Apomorphine: a potential modifier of amyloid deposition in Parkinson's disease?

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Abstract

Background: Evidence from clinical and pathological studies suggests a role for both α-synuclein and amyloid-β in the pathophysiology of dementia associated with PD. Recent work demonstrated improvement in memory and reduced amyloid-β burden in transgenic murine Alzheimer models given subcutaneous apomorphine.

Objective: To determine whether ante-mortem exposure to apomorphine was associated with lower levels of amyloid-β in brain tissue in a clinicopathological study of PD.

Methods: The case notes of donors with pathologically proven PD who had (n=36) and had not received apomorphine (n=35) during life for motor complications were reviewed to determine presence or absence of cognitive impairment. The four groups were well matched for disease duration, age at death, gender and apolipoprotein E4 genotype. The severity of amyloid-β mature/diffuse plaque load, tau pathology and α-synuclein pathology were all established. Cerebral amyloid angiopathy was determined based on a 4-tier grading system.

Results: Within the cognitively normal cases, significantly reduced amyloid-β deposition was present in those with ante-mortem apomorphine exposure; this finding was not replicated in those with cognitive impairment plus previous apomorphine use. In the apomorphine cognitively normal group only, a significant negative association was observed between maximum apomorphine dose received and amyloid-β burden. Early and maximum doses of apomorphine plus apolipoprotein genotype and gender were significant predictors of total plaque load in an explanatory model.

Conclusion: This exploratory study suggests that apomorphine may have a modifying effect on amyloid deposition in non-demented PD cases and thus may represent a potential therapy to reduce cognitive impairment in PD.

Introduction

Dementia occurs in up to 80% of Parkinson's disease (PD) subjects when followed longitudinally.[1-3] Current treatments for PD dementia (PDD) may improve cognitive and behavioural symptoms,[4] but have no effect on disease progression.

The pathophysiology of PDD is poorly understood and may vary between individuals, but evidence from clinical[5] and pathological studies[6-11] indicate that amyloid-β (Aβ), neurofibrillary tangle pathology and cortical Lewy body (LB) deposition are significant factors. Furthermore, a synergistic interaction between α-synuclein and Aβ accumulation has been found both *in vitro* [6, 7, 12] and in transgenic mice models,[13, 14] with concurrent evidence that Aβ promotes α-synuclein fibrillization and thus may influence the progression of cognitive decline.[14]

A therapy that prevents Aβ aggregation could conceivably be effective in the prevention or delay of Aβ-associated neurotoxicity. A new class of small molecules, structurally related to apomorphine, prevent Aβ fibril formation *in vivo* and undergo rapid autoxidation, suggesting that these autoxidation products may inhibit Aβ fibrillization.[15] More recently, transgenic Alzheimer disease (AD) mouse models treated with apomorphine demonstrated improvements in short-term memory function and significant reductions in intraneuronal Aβ accumulation, possibly due to antioxidative stress mechanisms.[16]

Given the role of Aβ aggregation in the etiology of PDD and the use of apomorphine in the management of motor complications in PD, we sought to determine whether apomorphine may have anti-amyloid effects in a clinicopathological study. We hypothesized that non-demented PD subjects who had received apomorphine ante-mortem would have reduced Aβ burden compared to a matched group who had not received this treatment.

Materials and Methods

Case selection

Forty three PD cases with exposure to apomorphine in life (Apo+) diagnosed using accepted neuropathological criteria[17] were identified from donors to Queen Square Brain Bank for Neurological Disorders, University College London. Apomorphine naïve cases (Apo-, n=38) were matched to age at disease onset, disease duration and age at death. Presence or absence of dementia was determined retrospectively by review of case notes and agreed by consensus of movement disorder specialists (A.J.Y, H.L., D.J.B.), according to established criteria.[18] Specifically, cognitive deficits severe enough to impact on activities of daily living, with a neuropsychological assessment (if available) was considered a positive history for PDD.[18] Other data collected included dose (early (within one month of commencement), one year, maximum dose), duration and method of delivery of apomorphine therapy; anticholinergic drug exposure (a potential confounder[19]); dopaminergic medication use; and clinical and demographic data. Lifetime cumulative levodopa dose was calculated using a previously described method.[20] An approximation for cumulative apomorphine dose was calculated using the following equation, approximating to the area under the curve: ((early apomorphine dose (mg) + one year dose)/2*1 + (one year + maximum apomorphine dose)/2*(duration of apomorphine use (years)-1))*365.25. All study subjects provided written informed consent ante-mortem. Cases were collected using a protocol approved by London Multicentre Research Ethics Committee and stored under the Human Tissue Authority licence.

