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Detection of proteolytic signatures for Parkinson’s disease

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Aim: To investigate if idiopathic Parkinson’s disease (IPD) is associated with distinct proteolytic signatures relative to non-neurodegenerative controls (NND) and patients with multiple system atrophy (MSA). Materials & methods: A subtiligase-based N-terminomics screening method was exploited for semiquantitative comparison of protein N-termini in cerebrospinal fluid for pooled samples of IPD (n = 6) and NND (n = 8) individuals. Subsequently, targeted selected reaction monitoring mass spectrometry measured the relative concentration of the proteolytic signature peptides in individual IPD (n = 22), NND (n = 11) and MSA (n = 18) samples. Results: The discovery screen detected 300 N-termini for 156 proteins. Selected reaction monitoring analysis revealed that two of these peptides differentiate IPD from NND while three peptides differentiate IPD from MSA. Conclusion: IPD is associated with distinct proteolytic signatures.

First draft submitted: 8 December 2015; Accepted for publication: 15 January 2016; Published online: 8 February 2016

Idiopathic Parkinson’s disease (IPD) is the most common neurodegenerative movement disorder [1]. The main clinical manifestations of IPD include bradykinesia, rigidity, tremor (at rest) and in later stages postural instability. These motor symptoms result from a depletion of the neurotransmitter dopamine in the striatum because the dopamine-producing neurons in the substantia nigra degenerate [2,3]. At present, no cure exists for IPD, but several potential disease-modifying therapies are being investigated [4]. Of these, immune therapy and neuroprotective treatments with, for example, antioxidants and neurotrophic factors have shown promise in animal models and other preclinical or clinical experiments, but so far the long-term clinical improvements in patients have failed to appear [5]. An explanation for the subtle effect could be that most neuroprotective trials have been performed on patients with pronounced loss of dopaminergic neurons. IPD is typically not diagnosed before the motor symptoms become apparent, but studies have suggested that nonmotor symptoms reflecting mainly nondopaminergic pathology like sleep and gastrointestinal (GI) symptoms may precede this by several years [6–8]. The effects of the neuroprotective therapies are likely to be stronger if the disease could be detected and treated in the initial nonmotor stages [9,10]. Another explanation for the lack of success in developing disease-modifying treatments may be that other parkinsonian disorders like multiple system atrophy (MSA) may confound the early diagnosis [11,12]. There is, therefore, a strong clinical need for biomarkers that can provide early detection and differential diagnosis of parkinsonian disorders.

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Proteolytic stress occurs when the level of misfolded or otherwise damaged protein material exceeds the capacity of the cellular clearance mechanisms. Proteolytic stress is strongly associated with IPD and suspected of being one of the early molecular events that trigger neurodegeneration in the brains of IPD subjects \[13,14\]. Nevertheless, no studies have investigated whether the proteolytic stress gives rise to distinct signatures that can be exploited as early biomarkers for IPD. A functional ubiquitin-proteasome system (UPS) is the most important degradation pathway in cells; however, during IPD the UPS is inhibited by aggregated protein species including misfolded α-synuclein \[15,16\]. This decline in the UPS activity hampers the ability to remove damaged and aggregated proteins, producing a self-perpetuating mechanism. The physiological evidence in the brain includes the appearance of large intracellular inclusions of protein aggregates termed Lewy bodies (LB). LB contains agitated forms of the synaptic protein α-synuclein. We hypothesized that protein aggregation along with proteolytic stress leads to the presence of aberrantly cleaved proteins in cerebrospinal fluid (CSF). When a protein is internally cleaved, this will give rise to a novel N-terminal (neoterminal), and thus it should be possible to detect potential proteolytic signatures for IPD by exploiting N-terminomics investigations. Our N-terminomics protocol relied on selective ligation of affinity tags to protein N-termini mediated by a re-engineered version of the subtilisin BPN’ protease, subtiligase \[17\]. We optimized this protocol for protein-sparse samples and further developed a robust relative quantification strategy with early incorporation of isotopic labels to identify N-termini that differentiate IPD from non-neurodegenerative subjects (NND). Subsequently we developed selected reaction monitoring (SRM) assays to measure prototypic signature peptides specific for the protein termini in question in a panel of individual patient samples, including patients with MSA as a control arm. As with IPD, MSA is a synucleinopathy associated with α-synuclein aggregation, LB and proteolytic stress \[18\] and, therefore, we expected that the proteolytic signatures of MSA would also differ from that of the NND controls.

**Materials & methods**

- **Patient samples for discovery study**

A total of six IPD patients and eight NND controls were included in the initial discovery study. IPD patients participating in the N-terminomics discovery study were recruited from the outpatient clinic at Aarhus University Hospital between December 2007 and May 2010. All included patients were examined by a movement disorder specialist and underwent dopamine transporter (DAT) scan to confirm loss of dopaminergic termini in the striatum as well as MRI to exclude parkinsonism caused by vascular or other structural lesions. All analyzed IPD patients displayed a stage 2.0 bilateral involvement without impairment of balance based on a modified Hoehn and Yahr scale assessment \[19\]. CSF samples were only included, if the individuals were otherwise healthy with no signs of dementia. All patients were screened for cognitive dysfunction using Mini Mental State Examination or Montreal Cognitive assessment as well as Addenbrooks Cognitive Assessment. In cases with signs of cognitive impairment, full neuropsychological evaluation was performed \[20,21\]. Samples from NND controls were obtained from healthy individuals with suspected neuroborreliosis. The inclusion criteria for the NND group required that an IgG test for borreliosis was negative and that the patients were otherwise healthy apart from idiopathic dizziness. CSF (4–10 ml) were collected by lumbar puncture into polypropylene tubes and immediately transferred to 4°C. Within 30 min, the samples were gently centrifuged to sediment cells (10 min; 2000 g; 4°C) before the supernatants were aliquoted and stored at -80°C. Cell counts, total protein concentration, albumin concentration, immunoglobulin concentration, glucose and salts levels were all within standard values \[22\]. Discard criteria were blood contamination, infection, blood–brain barrier leakage or inflammation – these criteria were examined by CSF–albumin, red blood cell counts and total protein measurements. In the N-terminomics protocol, sample pooling was applied in order to have sufficient CSF protein. Different sex- and age-matched pools were created to allow for two different quantitative approaches. For the 4-plex iTRAQ strategy the pools were: IPD pool 1 (68 ± 5 years), IPD pool 2 (71 ± 12 years), NND pool 1 (64 ± 20 years), NND pool 2 (62 ± 10 years). For the 2-plex light/heavy strategy the pools were: IPD pool (70 ± 8 years) and NND pool (57 ± 7 years). Additional information about the patient samples for the discovery study is available as Supplementary Information 1. Before inclusion, written informed consent was obtained from all patients. All analyses of CSF
samples were approved by the regional ethics committees.

- **Patient samples for targeted SRM study**
  A total of 22 IPD, 11 NND and 18 MSA CSF patients were part of the SRM verification study; the average age and standard deviation for these groups were 65.2 ± 4.5 (IPD), 60 ± 12.2 (NND) and 65.1 ± 7.4 (MSA). The CSF samples from Aarhus University Hospital (Noerrebrogade, 8000 Aarhus C) were included along with additional samples from Bispebjerg Movement Disorders Biobank (BMDB). All included patients at BMDB were examined by a movement disorder specialist and underwent DAT scan and MRI. Blood screening, orthostatic blood pressure measurement and urological tests were also performed. CSF from NND controls was obtained from patients recruited from the Department of Neurology, Bispebjerg Hospital (Copenhagen, Denmark), where they were admitted with acute onset of a headache. In all cases, a neurological examination, brain MRI scan, blood screening and lumbar puncture with routine CSF analyses were conducted. Besides a headache (<2%), no clinical or laboratory evidence of neurological disease were found. MSA patients were diagnosed according to consensus guidelines [23]. CSF sampling at BMDB was carried out with similar procedures as at Aarhus University Hospital as described previously [24]. Before inclusion, written informed consent was obtained from all patients and all analyses of CSF samples were approved by the regional ethics committees.

- **Purification of subtiligase**
  The subtiligase used in this study was a phage-display optimized subtilisin mutant [25] with eight point mutations including four mutations for increased ligase activity (S221C, P225A, M124L and S125A [25, 26]) and five point mutations introduced to increase the stability (M50F, N76D, N109S, K213R and N218S [27]). Expression and purification were essentially conducted as described before [26] except that, ‘helper’ subtilisin was omitted from the culture, and a cation exchange chromatography step was used to achieve the desired purity and concentration instead of size exclusion chromatography.

- **Tobacco Etch virus proteinase**
  Crude tobacco Etch virus proteinase (TEVpr) was a generous gift from Xavier Gomis-Rüth (Department of Structural Biology, Proteolysis laboratory, Barcelona Science Park, Barcelona, Spain). TEVpr was expressed in *Escherichia coli* and purified as described in Fang *et al.* 1997 [28]. The TEVpr used for N-terminomics was further purified by cation exchange chromatography.

- **Synthesis & purification of light & heavy peptide ester**
  The light biotinylated peptide ester (PE) has been described before [17]. In the present study, a more soluble (N-(3-(2-(2-(3-amino-propoxy)-ethoxy)-ethoxy)-propyl)succinamic acid (Ttds) linker was used, and the overall structure was thus biotin-Ttds-Thr-Glu-Asn-Leu-Tyr-Phe-Gln-Ser*-Tyr-C0-O-CH2-CO-Tyr-amide. In the light PE, the Ser* (the asterisk refers to a heavy-labeled amino acid [standard token in mass spectrometry]) residue was composed of natural isotopes while the +4 heavy PE, contained a Ser* composed of three $^{13}$C atoms and a single $^{15}$N atom (JPT peptides [Berlin, Germany]). The PE’s were purified by reverse-phase HPLC (μBondapak C18 125 Å 10 μM 3.9 × 150 mm, Waters Corp. [Milford, MA, USA]) using an Amersham Pharmacia Äkta Explorer HPLC system (GE Healthcare, Little Chalford, UK). The mass of the purified PEs were verified by Matrix Assisted Laser Desorption Ionization Time of Flight mass spectrometry using a Micromass Ultima Global (Micromass/Waters Corp.).

