Post-Doctoral Research

Nutritional and metabolic factors associated with the development and progression of Parkinsonian symptoms

Maria Maraki

Supervisor: Maria Yannakoulia

Associate Professor of Nutrition and Dietary Behavior

Department of Nutrition and Dietetics,

School of Health Science and Education, Harokopio University

In association with the 1st Department of Neurology,

Medical School, National and Kapodistrian University of Athens

State Scholarships Foundation, 2019

Nutritional and other determinants of Parkinson's Disease

Extended synopsis of Post-Doctoral Research

MARIA MARAKI, APRIL 2019

Abstract

Parkinson's Disease (PD) develops progressively remaining at a pre-diagnostic stage for many years. The International Parkinson and Movement Disorder Society (MDS) recently introduced a methodology for probability score calculation for prodromal PD (pPD). The aim of this postdoctoral research was to investigate the association of dietary (Mediterranean Diet adherence) and other factors (such as physical activity, cognitive function, motor function, frailty syndrome) with pPD (symptoms, probability, status). Data from a population-based cohort study of older adults (HEllenic Longitudinal Investigation of Aging and Diet–HELIAD) in Greece were used. Probability of pPD was calculated according to MDS research criteria. A detailed food frequency questionnaire was used to evaluate dietary intake and calculate Mediterranean diet adherence score. Physical activity was assessed with a physical activity questionnaire and, indirectly, with gait speed tests and a motor complaints questionnaire. Frailty was evaluated according to definitions of the phenotypic and multidomain approach. Cognitive performance in 5 cognitive domains was assessed by a detailed neuropsychological battery. Logistic and linear regression models were performed to investigate associations between each factor (MeDi score, frailty, physical activity etc) and probability of pPD, either continuous or dichotomous (≥30% probability score). The median probability of prodromal PD was 1.9%, ranging from 0.2 to 96.7% in 1731 PD-free individuals over the age of 65 (41% male). Lower probability for prodromal PD ($p<0.001$) in the higher Mediterranean diet adherence groups was noted, driven mostly by non-motor markers of prodromal PD, depression, constipation, urinary dysfunction and daytime somnolence. Each unit increase in the Mediterranean diet score was associated with a 2% decreased probability for prodromal PD ($p<0.001$). On the other hand, for each unit increase in motor complaints score and for each kcal/kg/day lower energy expenditure (corresponding to 20min of light walking/day for a 75-kg man) there was a 27 and 3% higher probability for prodromal PD, respectively ($P<0.001$). Higher probability of pPD was also

related to lower performance in all cognitive domains (memory, language, executive, attention, and visuospatial function) ($p<0.001$). In addition, frail participants had approximately 3 times higher pPD probability score ($p<0.001$). We concluded that adherence to the Mediterranean diet and physical activity is associated with lower probability of pPD in older people, while frailty status and lower cognitive performance was associated with pPD. Further studies are needed to elucidate the potential causality of these associations as well as the underlying neurobiological mechanisms.

INTRODUCTION

Due to increasing life expectancy, neurodegenerative diseases, such as **Parkinson's Disease (PD),** have continuously increased over the years[1-5]. PD develops progressively, presenting several motor and non-motor symptoms [6], and, currently, there is no fully treatment of the disease. As PD remains at a pre-diagnostic stage for many years, the International Parkinson and Movement Disorder Society (MDS) recently published research criteria and introduced a comprehensive method of calculating probability score for **prodromal PD (pPD)**[7]. Very limited data regarding pPD are available, while studies on protective lifestyle factors are warranted.

Meanwhile, several studies have investigated the role of diet and physical activity in PD [8-10]. Studies on specific nutrients and foods showed inconsistent results [11, 12]. On the other hand, people eat meals containing a variety of foods consisting of a variety of nutrients that may act interactively or synergistically[13]. Thus, using a whole-diet approach, i.e., dietary patterns, provides an alternative and complementary tool to understand the role of diet in chronic, including neurodegenerative, diseases [13, 14]. Adherence to the Mediterranean Diet (MeDi) is associated with reduced odds or risk for PD, in studies in the US population [15, 16]. However, whether MeDi is related to pPD, or some of its manifestations (motor and non-motor markers), is currently unknown. Similarly, results on the effects of cognitive status, physical activity, and other metabolic and motor factors on PD are limited and for some factors conflicted, while associations with pPD are unknown.

Frailty is a term used to describe the increased vulnerability levels observed mainly in older people caused by accumulation of multiple deficits [17, 18]. Frail people present a decline in various domains of human functioning (mobility, gait, muscle strength, cognition, physical activity) and are more susceptible to adverse health outcomes (including hospitalization, loss of autonomy, falls and mortality) [19]. Regarding the assessment of frailty, many instruments have been

developed and used but overall two approaches have prevailed: a) the biological or phenotypic approach, which focuses on the physical aspects of frailty, and b) the multidomain approach, as part of a broader perspective which includes a combination of physical measures, syndromes, diseases and psychosocial factors [20]. Some core features of frailty syndrome (low gait speed, age-dependency, and physical appearance of weakness) are also characteristics of PD. Similarities between frailty and PD probably underlie shared pathophysiological mechanisms. Data regarding the association between frailty syndrome and PD are limited, derived mostly from cohorts of people diagnosed with PD [21-25]. These studies showed that frailty prevalence is higher among people with PD [23, 24] or mild parkinsonian symptoms [22], compared to that observed in general older population. To our knowledge, there is no study examining the association between frailty syndrome and pPD.

To sum up, the purpose of this postdoctoral research was to investigate the association of dietary (Mediterranean Diet adherence) and other factors (such as physical activity, cognitive function, motor function, frailty syndrome) with pPD (symptoms, probability, status) and PD in a general population of community dwelling older population in Greece.

METHODS

Study Population and Design

The HEllenic Longitudinal Investigation of Aging and Diet (HELIAD) is a large-scale, population-based, multidisciplinary study, designed to assess the prevalence and incidence of several neuropsychiatric conditions of aging and possible associations with diet, in Greece. Participants in HELIAD were selected among community-dwelling population through random sampling (age≥65, no exclusion criteria) from two areas in Greece. Several demographic, medical,

environmental, clinical, nutritional, and neuropsychological determinants were collected. Qualified neurologists, trained neuropsychologists and dieticians administered all questionnaires and conducted face-to-face interviews. Details on HELIAD design, participation rates and clinical and neuropsychological evaluation have been previously published [26-31]. Below, we provide details that are relevant to the present research. All volunteers gave informed written consent prior to participation. Study procedures were approved by the relevant Institutional Review Boards.

We collected demographic characteristics of the participants (age, sex, years of education). A series of questions were posed to assess the type and amount of tobacco use. Pesticide exposure was assessed using a structured pretested questionnaire[32]. Participants provided information regarding all previous neurological conditions, medical problems, illnesses and current medications. We also collected information regarding the medical history of the participants' first degree relatives, with particular attention to neurological diseases. Clinical co-morbidities were recorded using a questionnaire containing 23 clinical conditions.

Participants were screened for depressive symptoms and anxiety over the past week, using the 15-item Geriatric Depression Scale[33] and the 7-item anxiety subscale of the Hospital Anxiety and Depression Scale[34], respectively. Quantitative and qualitative features of sleep during the last month were assessed using the 12-item Medical Outcomes Study Sleep Scale[35]. Perceived changes in performance of daily activities and self-care habits were assessed with the Blessed Dementia Scale[36].

Parkinsonian signs and symptoms were evaluated with the UPDRS motor part (part III)[37]. We also designed and administered a structured questionnaire to determine whether core (e.g, parkinsonism), suggestive [e.g., REM sleep behavior disorder (RBD)] or supportive features (e.g, systematised delusions) of

the revised diagnostic criteria for Dementia with Lewy Bodies were present[26, 38]. Finally, we administered the 12-item Neuropsychiatric Inventory[39].

Participants were examined by certified neurologists, or as an exception, on rare occasions, by neurology residents towards the end of their training (as part of a research project assignment). The obtained information was reviewed and clinical diagnosis for each participant was reached using published criteria at expert consensus meetings including the neurologists who examined the participants (junior neurologists, and ED, GMH, and NS as senior neurologists) and the neuropsychologists (psychometricians and MHK as senior neuropsychologist). PD diagnosis was reached through standard clinical research procedures: neurological clinical evaluation (past history, symptomatology, examination etc), UPDRS administration, inspection of medications etc[40]. The diagnosis of dementia was based on Diagnostic and Statistical Manual of Mental Disorders -IV-text revision criteria,[41] the diagnosis of probable or possible Alzheimer disease was made according to the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer Disease and Related Disorders Association criteria.[42] The diagnosis of vascular dementia was based on a history or clinical evidence of stroke, the presence of a clear temporal relation between stroke and the onset of dementia and the results of Hachinski Ischemia Scale score.[43] Lewy body and frontotemporal dementias were considered according to respective criteria.[44, 45] Mild cognitive impairment (MCI) was diagnosed according to Petersen Criteria.[46] Additionally a detailed neuropsychological battery was used to assess participants' cognitive status [27, 47-56] and standardized scales to evaluate functional status as previously described.[26, 27, 31]. For analyses purposes, we calculated a composite z-score constructed from cognitive tests.

MDS research criteria and calculation of pPD probability

Three independent neurologists (Nikolaos Skarmeas, Georgia Xiromerisiou and Maria Stamelou) reviewed questionnaires and clinical examinations included in

the HELIAD study to identify variables corresponding to MDS risk and prodromal markers. Data on all risk markers proposed by MDS, except occupational solvent exposure and substantia nigra hyperechogenicity, were available (i.e., sex, pesticides' exposure, caffeine use, smoking, first degree relatives with PD [genetics]). Moreover, information on the following prodromal markers included in the MDS research criteria for pPD were available: probable RBD, excessive daytime somnolence, orthostatic hypotension, possible subthreshold Parkinsonism, constipation, urinary dysfunction, erectile dysfunction, depression or anxiety without depression (for details, see **Tables 1 & 2**). Information on olfactory dysfunction and tracer uptake of the presynaptic dopaminergic system (SPECT/PET) were not available.

As recommended by MDS, pre-test probability of pPD was calculated according to participant age. Individualized likelihood ratios (LRs) were calculated for each risk and prodromal marker. Missing values were scored as 1.0. Total risk LR and total prodromal LR was calculated separately by multiplying the relevant markers and these LRs were then multiplied to generate the total LR. This total LR was combined with pre-test probability to calculate the final post-test probability of pPD.

$$\text{LR risk markers} = LR_{sex} \times LR_{pesticides} \times LR_{non\text{-}caffeine} \times LR_{non\text{-}smoking} \times LR_{genetics}$$

$$\text{LR prodromal markers} = LR_{probable\ RBD} \times LR_{daytime\ somnolence} \times LR_{constipation} \times LR_{subParkinsonism} \times LR_{urinary\ dysfunction} \times LR_{erectile\ dysfunction} \times LR_{depression/anxiety}$$

$$\text{Total LR} = \text{LR risk markers} \times \text{LR prodromal markers}$$

$$\text{Pre-test odds} = (\text{Pre-test probability}/100) \,/\, (1-(\text{Pre-test probability}/100))$$

$$\text{Post-test odds} = \text{Pre-test odds} \times \text{Total LR}$$

$$\text{Post-test probability of prodromal PD} = (\text{Post-test odds} \,/\, (\text{Post-test odds} + 1)) \times 100$$

For the purpose of the current analysis, we used post-test probability as a continuous variable, but also the cut-offs of 30% and 80%[7, 57].

Dietary assessment

Dietary intake was assessed with a semi-quantitative food frequency questionnaire (FFQ) validated for the Greek population[58]. FFQ questions referred to the last month. A trained researcher guided each participant or his/her caregiver in completing the questionnaire. Adherence to the MeDi was evaluated through the MeDi Score[26, 59]. Briefly, consumption of non-refined cereals, fruits, vegetables, legumes, potatoes, fish and olive oil is presumed to closely characterize the MeDi pattern and scored positively, while consumption of meat and meat products, poultry and full-fat dairy products is presumed to diverge from this dietary pattern and scored negatively, while for alcohol intake scoring has an inverted U shape. The total score ranges from 0 to 55, with higher values indicating greater adherence to the MeDi. For the purposes of the present study, the MeDi score was used either as a continuous variable, or as quartiles: the first quartile served as the reference group and was compared to the other quartiles, i.e., Q2, Q3 and Q4, with the latter indicating the greatest MeDi adherence [26, 29].

Physical activity assessment and other measures of motor function

Physical activity was assessed with the Athens Physical Activity Questionnaire (APAQ), validated for the Greek population [60]. APAQ questions referred to the last week. A trained researcher guided each participant or his/her caregiver in completing the questionnaire. APAQ was used to estimate total daily energy expenditure (TEE) as kcal/kg of body weight/day.

Physical activity was also assessed indirectly with a 12-item questionnaire on motor/walking complaints. The questions are designed to assess self-reported subjective symptoms of gait dysfunction and postural instability (patient-reported walking difficulties, freezing, small steps, dragging feet, imbalance and getting up from a chair/bed and sitting down). A trained researcher guided each participant in completing the questions. Ten questions were dichotomous (yes/no:1/0), while two questions had six possible answers that were

dichotomously categorized before scoring. The total motor complaints score ranges from 0 to 12, with higher scores indicating increased subjective motor impairment and therefore low physical activity.