Tissue collection

After fixation in 10% buffered formalin, brains were examined by a neuropathologist in accordance with QSBB protocol. Seven μm thick paraffin sections were cut and de-paraffinized, followed by pre-treatment with formic acid and pressure-cooking in citrate buffer at pH 6.0 and stained according to previously published methods.[21] Nonspecific binding was blocked using 10% non-fat milk in phosphate-buffered saline. Sections were incubated with antibodies recognizing Aβ, residues 17–26 (Biosource Int, 1:4,000), α-synuclein (Novocastra, 1:75) or tau (AT8, Autogen Bioclear, 1:600). The

secondary antibody (Dako, 1:200) was then applied, followed by incubation with ABC (Dako) and colour development by di-aminobenzidine/H_2O_2.

Quantification of pathology

The frequency of neocortical Aβ-positive plaques (mature and diffuse) was assessed using Aβ immunohistochemistry (performed by T.L., Y.C. and T.R., blinded to clinical data) using a previously described protocol.[7, 22] Using the approach recommended by CERAD, the severity of the cortical pathology for each plaque type was determined in a semi-quantitative manner as absent (grade 0), sparse (1), moderate (2) or frequent (3) for the following regions: frontal, temporal, occipital and parietal cortices; and entorhinal cortex.[7] Scores for both mature ($Plaque_{sum}$, range 0-15) and diffuse ($Diffuse_{sum}$, range 0-15) plaques were calculated, in addition to total Aβ plaque score ($Plaque_{sum}$+$Diffuse_{sum}$, range 0-30). α-synuclein pathology was assessed using a semi-quantitative grading system[23] and Braak LB pathology stage was given for each case based on the distribution of LB.[24]

A previously described and validated quantitative method was also used for determining the Aβ cortical load and LB counts.[7, 8] As we have previously found a good correlation between the cingulate and entorhinal LB densities and total cortical LB densities (Spearman's r = 0.985), and between temporal and parietal Aβ scores and total cortical Aβ scores (Spearman's r = 0.975),[7] using image analysis LB densities and Aβ load were also determined in these anatomical areas. Regions of interest were outlined and the surface area of each section measured using the NIH Image J software (available at http://rsb.info.nih.gov/ij/applets.html). In the selected areas all LBs were systematically counted and LB counts, adjusted to surface area, were expressed as LBs/mm^2, with Aβ load density expressed/ mm^2. Intra- and inter-rater repeatability studies revealed that 95% of measurements fell within two standard deviations of the mean difference, with a correlation coefficient of 0.996.

Cerebral amyloid angiopathy (CAA) was quantified based on a 4-tier grading system as described elsewhere[7] in the same cortical and subcortical regions. Using Aβ immunohistochemistry, the

presence or absence of Aβ-positive plaques was also measured in different brain regions and a Thal phase allocated to each case.[25]

Neurofibrillary tangle pathology was determined using Braak staging[26] and apolipoprotein E (APOE) genotype was determined for 24 Apo+ and 34 Apo- cases using previously described methods.[7]

Statistics

Subjects were classified by whether they had received apomorphine in life (Apo+) or not (Apo-) and cognitive status at the time of death (normal, CN, or impaired, CI), thus yielding four groups: Apo+CN, Apo-CN, Apo+CI and Apo-CI. Data were analysed as Apo+CN vs. Apo-CN and Apo+CI vs. Apo-CI groups. Statistical analyses were performed using SPSS 21.0 (SPSS, Chicago, IL). Means were compared using unpaired t-tests or Mann-Whitney tests, depending upon data distribution. Pearson Chi-Squared or Fisher's exact tests were used to analyze categorical data. One-tailed tests were used for the comparisons of Aβ deposition between groups only as our hypothesis was that Aβ load would be significantly less in the Apo+ cases. Spearman's rank correlation coefficients (two-tailed) were calculated to assess bivariate associations between Aβ scores and apomorphine dose. Due to non-Normality in the Aβ plaque scores, statistical package R (version 2.15.3) was used to find a suitable model. A negative binomial model in SPSS allowed comparison between groups whilst controlling for covariates. As this was an exploratory study, *a priori* correction for multiple comparisons was not made; if a parameter was significant, even if by chance, we felt it merited further analysis.[27]