- **N-terminomics protocol**
  The N-terminal positive enrichment strategy using subtiligase (Figure 1A) and the workflow for the discovery study (Figure 1B) was essentially conducted as described in [17]. A full protocol is provided as online Supplementary Information 2.

- **LC-MS/MS analysis of N-terminomics samples**
  For the light/heavy strategy, the purified N-terminal peptides were resuspended in 0.5 μl 100% formic acid and diluted to 20 μl in 0.05% TFA. An aliquot (5 μl) was loaded onto an EASY-nLC system (Proxeon, Denmark). Peptides were loaded directly onto a 20 mm length, 100 μm inner diameter, 360 μm outer diameter, ReproSil – Pur C18 AQ 3 μm (Dr Maisch, Ammerbuch-Entringen, Germany) reversed-phase capillary column. The peptides were eluted using a gradient from 100% phase A (0.1% formic acid) to 35% phase B (0.1% formic acid, 90% acetonitrile) over 90 min.
Subtiligase tagging

Tryptic digestion

Affinity capture

Sample collection

Clarification, inhibition of adventitious proteolysis, sample pooling and protein quantification

2-plex N-terminomics

4-plex N-terminomics

LC–MS/MS identification and quantification of N-terminal peptides

Targeted SRM study

Sample collection

Retention time (min)

Intensity (10^3)

0 50 100 150 200 250

0 10 20 30 40 50 60 70

TENLYFQ-SY

TENLYFQ-S*Y

iTRAQ 114

iTRAQ 115

iTRAQ 116

iTRAQ 117

N-terminomics discovery study

6 × IPD

8 × NND

22 × IPD

11 × NND

18 × MSA

N-terminomics discovery study
Figure 1. N-terminomics protocol and overview of discovery and verification study (see facing page). (A) Simplified illustration of the subtiligase method for purification of the N-terminal ends of proteins in complex mixtures. Initially (Ai) the subtiligase ligates a biotinylated peptide ester to the N-α primary amine group of a protein. Subsequently (Aii), trypsin digests at lysine and arginine residues, which leads to release of all internal peptides. Next (Aiii) the N-terminal peptides are enriched by avidin-agarose affinity capture of the biotin moieties, while the internal peptides are removed by rigorous washing procedures under denaturing conditions. Finally, tobacco etch virus protease is added in order to remove the biotin part prior to LC–MS/MS analysis (not shown here). The illustration has been simplified for clarity; for original reference see [17]. (B) Workflow for the discovery study, where N-termini from pooled IPD samples and pooled NND control samples were analyzed in a semiquantitative fashion by both 2-plex and 4-plex methodologies. The 2-plex method exploited stable isotope labeling with a natural isotope version of the peptide ester (light) and a peptide ester with a +4 heavy serine, S*. The 4-plex strategy relied on labeling of N-terminal peptides with 4-plex iTRAQ reagents. (C) Illustration of a method for relative quantification of the proteolytic signatures in single patient samples by LC–SRM MS. The chromatogram displays the retention time for each of the individual spiked-in heavy peptides used to quantify the endogenous cerebrospinal fluid-termini. For clarity, some of the peptide sequences have been omitted from the diagram.

IPD: Idiopathic Parkinson’s disease; NND: Non-neurodegenerative control; MSA: Multiple system atrophy; SRM: Selected reaction monitoring.

at 200 nl/min directly into an LTQ-Orbitrap XL (Thermo Scientific). The LTQ-Orbitrap XL was operated in a data-independent mode automatically switching between MS and MS/MS using a threshold of 20,000 for ion selection. For each MS scan, the five most abundant precursor ions were selected for fragmentation using CID (normalized collision energy 35; isolation window of 2 Da, activation time 10 ms and selection of 2+, 3+ and 4+ ions). iTRAQ labeled sample were analyzed as described above but with the following modifications: The LTQ-Orbitrap XL was operated in a data-independent mode automatically switching between MS and MS/MS using a threshold of 30,000 for ion selection. For each MS scan, the three most abundant precursor ions were selected for fragmentation using CID (normalized collision energy 35; isolation window of 3 Da, activation time 10 ms and selection of 2+, 3+ and 4+ ions) and higher energy collision induced dissociation (HCD; resolution 7500 in the Orbitrap, isolation window 3 Da, normalized collision energy 48, activation time 5 ms and FT first mass value set to m/z 110).

- **Peptide identification & quantitation**

All raw MS files for the 2-plex N-terminomics protocol were processed using Mascot Distiller 2.4.3.2 (Matrix Science, London, UK). After peak picking all scans, a search was performed against the human proteins in Swiss-Prot (version 2011-03) using Mascot 2.2 with search parameters allowing one missed semiTrypsin cleavage site. Carbamidomethyl and methionine oxidation were entered as fixed and variable modifications, respectively. The mass accuracy of the precursor and product ions were 10 ppm and 0.3 Da and the instrument setting was specified as ESI-TRAP. An identical search was done using ‘trypsin’ as the enzyme in order to provide improved identification of potential short, fully tryptic peptides (i.e. in the cases where the neo-terminal was downstream to a Lys/Arg residue).

Data processing of iTRAQ searches was performed using Proteome Discoverer (beta version 1.2.0.198) and Mascot 2.2 against the human proteins in Swiss-Prot (version 2010-03). The enzyme was specified to semiTrypsin, and one missed cleavage was allowed. Carbamidomethyl modification of cys residues and N-terminal 4-plex iTRAQ + serine tyrosine (N-term) were entered as fixed modifications. Oxidation of methionine, and 4-plex iTRAQ modification (K) were entered as variable modifications. The mass accuracy of the precursor and product ions were 10 ppm and 0.8 Da for MSA/ETD and 0.05 Da for HCD and the instrument setting was specified as CID-TRAP/HCV-FTICR. The false discovery rate was limited to 5% in all searches.

All Mascot hits were manually verified for: likelihood of missed cleavages; proline-directed fragmentation in MS/MS spectra; occurrence of uninterrupted ion series of at least four consecutive ions from the same series; and presence of ‘diagnostic’ a/b ions. For example, for iTRAQ-labeled samples the MS/MS spectra should contain the m/z peaks 367.21 and 395.21, corresponding to a 4-plex iTRAQ-label on N-terminal Ser-Tyr...
residues. For the 2-plex strategy, the spectra should contain either the a/<b> ion pair 223.1/251.1 (in case of the light peptide ester) or the 227.1/255.1 ion pair (in case of the heavy peptide ester). Both 2-plex and 4-plex data were normalized for overall protein concentration by median normalization. In the 2-plex quantification, label swapping was performed to eliminate random hits due to technical noise; that is, in the first experiment the IPD samples were labeled with the light peptide ester and the NND samples were labeled with the +4 heavy peptide ester. Subsequently, the labeling of samples was swapped. The quantitation was only accepted if the ratios of the light/heavy LC–MS envelopes for a given peptide returned reciprocal value in the label-swapped experiments. When available, 2-plex quantitation was preferred over 4-plex quantitation because in the 2-plex protocol the stable isotopes were introduced at the earliest possible step. To identify protein N-termini that differ in abundance between IPD and NND samples an arbitrary IPD/NND threshold was defined. A relatively low fold change was chosen, because the proteolytic differences would subsequently be confirmed by the SRM method. For the 2-plex quantitation, only N-termini with a 1.2-fold difference were accepted, and due to the possible higher technical variance in the 4-plex labeling, the threshold in this case was 1.8. All peptides that exceeded the thresholds were inspected for possible technical variation that might occur due to the primary structure. Therefore, peptides were not regarded as potential biomarkers if they contained, potential trypsin missed cleavage sites, possible Met oxidations, Glu and Asp near the cleavage site and possible post-translational modifications.

**Sample preparation for LC-SRM MS**

Synthetic peptides (SpikeTides_L and MaxiPrep_L, JPT Peptide Technologies, Berlin, Germany) were used for optimization of the SRM assay. The crude peptides were resuspended in 80% 0.1 M ammonium bicarbonate and 20% acetonitrile for 30 min in the 96-well plate, to a concentration of approximately 175 pmol/μl. Peptides were combined into a peptide pool by adding 10 μl aliquots from each peptide stock to 100 μl 0.1% formic acid. The peptide mixture was micropurified using Poros 50 R2 reversed phase column material (PerSeptive Biosystems, MA, USA) packed in GELoader Tips (Eppendorf, Hamburg, Germany), dried in a vacuum centrifuge, resuspended in 0.1% formic acid and stored at 4°C until analysis. After optimization of the SRM assay, one stock peptide mixture was prepared and all CSF samples were dissolved in this mixture.

Total protein concentration for all CSF samples was determined by the Bicinchoninic acid assay, BCA (Thermo Scientific, MA, USA). A total of 50 μg of each CSF sample was lyophilized and resuspended in 25 μl 8 M urea 0.2 M Tris-HCl pH 8.3, reduced in 15 mM DTT for 1 h and alkylated in 15 mM iodoacetamide for 1 h at 22°C. The samples were diluted five times with 0.1 M Tris-HCl pH 8.3 and digested overnight with 1:50 w/w trypsin (Sigma-Aldrich, MO, USA) at 37°C for 16 h. From each sample, 3 μg was desalted using Poros 50 R2 reverse phase column material (PerSeptive Biosystems) packed in GELoader Tips (Eppendorf), dried in a vacuum centrifuge, resuspended in 18 μl peptide mixture and stored at 4°C until analysis by LC–SRM MS.

**SRM assay & LC-SRM MS analysis**

The SRM assay was developed and optimized using Skyline v. 2.1.0.4936 [29]. Briefly, an MS discovery analysis of the synthetic peptide mixture was performed on a Qtrap 5500 instrument (AB Sciex, MA, USA). The peptides, associated precursor ions and fragment ions were then processed in Skyline. For peptides containing Met residues, both the oxidized and nonoxidized versions were analyzed. Multiple rounds of optimizations were performed by running SRM analyses, importing the data into Skyline and selecting optimal transitions. Once five optimal transitions had been selected in Skyline, the collision energy was optimized for all transitions using four steps of 1 V on each side of the calculated collision energy values, and the tree most intense transitions were kept for each peptide. Peptides for which three co-eluting transitions could not be found were excluded from the final assay. Finally, the identity and elution time of all peptides was confirmed by SRM-triggered MS/MS scans.