We also used two gait speed tests (for 1m and 4m) as indirect measures of fitness/physical activity [61-64], as they have been shown to be related to future gait and balance problems in older adults and in PD patients[61-64]. Each gait speed test was performed twice and time to completion (sec) was recorded and averaged, with greater time needed to perform the test being an indicator of motor impairment. For the 4m-test we also used the cutoff speed of 0.8m/s[65].

Anthropometrics

Height and weight were measured to the nearest 0.5cm and 0.5kg, respectively. Body Mass Index (BMI) was calculated by dividing the weight (kg) by the height (m^2). Waist circumference was measured using an anelastic tape in the midway between the lower rib margin and the top of the iliac crest. World Health Organization criteria for obesity[66] and abdominal obesity status[67] were used.

Frailty assessment

We took into account both the biological and the multidomain approaches of frailty [20]. Thus we used two measurements of frailty: the Fried definition [17] and the Frailty Index (FI) [18, 68]. Briefly, regarding the Fried definition, participants who met three or more criteria were considered as frail, those with one or two criteria present as pre-frail and those who met none of the Fried's criteria as non-frail The five criteria were the following: (1) Slow walking speed was defined as the lowest 20% of our study population for the 4 meters walking speed test (adjusted for sex and height); (2) Shrinking/weight loss was defined as Body Mass Index (BMI) <18.5kg/m^2; (3) Poor endurance/exhaustion was evaluated as a negative response to the question taken from the Geriatric Depression Scale "Do you feel full of energy?" [69]; (4) Low physical activity was

estimated based on TEE calculated from the APAQ [70]. The lowest 20% for each sex was assumed to be indicative of this frailty criterion; (5) Weakness was defined as grip strength in the lowest 20% adjusted for sex and BMI. Grip strength of the dominant hand was measured with an electronic dynamometer (model MG-4800, UK) and the mean strength of three trials was used in the current analysis. Details regarding the operationalization of this definition and specific cutoffs for walking time and grip strength criteria have been reported in previous publications from the HELIAD study [71].

Fried's criteria are mainly physical, thus frailty defined in this way is more likely to share similar characteristics with PD. Thus, we also operationalized frailty based on the multidomain definition proposed by Rockwood and Mitnitski [18, 68]. This approach is based on the calculation of a Frailty Index, defined as the ratio of deficits presented in a person to the total number of deficits considered in a medical evaluation. For the construction of FI we followed the standard procedure described by Searle et al [72]. In the current study, 38 variables regarding diseases, syndromes, functioning in activities of daily living, cognitive decline, mood disorders and performance on physical activities, were included for the assessment of frailty (Supplementary Table 1). To avoid circularity when investigating associations with PD, variables related to PD or included in the pPD calculation (e.g., PD diagnosis, intention tremor, family history of PD) were not included in the operationalization. According to this index, a score of 0.25 is the cut-off point for frailty, with higher scores indicating the presence of more "deficits", and, thus, a greater degree of frailty [18].

Statistical analysis

Normality of data was graphically explored using Q-Q plots. Values are presented as means±SD or medians (Q1, Q3) for continuous, normally and not normally distributed, respectively, and as frequencies (%) for categorical variables. Differences between sexes were tested by unpaired t-test or Mann Whitney rank tests for normally and not normally distributed continuous, respectively, and

Chi-Square tests for categorical variables. Differences between MeDi quartiles were tested with one-way ANOVA followed by post-hoc Student paired t-tests, or Kruskal Wallis followed by Mann Whitney rank tests, for normally and not normally distributed continuous, respectively, and Chi-Square tests for categorical variables. Post-hoc p-values were corrected for multiple comparisons (Bonferonni). In supplementary analyses, we used logistic regression analyses to investigate relations between MeDi and some individual elements (constipation or urinary dysfunction), adjusted for sex. Furthermore, we used linear regression models to investigate relations between MeDi and LR for prodromal markers (ranked data), adjusted for some risk factors (age, sex, smoking, or pesticides' use).

The associations between MeDi score, cognitive performance, TEE and other measures of motor function, frailty status (independent variables) and probability of pPD (dependent variable, log-transformed data) were evaluated with linear regression analyses. Logistic regression analyses were used for PD and when pPD probability was treated as a dichotomous outcome (i.e.≥30% probability). We used models unadjusted and adjusted for possible confounders; results are shown are shown as β (95% CI) for linear and OR (95% CI) for logistic regression analyses.

The MeDi score was entered into the models both as a continuous variable, as well as in categorical form (comparing the first-lowest *vs*. other quartiles). in the case of FI, frailty was entered into the models mentioned above both as a categorical (comparing frail to non-frail individuals), but also as a continuous variable (as the total criteria met by participants). Statistical significance was set at the 5% level ($p \leq 0.05$). All data were analysed using SPSS statistical software (SPSS 19.0, SPSS Inc., USA).

RESULTS

According to literature [3-5] we expected a PD prevalence of 1-2% in our sample. Out of 1765 volunteers, 34 (1.9%) were diagnosed with PD (for PD diagnosis see methods). By design, participants with PD were excluded from the following analyses regarding pPD. The distribution of the probability score for pPD in our PD-free sample is shown in **Figure 1A** (probability data) and **Figure 1B** (log-tranformed probability data that were used in the statistical models). Most participants (~79%) had less than 5% probability of pPD, while 3% (*n*=49) of the sample had 30% or more pPD probability. Demographics and anthropometrics of the study population are shown in **Table 3**.

Dietary intake and MeDi adherence

The MeDi score of the participants with PD was 32.2±3.4, compared to 33.2±4.6 for those without PD (p=0.083) but the power of such analyses is quite low. Dietary intake for PD-free volunteers is shown in **Table 4**. Females reported greater carbohydrate and fat intake, and lower alcohol intake (as % energy intake), resulting in a slightly lower MeDi score, compared to males (p<0.001).

Assessment of the pPD probability and its features by quartiles of MeDi score are shown in **Table 5**. Pretest probability was higher in the 1st quartile, compared to the 3rd and 4th quartiles. Male sex and regular pesticide exposure were more prevalent in the upper MeDi quartiles; however, non-use of coffee and non-smoking were more prevalent in the lower quartiles, resulting in lower LR from risk markers only in the 4th quartile compared to the 2nd one. Moreover, daytime sleepiness, depression, constipation, and urinary dysfunction were less prevalent in higher quartiles, resulting in lower pPD probability in the 3rd and 4th quartile compared to both the 1st and 2nd quartile of MeDi score (**Table 5** and **Figure 3**).

Results of linear regression analysis assessing the association of pPD probability with MeDi adherence are shown in **Table 6**. For each unit increase in MeDi

score, we observed a 2.9% lower probability score for pPD on unadjusted analysis, 1.9% when adjusted for years of education and 2.3% when further adjusted for energy intake ($p<0.01$). Compared to participants in the lowest quartile of MeDi adherence, those in the 3rd and 4th quartiles were associated with an ~18-27% lower probability for pPD.

We explored associations between MeDi and certain individual elements of pPD considering potential sex influences. Increasing MeDi score was associated with lower odds of constipation and urinary dysfunction even when adjusting for sex (data not shown). We further investigated the potential influence of constipation, the pPD feature more directly linked to dietary habits. Associations of pPD probability with MeDi adherence remained unchanged when the prodromal marker of "constipation" was excluded from the calculation of pPD probability (data not shown).

We explored the potential influence of cognitive dysfunction, which may lead to underreporting or misreporting. Excluding participants with dementia (*n*=80) or, additionally those with mild cognitive impairment (MCI, *n*=206) associations between MeDi and pPD probability remained unchanged (data not shown, for dementia and MCI diagnostic procedures in HELIAD see[26, 27]).

Cognitive performance

Cognitive z score analyses

Assessment of the probability of pPD and its individual components by quartiles of composite cognitive z score are shown in **Table 7**. Overall, higher probability of pPD was associated with lower cognitive z scores.

Non-smoking, daytime somnolence, depression, constipation, urinary dysfunction and sub threshold parkinsonism were more prevalent in participants with lower cognitive performance ($p<0.05$). Higher pretest probability, higher risk markers LR and higher prodromal markers LR were all

related to lower cognitive performance (p<0.001 for all comparisons). Overall, as probability of pPD increased, cognitive performance decreased (**Table 8 and Figure 4**). Possible/probable pPD (probability ≥30%) was related with lower cognitive performance (p=0.002) (**Table 8**).

Higher probability of pPD was related to lower cognitive performance in both adjusted (for education, SES and number of comorbidities) and unadjusted models (**Table 9**). Use of cognitive performance in quartile z score type suggested a linear association (see p for trend- **Table 9**).

In logistic regression models, for each one SD reduction in cognitive tests, odds of being diagnosed with possible/probable pPD doubled (OR=2.032; 95%CI: 1.403-2.942; p<0.001). Associations in adjusted for education, SES and number of comorbidities model were similar: OR: 1.721 (95%CI: 1.080-2.741; p=0.022) for decline of one SD of composite z-score.

When additionally adjusting for age and sex, associations between composite z score and probability for pPD remained unchanged (data not shown).

MCI analyses

Among 1.629 participants, 203 received an MCI diagnosis and 1426 were deemed cognitively unimpaired. Pretest probability of pPD, LRs for prodromal markers, total LR and overall probability for pPD differed among participants with MCI diagnosis and those with normal cognition (p<0.001 for all comparisons), with enrichment of MCI proportions in population with higher LR and higher PD probabilities.

Logistic regression analyses showed that compared to those with less than 30% probability score for pPD, those having possible/probable pPD (>=30% probability score) had triple odds of being diagnosed with MCI (OR= 3.148 ; 95%CI: 1.542-6.427; p=0.001; adjusted model).

Individual cognitive domains

Higher probability of pPD was associated with lower memory, executive, visual spatial, language and attention speed z-scores (r=-0.171 to -0.288, p<0.001) (**Table 10**). Moreover, all cognitive z-scores were lower in participants with ≥30% probability of pPD (**Table 10**).

Physical activity and motor function

Physical activity and other motor measures and pPD probability based on whether or not they had possible/probable pPD are shown in **Table 11**. Frequencies of each motor/walking complaint are shown in **Figure 4**. Complaints reported most frequently were walking difficulty indoors (6%) and outdoors (11%), poor balance (9%), while 7% of the study population reported use of a walking stick.

The possible/probable pPD group had a significantly higher total motor complaint score (p<0.001, Table 11) and reported having most of the motor complaints (10 out of 12, p<0.05, Figure 4) more frequently than the non-pPD group. They spent 1.6 fewer kcal/kg/day (p=0.010) in everyday physical activities (corresponding to ~30 min of light walking less per day for a 75 kg man). They also had low gait speed more frequently (p<0.001) and needed longer to complete the gait tests (p≤0.001). In addition, they had greater score in UPDRSIII (p<0.001).

For each unit increase in motor complaints score, there was a 27% higher pPD probability (p<0.001) and a 48% increase in the odds of having possible/probable pPD (p<0.001) (**Table 12**). Having at least one motor complaint was associated with more than double pPD probability (p<0.001) and approximately 5 times higher odds of having possible/probable pPD (p<0.001). The probability of pPD was associated mainly with dragging the feet or taking small steps when walking and poor balance (p<0.05; data not shown), and to a

lesser extent (only when probability was treated as a dichotomous variable, $p<0.05$; data not shown) with using a cane as a walking aid.

Decrease in physical activity (TEE) was associated with increased pPD probability, so that 1 kcal/kg/d lower TEE (corresponding to 20 min of light walking per day for a 75-kg man) was associated with 3% higher pPD probability ($p<0.001$) and a 9% increase in odds of having possible/probable pPD ($p=0.043$) (Table 12).

Frailty

Frailty prevalence in the total sample was 4.2% and 22.2% as measured with the Fried definition and the FI, respectively [73]. Frailty prevalence was higher among participants with PD or possible/probable pPD, compared to PD/pPD-free participants, irrespectively of the frailty definition used ($p<0.05$; **Figure 5**).

Results of regression analyses assessing the association of frailty with probability of pPD, possible/probable pPD (≥30% probability) and with PD are shown in **Table 13** and depicted in **Figure 6a and 7b**. Using the Fried definition, pre-frail participants had 47% higher pPD probability and double odds of having possible/probable pPD and PD, compared to non-frail participants. Frail participants had 2.9 times higher pPD probability score, approximately four times higher odds of having possible/probable pPD and approximately seven times higher odds of having PD, than the non-frail participants.

When frailty was measured with the FI, frail participants had 8 times higher odds of having possible/probable pPD ($p<0.001$) and 12 times higher odds of being diagnosed with PD ($p<0.001$), than the non-frail participants. It is remarkable that for each point increase in frailty score (out of 38) i.e. for each additional criterion of frailty in the FI, we observed approximately a 17% higher pPD probability ($p<0.001$), 37% higher odds of having possible/probable pPD ($p<0.001$) and 32% higher odds of being diagnosed with PD ($p<0.001$).