Results

Of the 43 Apo+ and 38 Apo- cases identified, eight and two, respectively, were excluded due to incomplete medical records or inadequate brain tissue samples. The Apo+ group comprised seven subjects who used a pen (i.e. bolus) only as method of drug delivery, 15 who used continuous subcutaneous infusion and 13 who used both. Nineteen of the Apo+ group (54.3%) and 17 of the Apo- group (47.2%) were classified as cognitively normal. Groups were well matched for age at disease onset, disease duration, age at death and APOE4 genotype, in addition to other coexisting

(non-Aβ) diagnostic pathologies (table 1 and 2). Cumulative levodopa dose and duration of other dopaminergic agents were similar across the groups, with the exception of significantly longer duration of selegiline use in the Apo-CI compared to Apo+CI group (mean 8.3 versus 4.4 years, p=0.044, Mann Whitney 2-sided test). Male cases had significantly lower Aβ scores than females (median $Plaque_{sum}$+$Diffuse_{sum}$ scores 1.00 *vs.* 9.50, p=0.018 and Aβ load total 0.34 *vs.* 1.39, p=0.049, Mann-Whitney 2-sided test), although gender was not significantly different across the groups.

Within the non-demented subjects, there was significantly reduced Aβ accumulation in Apo+ cases, with $Diffuse_{sum}$ scores and $Plaque_{sum}$+$Diffuse_{sum}$ scores lower in the Apo+CN compared to Apo-CN group (table 2). Total cortical LB density, however, was greater in the Apo-CN group, largely driven by a greater cingulate α-synuclein burden. Of note, those previously exposed to apomorphine also had greater exposure to anticholinergic drugs, although no difference was observed in proportions of subjects who had taken anticholinergics for over 2 years (X^2=1.69, p=0.311, Pearson Chi-Squared test). No significant differences were noted within the cognitively impaired groups.

APOE4 genotype is a risk factor for AD, dementia with Lewy bodies (DLB) and possibly also for PDD.[28] Data were unavailable for APOE status in 13 participants (largely from the Apo+ group), so analyses were repeated only in cases where APOE status was available (supplementary table 1). The groups remained well matched for general characteristics including age at death, disease duration and gender. Significant differences persisted between the Apo+CN and Apo-CN group with respect to $Diffuse_{sum}$ scores (median 0.00 *vs.* 3.00, respectively, p=0.046, Mann-Whitney 1-sided test) and $Plaque_{sum}$+$Diffuse_{sum}$ scores (0.00 *vs.* 3.00, p=0.029). Interestingly, no differences were seen in total cortical LB density within the cognitively normal subgroups (median score Apo+CN 0.34 *vs.* 2.48 Apo-CN, p= 0.179, Mann-Whitney 2-sided test).

Within the whole Apo+ group, early dose of apomorphine used tended to be greater in those who were cognitively normal compared to cognitively impaired at death but did not reach statistical significance (median dose 44 vs. 11 mg, p=0.093, Mann-Whitney 2-sided test). No significant differences were seen in one year dose, maximum dose, cumulative dose and duration of apomorphine

in those who were cognitively normal. No difference in amyloid burden was seen in those using pen versus infusion delivery method, which is likely to be due to the small numbers within each group (data not shown). In the Apo+CN group only, a significant negative correlation was seen between maximum apomorphine dose received and $Plaque_{sum}$+$Diffuse_{sum}$ score (r= -0.474, p=0.047, Spearman's rho, Figure 1A). Correlation between both maximum and early dose of apomorphine and $Diffuse_{sum}$ score did not reach significance (r= -0.450, p=0.070 and r= -0.465, p=0.052, respectively, Spearman's rho, Figure 1B and 1C). The analyses were repeated after removal of the obvious outlier (a case receiving a maximum dose of apomorphine 400mg). There was a significant association between early apomorphine dose and $Diffuse_{sum}$ score (r= -0.504, p=0.046), with no significant correlation between maximum dose of apomorphine and $Diffuse_{sum}$ score or $Plaque_{sum}$+$Diffuse_{sum}$ score (r= -0.431, p=0.084 and r= -0.444, p=0.076, respectively, Spearman's rho, Figure 1D and 1E).

