloid PET imaging.

[^{11}C]dihydrotetrabenazine PET imaging was performed the morning after withholding dopaminergic medications overnight. MRI was performed on a 3 Tesla Philips Achieva system (Philips, Best, The Netherlands) and PET imaging was performed in 3D imaging mode with an ECAT Exact HR+ tomograph (Siemens Molecular Imaging, Inc., Knoxville, TN) as previously reported ⁴.

[^{11}C]DTBZ, [^{11}C]PMP, and [^{11}C]PIB were prepared as described previously ^{15, 16}. Dynamic PET scanning was performed for 70 minutes using a bolus dose of 15 mCi [^{11}C]PMP dose. Bolus/infusion protocols were used for [^{11}C]DTBZ (15 mCi) in 60 minutes and for [^{11}C]PIB (18 mCi) in 70 minutes ¹⁷.

Analysis

All image frames were spatially coregistered within subjects with a rigid-body transformation to reduce the effects of subject motion during the imaging session ¹⁸. Interactive Data Language image analysis software (Research systems, Inc., Boulder, CO) was used to manually trace volumes of interest (VOI) on MRI images to include the thalamus, caudate nucleus, and putamen of each hemisphere. Total neocortical VOI were defined using semi-automated threshold delineation of the cortical gray matter signal on the MRI scan ⁴.

[^{11}C]dihydrotetrabenazine distribution volume ratios were estimated using the Logan plot graphical analysis method with the striatal time activity curves as the input function and the total neocortex as reference tissue, a reference region overall low in VMAT2 binding sites, with the assumption that the non-displaceable distribution is uniform across the brain at equilibrium ¹⁹.

Acetylcholinesterase [^{11}C]PMP hydrolysis rates (k_3) were estimated using the striatal volume of interest (defined by manual tracing on the MRI scan of the putamen and caudate nucleus) as the tissue reference for the integral of the precursor delivery ²⁰. There are two major brain cholinergic projection systems; the basal forebrain complex providing the principal cholinergic input to the cortical mantle and the PPN complex, providing cholinergic inputs to the thalamus, cerebellum, basal ganglia, other brainstem nuclei, and the spinal cord ²¹. Both degenerate variably in PD. Acetylcholinesterase PET imaging assesses cholinergic terminal integrity with cortical uptake reflecting largely basal forebrain neuron integrity and thalamic uptake principally reflecting PPN integrity.

[^{11}C]PIB PET data were analyzed using the Logan graphical method and the cerebellum as reference region ^{22, 23}. Global cortical PIB distribution volume ratio was calculated.

In order to determine the frequency of each of the two extra-nigral pathologies, we used normative data to dichotomize abnormal uptake or deficits.

We classified AChE PET scans as either below or within normal range due to the significant overlap of cholinergic innervation seen between subjects with PD and healthy controls. Abnormal neocortical and thalamic cholinergic innervation was based on a 5th percentile cut-off from non-PD elderly normative data (n=29, mean age 66.8 ± 10.9 years comparable to the current patient cohort) ⁴.

We used [¹¹C]PIB β -amyloid data from young to middle-aged healthy subjects (n = 11, 5 males/6 females), mean age 46.7±3.4 (range 40–50 yr) to define elevated [¹¹C]PIB binding in the patients, as significant fibrillary β -amyloid deposition occurs in many cognitive normal elderly individuals ²⁴. The maximum normal neocortical [¹¹C]PIB distribution volume ratio was 1.17 and PD patients with values exceeding this threshold were defined as exhibiting increased β -amyloid binding.

Standard pooled-variance t or Satterthwaite's method of approximate t tests (t_{approx}) were used for group comparisons. χ^2 testing were performed for comparison of proportions between groups and with Fisher Exact test in the presence of small cell populations. Cochran-Armitage trend test was performed to determine a dose-response relationship when assessing the association between no, single or dual extra-nigral pathologies and FoG status. In the subset of subjects who had completed all 3 PET measures, multiple logistic regression was performed using FoG status as the outcome parameter, and cholinopathy, amyloidopathy, striatal VMAT2 bindings as PET regressors and clinical confounder variables (motor, cognitive performance and duration of disease) that were shown to be different between freezers and non-freezers. Analyses were performed using SAS version 9.2, SAS institute, Cary, North Carolina).

Results

Patients with FoG

There were 20 subjects with observed FoG (14.0%). Freezers had longer duration of disease, more severe motor impairments and lower striatal dopaminergic activity compared to non-freezers (Table 1). There were no significant differences in age or gender distribution between groups. Freezers manifested a trend toward lower cognitive performance compared to non-freezers (Table 1).