When adjusted for age, sex, education and socioeconomic status the above associations remained strong for the most part (**Table 14**). Compared to non-frail participants, those with frailty, as identified with either the Fried definition or FI, had more than four (p=0.014) and 11 times (p<0.001), respectively, higher odds of having PD, while each additional criterion of frailty in the FI was associated with 1.3 times higher odds of having PD (p<0.001). In analyses where continuous pPD probability score was the outcome, frail participants had 63% (p<0.001) and 2.3 (p<0.001) times higher pPD probability score in the analyses with the Fried definition and FI respectively. Each additional criterion of frailty in the FI raised the pPD probability score by 14% (p<0.001). Compared to the non-frail by the Fried definition, pre-frail participants had 1.6 (95% confidence interval [CI] 0.84–3.14) times and frail participants 2.2 (95% confidence interval [CI] 0.72–7.23) times higher odds of having pPD. These non-significant results were possibly due to the relatively small number of participants diagnosed with pPD and either pre-frailty (n=29) or frailty (n=5). Frail participants as measured with FI had more than seven times higher odds of having possible/probable pPD (p<0.001). Each additional criterion of frailty in the FI was associated with 1.3 times higher odds of having pPD (p<0.001).

When we further explored the association between PD diagnosis, pPD diagnosis, pPD probability score and frailty status by using the frailty instruments FRAIL Scale, TFI and GFI, we found statistically significant associations in all models. Specifically, compared to that of non-frail participants, the odds of frail people being diagnosed with PD or pPD and the pPD probability score remained significantly increased, irrespectively of the definition used (data not shown).

DISCUSSION

In the present research we investigated the association of dietary (Mediterranean Diet adherence) and other factors (such as physical activity,

cognitive function, motor function, frailty syndrome) with pPD (symptoms, probability, status) in a general population of community dwelling older population in Greece.

Several studies have shown protective effects of the MeDi dietary pattern for numerous diseases, including some neurodegenerative ones, like Alzheimer's disease[74]. However, limited data on the MeDi and PD associations are available. A previous case-control study in the US population found that MeDi adherence was associated with later PD age-at-onset[15], while a prospective study showed that higher MeDi adherence tended to reduce the risk for established PD[16]. In the present work, we extended current knowledge by evaluating the probability of pPD (and its features) and possible associations with adherence to the MeDi pattern. **Adherence to the MeDi was associated with a lower overall probability score for pPD**. Exploring individual features of pPD, older adults who had a high level of MeDi adherence had a lower likelihood ratio for non-motor markers of pPD, mainly **depression** and **constipation** and, to a lesser extent, **daytime somnolence** and **urinary dysfunction**.

Several mechanisms may operate and explain our findings. High adherence to MeDi may protect from alpha-synuclein aggregation and early neuronal degeneration, presumably in the gut and several areas in the brain[75-78], decreasing odds or delaying manifestation of early features of pPD, such as constipation, daytime somnolence and depression, and, finally, delaying PD onset. The MeDi is characterized by high intake of plant foods (vegetables, fruits, legumes, whole grains), low to moderate intake of fish and wine in moderation, and components (phenolics, fibers, etc.), which have been shown to exert antioxidant and anti-inflammatory effects, and thereby may conceivably protect from neurodegeneration[74, 79-82]. Concomitantly, these MeDi components and the whole MeDi pattern have been associated with favorable gut microbiota characteristics[83-85], possibly ameliorating the gut-to-brain signaling and, therefore, beneficially affecting neuronal functioning in both the enteral and the

central nervous system[76-78, 86]. Nevertheless, since the association of MeDi with lower probability score of pPD in our study still remains when performing the analysis without constipation, raises the possibility that association of MeDi with other pPD markers may be also pertinent. In relation to the other features of pPD, MeDi adherence has been associated with lower risk of changes in sleep duration and with better sleep quality in older adults[87], with the latter possibly being related to reduced daytime somnolence seen in our study. Similarly, our results on the association of MeDi with lower likelihood of depression are in accordance with most, but not all, previous evidence [88-90]. We detected no association between MeDi adherence and motor exam. It could be that motor features of PD (even subtle ones) are exhibited in the latter stage of pPD, i.e. may be less present in the earlier pre-diagnostic/precursor stage, where other non-motor markers are more prevalent[6].

It is also possible that some of the non-motor markers of pPD, such as constipation, were reduced in the adherers to the MeDi, regardless of potential PD prodromal state. In other words conceivably MeDi affects constipation without interfering with PD pathogenetic process *per se*. In such case, adherence to the MeDi may have masked early features of pPD. Phrased differently, MeDi adherence may be influencing the probability of individual aspects of the PD risk/prodromal phenotype [see above[84, 88, 90]], but without being necessarily associated with the underlying biological mechanisms. MeDi may also have a role in modulating pathways that are related to aging process in general, such as brain atrophy[91-93] and telomere length[94]. Spurious associations cannot be entirely ruled out as there is no biomarker (e.g., DaTSCAN) to identify pPD cases. Studies with biopsies or prospective studies are needed to investigate whether MeDi adherence and the associated favorable non-motor markers' profile correspond to reduced alpha-synuclein aggregation, neurodegeneration and PD onset, or is just a "masking" effect.

We cannot rule out the possibility of reverse causality, i.e. that those with early features of pPD tend to adhere to the MeDi to a lesser extent due to circumstances associated with their incipient disease state. For instance, depression has been associated with increased consumption of unhealthy foods[95], while olfactory impairment due to aging or to pPD *per se* may exacerbate this behavior[96, 97]. Again, prospective studies are needed to reveal the direction of causality.

Even though we focused on pPD, the MeDi score of the diagnosed PD was non-significantly lower compared to those without PD (p=0.083). This could be due to limited power. Although there have been exceptions[10], previous studies with considerably higher power (PD cases between 257 and 600, i.e. 5 and 17.5 fold higher than ours) have reported lower MeDi adherence in PD patients[15, 16]. Lack of significant association of MeDi with pPD using cut-offs (vs. overall pPD probability) could be also related to power restrictions.

Regarding physical activity we found that **reduced <u>physical activity</u>, as measured by TEE (1kcal/kg/day, corresponding to 20 min of light walking per day for a 75-kg man), was associated with pPD**, extending our knowledge on the importance of lifestyle in the prevention of neurodegenerative diseases[26, 98]. However, prospective studies are needed to confirm these results. Other indicators of reduced physical activity such as **motor/walking complaints and low gait speed were also associated with a higher pPD probability score**. All these associations were significant in adjusted models, even when participants with dementia, MCI, or stroke or age>80 years were excluded, and when subthreshold Parkinsonism (i.e. UPDRSIII score without action tremor greater than 3) was excluded from the calculation of pPD probability. Nevertheless, the question of whether motor function measures (motor complaints, physical activity or gait speed tests) are predictors or indicators of pPD, needs to be further assessed in prospective studies.

Higher probability of pPD was also associated with lower cognitive performance in all domains and with higher probability of MCI. This was the case not only for executive function, as some previous studies on PD have suggested,[99] but for all studied cognitive domains, e.g. memory, language, attention-speed, executive, and visual-spatial. Among pPD markers, non-smoking status, daytime somnolence, urinary dysfunction, constipation, depression and sub threshold parkinsonism were associated with worse cognitive function.

Even though our results do not permit any firm conclusions regarding the etiology of lower cognitive scores in participants with higher probability of pPD, several previous data could explain these findings. In a recent pathological study, participants with minimal motor symptoms were noted to have significant decreases in dopaminergic neurons and terminals in the substantia nigra and putamen, with phosphorylated α-synuclein inclusions in the substantia nigra and considerable Lewy neuritic pathology in the putamen.[100] The inference, related to our findings, is that such dopaminergic deficits in subclinical PD may also influence cognitive abilities. Cognitive impairment in pPD may be also partially mediated by early extrastriatal disease pathology or non-dopaminergic pathways. Impairment of noradrenergic and cholinergic systems occur early in Braak's proposed pathologic staging, before the engagement of nigral or cortical neurons, involving these pathways as prime candidates for mediating lower cognitive function in pPD.[101] Additionally, progression of dopaminergic system related neuropathology in PD may not fully comply with Braak's staging model in all cases. No matter which model is the most reliable, the identification of onset of parkinsonian symptoms outside the substantia nigra may relate to cognitive changes in prodromal stage of PD.

To our knowledge, this is the first study to examine the association between frailty (as measured with various instruments) and pPD probability (as computed using the recently published MDS research criteria). Our results showed that irrespective of the frailty measurement used, **frail community-**

dwelling older adults had higher odds of being identified either with PD or pPD, compared to non-frail individuals (**Figure 6a and 6b**), implying that the mechanisms underlying this relationship may be independent of the specific frailty criteria used in each assessment tool.

Regarding the association between PD and frailty, our findings are in line with previous research. Ahmed et al. found that the prevalence of frailty in a sample of PD patients was much higher, compared to that reported in cohorts of older individuals [21]. Frailty prevalence was also found to be higher in PD patients compared to the general older population [23] or their spouses/siblings [24]. A higher prevalence of frailty has been reported in patients with mild parkinsonian symptoms as well [22]. We expand these findings by ascertaining associations in the pPD stage and using different frailty definitions.

The pathway lying beneath the relationship between frailty and PD is not fully understood. However, common underlying mechanisms between frailty and PD may be responsible for the observed association. Specifically, inflammation [102, 103], oxidative stress and free radicals [102, 104, 105] and mitochondrial dysfunction [106, 107] are common in both frailty and PD pathology. Frailty-related dysfunction of the gut–brain axis, may also contribute to PD pathology [108]. It has been also hypothesized that dopamine dysfunction may mediate this relationship [22].

Most of the aforementioned changes attributed to PD pathology are thought to occur early, years before PD diagnosis is reached. Our data support that notion, since we found that frailty is associated not only with PD but also with pPD, implying that the common characteristics between frailty and PD are present even in the prediagnostic stage of PD (pPD). Thus, frailty diagnosis early in the pPD stage may exacerbate the need for lifestyle changes to improve quality of life and, perhaps, prevent or delay PD onset. Supporting to this notion is the finding that a lifestyle factor, adherence to Mediterranean diet, is associated with lower odds for both clinical identities in studies with a cross-sectional design, such as ours [71, 98], suggesting common clinical strategies for their management.

However, the cross-sectional design of the current study does not allow us to establish any causal or temporal relationship between frailty and PD or pPD. According to our findings, frail individuals had higher odds of being identified with pPD, compared to non-frail individuals, but reverse causality cannot be rejected. Besides frailty and pPD symptoms are possibly co-occurred and triggered by the gradual multisystem decline of aging pathology. Future follow-up will permit us to further explore on the direction of the relationship between frailty syndrome and pPD probability or PD.

Our study has certain limitations. Due to the cross-sectional design, no causal relationship can be established as discussed above. A potential non-response bias, a phenomenon quite common in population-based cohorts of elderly with low education[109, 110] has to be considered. No major demographic differences were identified between those agreeing and those refusing to participate in HELIAD, except for a small age and sex difference: those who refused were ~4 years older and more likely male (46% vs. 41% males in participants)[31]. This may have contributed to underestimation of the overall pPD probability in our population.

Another possible limitation is the lack of data on some MDS criteria, such as olfactory loss and dopaminergic system PET/SPECT. In contrast to clinic-based samples, obtaining data for each and every MDS criterion would be very time-consuming, costly and not practical for large population-based cohorts. Overall, we collected data for the vast majority of markers: 13 out of 17 (8 out of 10 prodromal markers). Furthermore, according to MDS recommendations and recent studies[2, 111], pPD probability may be calculated using available markers in each cohort, keeping in mind that it may be underestimated if the number of markers is limited. Yet, pPD probability in our study was quite similar to that found in other cohort of older adults[2]. Another concern could be that some markers may have been assessed with tools not ideal, albeit practical for such large population-based cohort studies. For example, we used questionnaires

rather than polysomnography for RBD. Nevertheless, we noted a possible RBD prevalence of 7%, very much in line with published rates from similar populations[112, 113]. In addition, the MDS criteria allow for assignment of appropriate LR (i.e. 2.3) for possible RBD (*vs*. a LR of 130 for polysomnography proven RBD[7]), thereby protecting from a potentially unjustifiably high influence in pPD score calculation. We should keep in mind that MDS pPD criteria are still under validation, and each criteria may not be specific to pPD (e.g. urinary dysfunction) or may overlap with other neurodegenerative diseases (e.g. depression in dementia); however, prospective studies showed promising results, regarding their "combination", i.e., pPD probability score [114, 115].

At the same time, the current study has several strengths. We investigated for the first time the association between diet and other factors and prodromal state of PD, where preventive strategies may be more clinically valuable. Our sample is as representative as possible of the aging population in Greece. All subjects were fully examined by Neurologists (or rarely by Neurologists towards the end of their residency training). A multidisciplinary consensus expert group established the diagnoses based on uniform application of widely accepted criteria. Noteworthy, PD prevalence (1,9%) in our sample was similar to that found in other cohorts of older adults[3-5], strengthening both the representativeness of our sample and the validity of the diagnostic process used. PD prevalence in non-institutional men in Canada was 1.2% for those aged 65-79 years and 2.1% for those more than 80 years old[3]. In Europe, the overall prevalence in persons aged≥65 years was 1.8%[5]. Similarly, a meta-analysis of cohort studies in Europe, North America and Australia found PD prevalence of 0.5% (60-69 years), 1.6% (70-79 years) and 3.0% (80+ years)[4]. Along the same lines, median probability score for pPD (1,9%) was also within previously reported ranges[2]. Furthermore, the detailed evaluation of HELIAD subjects provided the ability to assess most pPD markers (8 out of 10).