To inform our selection of covariates that may predict Aβ in an explanatory model, we explored the association between Aβ score and gender, anticholinergic use, APOE status, age at disease onset, age at death, disease duration and cumulative levodopa dose (table 3). Of these, only gender, APOE positivity and age at death were significantly associated. These covariates were therefore entered into a negative binomial model with $Plaque_{sum}$+$Diffuse_{sum}$ score of the CN group as the dependent and early/maximum/one year/cumulative apomorphine dose; age at death; APOE positivity; and gender as independent variables. Early and maximum dose were stable significant predictors of $Plaque_{sum}$+$Diffuse_{sum}$ score, with greater dose predicting reduced Aβ. The other dose calculations were not significant (table 3).

Discussion

This is the first clinicopathological study to investigate the relationship between amyloid burden in PD subjects who had received apomorphine ante-mortem, following reports of putative disease-modifying effects in AD murine models.[16] It is possible that apomorphine treatment may have a modifying effect on Aβ peptide deposition in non-demented PD cases, with our results demonstrating decreased diffuse and total plaque load in the Apo+ compared to Apo- group, reduced Aβ

accumulation in subjects who had received greater earlier and maximum apomorphine doses and an inverse association between maximum drug dose and Aβ peptide deposition.

Mechanisms by which apomorphine may reduce Aβ accumulation include antioxidative prevention of apoptosis and synaptic injury, and greater degradation of intracellular Aβ, the latter potentially by upregulation of the proteasome system.[16] *In vitro* work indicating that apomorphine inhibits Aβ fibril formation and stabilizes Aβ oligomeric species via hydroxyl groups on the D-ring of apomorphine and related compounds adds further support to a mechanistic basis for the observed benefits.[15] Aβ42, containing 42 amino-acid residues, is a major constituent of mature amyloid plaques, and is highly prone to oligomerization and aggregation into fibrils.[29-31] Conversely, Aβ40 is not found within diffuse plaques, which are not fibrillar and contain almost exclusively Aβ42 proteins; these may represent precursors of mature, neuritic plaques.[31] Aβ42 is the more toxic of the species,[29] and hence we speculate that the positive findings in our study that relate to diffuse plaque and total plaque load may be attributable to apomorphine-induced inhibition of Aβ42 to a greater degree than Aβ40. This is substantiated by the observation that specifically it is diffuse plaque load which correlates with α-synuclein pathology.[7]

The finding of lower Aβ burden in the Apo+CN compared to Apo-CN group is strengthened by the fact that the former cases had greater anticholinergic exposure, which has been associated with increased Aβ plaque densities.[19] However, this could also be explained by inadvertent sampling bias. Postural instability gait difficulty (PIGD) phenotype is a recognised risk factor for the development of PDD[1, 32, 33] and has been associated with greater Aβ deposition than in tremor dominant subtypes,[34, 35] hence confounding-by-indication is a possibility. Anticholinergic drugs were previously used to treat tremor and hence anticholinergic use in this study may reflect a proxy for motor phenotype; unfortunately the retrospective nature of this study meant that we were unable to account for motor heterogeneity.

Within the Apo+CN group, total α-synuclein load was smaller than in the Apo-CN group, which is of significance as cortical Aβ deposition and LB load demonstrate a close association in Lewy body

diseases[6, 7, 11] and may have contributed to the greater amyloid plaque burden in the Apo-CN cases. Moreover, recent studies have suggested that cortical LB pathology may be the principal pathological substrate of dementia in PD.[9, 10] No differences were seen in α-synuclein load however, when analysis was limited to those cases where APOE status was available (supplementary table 1); significantly reduced Aβ deposition remained between the cognitively normal and impaired groups, thus strengthening our findings. One study has previously demonstrated an association between tau pathology and cerebrovascular disease, with greater cerebrovascular lesion burden in PDD cases compared to age-matched controls.[36] Others, however, have shown that cerebrovascular burden occurs in only a minority[3, 9, 37] and is not a significant predictor of cognitive impairment.[10] We believe that cerebrovascular disease is therefore unlikely to contribute to dementia in PD in a majority of patients.[38]