Cholinergic PET findings in freezers

Neocortical cholinergic denervation was heterogeneous with 46 PD patients (32.2%) exhibiting neocortical acetylcholinesterase activity below normal range activity levels. Below normal range PPN-thalamic acetylcholinesterase activity levels were seen in 23 patients (16.1%). There was a significantly higher proportion of FoG subjects in the below normal range compared to the normal range neocortical acetylcholinesterase group: 23.9% versus 9.3% ($\chi^2=5.56$, $P=0.018$, Figure 1A). There was no significant difference in the proportion of freezers in the below normal range versus normal range PPN-thalamic acetylcholinesterase group: 17.4% versus 13.3% ($\chi^2=0.26$, $P=0.61$; Figure 1B).

Subgroup analysis of β -amyloid PET findings in freezers

A subset of PD subjects ($n = 61$) underwent also [^{11}C]Pittsburgh compound B (PIB) β -amyloid PET imaging. Subjects in this subgroup were older (69.0 ± 6.3 versus 62.9 ± 7.1), had more severe cognitive impairments (composite z-score -0.87 ± 0.96 versus -0.04 ± 0.68), slightly higher Hoehn and Yahr stage (2.7 ± 0.5 versus 2.3 ± 0.6) compared to the remainder of the subjects ($n=82$). However, there was no significant difference in the frequency of FoG between these two subgroups (16.4% versus 12.2% ($\chi^2=0.51$, $P=0.44$)). Increased neocortical β -amyloid accumulation was present in 23 of the 61 subjects (37.7%) in the subgroup who completed the [^{11}C]PIB scan. There was a significantly higher proportion of patients with FoG in the “positive” compared to the “negative” neocortical β -amyloid group: 30.4% versus 7.9% (Fisher Exact test: $P = 0.032$; figure 1C).

Proportion of freezers in subgroup combinations of patients with cortical amyloidopathy and/or cholinopathy

There was no significant association between the presence of cortical cholinopathy and amyloidopathy ($\chi^2=0.32$, $P=0.58$) with 47.8% of subjects with amyloidopathy have normal range neocortical cholinergic innervation and 52.2% below normal range activity. Combined pathologies were present in 19.7% of patients, absence of both in 34.4%, with the remainder exhibiting either isolated cholinopathy (27.9%) or amyloidopathy (18%).

FoG frequency was lowest in subjects with absence of both pathologies (4.8%), intermediate in subjects with single extra-nigral pathology (14.3%) and highest with combined neocortical cholinopathy and amyloidopathy (41.7%; Fisher Exact test: $P=0.03$). There was a significant trend indicating a dose-response association between absence, presence of single and dual extranigral pathologies (Cochran-Armitage trend test $Z=2.63$, $P=0.015$; Figure 2). Within the group of freezers, 90% of all freezers had at least one of the two extra-nigral pathologies studied. Figure 3 illustrates low range cortical AChE and “positive” β -amyloid imaging findings in PD freezer compared to the non-freezer.

Multiple logistic regression analysis was performed to evaluate the relative effects of neocortical amyloidopathy versus cholinopathy while controlling for effects of potentially confounding variables, including striatal VMAT2 binding, estimated duration of motor disease, MDS-UPDRS motor score and composite cognitive z-score (Table 2). There was no significant difference in age between freezers (69.2 ± 6.7) and non-freezers (68.0 ± 4.1 ; $t=0.56$, $P=0.58$) in this subgroup. There was an overall significant model ($\chi^2=17.66$; $P=0.0072$) with neocortical amyloidopathy as the only significant predictor variable while neocortical cholinopathy was no longer significant. Nigrostriatal dopaminergic denervation had borderline significance in the model ($P=0.057$).

A *post hoc* analysis was performed to evaluate the prediction of FoG status based on absolute neocortical β -amyloid [^{11}C]PIB distribution volume ratio rather than as a binary classification variables. Results of the multivariate logistic regression analysis (total model $\chi^2 = 17.26$; $P = 0.0084$) using the absolute neocortical β -amyloid [^{11}C]PIB distribution volume ratio as a predictor variable confirmed the binary amyloidopathy parameter findings ($\chi^2 = 3.85$; $P = 0.049$).