Another advantage of our study is that we used an *a priori* MeDi score, instead of a population-specific one used in prior studies on MeDi and PD[10, 15, 16]; thus, our data may be compared with those of other populations in similar future studies. Using the whole-diet approach excluded the investigation of nutrients/food effects, of potential additive or contrasting food effects or the role of cooking byproducts. Dietary information was collected once and longstanding dietary habits capturing potential cumulative effects of changing dietary habits have not been explored. Nevertheless, isolating "parts" of diet may be misleading and not representative of real dietary behaviors possible associated with chronic diseases[13, 14]. Therefore, we believed that the use of dietary pattern is more an advantage rather than disadvantage of our study.

Moreover, the assessment of frailty with definitions belonging both to the phenotypic and multidomain approach is another major strength. Cognitive function was not estimated by self or proxy-report, or from previously recorded information (all, susceptible to recall and interviewer bias) but via a very extensive, detailed and validated neuropsychological battery designed to cover a very broad range of cognitive domains.[26, 28] Moreover, we used definitions including expert clinical judgment (e.g. MCI) apart from the objective cognitive tests. Finally, we adjusted for many potential confounders (energy intake, education, co-morbidities etc) and we performed all necessary supplementary analyses to increase confidence in our findings.

In summary, the present research has contributed substantial information to the growing literature demonstrating links between lifestyle and neurological diseases. Our results suggest that adherence to the MeDi and physical activity is associated with lower probability of pPD and some of its features in older people. More studies are needed to elucidate the potential causality and the underlying mechanisms. Clinicians may find it useful to evaluate diet and physical activity in highly vulnerable populations, such as those with a PD genetic predisposition or other pPD risk factors. Recommending the MeDi pattern and the Mediterranean

active lifestyle, either to reduce the risk or lessen the effects (e.g., constipation, depression) of pPD needs to be considered and further explored. Moreover, it seems important for clinicians to be aware of the increased prevalence of frailty when planning treatment not only in PD, but also in pPD stages. In addition, the association between cognitive function and probability of pPD noted in our data, should alert clinicians to look for motor symptoms in patients with MCI but also for further relevant non-motor symptoms that may cluster together with lower cognitive function such as RBD, autonomic dysfunction etc[116] and inform about probability of future PD. As research is concerned, it would be important to further investigate the underlying biologic pathways of the association between the investigated and other factors and pPD and even search for potential biological causal links between these factors / conditions. Future longitudinal studies with multiple timepoints of assessments may bring us closer to this target.

REFERENCES

1. Dorsey ER, Constantinescu R, Thompson JP, et al. Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. Neurology 2007;68:384-386.
2. Mahlknecht P, Gasperi A, Willeit P, et al. Prodromal Parkinson's disease as defined per MDS research criteria in the general elderly community. Mov Disord 2016;31:1405-1408.
3. Wong SL, Gilmour H, Ramage-Morin PL. Parkinson's disease: Prevalence, diagnosis and impact. Health reports 2014;25:10-14.
4. Pringsheim T, Jette N, Frolkis A, Steeves TD. The prevalence of Parkinson's disease: a systematic review and meta-analysis. Movement disorders : official journal of the Movement Disorder Society 2014;29:1583-1590.
5. de Rijk MC, Launer LJ, Berger K, et al. Prevalence of Parkinson's disease in Europe: A collaborative study of population-based cohorts. Neurologic Diseases in the Elderly Research Group. Neurology 2000;54:S21-23.
6. Chen H, Burton EA, Ross GW, et al. Research on the premotor symptoms of Parkinson's disease: clinical and etiological implications. Environmental health perspectives 2013;121:1245-1252.
7. Berg D, Postuma RB, Adler CH, et al. MDS research criteria for prodromal Parkinson's disease. Mov Disord 2015;30:1600-1611.
8. Agim ZS, Cannon JR. Dietary factors in the etiology of Parkinson's disease. BioMed research international 2015;2015:672838.
9. Shen L. Associations between B Vitamins and Parkinson's Disease. Nutrients 2015;7:7197-7208.
10. Cassani E, Barichella M, Ferri V, et al. Dietary habits in Parkinson's disease: Adherence to Mediterranean diet. Parkinsonism & related disorders 2017;42:40-46.
11. Liu R, Guo X, Park Y, et al. Alcohol Consumption, Types of Alcohol, and Parkinson's Disease. PloS one 2013;8:e66452.

12. Zhang D, Jiang H, Xie J. Alcohol intake and risk of Parkinson's disease: a meta-analysis of observational studies. Mov Disord 2014;29:819-822.

13. Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. Curr Opin Lipidol 2002;13:3-9.

14. Gu Y, Scarmeas N. Dietary patterns in Alzheimer's disease and cognitive aging. Curr Alzheimer Res 2011;8:510-519.

15. Alcalay RN, Gu Y, Mejia-Santana H, Cote L, Marder KS, Scarmeas N. The association between Mediterranean diet adherence and Parkinson's disease. Mov Disord 2012;27:771-774.

16. Gao X, Chen H, Fung TT, et al. Prospective study of dietary pattern and risk of Parkinson disease. The American journal of clinical nutrition 2007;86:1486-1494.

17. Fried LP, Tangen CM, Walston J, et al. Frailty in older adults: evidence for a phenotype. The journals of gerontology Series A, Biological sciences and medical sciences 2001;56:M146-156.

18. Rockwood K, Mitnitski A. Frailty defined by deficit accumulation and geriatric medicine defined by frailty. Clinics in geriatric medicine 2011;27:17-26.

19. Morley JE, Vellas B, van Kan GA, et al. Frailty consensus: a call to action. Journal of the American Medical Directors Association 2013;14:392-397.

20. de Vries NM, Staal JB, van Ravensberg CD, Hobbelen JS, Olde Rikkert MG, Nijhuis-van der Sanden MW. Outcome instruments to measure frailty: a systematic review. Ageing research reviews 2011;10:104-114.

21. Ahmed NN, Sherman SJ, Vanwyck D. Frailty in Parkinson's disease and its clinical implications. Parkinsonism & related disorders 2008;14:334-337.

22. Seiffert P, Derejczyk J, Kawa J, et al. Frailty phenotype and the role of levodopa challenge test in geriatric inpatients with mild parkinsonian signs. Biogerontology 2017;18:641-650.

23. Peball M, Mahlknecht P, Werkmann M, et al. Prevalence and Associated Factors of Sarcopenia and Frailty in Parkinson's Disease: A Cross-Sectional Study. Gerontology 2018:1-13.

24. Tan AH, Hew YC, Lim SY, et al. Altered body composition, sarcopenia, frailty, and their clinico-biological correlates, in Parkinson's disease. Parkinsonism & related disorders 2018.

25. Roland KP, Jakobi JM, Jones GR, Powell C. Quality of life as a determinant of frailty phenotype in community-dwelling persons with Parkinson's disease. Journal of the American Geriatrics Society 2012;60:590-592.

26. Anastasiou CA, Yannakoulia M, Kosmidis MH, et al. Mediterranean diet and cognitive health: Initial results from the Hellenic Longitudinal Investigation of Ageing and Diet. PloS one 2017;12:e0182048.

27. Tsapanou A, Gu Y, O'Shea DM, et al. Sleep quality and duration in relation to memory in the elderly: Initial results from the Hellenic Longitudinal Investigation of Aging and Diet. Neurobiol Learn Mem 2017;141:217-225.

28. Dardiotis E, Kosmidis MH, Yannakoulia M, Hadjigeorgiou GM, Scarmeas N. The Hellenic Longitudinal Investigation of Aging and Diet (HELIAD): rationale, study design, and cohort description. Neuroepidemiology 2014;43:9-14.

29. Ntanasi E, Yannakoulia M, Kosmidis MH, et al. Adherence to Mediterranean Diet and Frailty. Journal of the American Medical Directors Association 2017.

30. Vlachos GS, Kosmidis MH, Anastasiou CA, et al. Definition, prevalence and predictors of subjective cognitive decline in the Greek elderly population. Alzheimer Disease & Associated Disorders accepted for publication.

31. Kosmidis MH, Vlachos GS, Anastasiou CA, et al. Dementia Prevalence in Greece: The Hellenic Longitudinal Investigation of Aging and Diet (HELIAD). Alzheimer Dis Assoc Disord 2018;32:232-239.

32. Kokouva M, Bitsolas N, Hadjigeorgiou GM, Rachiotis G, Papadoulis N, Hadjichristodoulou C. Pesticide exposure and lymphohaematopoietic cancers: a case-control study in an agricultural region (Larissa, Thessaly, Greece). BMC public health 2011;11:5.

33. Fountoulakis KN, Tsolaki M, Iacovides A, et al. The validation of the short form of the Geriatric Depression Scale (GDS) in Greece. Aging (Milano) 1999;11:367-372.

34. Michopoulos I, Douzenis A, Kalkavoura C, et al. Hospital Anxiety and Depression Scale (HADS): validation in a Greek general hospital sample. Annals of general psychiatry 2008;7:4.

35. Hays RD, Martin SA, Sesti AM, Spritzer KL. Psychometric properties of the Medical Outcomes Study Sleep measure. Sleep medicine 2005;6:41-44.

36. Blessed G, Tomlinson BE, Roth M. The association between quantitative measures of dementia and of senile change in the cerebral grey matter of elderly subjects. Br J Psychiatry 1968;114:797-811.

37. Fahn S, Elton RL. Unified Parkinson's disease rating scale. In: Fahn S, Marsden CD, Calne DB, Goldstein M, eds. Recent Developments in Parkinson's Disease. Florham Park, N.J: Macmillan Healthcare Information, 1987: 153-163.

38. McKeith IG, Dickson DW, Lowe J, et al. Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. Neurology 2005;65:1863-1872.

39. Cummings JL. The Neuropsychiatric Inventory: assessing psychopathology in dementia patients. Neurology 1997;48:S10-16.

40. Postuma RB, Berg D, Stern M, et al. MDS clinical diagnostic criteria for Parkinson's disease. Mov Disord 2015;30:1591-1601.

41. AmericanPsychiatricAssociation. Diagnostic and Statistical Manual of Mental Disorders, ed 4th ed. Washington, DC. 2000.

42. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939-944.

43. Hachinski VC, Iliff LD, Zilhka E, et al. Cerebral blood flow in dementia. Arch Neurol 1975;32:632-637.

44. McKeith IG, Galasko D, Kosaka K, et al. Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies (DLB): report of the consortium on DLB international workshop. Neurology 1996;47:1113-1124.

45. Neary D, Snowden JS, Gustafson L, et al. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. Neurology 1998;51:1546-1554.

46. Petersen RC, Lopez O, Armstrong MJ, et al. Practice guideline update summary: Mild cognitive impairment: Report of the Guideline Development, Dissemination, and Implementation Subcommittee of the American Academy of Neurology. Neurology 2018;90:126-135.

47. Vlahou CH, Kosmidis MH. The greek trail making test: Preliminary norms for clinical and research use. Psychology 2002;9:336–352.

48. Lezak MD, Howieson DB, Loring DW. Neuropsychological Assessment. New York, NY, USA: Oxford University Press, 2004.

49. Giaglis G, Kyriazidou S, Paraskevopoulou E, Tascos N, Kosmidis MH. Evaluating premorbid level: Preliminary findings regarding the vulnerability of scores on cognitive measures in patients with MS (abstract). Journal of the International Neuropsychological Society : JINS 2010;165:159.

50. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. Journal of psychiatric research 1975;12:189-198.

51. Vlahou CH, Kosmidis MH, Dardagani A, et al. Development of the Greek Verbal Learning Test: reliability, construct validity, and normative standards. Archives of clinical neuropsychology : the official journal of the National Academy of Neuropsychologists 2013;28:52-64.

52. Tsapkini K, Vlahou CH, Potagas C. Adaptation and validation of standardized aphasia tests in different languages: Lessons from the Boston Diagnostic Aphasia Examination - Short Form in Greek. Behavioural neurology 2010;22:111-119.

53. Kosmidis MH, Vlahou CH, Panagiotaki P, Kiosseoglou G. The verbal fluency task in the Greek population: normative data, and clustering and switching strategies. Journal of the International Neuropsychological Society : JINS 2004;10:164-172.

54. Kosmidis MH, Tsotsi S, Karambela O, Takou E, Vlahou CH. Cultural factors influencing performance on visuoperceptual neuropsychological tasks. Behavioural neurology 2010;23:245-247.

55. Benton AL, Sivan AB, Hamsher Kd, Varney NR, Spreen O. Contributions to neuropsychological assessment, 2nd ed. New York: Oxford University Press, 1994.

56. Bozikas VP, Giazkoulidou A, Hatzigeorgiadou M, Karavatos A, Kosmidis MH. Do age and education contribute to performance on the clock drawing test? Normative data for the Greek population. Journal of clinical and experimental neuropsychology 2008;30:199-203.