SRM analyses were performed on an EASY-nLC system (Thermo Scientific) connected inline to a Qtrap 5500 mass spectrometer (AB Sciex) equipped with a NanoSpray III source (AB Sciex) and operated under Analyst 1.6.2 control. The samples were injected, trapped and desalted using an isocratic elution on a Biosphere C18 precolumn (ID 100 μm × 2 cm, 5 μm, 120 Å, NanoSeparation, Nieuwkoop, The Netherlands).
after which the peptides were eluted from the trap column and separated on a 15 cm analytical column (75 μm i.d.), which was pulled in-house using a P2000 laser puller (Sutter Instrument Co.), and packed with ReproSil-Pur C18-AQ 3 μm resin (Dr Marisch GmbH, Ammerbuch-Entringen, Germany). Peptides were eluted at a flow rate of 250 nl/min employing a 80 min gradient from 5 to 35% B (0.1% formic acid, 90% acetonitrile), followed by re-equilibration for 10 min back to the starting conditions. The Qtrap was operated in positive ion mode with 2500 V ion spray voltage, curtain gas setting of 30, ion source gas setting of 5 and an interface heater temperature of 150°C. The eluted peptides were measured with a 90 min scheduled SRM assay using a 5 min detection window and a target scan time of 2.2 s, resulting in a minimum of 25 ms dwell time and 10 data points over the elution peak for all transitions (432 in total). The resolution in Q1 and Q3 was set to unit (0.7 amu FWHM). All samples were measured in triplicates. The mass spectrometry data have been deposited to the PeptideAtlas SRM Experiment Library (PASSEL) with the dataset identifier PASS00549.

- **Statistical analysis of SRM data**
  All SRM samples were imported into Skyline, the peak boarders were manually verified and, where needed, corrected for all samples. The ratios between the endogenous and exogenous (spiked) peptides were exported to Microsoft Excel, and average values were calculated from the triplicate measures. Finally, a student’s t-test was performed on the average values to identify peptides differing in prevalence between the control groups (online Supplementary Information 3).

**Results**

- **Discovery of proteolytic signatures using N-terminomics**
  The aim of the present study was to investigate if Parkinson’s disease is associated with distinct proteolytic signatures. In essence, this was analyzed by a semiquantitative N-terminomics protocol to determine if the protein N-termini in CSF samples varies between IPD subjects and NND controls. One of the challenging aspects of N-terminomics is that the reactivity of the ε-group of lysine residues very closely resemble the reactivity of the N-terminal amino-group of a protein, and therefore it can be difficult to obtain the desired selectivity. A solution to this problem is to exploit the re-engineered enzyme subtiligase because the particular structure and mechanism of the enzyme mean that it is exquisitely specific for the N-terminal amino group. In brief, the N-terminomics protocol (Figure 1A) is based on an initial subtiligase-mediated ligation of a biotin-containing peptide ester to the N-terminus of a protein. Subsequently, trypsin digestion leads to release of N-terminal peptides, internal fully tryptic peptides and C-terminal peptides. The biotin-tag – originally on the peptide ester and now ligated to the N-terminal peptides – is exploited for affinity purification, while the internal and C-terminal peptides are washed away under denaturing conditions. Finally, TEV protease is used to release the N-terminal peptide from the affinity resins, and LC–MS/MS is performed to identify the sequence of the peptide. A small MS signature from the peptide will reside on the N-terminal peptide after the N-terminomics protocol and thus the existence of N-terminal peptides were verified by two characteristics including: the presence of a doublet in the LC–MS chromatogram in the case of 2-plex light/heavy quantitation (Figure 2A); and, a specific N-terminal Ser-Tyr a_1 and b_1 ion signature in the LC–MS/MS spectrum (Figure 2B). By exploiting these two traits, non-N-terminal peptides can be excluded from the analysis, producing a list of 300 different N-termini in 156 proteins (online Supplementary Information 4).

Several of the detected proteins contained more than one N-terminal including ALBU (31 termini), CYTC (six termini), immunoglobulin chains (six termini) and PTGDS (eight termini). Western blots of 2D gels using a polyclonal antibody specific for ALBU confirmed the presence of truncated versions of albumin in both IPD and NND samples (online Supplementary Information 5). ALBU, CYTC and immunoglobulins all have high protein concentration in CSF, for example, ALBU constitutes approximately 75% of the total CSF protein by weight [31]. The many observed termini in high-abundance proteins are likely due to the availability of these proteins as substrates for both endogenous proteolytic events and for subtiligase tagging during the N-terminomics protocol. Conversely, some proteins with low concentration in CSF were detected with several N-termini including secretogranin-1 (11 N-termini) even though the protein only constitutes approximately 0.09% of the total protein content in CSF [31]. In this case, the many detected N-termini are likely to present because secretogranin-1 is reported to be extensively processed by
Figure 2. Higher prevalence of specific CD99 antigen N-termini in idiopathic Parkinson’s disease patients. (A) LC–MS spectrum of the light and heavy SY-tagged peptide SFSDADLADGVGVSGGEGK. The 2-plex N-terminomics protocol enables quantification of the relative prevalence of specific N-terminal proteins by integrating the area under the curve for the light (NND) and the heavy (IPD) envelope. (B) LC–MS/MS spectrum of the peptide from A. True, N-terminal peptides are confirmed by verifying the existence of fragments from the subtiligase-tag serine-tyrosine separated with a spacing of 28 u corresponding to a carbonyl group. (C) Summary of the specific sequences as well as the semi-quantitative information for the detected CD99 N-terminal peptides. “IPD N-terminal AA” corresponds to the Uniprot residue number. The anticipated N-terminal according to Uniprot protein knowledgebase is also shown. (D) Truncation plot displaying the IPD/NND ratio as a function of the detected CD99 antigen N-termini. All N-termini are mapping to the extracellular domain of CD99. Note that a single N-terminal detected (arrow) was detected with a 1:1 prevalence in IPD and NND samples while the other peptides all exceeded and IPD/NND ratio of 1.34.
limited proteolysis at basic residues by prohormone convertases, which leads to the generation of several biologically active peptides [32]. Six N-termini were also detected for Aβ protein, which is similarly present with relatively low concentration in CSF. Thus in some cases the number of identified termini per protein is in accordance with the protein abundance in CSF, while in other cases a high number of identified termini can be explained by expected biological processing and high susceptibility to proteolysis. Apart from these observations, unexpected proteolytic activities were also observed in specific proteins. Interestingly, several of these differed in prevalence when comparing IPD patients and NND controls.

**Proteolytic signatures for IPD**

In the present study, two semiquantitative approaches were explored to detect proteolytic incidents with elevated prevalence in IPD CSF samples, namely 4-plex iTRAQ-labeling and a 2-plex light/heavy strategy. The advantage of the iTRAQ strategy is that the peptide identification yield is boosted because the isobaric tags give identical m/z value of the precursor ions and the fragments in the MS/MS spectrum. On the other hand, a drawback with the iTRAQ approach is that the quantitative labels are introduced relatively late in the N-terminomics protocol and therefore the quantitation will likely exhibit relatively high standard deviation. Conversely, the 2-plex light/heavy strategy introduces the quantitative labels very early in the N-terminomics protocol with the natural isotopes (light) and the +4 Ser* residue (heavy) at the subtiligase biotinylation step. Subsequent to this step, the light- and heavy-labeled CSF protein termini from IPD and NND subjects can be pooled and thus the termini will exhibit the same technical variances for the remainder of the protocol, which means that the quantitation will be more robust. Label-swap experiments were used to assess the robustness of the 2-plex method. Thus in the first experiment, the pooled CSF samples from IPD subjects were labeled with heavy peptide ester, and CSF samples from NND subjects were labeled with light peptide ester. Conversely in the second experiment, the labeling was interchanged. These label-swap experiments show that the technical variance is extremely low (Table 1), for example, for some peptides the standard deviation between label-swap experiments was ±0.09 (GASQAGAPQGR, GELS), ±0.02 (SNLDEDIIAENIVSR, CO3 (C3b alpha’ chain)) and ±0.00 (NILTEEPK, PON1).

As observed in the truncation plot for CD99 antigen protein (Figure 2C) some of the detected N-terminal peptides differed in abundance between IPD patients and NND controls. A total of 38 N-terminal peptides in 25 different proteins were observed that exceeded the defined thresholds of 1.2-fold difference for the 2-plex analysis and 1.8-fold difference for the 4-plex analysis (Table 1). In most cases, the difference in abundance in IPD and NND pools was relatively modest, while, for example, for PON1 the incidence of N-terminal at position 309 was much higher in the NND pool (2.8-fold difference). Several of the detected N-termini with higher abundance in the IPD samples showed signs of possible degradation intermediates. This was especially evident for CYTC and PTGDS where many IPD-specific N-terminal peptides were differing only by single residues (online Supplementary information 6). These N-terminal trimming events could likely be due to sequential removal of amino acids by the amino peptidases that are known to be involved in the degradation and recycling of proteins [33].

**Verification by SRM**

The purpose of the N-terminomics study was to serve as an initial, in-depth, discovery screen for detection of IPD-specific proteolytic signatures in pooled CSF samples, which subsequently could be analyzed for biological variance and validity in individual CSF samples by SRM assays. In the SRM approach, synthetic, heavy-labeled +10 Arg or +6 Lys semitryptic peptides corresponding to the proteolytic signature peptides from the N-terminomics study were spiked into the individual CSF samples after these have been digested with trypsin.

As illustrated in the flow chart (Figure 1C) the heavy peptides elute from a C18 reversed phase column during a long, shallow, acetonitrile gradient. The exogenously added heavy peptides will have the same retention time behavior as the endogenous semitryptic N-terminal CSF peptides. Thus, by having a fixed spike-in of heavy peptides relative to the CSF protein concentration, relative quantification of the 38 CSF N-termini in individual patient samples can be performed highly reproducibly in a single LC–MS/MS run.