Discussion

FoG is a debilitating feature of PD, with poorly defined pathophysiological mechanisms. Sudden freezing may be related to altered neocortical regulation of movement, perhaps exacerbated by progressive impairment of subcortical locomotor centers function²⁵. Several studies explored the sources of altered cerebral functions associated with FoG. These studies show evidence of disruption of cortical function, including neocortical networks involved in executive functions and sensorimotor perception in subjects who tend to have severe nigrostriatal denervation, particularly of the caudate nucleus^{25–27}. A recent resting state functional MRI brain connectivity study identified reduced cortical network connectivity in PD with FoG, consistent with a role for cortical dysfunction in FoG²⁷. Localization of the key nodes within the locomotor network whose dysfunction is responsible for FoG remains unclear. Recent studies emphasize altered interactions between subcortical, in particular the PPN, and cortical regions. For example, one recent functional MRI study of PD subjects with known FoG during a virtual reality timed ‘up and go’ gait task provides evidence of dysfunction across coordinated neural networks, including the caudate nucleus, globus pallidus pars interna, thalamus, and mesencephalic locomotor center²⁸. A recent diffusion tensor imaging study showed evidence of reduced connectivity of the PPN and cortical regions²⁹. Similarly, a PPN deep brain stimulation study showed significant regional cerebral blood flow increments not only to subcortical but also to cortical regions, including the sensorimotor and supplemental motor cortices³⁰.

The main findings in this study reflect an association between presence of observed FoG and extra-nigral pathologies. However, mechanistic or etiopathogenetic inferences cannot be drawn from these observations. Furthermore, our PET markers may indicate the presence of cortical pathology but cannot explain the episodic nature of the FoG movement disorder. In this respect, our findings may identify a weak link within a neural circuit where freezing behavior in PD may occur because of impaired communication between complimentary yet competing neural networks^{31, 32}.

Our findings support the role of neocortical changes in PD patients with FoG. We found that the effects of cholinergic projection system deficits associated with FoG were driven by neocortical denervation but not by PPN-thalamic degeneration. Degeneration of cholinergic PPN-thalamic projections is associated with postural reflex impairments, whose underlying pathophysiology may differ from that leading to FoG^{33, 34}. However, PPN-thalamic and forebrain cortical cholinergic denervation do partially overlap⁴ and a potential role of PPN-thalamic cholinergic denervation cannot be excluded.

We recently reported a relationship between postural instability and gait difficulty feature severity and neocortical β -amyloid burden in PD patients at risk for development of dementia⁸. Similar findings have been reported for lower cerebrospinal fluid β -amyloid concentrations in patients with PD and the postural instability and gait difficulty motor phenotype³⁵. These associations of cerebrospinal fluid β -amyloid concentrations with severity of postural instability, gait difficulty and lower limb bradykinesia were also independent from age and cognitive functions in PD patients and no association between cerebrospinal fluid markers and other motor features were found³⁵.

Findings of lack of significant cholinergic status predictor of FoG in the multivariate model limited to the subgroup of 61 patients who completed all three PET scans should be considered as preliminary, given the smaller sample size and because concurrent presence of amyloidopathy and cholinopathy showed a significant dose-response effect in a trend analysis comparing FoG status relative to absence or presence of one or two of these extra-nigral pathologies. The preliminary multivariate logistic regression analysis in the subset of 61 subjects who completed all three PET scans identified amyloidopathy as a predictor of FoG independent of the degree of cognitive impairment and other clinical confounder variables. Although the extra-nigral pathologies of cortical cholinopathy and amyloidopathy are common in subjects with FoG they are not exclusively present in FoG and that other pathologies or mechanisms may also play a role. These preliminary observations need to be confirmed in future prospective studies with semi-quantitative assessment of FoG using dedicated provocation protocols ¹², preferably performed in both the dopaminergic medication 'on' and 'off' states.

We found that freezers had more severe nigrostriatal denervation compared to non-freezers in our study and nigrostriatal denervation had borderline significant prediction of FoG in the multifactorial model. This is in keeping with the fact that FoG can respond to dopaminergic medications in some subjects ³⁶, such as in patients with less severe disease. These findings emphasize multi-system changes underlying FoG in PD, which is supported by our findings of a dose-response relationship between the higher frequency of FoG in subjects with combined extra-nigral pathologies, compared to a single presence or absence of both. Relatively isolated impairment of a single system in PD (i.e., nigrostriatal denervation) may not lead to the development of some motor impairments because of adaptive plasticity in remaining intact systems. The degree of dopamine responsiveness of gait freezing may depend on the integrity and adaptive plasticity of the remainder of the neural networks supporting mobility functions.