In contrast to AD mouse models,[16] we did not find differences in tau pathology between those who had and had not received apomorphine, which may represent differing underlying disease processes. It is interesting that differences were only seen within the cognitively normal group and not in those with dementia at the time of death. Indeed, although significance was not reached, those in the Apo+CI group tended to have higher Aβ plaque densities than Apo-CI cases (table 2); we hypothesize that when apomorphine was stopped in the cognitively impaired group there may have been acceleration of Aβ deposition, or that the doses used in this population were insufficient to inhibit Aβ fibril formation.[15] We note, however, that overall there were no differences in median doses of apomorphine between the groups, although those who were cognitively normal tended to have a higher early dose. It is noteworthy that murine models used 5 to 10mg/kg of apomorphine per week (corresponding to 0.4-0.8mg/kg in humans)[16] and whilst not reaching significance, the Apo+CN group in our study tended towards greater early, one year and maximum doses of apomorphine. Additionally, *in vitro* work has indicated that if apomorphine was added to cell culture after the neurotoxic insult, the antioxidant effect of the drug was significantly lessened;[39] hence we speculate that apomorphine may have been commenced in the Apo+CI after the deposition of toxic Aβ and/or LB species and thus too late to observe a clinical effect.

Although this study has yielded some promising results, the data are exploratory and clearly require confirmation in larger cohorts and *in vivo*. We acknowledge that one-tailed statistics, used due to the hypothesis-driven study, without correction for multiple hypotheses, could possibly render the associations observed as due to chance. However, the fact that greater apomorphine doses remained a significant predictor of Aβ load in a model after adjusting for other covariates adds consistency and strength to our argument, as do the two-tailed Spearman's correlations. The study design at present cannot answer whether apomorphine prevents cognitive impairment, and would necessitate a prospective *in vivo* study. The retrospective nature of our study mean that there are limitations as regards the cognitive state of individuals at time of death. Furthermore, it is possible that there was an implicit bias in the decision not to use apomorphine in the Apo-CN group in subjects with subtle early signs of cognitive impairment that was not identified on case note review. However, the groups were well matched for use of oral dopamine agonists and amantadine, which in clinical practice are more likely to be avoided in the face of cognitive decline. Other limitations include ascertaining clinical information retrospectively, missing APOE genotype data and lack of precision in determining apomorphine cumulative dosages. Whilst cumulative lifetime levodopa dose calculation is well established,[20] apomorphine cumulative doses are more difficult to estimate and hence may have contributed to the lack of effect demonstrated in our work. In conclusion, we postulate that apomorphine may represent a novel therapeutic agent to reduce the burden of cognitive impairment in PD.

Author roles

AJ Yarnall – Project execution and organization; Statistical analysis; Writing first manuscript draft

T Lashley – Project execution and organization; Review and critique of manuscript

H Ling – Project execution and organization; Review and critique of manuscript

A Lees – Project conception; Review and critique of manuscript

S Coleman – Statistical analysis; Review and critique of manuscript

S Sullivan - Project execution; Review and critique of manuscript

Y Compta - Project execution; Review and critique of manuscript

T Revesz - Project execution; Review and critique of manuscript

D Burn – Project conception and organization; Review and critique of manuscript

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Conflicts of interest

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served on scientific advisory boards for Britannia, Roche, Novartis, Boehringer Ingelheim, Lundbeck, GE Healthcare, Servier, Teva, Solvay, GSK, Ipsen, Allergan, Orion, Bial and AbbVie Lucid. Dr Coleman has no disclosures to add. Dr O'Sullivan has received honoraria from Teva, Lundbeck, UCB, AbbVie and Eisai for attending conferences. He has received Consultancy fees from Britannia Pharmaceuticals for work unrelated to this study. Dr Compta has received honoraria, funding and/or sponsorship from Lundbeck, Novartis, Boehringer, GSK, UCB, Abbvie and Medtronic. Professor Revesz has been supported in the past two years by the National Institute for Health Research (NIHR) Biomedical Research Unit in Dementia based at University College London Hospitals (UCLH), University College London (UCL), CBD Solutions and Parkinson's UK. Professor Burn has received grant support from Parkinson's UK, Newcastle University Lockhart Parkinson's disease Research Fund, the National Institute for Health Research, Wellcome Trust, Michael J Fox Foundation and GlaxoSmithKline over the past 2 years.