57. Berg D, Postuma RB, Bloem B, et al. Time to redefine PD? Introductory statement of the MDS Task Force on the definition of Parkinson's disease. Mov Disord 2014;29:454-462.

58. Bountziouka V, Bathrellou E, Giotopoulou A, et al. Development, repeatability and validity regarding energy and macronutrient intake of a semi-quantitative food frequency questionnaire: methodological considerations. Nutr Metab Cardiovasc Dis 2012;22:659-667.

59. Panagiotakos DB, Pitsavos C, Stefanadis C. Dietary patterns: a Mediterranean diet score and its relation to clinical and biological markers of cardiovascular disease risk. Nutr Metab Cardiovasc Dis 2006;16:559-568.

60. Kavouras SA, Maraki MI, Kollia M, Gioxari A, Jansen LT, Sidossis LS. Development, reliability and validity of a physical activity questionnaire for estimating energy expenditure in Greek adults. Science and Sports 2017;31:e47-e53.

61. Schoon Y, Bongers K, Van Kempen J, Melis R, Olde Rikkert M. Gait speed as a test for monitoring frailty in community-dwelling older people has the highest diagnostic value compared to step length and chair rise time. Eur J Phys Rehabil Med 2014;50:693-701.

62. Lindholm B, Nilsson MH, Hansson O, Hagell P. The clinical significance of 10-m walk test standardizations in Parkinson's disease. J Neurol 2018;265:1829-1835.

63. Wrisley DM, Kumar NA. Functional gait assessment: concurrent, discriminative, and predictive validity in community-dwelling older adults. Phys Ther 2010;90:761-773.

64. Kubicki A. Functional assessment in older adults: should we use timed up and go or gait speed test? Neurosci Lett 2014;577:89-94.

65. Abellan van Kan G, Rolland Y, Andrieu S, et al. Gait speed at usual pace as a predictor of adverse outcomes in community-dwelling older people an International Academy on Nutrition and Aging (IANA) Task Force. J Nutr Health Aging 2009;13:881-889.

66. World Health Organization. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee Geneva1995.

67. World Health Organization. Waist Circumference and Waist–Hip Ratio: Report of a WHO Expert Consultation, Geneva, 8-11 December 2008. Geneva2011.

68. Rockwood K, Mitnitski A. Frailty in relation to the accumulation of deficits. The journals of gerontology Series A, Biological sciences and medical sciences 2007;62:722-727.

69. Yesavage JA. Geriatric Depression Scale. Psychopharmacol Bull 1988;24:709-711.

70. Kavouras SA, M.I. M, M. K, A. G, L.T. J, L.S. S. Development, reliability and validity of a physical activity questionnaire for estimating energy expenditure in Greek adults. Science & Sports 2016;31:e47-e53.

71. Ntanasi E, Yannakoulia M, Kosmidis MH, et al. Adherence to Mediterranean Diet and Frailty. Journal of the American Medical Directors Association 2018;19:315-322 e312.

72. Searle SD, Mitnitski A, Gahbauer EA, Gill TM, Rockwood K. A standard procedure for creating a frailty index. BMC geriatrics 2008;8:24.

73. Ntanasi E, Yannakoulia M, Mourtzi N, et al. Prevalence and Risk Factors of Frailty in a Community-Dwelling Population: The HELIAD Study. Journal of aging and health 2018:898264318801735.

74. Gu Y, Luchsinger JA, Stern Y, Scarmeas N. Mediterranean diet, inflammatory and metabolic biomarkers, and risk of Alzheimer's disease. Journal of Alzheimer's disease : JAD 2010;22:483-492.

75. Sampson TR, Debelius JW, Thron T, et al. Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease. Cell 2016;167:1469-1480.e1412.

76. Erro R, Brigo F, Tamburin S, Zamboni M, Antonini A, Tinazzi M. Nutritional habits, risk, and progression of Parkinson disease. Journal of neurology 2017.

77. Stocchi F, Torti M. Constipation in Parkinson's Disease. International review of neurobiology 2017;134:811-826.

78. Klingelhoefer L, Reichmann H. The Gut and Nonmotor Symptoms in Parkinson's Disease. International review of neurobiology 2017;134:787-809.

79. Puspita L, Chung SY, Shim JW. Oxidative stress and cellular pathologies in Parkinson's disease. Molecular brain 2017;10:53.

80. Jackson PA, Pialoux V, Corbett D, et al. Promoting brain health through exercise and diet in older adults: a physiological perspective. The Journal of Physiology 2016;594:4485-4498.

81. Caruana M, Cauchi R, Vassallo N. Putative Role of Red Wine Polyphenols against Brain Pathology in Alzheimer's and Parkinson's Disease. Frontiers in nutrition 2016;3:31.

82. Sureda A, Bibiloni MDM, Julibert A, et al. Adherence to the Mediterranean Diet and Inflammatory Markers. Nutrients 2018;10.

83. Del Chierico F, Vernocchi P, Dallapiccola B, Putignani L. Mediterranean Diet and Health: Food Effects on Gut Microbiota and Disease Control. International journal of molecular sciences 2014;15:11678-11699.

84. Mitsou EK, Kakali A, Antonopoulou S, et al. Adherence to the Mediterranean diet is associated with the gut microbiota pattern and gastrointestinal characteristics in an adult population. The British journal of nutrition 2017;117:1645-1655.

85. De Filippis F, Pellegrini N, Vannini L, et al. High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome. Gut 2016;65:1812-1821.

86. Perez-Pardo P, Kliest T, Dodiya HB, et al. The gut-brain axis in Parkinson's disease: Possibilities for food-based therapies. European journal of pharmacology 2017;817:86-95.

87. Campanini MZ, Guallar-Castillon P, Rodriguez-Artalejo F, Lopez-Garcia E. Mediterranean Diet and Changes in Sleep Duration and Indicators of Sleep Quality in Older Adults. Sleep 2017;40.

88. Adjibade M, Assmann KE, Andreeva VA, et al. Prospective association between adherence to the Mediterranean diet and risk of depressive symptoms in the French SU.VI.MAX cohort. European journal of nutrition 2017.

89. Lai JS, Oldmeadow C, Hure AJ, McEvoy M, Byles J, Attia J. Longitudinal diet quality is not associated with depressive symptoms in a cohort of middle-aged Australian women. The British journal of nutrition 2016;115:842-850.

90. Sanchez-Villegas A, Martinez-Gonzalez MA, Estruch R, et al. Mediterranean dietary pattern and depression: the PREDIMED randomized trial. BMC medicine 2013;11:208.

91. Staubo SC, Aakre JA, Vemuri P, et al. Mediterranean diet, micronutrients and macronutrients, and MRI measures of cortical thickness. Alzheimer's & dementia : the journal of the Alzheimer's Association 2017;13:168-177.

92. Gu Y, Brickman AM, Stern Y, et al. Mediterranean diet and brain structure in a multiethnic elderly cohort. Neurology 2015;85:1744-1751.

93. Berti V, Walters M, Sterling J, et al. Mediterranean diet and 3-year Alzheimer brain biomarker changes in middle-aged adults. Neurology 2018.

94. Crous-Bou M, Fung TT, Prescott J, et al. Mediterranean diet and telomere length in Nurses' Health Study: population based cohort study. Bmj 2014;349:g6674.

95. Kilian R, Becker T, Kruger K, Schmid S, Frasch K. Health behavior in psychiatric in-patients compared with a German general population sample. Acta psychiatrica Scandinavica 2006;114:242-248.

96. Gopinath B, Russell J, Sue CM, Flood VM, Burlutsky G, Mitchell P. Olfactory impairment in older adults is associated with poorer diet quality over 5 years. European journal of nutrition 2016;55:1081-1087.

97. Ponsen MM, Stoffers D, Booij J, van Eck-Smit BL, Wolters E, Berendse HW. Idiopathic hyposmia as a preclinical sign of Parkinson's disease. Annals of neurology 2004;56:173-181.

98. Maraki MI, Yannakoulia M, Stamelou M, et al. Mediterranean diet adherence is related to reduced probability of prodromal Parkinson's disease. Mov Disord 2019;34:48-57.

99. Ross GW, Abbott RD, Petrovitch H, Tanner CM, White LR. Pre-motor features of Parkinson's disease: the Honolulu-Asia Aging Study experience. Parkinsonism & related disorders 2012;18 Suppl 1:S199-202.

100. Chu Y, Buchman AS, Olanow CW, Kordower JH. Do subjects with minimal motor features have prodromal Parkinson disease? Ann Neurol 2018;83:562-574.

101. Bohnen NI, Albin RL. The cholinergic system and Parkinson disease. Behavioural brain research 2011;221:564-573.

102. Soysal P, Stubbs B, Lucato P, et al. Inflammation and frailty in the elderly: A systematic review and meta-analysis. Ageing research reviews 2016;31:1-8.

103. Umemura A, Oeda T, Yamamoto K, et al. Baseline Plasma C-Reactive Protein Concentrations and Motor Prognosis in Parkinson Disease. PLoS One 2015;10:e0136722.

104. Farooqui T, Farooqui AA. Lipid-mediated oxidative stress and inflammation in the pathogenesis of Parkinson's disease. Parkinsons Dis 2011;2011:247467.

105. Khan Z, Ali SA. Oxidative stress-related biomarkers in Parkinson's disease: A systematic review and meta-analysis. Iranian journal of neurology 2018;17:137-144.

106. Rocha EM, De Miranda B, Sanders LH. Alpha-synuclein: Pathology, mitochondrial dysfunction and neuroinflammation in Parkinson's disease. Neurobiology of disease 2018;109:249-257.

107. Andreux PA, van Diemen MPJ, Heezen MR, et al. Mitochondrial function is impaired in the skeletal muscle of pre-frail elderly. Sci Rep 2018;8:8548.

108. Di Sabatino A, Lenti MV, Cammalleri L, Corazza GR, Pilotto A. Frailty and the gut. Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver 2018;50:533-541.

109. Keeter S, Miller C, Kohut A, Groves RM, Presser S. Consequences of reducing nonresponse in a national telephone survey. Public opinion quarterly 2000;64:125-148.

110. Heegaard KM, Holm-Pedersen P, Bardow A, Hvidtfeldt UA, Gronbaek M, Avlund K. The Copenhagen Oral Health Senior Cohort: design, population and dental health. Gerodontology 2011;28:165-176.

111. Skorvanek M, Ladomirjakova Z, Han V, et al. Prevalence of Prodromal Parkinson's Disease as Defined by MDS Research Criteria among Elderly Patients Undergoing Colonoscopy. J Parkinsons Dis 2017;7:481-489.

112. Ma JF, Hou MM, Tang HD, et al. REM sleep behavior disorder was associated with Parkinson's disease: a community-based study. BMC neurology 2016;16:123.

113. Boot BP, Boeve BF, Roberts RO, et al. Probable rapid eye movement sleep behavior disorder increases risk for mild cognitive impairment and Parkinson disease: a population-based study. Ann Neurol 2012;71:49-56.

114. Mahlknecht P, Gasperi A, Djamshidian A, et al. Performance of the Movement Disorders Society criteria for prodromal Parkinson's disease: A population-based 10-year study. Mov Disord 2018;33:405-413.

115. Heinzel S, Kasten M, Behnke S, et al. Age- and sex-related heterogeneity in prodromal Parkinson's disease. Mov Disord 2018;33:1025-1027.

116. Fereshtehnejad SM, Romenets SR, Anang JB, Latreille V, Gagnon JF, Postuma RB. New Clinical Subtypes of Parkinson Disease and Their Longitudinal Progression: A Prospective Cohort Comparison With Other Phenotypes. JAMA neurology 2015;72:863-873.

Figure 1 Distribution of the probability score for Prodromal Parkinson's Disease

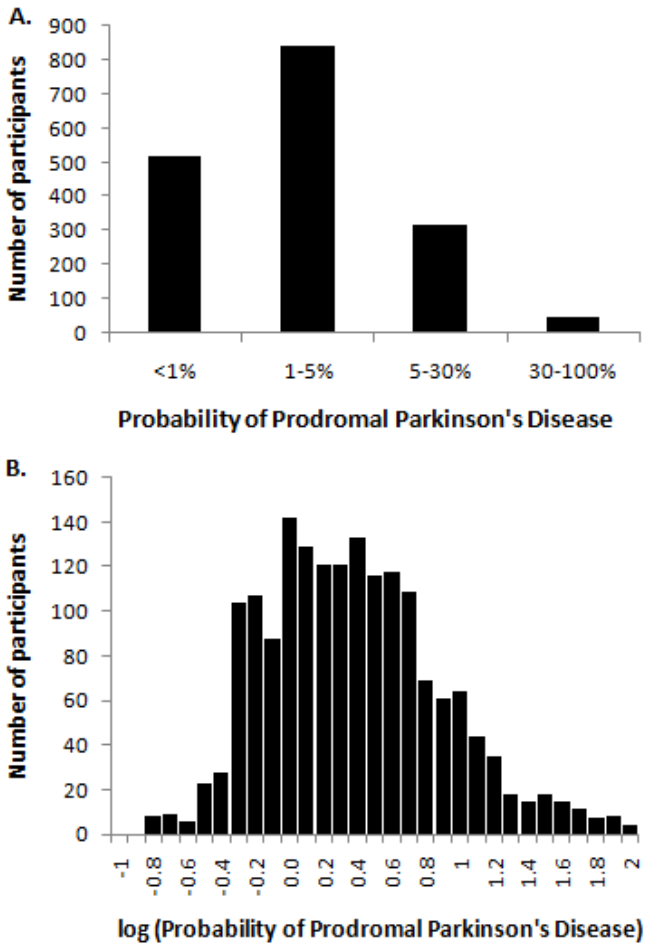

Figure 1 legend A. probability data and B: log-transformed probability data for prodromal Parkinson's Disease (PD) according to the International Parkinson and Movement Disorders Society's research criteria for prodromal PD in older adults.