25 IPD samples and 18 NND samples were included in the targeted SRM study. Furthermore, to test if the proteolytic signatures could differentiate IPD subjects from
### Table 1. 38 N-termini with altered abundance in Parkinson’s disease patients.

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<td></td>
</tr>
<tr>
<td>P51693</td>
<td>APLP1</td>
<td>GSLA.GGSPGAAEAPSAQVAGLCGR.LTLH</td>
<td>65</td>
<td>1.44 ± 0.35</td>
<td>42</td>
<td>39</td>
</tr>
<tr>
<td>Q06648</td>
<td>APLP2</td>
<td>PMKK.GSGVGEQDGGGIGAEK.VINS</td>
<td>104</td>
<td>1.27 ± 0.07</td>
<td>625</td>
<td>32</td>
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<tr>
<td>P14209</td>
<td>CD99</td>
<td>PNHP.SSSGFDADLADGVSQGEGK.GGS</td>
<td>68</td>
<td>1.34 ± 0.02</td>
<td>85</td>
<td>23/228</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SSSG.FSDADLADGVSQGEGK.GGS</td>
<td>108</td>
<td>1.44 ± 0.08</td>
<td>89</td>
<td></td>
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<td></td>
<td></td>
<td>SSSG.FSDADLADGVSQGEGK.GGS</td>
<td>31</td>
<td>1.74</td>
<td>90</td>
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<tr>
<td>P10909</td>
<td>CLUS</td>
<td>VTTY.ASHTSDSVPGVTEVVK.LFDS</td>
<td>61</td>
<td>1.41 ± 0.13</td>
<td>390</td>
<td>23</td>
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<tr>
<td>P10104</td>
<td>CO3 (C3b α’ chain)</td>
<td>GLAR.SNLDEDIAEBNIVSR.SEPF</td>
<td>43</td>
<td>0.24 ± 0.02</td>
<td>749</td>
<td>749</td>
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<tr>
<td>P02671</td>
<td>FIBA (fibrin peptide A)</td>
<td>TAW.TADSGEDFLAEFGVR.GPRV</td>
<td>88</td>
<td>2.09 ± 0.08</td>
<td>20</td>
<td>20</td>
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<tr>
<td></td>
<td>FIBA (fibrinogen α-chain)</td>
<td>EITR.GGSTSVGTSETSPR.NPSS</td>
<td>76</td>
<td>0.38 ± 0.08</td>
<td>28</td>
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<tr>
<td>P06396</td>
<td>GELS</td>
<td>TATR.GASQAGAPGR.VPFA</td>
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<td>1.43 ± 0.09</td>
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<td>28</td>
</tr>
<tr>
<td>P00738</td>
<td>HPT</td>
<td>QLFV.GDSVNDVTIDADGDGCPPEIAHGY VEHSV.R.YQCK</td>
<td>73</td>
<td>0.37</td>
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<td>19</td>
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<tr>
<td>Q16650</td>
<td>MOG</td>
<td>HPIR.LVGDVEIPLCR.IJSVP</td>
<td>82</td>
<td>1.55 ± 0.07</td>
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<tr>
<td></td>
<td></td>
<td>PIRL.LVGDVEIPLCR.IJSVP</td>
<td>70</td>
<td>1.35</td>
<td>44</td>
<td>30</td>
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<tr>
<td>P10451</td>
<td>OSTP</td>
<td>DAVA.TWNLPDSQPK.QNLL</td>
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<td>42</td>
<td>17</td>
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<td></td>
<td></td>
<td>AVAT.WLNPDSQPK.QNLL</td>
<td>36</td>
<td>1.23 ± 0.11</td>
<td>43</td>
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<td></td>
<td>KAIPVQDLNAPSWDWSR.GKDS</td>
<td>68</td>
<td>1.62 ± 0.02</td>
<td>207</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>AIPV.AQDLNAPSWDWSR.GKDS</td>
<td>87</td>
<td>1.54 ± 0.15</td>
<td>208</td>
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<tr>
<td>Q6UX71</td>
<td>PLXDC2</td>
<td>TNRA.SVGQDSPEPR. SFTD</td>
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<td>1.33 ± 0.05</td>
<td>81</td>
<td>31</td>
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<tr>
<td>P41222</td>
<td>PTGDS</td>
<td>ALLG.VLGDQDAPEAQSVVPQPNFQQDK.FLGR</td>
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<td>1.46</td>
<td>16</td>
<td>23</td>
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<tr>
<td></td>
<td></td>
<td>DLQ2.APEAQSVVPQPNFQQDK.FLGR</td>
<td>76</td>
<td>1.31 ± 0.09</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>QAA.PEAVSVVPQFNQQDK.FLGR</td>
<td>50</td>
<td>1.30 ± 0.15</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

Summary of identified N-terminal peptides that exceed the required IPD/NND threshold (see ‘Materials & methods’ section). Uniprot accession number and the corresponding protein name is indicated for each identified N-terminal. ‘Sequence’ denotes the identified sequence (bold) along with the flanking four C- and N-terminal residues. IPD/NND is the relative ratio of the identified N-terminal peptide in the IPD sample relative to the NND sample as determined by either the 2-plex or 4-plex analysis. Standard deviations are reported if the same peptide was detected in both 2-plex label-swap experiments, in these cases the average and standard deviation are reported based on the total number of accepted LC–MS observations. ‘N-terminal position’ denotes the residue number that the identified N-terminal corresponds to, while ‘Regular N-terminus’ denotes the usual protein N-terminal residue, as indicated in Uniprot.

† Indicates that the quantitation was based on 4-plex iTRAQ quantitation – all other ratios are in the table are based on 2-plex light/heavy quantitation.

‡ Indicates that the N-terminal assignment is based on experimental findings.

IPD: Idiopathic Parkinson’s disease; NND: Non-neurodegenerative control.
Detection of proteolytic signatures for Parkinson’s disease

**Discussion**

Proteolytic dysfunction is suspected to occur early in the disease progression of IPD. Aberrant N-terminomics screening for Parkinson’s disease biomarkers

**Results**

### Table 1. 38 N-termini with altered abundance in Parkinson’s disease patients (cont.)

<table>
<thead>
<tr>
<th>Accession</th>
<th>Protein name</th>
<th>Sequence</th>
<th>Peptide Score</th>
<th>IPD/NND ratio</th>
<th>N-terminal position</th>
<th>Regular N-terminal</th>
</tr>
</thead>
<tbody>
<tr>
<td>P07602</td>
<td>SAP</td>
<td>DTVK.SLP.CDICK.DVVT</td>
<td>33</td>
<td>1.24</td>
<td>60</td>
<td>60†</td>
</tr>
<tr>
<td>P027643</td>
<td>HTRA1</td>
<td>RAG.R.SAPLAAGCPD.RCPD</td>
<td>62</td>
<td>1.20 ± 0.08</td>
<td>30</td>
<td>23</td>
</tr>
<tr>
<td>P02768</td>
<td>ALBU</td>
<td>YKAA.FTECCOA.ADK.AACL</td>
<td>52</td>
<td>0.47†</td>
<td>189</td>
<td>25†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LEKC.CAADDH.PECYAK.VFDE</td>
<td>50</td>
<td>0.18</td>
<td>385</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>LIQQ.NC.FEQ.GEYK.FQNA</td>
<td>36</td>
<td>0.39</td>
<td>415</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>VTWC.CTESSVNR.RPCF</td>
<td>57</td>
<td>0.40 ± 0.10</td>
<td>501</td>
<td></td>
</tr>
<tr>
<td>P27169</td>
<td>PON1</td>
<td>LRLQ.NILTEEPK.VTOV</td>
<td>45</td>
<td>0.36 ± 0.01</td>
<td>309</td>
<td>2†</td>
</tr>
<tr>
<td>P02766</td>
<td>TTHY</td>
<td>DYSY.STTAVTPK.E (C-terminal)</td>
<td>33</td>
<td>1.81†</td>
<td>137</td>
<td>21†</td>
</tr>
</tbody>
</table>

*Summary of identified N-terminal peptides that exceed the required IPD/NND threshold (see ‘Materials & methods’ section). Uniprot accession number and the corresponding protein name is indicated for each identified N-terminal. ‘Sequence’ denotes the identified sequence (bold) along with the flanking four C- and N-terminal residues. ‘IPD/NND’ is the relative ratio of the identified N-terminal peptide in the IPD sample relative to the NND sample as determined by either the 2-plex or 4-plex analysis. Standard deviations are reported if the same peptide was detected in both 2-plex label-swap experiments, in these cases the average and standard deviation are reported based on the total number of accepted LC-MS observations. ‘N-terminal position’ denotes the residue number that the identified N-terminal corresponds to, while ‘Regular N-terminus’ denotes the usual protein N-terminal residue, as indicated in Uniprot.

† Indicates that the quantitation was based on 4-plex iTRAQ quantitation – all other ratios are in the table are based on 2-plex light/heavy quantitation.

‡ Indicates that the N-terminal assignment is based on experimental findings.

IPD: Idiopathic Parkinson’s disease; NND: Non-neurodegenerative control.

As exemplified in the SRM chromatogram for the SODE peptide WTGCA.E.SADW.AEIR and NND control, the light/heavy ratio is 0.45 while SRM analysis of the NND control returns the light/heavy ratio of 0.27 and final IPD/NND ratio 390 (0.45/0.37).

By comparing the area under the curve for the peptide WTGCA.E.SADW.AEIR and NND control, it can be seen that the area under the curve for the peptide WTGCA.E.SADW.AEIR is higher in the IPD sample compared to the NND sample. The difference in area under the curve is due to the higher abundance of the N-terminal peptide WTGCA.E.SADW.AEIR in the IPD sample compared to the NND sample.

A statistical t-test analysis (p < 0.05) of the SRM results confirmed that two peptides had higher prevalence in IPD compared with NND, while three termini were more abundant in IPD compared with MSA subjects (Table 2).