References

1. Aarsland D, Andersen K, Larsen JP, Lolk A, Kragh-Sorensen P. Prevalence and characteristics of dementia in Parkinson disease: an 8-year prospective study. Arch Neurol-Chicago 2003;60(3):387-392.

2. Buter TC, van den Hout A, Matthews FE, Larsen JP, Brayne C, Aarsland D. Dementia and survival in Parkinson disease - A 12-year population study. Neurology 2008;70(13):1017-1022.

3. Hely MA, Reid WG, Adena MA, Halliday GM, Morris JG. The Sydney multicenter study of Parkinson's disease: the inevitability of dementia at 20 years. Mov Disord 2008;23(6):837-844.

4. Pagano G, Rengo G, Pasqualetti G, et al. Cholinesterase inhibitors for Parkinson's disease: a systematic review and meta-analysis. Journal Of Neurology, Neurosurgery, And Psychiatry 2014.

5. Siderowf A, Xie SX, Hurtig H, et al. CSF amyloid {beta} 1-42 predicts cognitive decline in Parkinson disease. Neurology 2010;75(12):1055-1061.

6. Pletnikova O, West N, Lee MK, et al. Abeta deposition is associated with enhanced cortical alpha-synuclein lesions in Lewy body diseases. Neurobiol Aging 2005;26(8):1183-1192.

7. Lashley T, Holton JL, Gray E, et al. Cortical alpha-synuclein load is associated with amyloid-beta plaque burden in a subset of Parkinson's disease patients. Acta Neuropathol 2008;115(4):417-425.

8. Compta Y, Parkkinen L, O'Sullivan SS, et al. Lewy- and Alzheimer-type pathologies in Parkinson's disease dementia: which is more important? Brain 2011;134(Pt 5):1493-1505.

9. Irwin DJ, White MT, Toledo JB, et al. Neuropathologic substrates of Parkinson disease dementia. Ann Neurol 2012;72(4):587-598.

10. Horvath J, Herrmann FR, Burkhard PR, Bouras C, Kovari E. Neuropathology of dementia in a large cohort of patients with Parkinson's disease. Parkinsonism Relat Disord 2013;19(10):864-868; discussion 864.

11. Kotzbauer PT, Cairns NJ, Campbell MC, et al. Pathologic accumulation of α-synuclein and Aβ in Parkinson disease patients with dementia. Arch Neurol-Chicago 2012;69(10):1326-1331.

12. Compta Y, Parkkinen L, Kempster P, et al. The significance of α-synuclein, amyloid-β and tau pathologies in Parkinson's disease progression and related dementia. Neurodegener Dis 2014;13(2-3):154-156.

13. Clinton LK, Blurton-Jones M, Myczek K, Trojanowski JQ, LaFerla FM. Synergistic Interactions between Abeta, tau, and alpha-synuclein: acceleration of neuropathology and cognitive decline. J Neurosci 2010;30(21):7281-7289.

14. Masliah E, Rockenstein E, Veinbergs I, et al. beta-amyloid peptides enhance alpha-synuclein accumulation and neuronal deficits in a transgenic mouse model linking Alzheimer's disease and Parkinson's disease. Proc Natl Acad Sci U S A 2001;98(21):12245-12250.

15. Lashuel HA, Hartley DM, Balakhaneh D, Aggarwal A, Teichberg S, Callaway DJ. New class of inhibitors of amyloid-beta fibril formation. Implications for the mechanism of pathogenesis in Alzheimer's disease. J Biol Chem 2002;277(45):42881-42890.

16. Himeno E, Ohyagi Y, Ma L, et al. Apomorphine treatment in Alzheimer mice promoting amyloid-beta degradation. Ann Neurol 2011;69(2):248-256.