A limitation of our study is that assessment of FoG was based on an unprovoked protocol with no special maneuvers like walking through narrow passages or making turns. This may limit the sensitivity of our FoG assessment and may explain the relatively low frequency of FoG in our study population. This low sensitivity may also result in a relative underestimation of effect size estimates. A relative advantage is the high specificity of FoG classification. Patients on cholinesterase inhibitors were not eligible for this study since we were performing acetylcholinesterase PET imaging. This exclusion criterion resulted in a predominantly non-demented cohort and may provide an alternative explanation for the relatively low frequency of FoG in our study. Another limitation of the study is that the subset of 61 PD subjects who underwent all three PET scans were older, had greater cognitive impairments, and slightly higher Hoehn and Yahr stage compared to the remainder of the subjects. However, there was no significant difference in the frequency of FoG between these two subgroups. Furthermore, despite that fact that all subjects in this subgroup had more significant cognitive impairment the frequency of amyloidopathy in subjects with FoG was nearly 4-fold higher compared to the non-FoG at risk subjects. We conclude that cortical cholinopathy and amyloidopathy are common extra-nigral pathologies in PD with FoG and in the presence of more severe nigrostriatal degeneration may contribute to its pathophysiology.

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Abbreviations

FoG	freezing of gait
MDS-UPDRS	Movement Disorder Society Revised Unified Parkinson's Disease Rating Scale
PD	Parkinson's disease
PIB	Pittsburgh compound B
PMP	methyl-4-piperidinyl propionate
PPN	pedunculopontine nucleus
VMAT2	vesicular monoamine transporter type 2

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Figure 1.

Proportion of patients with FoG in normal range versus below range cortical (Figure 1A), and PPN-thalamic cholinergic activity (Figure 1B). FoG was more common in patients with diminished neocortical cholinergic innervation but not in the thalamic cholinergic denervation group. Subgroup analysis of patient who completed also β -amyloid PET showed higher frequency of FoG in patients with positive compared to negative neocortical β -amyloid deposition (Figure 1C). Note: * indicates statistical significant group difference $P < 0.05$.

Figure 2.

Proportion of freezers in subgroups of patients with absence, single or combined presence of cortical amyloidopathy and/or cholinopathy. There was a significant trend effect with FoG frequency being lowest with absence of both pathology, intermediate in subjects with single extra-nigral pathology and highest with combined neocortical cholinopathy and amyloidopathy (Cochran-Armitage trend test $Z=2.63$, $P=0.015$). Note: * indicates statistical significance of trend $P < 0.05$.

Figure 3.

AChE (upper row) and β -amyloid imaging (lower row) findings in PD non-freezer (left) and freezer (right). Images illustrate reduced cortical cholinergic activity and increased β -amyloid deposition in the freezer compared to the non-freezer.

Table 1

Mean ($\pm$ SD) values of demographic, clinical, cognitive and striatal [^{11}C]dihydrotetrabenazine VMAT2 distribution volume ratios in the patients with PD without versus with FoG. Gender distribution is presented as proportions. Levels of statistical difference between groups are also presented.

	PD without FoG (n = 123)	PD with FoG (n = 20)	Statistical significance
Age	65.4 $\pm$ 7.7	66.4 $\pm$ 6.0	t = 0.58; P = 0.56
Gender (females/males)	34/89	3/17	$\chi^2 = 1.43$; P = 0.23
Duration of motor disease (years)	5.6 $\pm$ 4.1	8.3 $\pm$ 4.7	t = 2.65; P = 0.009
Hoehn and Yahr stage	2.4 $\pm$ 0.5	3.0 $\pm$ 0.7	$t_{\text{approx}} = 5.23$; P < 0.0001
Motor MDS-UPDRS	30.3 $\pm$ 12.9	46.7 $\pm$ 14.0	t = 5.21; P < 0.0001
Montreal Cognitive Assessment	26.0 $\pm$ 2.6	25.3 $\pm$ 2.7	t = 1.18; P = 0.24
Global cognitive composite z- score	-0.34 $\pm$ 0.87	-0.75 $\pm$ 1.06	t = 1.92; P = 0.058
Striatal VMAT2 binding	1.96 $\pm$ 0.27	1.81 $\pm$ 0.27	t = 2.53, P = 0.013

Note: t_{approx} = Satterthwaite's method of approximate t tests.

Table 2

Results of multiple logistic regression analysis and FoG in PD patients. Overall model was significant ($\chi^2 = 17.66$; $P = 0.0072$).

Variable	Logit regression coefficient	Standard Error	χ^2 ; P value
Cortical acetylcholinesterase group status (below versus normal range)	0.31	0.48	0.41; $P = 0.52$
Cortical β -amyloid status (positive versus negative)	0.95	0.48	3.97; $P = 0.046$
Striatal VMAT2 distribution volume ratio	-6.40	3.36	3.63; $P = 0.057$
Duration of motor disease	-0.05	0.09	0.28; $P = 0.59$
Motor MDS-UPDRS score	0.05	0.03	2.23; $P = 0.14$
Global cognitive composite z-score	0.48	0.55	0.76; $P = 0.38$