<table>
<thead>
<tr>
<th>Accession</th>
<th>Protein name</th>
<th>Sequence</th>
<th>Peptide Score</th>
<th>IPD/NND ratio</th>
<th>N-terminal position</th>
<th>Regular N-terminal</th>
</tr>
</thead>
<tbody>
<tr>
<td>P07602</td>
<td>SAP</td>
<td>DTVK.SLP.CDICK.DVVT</td>
<td>33</td>
<td>1.24</td>
<td>60</td>
<td>60†</td>
</tr>
<tr>
<td>P027643</td>
<td>HTRA1</td>
<td>RAG.R.SAPLAAGCPD.RCPD</td>
<td>62</td>
<td>1.20 ± 0.08</td>
<td>30</td>
<td>23</td>
</tr>
<tr>
<td>P02768</td>
<td>ALBU</td>
<td>YKAA.FTECCOA.ADK.AACL</td>
<td>52</td>
<td>0.47†</td>
<td>189</td>
<td>25†</td>
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<tr>
<td></td>
<td></td>
<td>LEKC.CAADDH.PECYAK.VFDE</td>
<td>50</td>
<td>0.18</td>
<td>385</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>LIQQ.NC.FEQ.GEYK.FQNA</td>
<td>36</td>
<td>0.39</td>
<td>415</td>
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<tr>
<td></td>
<td></td>
<td>VTWC.CTESSVNR.RPCF</td>
<td>57</td>
<td>0.40 ± 0.10</td>
<td>501</td>
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<tr>
<td>P27169</td>
<td>PON1</td>
<td>LRLQ.NILTEEPK.VTOV</td>
<td>45</td>
<td>0.36 ± 0.01</td>
<td>309</td>
<td>2†</td>
</tr>
<tr>
<td>P02766</td>
<td>TTHY</td>
<td>DYSY.STTAVTPK.E (C-terminal)</td>
<td>33</td>
<td>1.81†</td>
<td>137</td>
<td>21†</td>
</tr>
</tbody>
</table>
Figure 3. Targeted relative quantification of proteolytic signature peptides in individual samples by selected reaction monitoring. (A) Selected reaction monitoring transitions used for relative quantification of the SODE peptide WTGEDSAEPNSDSAEWIR in a cerebrospinal fluid (CSF) sample from a patient with IPD, ‘330 IPD’. The left chromatogram shows the individual transitions for the light peptide (endogenous CSF peptide; solid line) and the heavy peptide (exogenously spiked-in synthetic peptide; dashed line). The right chromatogram displays the cumulated total area for all three transitions. Integration of the area under curve results in a light/heavy ratio of 0.44. (B) The two chromatograms are analogous to the chromatograms displayed in panel A, but derive from a NND, ‘02-NND’ instead of an IPD patient. In this case, the light/heavy ratio between the endogenous and spiked-in peptide is lower, that is, 0.37. Thus when comparing the relative quantification of this particular terminal, the IPD/NND ratio will be 0.44–0.37, that is, 1.19. Three technical replicates were performed for each CSF sample included in the study. Savitzky-Golay smoothing was applied to all curves.

IPD: Idiopathic Parkinson’s disease; NND: Non-neurodegenerative control.
cleavage of proteins will lead to occurrence of novel protein termini, and therefore an N-terminomics protocol, was optimized to perform a semiquantitative comparison of the N-termini of IPD patients and NND controls.

An N-terminomics protocol based on subtiligase was used because this engineered enzyme has an unrivaled specificity for N_termini [17]. Another advantage of this protocol is that it is performed under near-native conditions, which means that the subtiligase will have similar accessibility for the CSF protein tails as the endogenous proteases. The subtiligase protocol has hitherto been limited to biological matrices with high protein content because the subtiligase has limited labeling efficiency [34], but with the sample work-up and adaptations in the present protocol it is now possible to use the N-terminomics method down to 500 μg and perform analyses on protein-scarce samples. Nevertheless, 300 peptides detected is a relatively low number and since these studies were conducted, robust, selective and more sensitive N-terminomics protocols based on chemical approaches have been established, for example, terminal amine isotopic labeling of substrates (TAILS) and COmbined FRActional Diagonal Chromatography (COFRADIC) [35,36]. Strategies like these could increase the number of N-termini detected and moreover provide identification and quantification of N-termini with post-translational modifications (e.g., acetylated amino groups at the N-terminus).

- **N-termini detected for amyloidogenic proteins**

  N-terminomics comparison of CSF from IPD and NND subjects revealed a higher prevalence of N-termini for several amyloidogenic proteins. In fact, nine of the 25 detected proteins with different abundance of N-terminal isoforms (Table 1) are known to be involved in protein aggregation including A4 [37], APLP1 and APLP2 [38], CYTC [39], FIBA [40], GELS [41], HTRA1 [42], TTHY [43] and CLUS [44]. It is often mutations that give rise to the amyloid diseases, although the nonmutated variants also display amyloidogenic properties and may accumulate in *vivo* in the nonhereditary forms of amyloid diseases [45]. During normal physiological conditions the baseline level of misfolded proteins are removed but when the UPS is compromised, degradation intermediates accumulate in the CSF. In addition, it has been shown that aggregated protein can be propagated from one generation by a mechanism referred to as seeding [46–49]. This process may further add to the accumulation of proteolytic fragments of amyloid-related proteins in CSF, but the validity of this hypothesis remains to be further investigated.

- **Collateral damage in high abundance proteins**

  Apart from the above, aggregation-related proteins, quantitative differences between IPD patients and NND controls were observed in high-abundance proteins, for example, ALBU, FIBA (alpha chain) and complement factors. Of these, ALBU and FIBA were observed with inconsistent ratios in the discovery study and the validation study. The cause of the anomaly is not certain, but it is possible that proteolytic events acting on these proteins are not specific for IPD, but rather the consequence of “collateral damage” in high-abundance proteins.

- **Proteolytic signatures for neurodegenerative diseases**

  As we have shown previously, SRM can be exploited to verify the individual proteolytic signature peptides in single patient samples [50,51]. In the present study, several of the peptides from the discovery study were statistically confirmed by the SRM assay (Table 2), which further strengthened the reliability of the N-terminomics protocol. The majority of peptides were consistent in trend when comparing the N-terminomics discovery study and the SRM-based targeted MS-analysis. Of the peptides with significant fold change between IPD and NND, the N-terminal SODE peptide (WTGEDSAEPNSDASEWIR) displayed the best discriminatory power. The CD99 peptide SFSADLADGVSGGEGK also differed significantly in prevalence when comparing IPD and NND. To our knowledge, CD99 has not previously been associated with IPD. CD99 is involved in many physiological conditions, like intracellular trafficking, cell adhesion and differentiation. Under pathological conditions, CD99 can induce apoptosis [52] and is pathogenically mainly associated with Ewing’s sarcoma tumors, where it can induce massive cell death [53]. The association with CD99 and Parkinson’s disease remains elusive, but a recent study has reported that CD99 is an
CD99 may have a similar role in IPD.

- An SRM assay to distinguish Parkinson’s disease & multiple system atrophy

Because IPD and MSA are both synucleinopathies associated with proteolytic stress and protein misfolding, the proteolytic signatures of these diseases were not expected to differ substantially. In line with this assumption, the SRM study revealed only a single peptide from the IPD versus NND discovery study that differed between the MSA and IPD, namely the peptide FTECCQAADK (Table 2, Serum Albumin). Therefore, as expected, no dramatic proteolytic differences were observed between IPD and MSA. On the other hand, SRM-differences were observed that could be ascribed to differences in protein concentration, namely for the two fully tryptic proteotypic SRM peptides designed in silico for the Amyloid beta A4 protein, Abeta (VESLEQEAANER and THPHFVIPYR). Abeta has long been associated with neurodegeneration, especially with respect to the amyloidogenic Alzheimer’s disease peptide Abeta$_{1-42}$ (residue 672 to residue 713) [37]. Abeta$_{1-42}$ has also been implicated in Parkinson’s disease in antibody-based measurements of the concentration of the peptide in CSF. For example, Hall et al. reported that Abeta$_{1-42}$ is a useful marker for distinguishing Parkinsonian disorders [55]. Abeta$_{1-42}$ has also very recently been suggested as an early prognostic biomarker of dementia associated with Parkinson’s disease [56]. In the present study, the proteotypic peptides that distinguish MSA and IPD do not map to the Abeta$_{1-42}$ region, but instead belong to the N-terminal and central region of Abeta (THPHFVIPYR, N-terminal residue no. 107 and VESLEQEAANER, N-terminal residue no. 439). This could mean that differential proteolytic processing of Abeta occurs in IPD and MSA. We have previously shown that the Abeta$_{1-42}$ concentration is lower in MSA compared with IPD [57], but it is not known whether this is reflected in the present data. Discriminatory markers for Parkinsonian disorders are strongly needed for stratification and monitoring purposes in order to develop better treatments for IPD and atypical parkinsonism. This is especially the case for MSA, where the neurologist’s sensitivity can be as low as 56% in the early

### Table 2. Peptides differing significantly (p < 0.05) between idiopathic Parkinson’s disease, multiple system atrophy and non-neurodegenerative control groups.