Figure 2 Probability of prodromal Parkinson's disease (PD) in relation to cognitive function

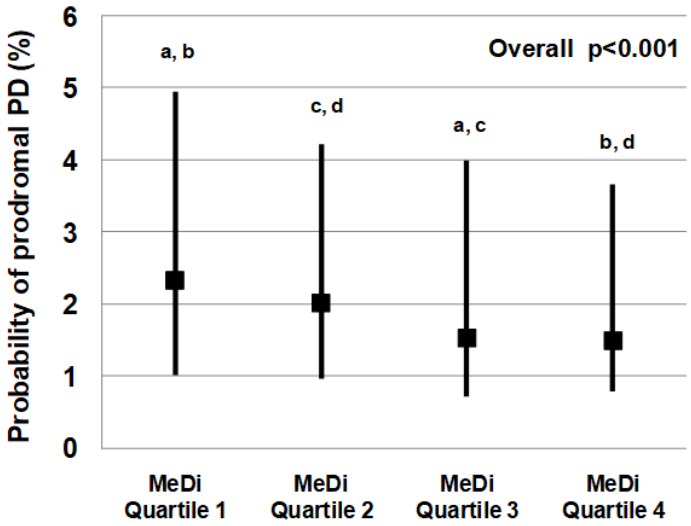

Figure 2 legend Probability of Prodromal Parkinson's Disease (PD) according to the International Parkinson and Movement Disorders Society's research criteria, in older people by quartiles of the Mediterranean Diet score (MeDi). Values are medians (Q1, Q3). P-value from a Kruskal-Wallis test is shown. Values sharing the same superscript letter are statistically significantly different from each other, according to post-hoc Mann Whitney rank tests (Bonferonni corrected for multiple comparisons. MeDi score ranges: Quartile 1: 17-30, Quartile 2: 31-33, Quartile 3: 34-36, Quartile 4: 37-46.

Figure 3 Probability of prodromal Parkinson's disease (PD) in relation to cognitive function

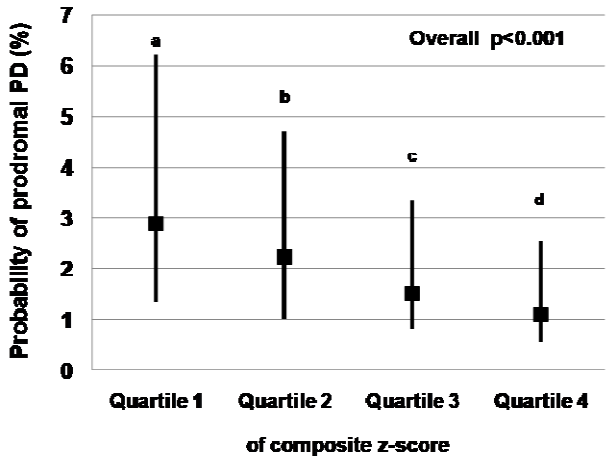

Figure 3 legend Probability of prodromal Parkinson's disease (PD) according to the International Parkinson and Movement Disorders Society's research criteria, by quartiles of the cognitive composite z score. Values are medians (Q1, Q3). P-value from a Kruskal-Wallis test is shown. Values bearing different superscript letters differ significantly, according to post-hoc Mann Whitney rank tests (Bonferroni corrected for multiple comparisons). E.g., each quartile of composite z score has different superscript (Q1 has [a], Q2 [b], Q3 [c] and Q4 [d]), meaning that they are all different from each other in the probability of prodromal PD.

Figure 4 Motor complaints in relation to prodromal Parkinson's Disease

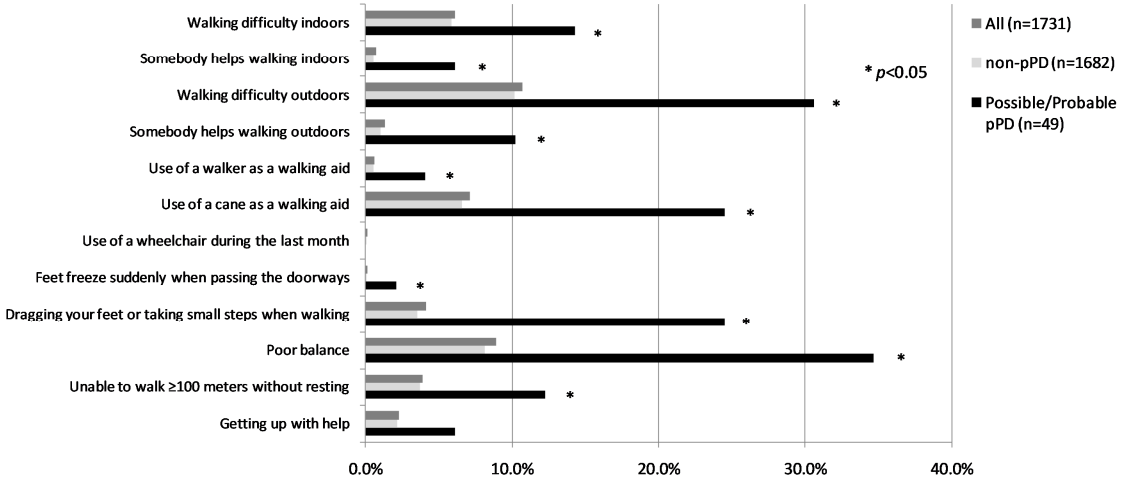

Figure 4 legend: Frequencies (%) of motor complaints of the study population (*n*=1,731, 41% males, Parkinson's Disease [PD]-free) and according to prodromal PD (pPD) status. Abbreviations: PD: Parkinson's Disease; pPD: prodromal PD. *P*-values were obtained with chi-square tests between pPD and non-pPD groups. **p*<0.05.

Figure 5 Frequencies (%) of frailty status according to Parkinson's disease (PD) status.

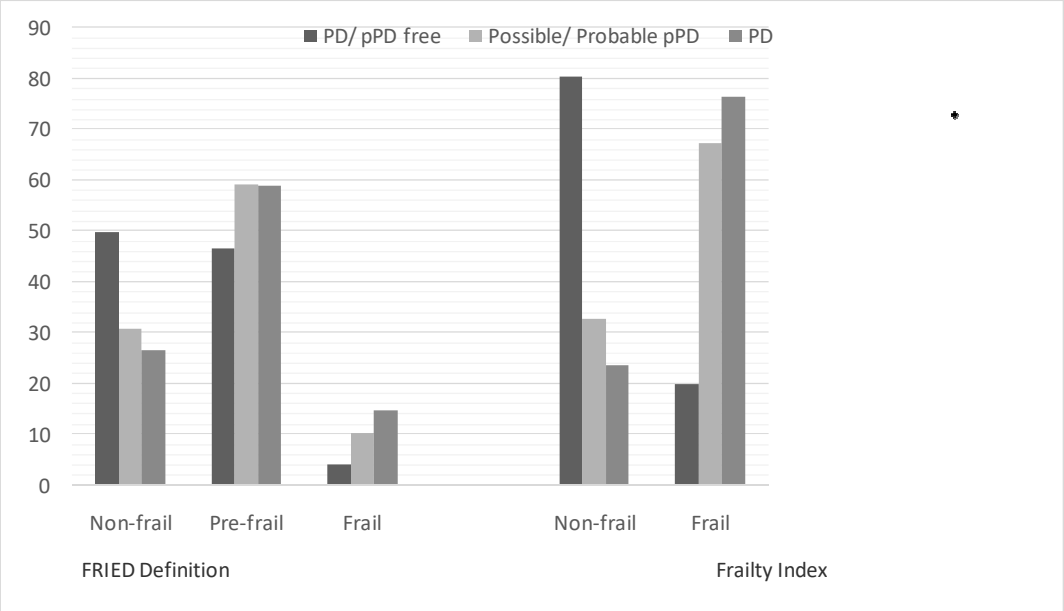

Figure 5 legend Abbreviations: PD: Parkinson's Disease; pPD: possible/probable prodromal PD (i.e. pPD probability ≥30%), *P*-values were obtained with logistic regression analyses. *p<0.05 vs. participants without PD or pPD.

Figure 6 Odds-ratio and confidence intervals of the unadjusted association between frailty status and diagnosis of PD and possible/probable pPD (≥30% probability).

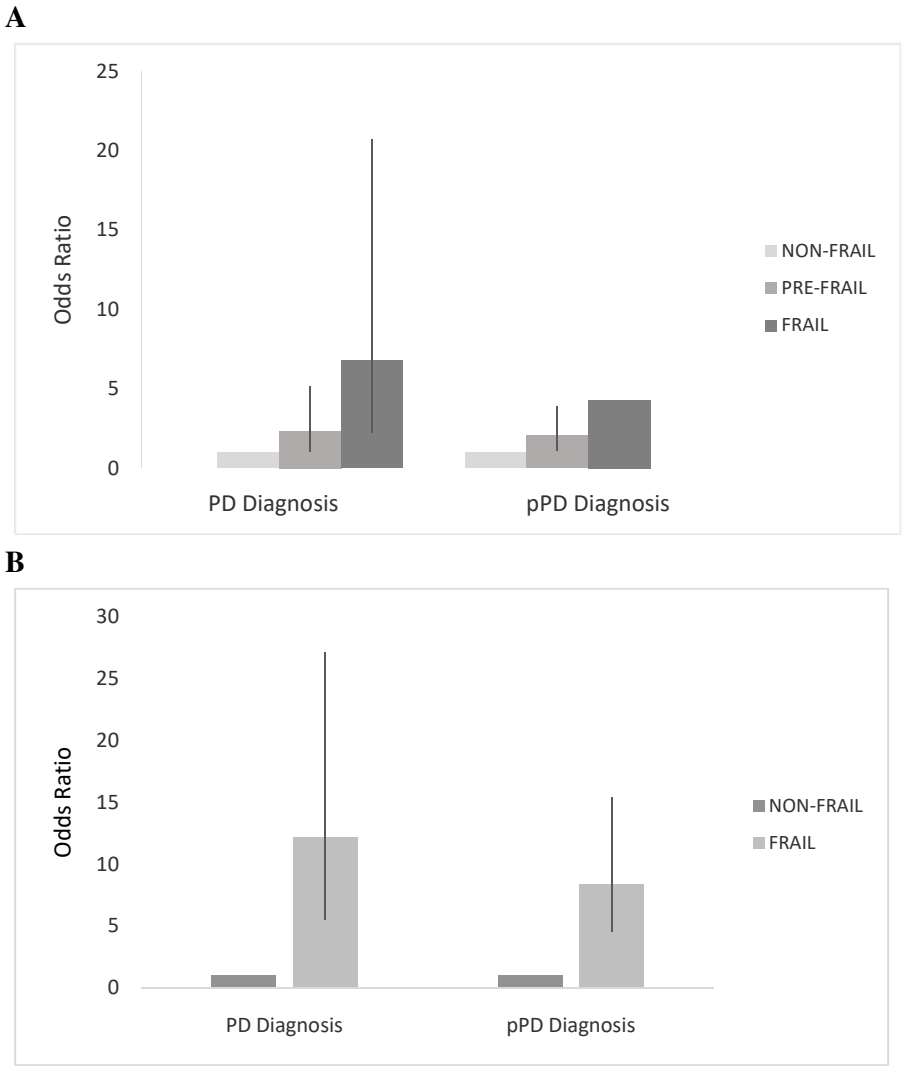

Figure 6 legend (A) Frailty status measured with Fried Definition and (B) frailty status measured with Frailty Index. Abbreviations: PD: Parkinson's Disease; pPD: possible/probable prodromal PD (i.e. pPD probability ≥30%),

Table 1

Risk markers and assigned likelihood ratios (LR) based on the International Parkinson and Movement Disorders Society's Research Criteria for Prodromal Parkinson's Disease. LR: likelihood ratio.

Marker	Assessment tool used	Answer for +	Number of cases	LR+/LR-
1. Sex, male	Structured questionnaire for demographics	Yes	757	1.2/0.8
2. Regular exposure to pesticides	Structured pretested questionnaire [32]	Regular occupational exposure to pesticides or >100 episodes of non-occupational exposure	184	1.5/1.0
3. Nonuse of coffee	Semi-quantitative food frequency questionnaire [58]	<3 cups of coffee/week	362	1.35/0.88
4. Non smoking status	Detailed questionnaire for tobacco use	Never smoker	1156	1.25 /0.45 0.8 for former smoker (minimum 1 pack-year)
5. Genetics	Medical history of the participants' first degree relatives	At least one first degree relative with Parkinson's Disease	86	2.5/1.0

Table 2

Clinical non-motor and motor markers and assigned likelihood ratios based on the International Parkinson and Movement Disorders Society's Research Criteria for Prodromal Parkinson's Disease. DLB: Dementia with Lewy Bodies; LR: likelihood ratio; PD: Parkinson's disease; RBD: Rapid-eye-movement sleep behavior disorder.