17. Ince PG, Clark B, Holton JL, Revesz T, Wharton SB. Disorders of movement and system degenerations. In: Love S, Louis DN, Ellison DW, eds. Greenfield's neuropathology. 8th edition ed. London: Edward Arnold, 2008:889-1030.

18. Emre M, Aarsland D, Brown R, et al. Clinical diagnostic criteria for dementia associated with Parkinson's disease. Mov Disord 2007;22(12):1689-1707.

19. Perry EK, Kilford L, Lees AJ, Burn DJ, Perry RH. Increased Alzheimer pathology in Parkinson's disease related to antimuscarinic drugs. Ann Neurol 2003;54(2):235-238.

20. Parkkinen L, O'Sullivan SS, Kuoppamaki M, et al. Does levodopa accelerate the pathologic process in Parkinson disease brain? Neurology 2011;77(15):1420-1426.

21. Lashley T, Rohrer JD, Bandopadhyay R, et al. A comparative clinical, pathological, biochemical and genetic study of fused in sarcoma proteinopathies. Brain 2011;134(Pt 9):2548-2564.

22. Mirra SS, Heyman A, McKeel D, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 1991;41(4):479-486.

23. 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(12):1863-1872.

24. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 2003;24(2):197-211.

25. Thal DR, Rub U, Orantes M, Braak H. Phases of A beta-deposition in the human brain and its relevance for the development of AD. Neurology 2002;58(12):1791-1800.

26. Braak H, Alafuzoff I, Arzberger T, Kretzschmar H, Del Tredici K. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. Acta Neuropathol 2006;112(4):389-404.

27. Perneger TV. What's wrong with Bonferroni adjustments. Bmj 1998;316(7139):1236-1238.

28. Tsuang D, Leverenz JB, Lopez OL, et al. APOE ε4 increases risk for dementia in pure synucleinopathies. JAMA Neurology 2013;70(2):223-228.

29. Jarrett JT, Berger EP, Lansbury PT, Jr. The carboxy terminus of the beta amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease. Biochemistry 1993;32(18):4693-4697.

30. Selkoe DJ. Amyloid beta-protein and the genetics of Alzheimer's disease. The Journal of biological chemistry 1996;271(31):18295-18298.

31. Irvine GB, El-Agnaf OM, Shankar GM, Walsh DM. Protein aggregation in the brain: the molecular basis for Alzheimer's and Parkinson's diseases. Molecular medicine 2008;14(7-8):451-464.

32. Alves G, Larsen JP, Emre M, Wentzel-Larsen T, Aarsland D. Changes in motor subtype and risk for incident dementia in Parkinson's disease. Mov Disord 2006;21(8):1123-1130.

33. Burn DJ, Rowan EN, Allan LM, Molloy S, O'Brien JT, McKeith IG. Motor subtype and cognitive decline in Parkinson's disease, Parkinson's disease with dementia, and dementia with Lewy bodies. J Neurol Neurosur Ps 2006;77(5):585-589.

34. Alves G, Pedersen KF, Bloem BR, et al. Cerebrospinal fluid amyloid-beta and phenotypic heterogeneity in de novo Parkinson's disease. J Neurol Neurosur Ps 2013;84(5):537-543.

35. Selikhova M, Williams DR, Kempster PA, Holton JL, Revesz T, Lees AJ. A clinico-pathological study of subtypes in Parkinson's disease. Brain 2009;132:2947-2957.

36. Jellinger KA, Attems J. Prevalence and impact of vascular and Alzheimer pathologies in Lewy body disease. Acta Neuropathol 2008;115(4):427-436.

37. Jellinger KA. Morphological substrates of parkinsonism with and without dementia: a retrospective clinico-pathological study. J Neural Transm Suppl 2007(72):91-104.

38. Irwin DJ, Lee VMY, Trojanowski JQ. Parkinson's disease dementia: convergence of alpha-synuclein, tau and amyloid-beta pathologies. Nat Rev Neurosci 2013;14(9):626-636.

39. Gassen M, Gross A, Youdim MB. Apomorphine enantiomers protect cultured pheochromocytoma (PC12) cells from oxidative stress induced by H2O2 and 6-hydroxydopamine. Mov Disord 1998;13(4):661-667.