<table>
<thead>
<tr>
<th>Accession</th>
<th>Protein name</th>
<th>Peptide sequence</th>
<th>N-terminal position</th>
<th>IPD vs NND</th>
<th>MSA vs NND</th>
<th>MSA vs IPD</th>
<th>Trend</th>
<th>t-test</th>
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<tbody>
<tr>
<td>P08294</td>
<td>SODE</td>
<td>WTGESAPSQSEWIR</td>
<td>19</td>
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<td>0.0234</td>
<td>0.0424</td>
<td>0.0239</td>
<td>1.277</td>
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<tr>
<td>P08294</td>
<td>CD99</td>
<td>SFSDADLADGVSGGK</td>
<td>89</td>
<td>↑</td>
<td>0.0284</td>
<td>0.0293</td>
<td>0.0297</td>
<td>1.295</td>
</tr>
<tr>
<td>P14209</td>
<td>CD99</td>
<td>SFSDADLADGVSGGK</td>
<td>89</td>
<td>↑</td>
<td>0.0284</td>
<td>0.0293</td>
<td>0.0297</td>
<td>1.295</td>
</tr>
<tr>
<td>P05067</td>
<td>P05067</td>
<td>VESLEQEAANER</td>
<td>621</td>
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<td>0.754</td>
<td>1.081</td>
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<tr>
<td>P05067</td>
<td>A4</td>
<td>THPHFVIPYR</td>
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<td>↑</td>
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<td>0.8445</td>
<td>1.354</td>
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<tr>
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<td>THPHFVIPYR</td>
<td>107</td>
<td>↑</td>
<td>0.8376</td>
<td>0.8445</td>
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</tr>
<tr>
<td>P02768</td>
<td>ALBU</td>
<td>FTECCQAADK</td>
<td>189</td>
<td>–</td>
<td>0.0819</td>
<td>0.1274</td>
<td>0.1274</td>
<td>1.560</td>
</tr>
<tr>
<td>P02768</td>
<td>ALBU</td>
<td>FTECCQAADK</td>
<td>189</td>
<td>–</td>
<td>0.0819</td>
<td>0.1274</td>
<td>0.1274</td>
<td>1.560</td>
</tr>
<tr>
<td>P02671</td>
<td>FIBA (α-chain)</td>
<td>GGSTSYGTGSETESPR</td>
<td>272</td>
<td>↑</td>
<td>0.0952</td>
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<td>1.444</td>
</tr>
<tr>
<td>P02671</td>
<td>FIBA (α-chain)</td>
<td>GGSTSYGTGSETESPR</td>
<td>272</td>
<td>↑</td>
<td>0.0952</td>
<td>0.1465</td>
<td>0.1465</td>
<td>1.444</td>
</tr>
</tbody>
</table>
Detection of proteolytic signatures for Parkinson’s disease

phases, but increasing as the condition progresses into phases where rational (pharmacoc-) therapy has little effects [11,12]. So far the most promising markers reported for discriminating IPD and MSA are neurofilament light chain (NF-L), DJ-1 and SAPPα (reviewed in Magdalino et al. and Jiménez-Jiménez et al. [58,59]). Nevertheless, conflicting and inconsistent results have been reported and there remains a strong need for novel, robust markers that discriminate IPD and MSA. A promising approach appears to be to combine measured biomarkers into panels that offer high power for discriminating between IPD and atypical Parkinsonism [55,60]. In this regard, it could prove fruitful to develop a multiplexed SRM assay that enables quantification of the most promising biomarkers along with proteolytic signature peptides for high-confidence disease stratification.

The present study also identified a single FIBA peptide that can distinguish MSA patients from NND controls, namely GGSTSYTGSETESPR. The exact mechanisms underlying the differences in termini are as yet not clear. Nonetheless, the data show that SRM measurements of specific proteolytic signature peptides may be exploited to perform differential diagnosis and prognosis of Parkinsonian disorders.

Future studies
The present work has highlighted interesting findings of proteolytic signatures in a relatively restricted number of patient and control samples. A limitations of the study is that the average age of NND group is lower than that of the IPD group and MSA group both in the discovery and targeted SRM study. Although the difference in average age at CSF tap is not statistically significant, there is a risk that the markers may reflect differences in age in addition to disease-specific markers. Therefore, if these results are to be translated into clinically applicable early biomarkers for differentiation of Parkinsonian disorders, SRM studies in well-validated large cohorts are needed in addition to longitudinal studies.

Conclusion
The present study has demonstrated that neurodegenerative diseases like IPD and MSA are associated with distinct proteolytic signatures

EXECUTIVE SUMMARY
The need for novel biomarkers for Parkinson’s disease
- Early and accurate diagnosis of Parkinson’s disease is important for future clinical trials aiming to develop novel disease-modifying strategies.

Proteolytic stress could be an early event in Parkinson’s disease
- Defects in the protein degradation machinery and proteolytic stress may be some of the early events in the etiopathogenesis of Parkinson’s disease. Thus, molecular indications of proteolysis-gone-wrong may be a source of future, early biomarkers.

Proteolytic signatures for neurodegenerative diseases
- Enrichment and LC–MS/MS-based identification of the N-termini of proteins (N-terminomics) can be used in combination with selected reaction monitoring (SRM) to detect distinct proteolytic signatures for neurodegenerative diseases.

Discovery of proteolytic signatures for Parkinson’s disease
- This study represents the first N-terminomics investigation of human cerebrospinal fluid from patients with idiopathic Parkinson’s disease. In total, 300 N-terminal peptides in 158 different proteins were detected.

SRM enables quantification of proteolytic signature peptides
- Analysis by SRM returned two significant peptides (p < 0.05) from SODE and CD99 that could be used to differentiate idiopathic Parkinson’s disease from non-neurodegenerative controls. In addition, three significant peptides – two from A4 and one from ALBU - could be used to differentiate idiopathic Parkinson’s disease from multiple system atrophy.

Longitudinal studies warranted
- The results must be validated in larger, longitudinal, cohorts of patients and non-neurodegenerative controls before it is known whether the proteolytic signature peptides can be exploited as early biomarkers for parkinsonian disorders.
and that these can be quantified using targeted MS-based SRM assays. More studies in larger cohorts are warranted to validate if the proteolytic signatures are applicable as discriminatory, early biomarkers for Parkinsonian disorders.

Future perspective

Improved techniques for early detection and differentiation of Parkinsonian disorders are strongly needed. Quantitative mass spectrometry has undergone a remarkable evolution for clinical applicability in the past decade, and it has thus become feasible to complement traditional ELISA assays with robust, multiplexed SRM assays. The present study serves to give an illustration of how mass spectrometry can be exploited to detect proteolytic signatures detected by N-terminomics instead of merely quantifying intact proteins as biomarkers of disease. As Overall et al. have recently suggested, the approach with characterization of proteolytic signatures holds promise for detection of biomarkers and development of precision medicine in other protease-related disease areas like inflammation and cancer [61].

Supplementary Data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/full/10.2217/fnl.16.3.

Acknowledgments

The authors acknowledge Sami Mahrus and David Wildes for skilled assistance in optimizing experimental conditions for subtiligase-mediated biotinylation of protein N-termini.

Financial & competing interests disclosure

This study was supported by the Michael J Fox Foundation and the Danish Agency for Science, Technology and Innovation (Innovation Consortium CureND). Bispebjerg Movement Disorders Biobank is supported by The John and Birthe Meyer Foundation and the ANT Foundation. MR Larson was funded by the Lundbeck Foundation (Junior Group Leader Fellowship). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest


•• Provides a solid background for understanding the proteolytic stress associated with idiopathic Parkinson’s disease and other neurodegenerative disorders.


Detection of proteolytic signatures for Parkinson’s disease  

**RESEARCH ARTICLE**


• Illustrates how N-terminomics can be used in combination with selected reaction monitoring to identify cell death-associated signatures over time in plasma.


Highlights the challenges related to discriminating parkinsonian disorders. Figure 1 (box plot) provides a good overview of the concentrations and spans of promising biomarkers for the respective parkinsonian disorders.

Excellent introduction to terminomics tools and protocols available for positional proteomics and their advantages.
Electroconvulsive therapy for depression in Parkinson’s disease: systematic review of evidence and recommendations

Anna Borisovskaya*1,2, William Culbertson Bryson1, Jonathan Buchholz1,2, Ali Samii1,2 & Soo Borson1

Aim: We performed a systematic review of evidence regarding treatment of depression in Parkinson’s disease (PD) utilizing electroconvulsive therapy. Methods: The search led to the inclusion of 43 articles, mainly case reports or case series, with the largest number of patients totaling 19. Results: The analysis included 116 patients with depression and PD; depression improved in 93.1%. Where motor symptoms’ severity was reported, 83% of patients improved. Cognition did not worsen in the majority (94%). Many patients experienced delirium or transient confusion, sometimes necessitating discontinuation of electroconvulsive therapy (ECT). Little is known about maintenance ECT in this population. Conclusion: ECT can benefit patients suffering from PD and depression. We recommend an algorithm for treatment of depression in PD, utilizing ECT sooner rather than later.

First draft submitted: 5 January 2016; Accepted for publication: 8 February 2016; Published online: 1 April 2016

Background
Depression is a frequently encountered neuropsychiatric disorder in patients with Parkinson’s disease (PD). Estimates in prevalence range widely in the literature with studies reporting 2.7–70% [1]. Inconsistent measurement techniques and sampling methods may account for these discrepancies. Further, depressed patients with PD may not be a homogeneous group. A rigorous systematic review reported clinically significant depressive symptoms in 35% of patients with prevalence rates of 17%

Practice points
- All patients with Parkinson’s disease (PD) should be screened for depression. When treating depression in PD, a clinician should not be satisfied with nonresponse or partial response.
- Electroconvulsive therapy (ECT) is a safe and effective treatment for most patients with PD and depression, offering advantage in that it also may improve motor symptoms.
- Cognitive impairment at baseline should not be a contraindication for ECT, though all patients should be counseled regarding the potential for transient cognitive worsening.
- ECT should be included in the guidelines for treatment of patients with PD and depression.
- Prospective studies of ECT in patients with PD and psychiatric comorbidities will help determine what ECT parameters are most likely to lead to remission of symptoms with minimal side effects.
- We call for more publications regarding clinical experience of maintenance ECT in patients with PD, which will advance this area of scientific inquiry.