Marker	Assessment tool used	Relevant questions/score	Answer for +	n	Number of cases (at least one alternative in each marker)	LR+/LR-
1. Possible REM Behavior disorder	DLB diagnostic tool [38]	RBD. Acting out their dreams.	Yes	110	121	2.3/0.76
		RBD. Intense movements during sleep	Yes	37		
2. Excessive daytime somnolence	Sleep Scale of the Medical Outcomes Study [35]	How often during the past 4 weeks did you feel drowsy or sleepy during the day?	All or most or a good bit of the time	305	773	2.2/0.88
		How often during the past 4 weeks did you have trouble staying awake during the day?		126		
		How often during the past 4 weeks did you take naps (5 minutes or longer) during the day?		370		
		Daytime somnolence score (sum of the revised score in the above questions)	>median score	763		
	Neuropsychiatric Inventory (NPI-12) [39]	Does the patient sleep excessively during the day?	Yes	5		
3a. Depression	Geriatric Depression Scale (GDS)[33]	GDS score	>5	268	383	1.8 /0.85
	Clinical evaluation	Depression as major/secondary cause of dementia diagnosis or not contributory to mental state	Yes	251		
	Medical History-Use of medication	Use of antidepressants	Yes	137		
3b. Anxiety without depression	7-item anxiety subscale of the Hospital Anxiety and Depression Scale	HADS score	>7	223	438 (246 without 3a depression)	1.0: for anxiety without depressio

	(HADS) [34]					n
	Clinical evaluation	Anxiety as major/secondary cause of dementia diagnosis or not contributory to mental state	Yes	278		
	Medical History-Use of medication	Use of anxiolytics	Yes	219		
4. Orthostatic hypotension	DLB diagnostic tool [38]	Does the participant suffer from orthostatic hypotension?	Yes	85	85	2.1/0.9
5. Constipation	DLB diagnostic tool [38]	Does the participant suffer from constipation?	Yes	313	313	2.2/0.8
6. Sexual dysfunction	DLB diagnostic tool [38]	Does the participant suffer from impotence?	Yes	4	4	2.0/0.9
7. Urinary dysfunction	DLB diagnostic tool [38]	Does the participant suffer from bladder incontinence?	Yes	322	363	1.9/0.9
	Blessed Dementia Scale [36].	Sphincter control	Occasional or frequent wet bed or completely incontinent	315		
8. Sub-threshold parkinsonism	Motor part of Unified Parkinson's Disease Rating Scale (UPDRS) [37]	UPDRS score excluding action tremor	>3	62	62	10/0.7

Table 3. Demographics and anthropometrics of the study population (*n*=1731)

Age, median (Q1, Q3), years	73 (69, 77)
Education, median (Q1, Q3), years	6 (5, 12)
Sex, male, %	40.7
Smoking, %	10.4
Socioeconomic status, lower, %	45.8
Number of clinical co-morbidities, median (Q1, Q3)	1 (1, 2)
BMI, median (Q1, Q3), kg/m^2	28.4 (25.7, 31.6)
BMI status	
Underweight, %	0.4
Normal weight, %	19.1
Overweight , %	43.8
Obese , %	36.7
Waist circumference, cm	101.0 (93.0, 109.0)
Abdominal obese , %	69.7

Values are means ± SD or median (Q1, Q3) or frequencies (%) for normally and not normally distributed continuous and categorical variables, respectively.

Table 4 Dietary characteristics of the study population

	All (***n*=1,731**)	**Males** (***n*=705**)	**Females** (***n*=1026**)	**p-value**
Energy Intake, kcal/d	1972 ± 534	2080 ± 564	1899 ± 500	<0.001
Carbohydrates, % energy	38.0 ± 6.0	37.0 ± 6.0	38.7 ± 5.9	<0.001
Proteins				
% of energy	15.4 ± 2.8	15.3 ± 2.7	15.5 ± 2.8	0.258
g/kg/d	1.03 ± 0.34	1.00 ± 0.31	1.05 ± 0.36	0.004
Lipids, % of energy	44.7 ± 5.8	44.2 ± 5.6	44.9 ± 5.9	0.016
Alcohol, % of energy	0.0 (0.0 , 1.9)	0.0 (1.5 , 5.7)	0.0 (0.0 , 0.7)	<0.001
MeDi score	33.2 ± 4.6	34.3 ± 4.3	32.5 ± 4.7	<0.001

Values are means ± SD or median (Q1, Q3) or frequencies (%) for normally and not normally distributed continuous and categorical variables, respectively. P-values obtained with unpaired t-test or Mann Whitney rank test (continuous, normally and not normally distributed, variables) or Chi-square test (categorical variables).

Table 5

Prevalence of LR positive risk, clinical motor and non-motor markers according to the International Parkinson and Movement Disorder Society, in older people by quartiles of the Mediterranean Diet score (MeDi).

	All (*n*=1,731; score range: 17-46)	**MeDi Quartile 1 (*n*=462; score range: 17-30)**	**MeDi Quartile 2 (*n* =375; score range: 31-33)**	**MeDi Quartile 3 (*n* =413; score range: 34-36)**	**MeDi Quartile 4 (*n* =424; score range: 37-46)**	**p-value**
Age (years)	73 (69, 77)	74 (70, 80) [a, b]	73 (69, 77)	72 (69, 76) [a]	73 (69, 76) [b]	<0.001
Pretest Probability of prodromal PD	2.83±0.72	2.93±0.74 [a, b]	2.83±0.71	2.78±0.81 [a]	2.73±0.70 [b]	<0.001
Risk markers						
Sex, % male	40.7	26.8	38.7	46.0	51.4	<0.001
Regular pesticide exposure, %	12.7	5.7	13.4	14.3	16.4	<0.001
Nonuse of coffee, %	19.5	24.7	20.5	17.7	15.1	0.003
Nonsmoking, %	63.1	70.7	65.3	59.4	55.9	<0.001
First degree relative with PD, %	4.5	5.2	5.1	4.6	3.1	0.420
LR risk markers	1.08±0.56	1.06±0.50	1.11±0.58 [a]	1.09±0.57	1.07±0.60 [a]	0.032
Prodromal markers						
Possible RBD, %	7.0	8.7	5.9	4.8	7.4	0.125
Daytime somnolence, %	44.7	49.6	47.2	40.2	43.2	0.029
Depression, %	22.1	29.4	22.9	20.1	16.7	<0.001
Orthostatic Hypotension, %	5.0	4.9	5.8	4.2	4.3	0.721
Constipation, %	18.2	22.1	18.4	16.0	14.7	0.022

Impotence, %	0.8	0.7	0.0	1.4	0.8	0.721
Urinary Dysfunction, %	21.3	25.5	23.2	17.2	18.4	0.007
Sub threshold parkinsonism, %	3.6	2.6	2.9	4.4	4.5	0.321
LR prodromal markers	0.64 (0.27, 1.37) range: 0.23- 799.95	0.66 (0.49, 1.51) [a, b] range: 0.23- 342.64	0.64 (0.30, 1.37) [c] range: 0.23- 125.14	0.58 (0.26, 1.37) [a, c] range: 0.23- 178.84	0.64 (0.26, 1.36) [b] range: 0.23- 799.95	<0.001
Total LR for prodromal PD	0.66 (0.31, 1.50)	0.77 (0.40, 1.76) [a, b]	0.71 (0.36, 1.48) [c]	0.54 (0.23, 1.31) [a, c]	0.56 (0.30, 1.32) [b]	<0.001
Probability of prodromal PD	1.92 (0.87, 4.32)	2.33 (1.01, 4.96) [a, b]	2.02 (0.96, 4.22) [c, d]	1.54 (0.72, 4.00) [a, c]	1.50 (0.79, 3.66) [b, d]	<0.001
≥80% probability of prodromal PD *n* (%)	*4* (0.2)	*3* (0.6)	*0* (0.0)	*0* (0.0)	*1* (0.2)	0.161
≥50% probability of prodromal PD *n* (%)	*19* (1.1)	*8* (1.7)	*3* (0.8)	*5* (1.2)	*3* (0.7)	0.466
≥30% probability of prodromal PD *n* (%)	*49* (2.8)	*17* (3.7)	*7* (1.9)	*10* (2.4)	*14* (3.3)	0.388

Values are median (Q1, Q3) or means ± SD or frequencies (%) unless otherwise stated. LR: likelihood ratio; PD: Parkinson's disease; RBD: Rapid-eye-movement sleep behavior disorder. P-values obtained with Kruskal Wallis tests (continuous, not normally distributed, variables) or chi-square tests (categorical variables). [a, b, c, d] values in the same row sharing the same superscript letter are statistically significantly different from each other according to post-hoc Mann Whitney rank tests corrected for multiple comparisons (Bonferonni).

Table 6 Associations between the Mediterranean Diet score and probability of prodromal Parkinson's disease.

	MeDi score as a continuous variable		Quartiles of MeDi score		
	β (95%CI)	p-value	β (95%CI)	p-value	p for trend
Un-adjusted	0.971 (0.959, 0.983)	<0.001	Q1: 1 (reference)		<0.001
			Q2: 0.896 (0.766, 1.049)	0.173	
			Q3: 0.728 (0.625, 0.849)	<0.001	
			Q4: 0.731 (0.628, 0.851)	<0.001	
Adjusted for years of education	0.981 (0.969, 0.993)	0.002	Q1: 1 (reference)		0.004
			Q2: 0.915 (0.785, 1.067)	0.259	
			Q3: 0.797 (0.686, 0.926)	0.003	
			Q4: 0.819 (0.704, 0.951)	0.009	
Adjusted for years of education and energy intake	0.977 (0.965, 0.990)	<0.001	Q1: 1 (reference)		0.001
			Q2: 0.916 (0.782, 1.072)	0.275	
			Q3: 0.796 (0.681, 0.930)	0.004	
			Q4: 0.793 (0.678, 0.929)	0.004	

Results from linear regression analyses using log-transformed data for probability of prodromal Parkinson's disease. For clarity purposes, β (95%CI) were back-transformed from log scale to their original scale.

Table7 Demographic and clinical characteristics of the study population by quartiles of cognitive composite z score

	Composite z score quartile 1 (*n*=368; range: -3.10 to -0.69)	**Composite z score quartile 2 (*n*=423; range: -0.69 to -0.10)**	**Composite z score quartile 3 (*n*=428; range: -0.10 to 0.38)**	**Composite z score quartile 4 (*n*=410; range: 0.38 to 1.38)**	***P*-value**
Sex, *n* (%)					
Male	141 (38.3)	161 (38.1)	187 (43.7)	165 (40.2)	0.318+
Female	227 (61.7)	262 (61.9)	241 (56.3)	245 (59.8)	
Age (years), median (Q1, Q3)	77 (73, 80) [a]	73 (70, 77) [b]	71 (69, 75) [c]	70 (67, 73) [d]	**<0.001‡‡**
Years of education, median (Q1, Q3)	4 (2, 6) [a]	6 (4, 6) [b]	6 (6, 12) [c]	12 (9, 16) [d]	**<0.001‡‡**
MMSE, median (Q1,Q3)	25 (22, 26) [a]	27 (25, 28) [b]	28 (27, 29) [c]	29 (28, 30) [d]	**<0.001‡‡**
Clinical comorbidities ^, *n* (%)					
No	52 (14.2)	61 (14.6)	93 (21.9)	96 (23.5)	**<0.001+**
1	125 (34.1)	143 (34.1)	147 (34.6)	142 (34.8)	
2	113 (30.8)	114 (27.2)	110 (25.9)	111 (27.2)	
≥3	77 (21.0)	101 (24.1)	75 (17.6)	59 (14.5)	
SES, *n* (%)					
Lower	230 (62.5)	207 (48.9)	169 (39.5)	122 (29.8)	**<0.001+**
Higher	138 (37.5)	216 (51.1)	259 (60.5)	288 (70.2)	

Table 8 Probability of pPD, prevalence of LR positive risk, clinical motor and non-motor markers according to the International Parkinson and Movement Disorders Society's research criteria for pPD, in older people, by quartiles of the cognitive composite z score