KEYWORDS
- electroconvulsive therapy
- major depressive disorder
- Parkinson’s disease

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1University of Washington Medical Center, Seattle, Washington, USA
2Veterans’ Affairs Medical Center, Seattle, Washington, USA

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<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Number of patients</th>
<th>Depression measure at baseline</th>
<th>Depression measure after ECT</th>
<th>Motor symptoms measure at baseline</th>
<th>Motor symptoms measure after ECT</th>
<th>Side effects</th>
<th>Duration of treatment effect</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scicutella (2015)</td>
<td>1</td>
<td>Psychotic depression</td>
<td>Improvement</td>
<td>NA</td>
<td>NA</td>
<td>Delirium leading to ECT discontinuation</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Nishioka (2014)</td>
<td>2</td>
<td>BDI 31; HAM-D 30; BDI 10; HAM-D 7</td>
<td>BDI 18; HAM-D 5; BDI 18; HAM-D 2</td>
<td>H&amp;Y 5; UPDRS(III) 47</td>
<td>H&amp;Y 5; UPDRS(III) 46</td>
<td>None reported</td>
<td>None reported</td>
<td>NA</td>
</tr>
<tr>
<td>Gadit (2012)</td>
<td>1</td>
<td>MDD and OCD</td>
<td>'Marked' improvement</td>
<td>NA</td>
<td>NA</td>
<td>None reported</td>
<td>None reported</td>
<td>NA</td>
</tr>
<tr>
<td>Berg (2011)</td>
<td>1</td>
<td>BDI 20</td>
<td>BDI 14</td>
<td>H&amp;Y 4-5</td>
<td>H&amp;Y 2</td>
<td>Transient confusion</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Bui (2011)</td>
<td>1</td>
<td>HAM-D 21</td>
<td>HAM-D 13</td>
<td>UPDRS(III) 22</td>
<td>UPDRS(III) 10</td>
<td>None reported</td>
<td>At least 5 months</td>
<td>NA</td>
</tr>
<tr>
<td>Ducharme (2011)</td>
<td>1</td>
<td>MDD</td>
<td>'Adequate' response</td>
<td>NA</td>
<td>NA</td>
<td>None reported</td>
<td>At least 1 year</td>
<td>NA</td>
</tr>
<tr>
<td>Nasr (2011)</td>
<td>1</td>
<td>MDD with psychotic symptoms</td>
<td>Remission</td>
<td>NA</td>
<td>NA</td>
<td>Depression returns after an unspecified period of time</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Ueda (2010)</td>
<td>2</td>
<td>HAM-D 23; HAM-D 25</td>
<td>HAM-D 2; HAM-D 1</td>
<td>H&amp;Y 4; H&amp;Y 5</td>
<td>H&amp;Y 3; H&amp;Y 4</td>
<td>Well tolerated</td>
<td>Well tolerated</td>
<td>At least 1 week; At least 1 week</td>
</tr>
<tr>
<td>Virit (2010)</td>
<td>3</td>
<td>HAM-D 40; HAM-D 28; HAM-D 30</td>
<td>HAM-D 16; HAM-D 8; HAM-D 6</td>
<td>UPDRS(III) 49; UPDRS(III) 60; UPDRS(III) 39</td>
<td>UPDRS(III) 39; UPDRS(III) 24; UPDRS(III) 21</td>
<td>None reported</td>
<td>At least 5 months</td>
<td>NA</td>
</tr>
<tr>
<td>Bailine (2008)</td>
<td>1</td>
<td>MDD with psychotic symptoms</td>
<td>'Significant' improvement</td>
<td>NA</td>
<td>NA</td>
<td>Transient confusion</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Belzeaux (2007)</td>
<td>1</td>
<td>MDD with catatonic symptoms</td>
<td>Remission</td>
<td>NA</td>
<td>NA</td>
<td>None reported</td>
<td>MECT done every 1-2 weeks with sustained effect</td>
<td>NA</td>
</tr>
<tr>
<td>Chou (2005)</td>
<td>1</td>
<td>MDD with psychotic symptoms</td>
<td>Remission</td>
<td>NA</td>
<td>NA</td>
<td>Transient confusion</td>
<td>At least 6 months</td>
<td>NA</td>
</tr>
<tr>
<td>Ozer (2005)</td>
<td>1</td>
<td>'Depression and psychosis' for all three courses</td>
<td>None reported after 1st course</td>
<td>Remission after 2nd course</td>
<td>'Cured' after 3rd course</td>
<td>Transient confusion</td>
<td>Depression returns 5 years after 1st course and 4 years after 2nd course. Patient 'cured' after 3rd course</td>
<td>NA</td>
</tr>
<tr>
<td>Shulman (2003)</td>
<td>1</td>
<td>MDD</td>
<td>Remission</td>
<td>NA</td>
<td>NA</td>
<td>None reported</td>
<td>None reported</td>
<td>NA</td>
</tr>
<tr>
<td>Benito (2002)</td>
<td>3</td>
<td>HAM-D 42; HAM-D 46; HAM-D 45</td>
<td>HAM-D 42; HAM-D 36; HAM-D 14</td>
<td>NA</td>
<td>NA</td>
<td>Transient confusion</td>
<td>None reported</td>
<td>NA</td>
</tr>
</tbody>
</table>

AIMS: Abnormal Involuntary Movement Scale; BDI: Beck Depression Inventory; CORSD: Cronholm & Orattosson Depression Rating Scale; CRSD: Carroll Rating Scale for Depression; Duvoisin: Columbia University Rating Scale; ECT: electroconvulsive therapy; EPS: Extrapyramidal symptoms; HAM-D: Hamilton Rating Scale for Depression; H&Y: Hoehn and Yahr Scale; MADRS: Montgomery–Asberg Depression Rating Scale; MDD: Major Depressive Disorder; MECT: Maintenance Electroconvulsive Therapy; MMSE: Mini-Mental Status Exam; NA: Not applicable; NOS: Not otherwise specified; OCD: Obsessive-compulsive disorder; SCRB: Short Clinical Rating Scale; SDS: Zung Self-Rating Depression Scale; Simpson–Angus Scale; UPDRS: Unified Parkinson's Disease Rating Scale; UPDRS(III): Motor Section (Part 3) of the UPDRS.
<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Number of patients</th>
<th>Depression measure at baseline</th>
<th>Depression measure after ECT</th>
<th>Motor symptoms measure at baseline</th>
<th>Motor symptoms measure after ECT</th>
<th>Side effects</th>
<th>Duration of treatment effect</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fall (2000)</td>
<td>3</td>
<td>MADRS 8</td>
<td>MADRS 4</td>
<td>H&amp;Y; UPDRS(III) 67</td>
<td>UPDRS(III) 62</td>
<td>None reported</td>
<td>NA</td>
<td>[44]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MADRS 12</td>
<td>MADRS 8</td>
<td>H&amp;Y; UPDRS(III) 11</td>
<td>UPDRS(III) 0</td>
<td>None reported</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MADRS 19</td>
<td>MADRS 8</td>
<td>H&amp;Y; UPDRS(III) 26</td>
<td>UPDRS(III) 14</td>
<td>None reported</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Amann (1999)</td>
<td>1</td>
<td>‘Delusional depression’</td>
<td>‘No success’</td>
<td>NA</td>
<td>NA</td>
<td>None reported</td>
<td>No effect</td>
<td>[45]</td>
</tr>
<tr>
<td>Fall (1999)</td>
<td>1</td>
<td>MADRS 19</td>
<td>MADRS 8</td>
<td>H&amp;Y; UPDRS(III) 26</td>
<td>UPDRS(III) 14</td>
<td>Transient confusion</td>
<td>NA</td>
<td>[46]</td>
</tr>
<tr>
<td>Kramer (1999)</td>
<td>10</td>
<td>Includes DSM-IIIR or DSM-IV defined MDD, atypical depression, or depression not otherwise specified</td>
<td>5 patients ‘much improved’</td>
<td>NA</td>
<td>NA</td>
<td>None reported</td>
<td>Followed for 4 years: 5 patients had sustained effect; 4 patients had some depression but still better than pre-ECT; 1 patient had no effect</td>
<td>[47]</td>
</tr>
<tr>
<td>Avila (1997)</td>
<td>2</td>
<td>‘Psychotic depression’</td>
<td>Remission</td>
<td>UPDRS(III) 61</td>
<td>UPDRS(III) 30</td>
<td>None reported</td>
<td>MECT for 2 months following index course with sustained effect</td>
<td>[48]</td>
</tr>
<tr>
<td>Nymeyer (1997)</td>
<td>1</td>
<td>MDD</td>
<td>Improvement</td>
<td>NA</td>
<td>NA</td>
<td>Transient delirium</td>
<td>NA</td>
<td>[49]</td>
</tr>
<tr>
<td>Factor (1995)</td>
<td>1</td>
<td>MDD with psychotic symptoms</td>
<td>Improvement</td>
<td>NA</td>
<td>NA</td>
<td>Transient confusion</td>
<td>NA</td>
<td>[50]</td>
</tr>
<tr>
<td>Sandyk (1993)</td>
<td>1</td>
<td>‘Delusional unipolar depression’</td>
<td>‘Modest’ improvement</td>
<td>NA</td>
<td>NA</td>
<td>Agitation</td>
<td>Depression returns after an unspecified period of time</td>
<td>[51]</td>
</tr>
<tr>
<td>Friedman (1992)</td>
<td>5</td>
<td>‘Severe depression’</td>
<td>Remission</td>
<td>H&amp;Y 4</td>
<td>H&amp;Y 3</td>
<td>None reported</td>
<td>At least 10 weeks Depression returns after 1-2 months</td>
<td>[52]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>‘Severe depression’</td>
<td>Improvement</td>
<td>H&amp;Y 3–4</td>
<td>H&amp;Y 3</td>
<td>Delirium</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>‘Severe depression’</td>
<td>Improvement</td>
<td>H&amp;Y 3</td>
<td>NA</td>
<td>PVCs during induction</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>‘Severe depression’</td>
<td>‘Mild’ improvement</td>
<td>H&amp;Y 4</td>
<td>NA</td>
<td>None reported</td>
<td>Died 2 days after last ECT Depression returns after 8 weeks</td>
<td></td>
</tr>
<tr>
<td>Holzer (1992)</td>
<td>1</td>
<td>‘Major depressive episode’</td>
<td>Remission</td>
<td>NA</td>
<td>NA</td>
<td>Stuttering, slurred speech</td>
<td>NA</td>
<td>[53]</td>
</tr>
</tbody>
</table>

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## Table 1. Depression severity, motor symptom severity, side effects and duration of treatment effect (cont.).