	Composite z score quartile 1 (*n*=368; range: -3.10 to -0.69)	**Composite z score quartile 2 (*n*=423; range: -0.69 to -0.10)**	**Composite z score quartile 3 (*n*=428; range: -0.10 to 0.38)**	**Composite z score quartile 4 (*n*=410; range: 0.38 to 1.38)**	***P*-value**
Pretest					
Pretest Probability of pPD, median (Q1,Q3) mean ±SD	3.5 (2.5,4.0)[a] 3.27±0.66	2.5 (2.5, 3.5)[b] 2.87±0.71	2.5 (2.0, 3.5)[c] 2.64±0.64	2.5 (2.0, 2.5)[d] 2.45±0.57	**<0.001‡‡**
Risk Markers					
Regular pesticide exposure, *n* (%)	45 (12.6)	56 (15.0)	34 (9.9)	24 (10.6)	0.167+
Nonuse of coffee, *n* (%)	66 (18.3)	75 (18.1)	85 (20.0)	78 (19.0)	0.886+
Nonsmoking, *n* (%)	260 (72.2)	284 (67.8)	256 (60.0)	211 (52.5)	**<0.001+**
Genetics, *n* (%)	15 (4.1)	19 (4.5)	19 (4.4)	21 (5.2)	0.910+
LR Risk Markers, median (Q1,Q3) mean±SD	0.88 (0.88, 1.32)[a] 1.13±0.64	0.88 (0.88, 1.32)[a] 1.11±0.60	0.88 (0.84, 1.32)[a,b] 1.07±0.52	0.88 (0.84, 1.30)[b] 1.00± 0.50	**<0.001‡‡**
Prodromal Markers					
Possible RBD, *n* (%)	24 (6.5)	34 (8.1)	29 (6.8)	24 (5.9)	0.663+
Daytime somnolence, *n* (%)	166 (45.1)	209 (49.4)	190 (44.4)	155 (37.8)	**0.009+**
Depression, *n* (%)	99 (26.9)	92 (21.7)	75 (17.5)	90 (22.0)	**0.017+**
Orthostatic hypotension, *n* (%)	21 (5.9)	19 (4.6)	21 (5.0)	20 (5.0)	0.887+
Constipation, *n* (%)	83 (22.6)	97 (23.0)	73 (17.1)	41 (10.1)	**<0.001+**
Impotence, *n* (%)	1 (1.8)	0 (0.0)	2 (1.6)	0 (0.0)	0.168+

Urinary dysfunction, *n* (%)	115 (31.3)	98 (23.2)	76 (17.8)	48 (11.7)	**<0.001**+
Sub threshold parkinsonism, *n* (%)	18 (4.9)	15 (3.6)	12 (2.8)	4 (1.0)	**0.013**+
LR Prodromal Markers, median (Q1,Q3) mean±SD	0.71 (0.54, 1.60)[a] 3.09±13.07	0.64 (0.49, 1.66)[a] 3.90±39.06	0.64 (0.26, 1.36)[b] 2.36±10.50	0.55 (0.26, 1.15)[c] 0.93±1.28	**<0.001**‡‡
Total					
Total LR for pPD, median (Q1, Q3) mean±SD	0.85 (0.48, 1.84)[a] 3.47±14.56	0.82 (0.37, 1.76)[a] 3.87±34.47	0.57 (0.31, 1.28)[b] 2.52±12.27	0.46 (0.22, 1.06)[c] 0.98±1.63	**<0.001**‡‡
Probability of pPD, median (Q1, Q3) mean±SD	2.89 (1.34, 6.24)[a] 6.35±10.83	2.24 (1.03, 4.72)[b] 5.17 ± 9.39	1.51 (0.82, 3.36)[c] 3.94 ± 8.99	1.09 (0.56, 2.54)[d] 2.35 ± 3.76	**<0.001**‡‡
Possible or probable pPD (≥30%probability), *n* (%)	13 (3.5)	16 (3.8)	8 (1.9)	1 (0.2)	**0.002**+

Abbreviations: PD=Parkinson's Disease; pPD= prodromal PD.

Lower quartile:1 - Upper quartile:4

Coffee consumption was assessed with a semi-quantitative food frequency questionnaire [58] and non-use of coffee was assigned when participant consumed less than <3 cups of coffee/week [7, 98].

+Pearson's chi-square test

‡‡Kruskal- Wallis test. Values in the same row bearing different superscript letters differ significantly, according to Mann Whitney rank tests (not normally distributed continuous variables, Bonferroni corrected for multiple comparisons).

e.g, for Pretest Probability of pPD: each quartile of composite z score has different superscript (Q1 has [a], Q2 [b], Q3 [c] and Q4 [d]), meaning that they are all different from each other; for LR Risk Markers: Q1 and Q2 have [a] , meaning that they do not differ from each other, Q4 has [b], meaning that differs from both Q1 and Q2 (which have [a]), while Q3 has both [a] and [b], meaning that it does not differ from any quartile)

Table 9 Associations between the cognitive composite z-score and probability of pPD.

Model	**Composite z-score as a continuous variable**		**Quartiles of Composite z-score**		
	Beta (95%CI)	**p-value**	**Beta (95%CI)**	**p-value**	**p for trend**
Un-adjusted	0.646 (0.591, 0.684)	**<0.001**	Q1: 0 (reference)		**<0.001**
			Q2: 0.796 (0.681, 0.932)	**0.005**	
			Q3: 0.560 (0.479, 0.655)	**<0.001**	
			Q4: 0.403 (0.345, 0.471)	**<0.001**	
Adjusted for years of education, SES and number of clinical comorbidities	0.725 (0.660, 0.796)	**<0.001**	Q1: 0 (reference)		**<0.001**
			Q2: 0.835 (0.712, 0.980)	**0.027**	
			Q3: 0.633 (0.535, 0.749)	**<0.001**	
			Q4: 0.520 (0.427, 0.635)	**<0.001**	

Abbreviations: SES=socioeconomic status; pPD= prodromal Parkinson's Disease.

Results from linear regression analyses using log-transformed data for probability of pPD. For clarity purposes, βs (95%CI) were back-transformed from log scale to their original scale. Lower quartile:Q1 - Upper quartile:Q4.

Table 10 Participants' z-scores in individual cognitive domains in relation to probability of pPD

	Memory z-score	**p**	**Executive z-score**	**p**	**Visual Spatial z-score**	**p**	**Language z-score**	**p**	**Attention Speed z-score**	**p**
Continuous pPD probability										
Spearman's Correlation Coefficient of association with probability of pPD	-0.270	**<0.001**	-0.272	**<0.001**	-0.171	**<0.001**	-0.288	**<0.001**	-0.250	**<0.001**
Dichotomous pPD probability (possible or probable; ≥30% probability)										
Total sample median (Q1, Q3)	-0.16 (-0.77, 0.51)		-0.08 (-0.61, 0.37)		0.09 (-0.50, 0.56)		-0.07 (-0.71, 0.56)		0.04 (-0.72, 0.57)	
No pPD median (Q1, Q3)	-0.14 (-0.74, 0.51)	**0.002***	-0.07 (-0.60, 0.38)	**0.003***	0.09 (-0.50, 0.56)	**0.020***	-0.06 (-0.69 , 0.57)	**<0.001***	0.05 (-0.72, 0.57)	**0.035***
Yes pPD median (Q1, Q3)	-0.66 (-1.19, -0.13)		-0.36 (-1.28, 0.07)		-0.12 (-0.94, 0.33)		-0.67 (-1.29, 0.04)		-0.24 (-1.48, 0.49)	

Abbreviations: PD=Parkinson's Disease; pPD= prodromal PD.

*Mann-Whitney rank test

Table 11. Physical activity and motor measures according to prodromal PD (pPD) status

	All (*n*=1731 PD-free)	**Non-pPD group (i.e. pPD probability<30) (*n*=1682)**	**Possible/Probable pPD (i.e. pPD probability ≥30) (*n*=49)**	***p*-value**
Motor complaints score, median (Q1, Q3)	0 (0, 0)	0 (0, 0)	1 (0, 2)	**<0.001**
At least one motor complaint, No. (%)	349 (20.2)	321(19.1)	28 (57.1)	**<0.001**
TEE, median (Q1, Q3), kcal/kg/day	27.6 (25.2, 30.9)	27.6 (25.2, 30.9)	26.0 (24.5, 27.6)	**0.010**
Gait speed, median (Q1, Q3), sec for 1m	1.20 (0.98, 1.53)	1.20 (0.97, 1.51)	1.43 (1.08, 1.90)	**0.001**
Gait speed, median (Q1, Q3), sec for 4m	3.95 (3.24, 4.95)	3.92 (3.24, 4.90)	4.93 (3.76, 6.63)	**<0.001**
Low gait speed (<0.8m/s, 4m test), No. (%)	408 (24.1)	384 (23.4)	24 (49.0)	**<0.001**
UPRDS score, median (Q1, Q3)	0 (0, 0)	0 (0, 0)	1 (7, 18)	**<0.001**

TEE=total energy expenditure; BMI=body mass index; PD=Parkinson's Disease; pPD=prodromal PD.

p-values were obtained with Mann Whitney rank tests (continuous, not normally distributed, variables) or chi-square tests (categorical variables) between pPD and non-pPD groups. Significant *p*-values are given in bold.

Table 12: Associations between physical activity and other motor measures and prodromal Parkinson's Disease (pPD)

	Models adjusted for BMI, years of education, socioeconomic status and number of clinical comorbidities					
	Associations with probability of pPD (continuous variable) (R^2 of confounders 0.075)			**Associations with possible/probable pPD (≥30% probability) (R^2 of confounders 0.040)**		
	R^2	*p*-value	β (95%CI)	R^2	*p*-value	OR (95%CI)
Motor complaints score	0.124	<0.001	1.268 (1.209, 1.331)	0.102	<0.001	1.484(1.287, 1.713)
At least one motor complaint	0.127	<0.001	2.053 (1.783, 2.363)	0.105	<0.001	5.042 (2.692, 9.442)
-1.0 * TEE (kcal/kg/day)	0.089	<0.001	1.030 (1.017, 1.043)	0.051	0.043	1.088 (1.003, 1.181)

TEE=total energy expenditure; BMI=body mass index; pPD=prodromal Parkinson's Disease.

Results from linear regression analyses using log-transformed data for probability of prodromal PD. For clarity purposes, β (95%CI) were back-transformed from log scale to their original scale.

Table 13. Associations between frailty (as measured with Fried definition and FI) and PD diagnosis, possible/probable pPD (≥30% probability) and probability of pPD (continuous variable) in older population.

	Participants with PD (n=34) vs participants without PD (*n*=1731)		Participants with pPD (n=49) vs participants without pPD (*n*=1682)		Associations with probability of pPD (n=1731 PD-free)	
	OR (95%CI)	p-value	OR (95%CI)	p-value	β (95%CI)	p-value
Association with Fried definition						
Non-frail	1 (reference)		1 (reference)			
Pre-frail	2.34 (1.06, 5.17)	**0.035**	2.08 (1.10, 3.90)	**0.023**	1.48 (1.32, 1.65)	**<0.001**
Frail	6.76 (2.21, 20.73)	**0.001**	4.29 (1.51, 12.18)	**0.006**	2.92 (2.20, 3.87)	**<0.001**
Association with FI (as a dichotomous variable)						
Non-frail	1 (reference)		1 (reference)			
Frail	12.16 (5.46, 27.09)	**<0.001**	8.39 (4.56, 15.42)	**<0.001**	2.86 (2.51, 3.25)	**<0.001**
Association with FI (as a continuous variable)						
Total continuous	1.33	**<0.001**	1.37	**<0.001**	1.17	**<0.001**

score	(1.23, 1.43)	(1.28, 1.47)	(1.16, 1.19)

Abbreviations: PD=Parkinson's Disease, pPD= prodromal PD.

Results for probability of PD and pPD came from logistic regression analyses. Results for probability of pPD came from linear regression analyses using log-transformed data. For clarity purposes, β (95%CI) were back-transformed from log scale to their original scale.

All models were unadjusted.

Table 14. Supplementary Analyses: Associations between frailty (as measured with Fried definition and FI) and PD diagnosis, possible/probable pPD (≥30% probability) and probability of pPD (continuous variable) in older population. Models were adjusted for gender, age, education and socioeconomic status.

	Participants with PD (n=34) vs participants without PD (*n*=1731)		Participants with pPD (n=49) vs participants without pPD (*n*=1682)		Associations with probability of pPD (n=1731 PD-free)	
	OR (95%CI)	p-value	OR (95%CI)	p-value	β (95%CI)	p-value
Association with Fried definition						
Non-frail	1 (reference)		1 (reference)			
Pre-frail	2.02 (0.90, 4.57)	0.093	1.63 (0.84, 3.14)	0.146	1.19 (1.07, 1.33)	**0.002**
Frail	4.76 (1.36, 16.64)	**0.014**	2.28 (0.72, 7.23)	0.160	1.63 (1.58, 3.91)	**0.001**
Association with FI (as a dichotomous variable)						
Non-frail	1 (reference)		1 (reference)			
Frail	11.76 (5.12, 27.01)	**<0.001**	7.55 (4.00, 14.25)	**<0.001**	2.393 (1.233, 2.158)	**<0.001**
Association with FI (as a continuous variable)						
Total continuous score	1.35 (1.24, 1.46)	**<0.001**	1.39 (1.28, 1.50)	**<0.001**	1.14 (1.13, 1.16)	**<0.001**

Abbreviations: PD=Parkinson's Disease, pPD= prodromal PD.

Results for probability of PD and pPD came from logistic regression analyses. Results for probability of pPD came from linear regression analyses using log-transformed data. For clarity purposes, β (95%CI) were back-transformed from log scale to their original scale.

Models were adjusted for gender, age, education and socioeconomic status.