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Number of patients</th>
<th>Depression measure at baseline</th>
<th>Depression measure after ECT</th>
<th>Motor symptoms measure at baseline</th>
<th>Motor symptoms measure after ECT</th>
<th>Side effects</th>
<th>Duration of treatment effect</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oh (1992)</strong></td>
<td>11</td>
<td>5 patients with MDD</td>
<td>3 patients ‘marked relief’</td>
<td>NA</td>
<td>NA</td>
<td>Delirium in 7 patients, leading to treatment discontinuation in 6 patients</td>
<td>NA</td>
<td>[54]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 patients with psychotic MDD</td>
<td>6 patients ‘some relief’</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 patient with depression NOS</td>
<td>2 patients no effect</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 patient with bipolar mania</td>
<td></td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td><strong>Zwil (1992)</strong></td>
<td>8</td>
<td>MDD with psychotic symptoms</td>
<td>Improvement</td>
<td>NA</td>
<td>NA</td>
<td>‘Disorientation’ arose in 8 out of 27 patients, but side effects in the subset of patients with Parkinson’s disease (n = 8) are not described separately.</td>
<td>NA</td>
<td>[29]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MDD</td>
<td>Improvement</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CRSD 31</td>
<td>CRSD 32</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MDD with psychotic symptoms</td>
<td>Improvement</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HAM-D 22</td>
<td>HAM-D 3</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HAM-D 30</td>
<td>HAM-D 6</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>‘Organic mood disorder, depressed’</td>
<td>Improvement</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MDD</td>
<td>Improvement</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td><strong>Figiel (1991)</strong></td>
<td>7</td>
<td>‘Major depressive episode’</td>
<td>‘Very much’ improvement</td>
<td>NA</td>
<td>NA</td>
<td>Delirium</td>
<td>NA</td>
<td>[55]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>‘Major depressive episode’</td>
<td>No effect</td>
<td>NA</td>
<td>NA</td>
<td>Delirium</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MDD</td>
<td>‘Very much’ improvement</td>
<td>NA</td>
<td>NA</td>
<td>Delirium</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MDD</td>
<td>‘Very much’ improvement</td>
<td>NA</td>
<td>NA</td>
<td>Delirium</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bipolar disorder, depressed</td>
<td>‘Very much’ improvement</td>
<td>NA</td>
<td>NA</td>
<td>Delirium</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>‘Major depressive episode’</td>
<td>Improvement</td>
<td>NA</td>
<td>NA</td>
<td>Delirium</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>‘Major depressive episode’</td>
<td>‘Much’ improvement</td>
<td>NA</td>
<td>NA</td>
<td>Delirium</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td><strong>Stern (1991)</strong></td>
<td>1</td>
<td>‘Anxious and depressed’</td>
<td>Improvement</td>
<td>NA</td>
<td>NA</td>
<td>None reported</td>
<td>NA</td>
<td>[56]</td>
</tr>
<tr>
<td><strong>Liberzon (1990)</strong></td>
<td>1</td>
<td>Depression and ‘paranoid ideation’</td>
<td>Marked’ improvement</td>
<td>NA</td>
<td>NA</td>
<td>‘No significant confusion’</td>
<td>NA</td>
<td>[57]</td>
</tr>
</tbody>
</table>

AIMS: Abnormal Involuntary Movement Scale; BDI: Beck Depression Inventory; CORSD: Cronholm & Ottoson Depression Rating Scale; CRSD: Carroll Rating Scale for Depression; Duvoisin: Columbia University Rating Scale; ECT: electroconvulsive therapy; EPS: Extrapyramidal symptoms; HAM-D: Hamilton Rating Scale for Depression; H&Y: Hoehn and Yahr Scale; MADRS: Montgomery–Åsberg Depression Rating Scale; MDD: Major Depressive Disorder; MEIC: Maintenance Electroconvulsive Therapy; MMSE: Mini-Mental Status Exam; NA: Not applicable; NOS: Not otherwise specified; OCD: Obsessive-compulsive disorder; SCOS: Short Clinical Rating Scale; SDS: Zung Self-Rating Depression Scale; Simpson–Angus Scale; UPDRS: Unified Parkinson’s Disease Rating Scale; UPDRS(III): Motor Section (Part 3) of the UPDRS.
<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Number of patients</th>
<th>Depression measure at baseline</th>
<th>Depression measure after ECT</th>
<th>Motor symptoms measure at baseline</th>
<th>Motor symptoms measure after ECT</th>
<th>Side effects</th>
<th>Duration of treatment effect</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Douyon (1989)</td>
<td>7</td>
<td>HAM-D range 21-33 in these seven patients; mean 27 with s.d. 4</td>
<td>Improvement</td>
<td>HAM-D 27% decrease</td>
<td>NYUPD 79</td>
<td>NYUPD 46% decrease</td>
<td>Delirium</td>
<td>NA</td>
</tr>
<tr>
<td>Burke (1988)</td>
<td>3</td>
<td>MDD</td>
<td>Remission</td>
<td>NA</td>
<td>NA</td>
<td>None reported</td>
<td>At least 1 month</td>
<td>[59]</td>
</tr>
<tr>
<td>Young (1985)</td>
<td>1</td>
<td>HAM-D 22</td>
<td>HAM-D 24</td>
<td>NA</td>
<td>NA</td>
<td>None reported</td>
<td>At least 1 month</td>
<td>[60]</td>
</tr>
<tr>
<td>Holcomb (1983)</td>
<td>1</td>
<td>SCRS 8</td>
<td>SCRS 2</td>
<td>EPS scale 3; AIMS 9</td>
<td>EPS scale 1; AIMS 14</td>
<td>None reported</td>
<td>Depression returns after 1 week</td>
<td>[61]</td>
</tr>
<tr>
<td>Levy (1983)</td>
<td>1</td>
<td>‘Severe depression’</td>
<td>‘Remarkable’ improvement</td>
<td>NA</td>
<td>NA</td>
<td>None reported</td>
<td>NA</td>
<td>[62]</td>
</tr>
<tr>
<td>Balldin (1980)</td>
<td>3</td>
<td>CORSD 4</td>
<td>CORSD 0</td>
<td>NA</td>
<td>NA</td>
<td>Well tolerated</td>
<td>NA</td>
<td>[63]</td>
</tr>
<tr>
<td>Yudofsky (1979)</td>
<td>1</td>
<td>‘Delusional depression’</td>
<td>Improvement</td>
<td>NA</td>
<td>NA</td>
<td>None reported</td>
<td>At least 3 weeks</td>
<td>[64]</td>
</tr>
<tr>
<td>Barcia (1978)</td>
<td>1</td>
<td>MDD with psychotic symptoms</td>
<td>Remission</td>
<td>NA</td>
<td>NA</td>
<td>None reported</td>
<td>NA</td>
<td>[65]</td>
</tr>
<tr>
<td>Asnis (1977)</td>
<td>1</td>
<td>SDS 70</td>
<td>SDS 40</td>
<td>Duvoisin 22; Simpson 15</td>
<td>Duvoisin 6; Simpson 9</td>
<td>Transient confusion</td>
<td>Depression returns after 7 weeks</td>
<td>[66]</td>
</tr>
<tr>
<td>Dysken (1976)</td>
<td>1</td>
<td>MDD</td>
<td>Improvement</td>
<td>NA</td>
<td>NA</td>
<td>None reported</td>
<td>NA</td>
<td>[67]</td>
</tr>
<tr>
<td>Lebensohn (1975)</td>
<td>2</td>
<td>MDD with psychotic symptoms, Bipolar disorder, depressed</td>
<td>‘Remarkable’ improvement</td>
<td>Remission</td>
<td>NA</td>
<td>NA</td>
<td>None reported</td>
<td>NA</td>
</tr>
</tbody>
</table>
for major depression, 22% for minor depression and 13% for dysthymia [2]. Depression is associated with poorer quality of life and increased morbidity and mortality in patients with PD, and it is the most important risk factor for suicide in this population [3]. Despite conventional thought that suicide is uncommon in PD, recent studies suggest that suicidal ideation may be present in up to 30% of patients with PD [4]. Unfortunately, despite the high prevalence, depression in PD is often unrecognized and undertreated [5].

Overlapping symptoms may make it difficult to discern problems expected in PD, such as apathy, sleep disturbance, masked facies and bradykinesia, from symptoms of depression, such as anhedonia, depressed affect and psychomotor slowing. According to the American Academy of Neurology guidelines, screening of depression in PD should include the use of a standardized measure such as the Beck Depression Inventory or the Hamilton Depression Rating Scale. A recent taskforce from the Movement Disorders Society advocated for use of the Geriatric Depression Scale [6,7]. Once accurately detected, treatment can still be a challenge.

Antidepressant therapy is the most common treatment of depression in PD with studies showing that up to 25% of patients with PD are on an antidepressant treatment at any given time [4]. Serotonin selective reuptake inhibitors (SSRIs) are more often prescribed than tricyclic antidepressants (TCAs). A national study from the Veteran’s Administration showed rates for treatment of depression in PD at 63% for SSRIs compared with 7.5% for TCAs [8]. Despite these statistics, there are relatively few randomized trials showing efficacy of SSRIs and TCAs in the treatment of depression in PD. According to the most recent practice parameter from the American Academy of Neurology, there was insufficient evidence to support or refute effectiveness of antidepressants aside from small results indicating amitriptyline may be useful [6].

In 2011, the Movement Disorder Society Task Force on Evidence-Based Medicine published a review of the literature. They argued that desipramine and nortriptyline were likely efficacious and possibly clinically useful but found insufficient evidence to support SSRIs and rated them investigational [9]. Since that time a few randomized trials have been performed; the largest showed that both paroxetine and venlafaxine improved depression and did not worsen motor function [10]. Dopamine agonists

### Table 1. Depression severity, motor symptom severity, side effects and duration of treatment effect (cont.)

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Number of patients</th>
<th>Depression measure at baseline</th>
<th>Depression measure after ECT</th>
<th>Motor symptoms measure at baseline</th>
<th>Motor symptoms measure after ECT</th>
<th>Side effects</th>
<th>Duration of treatment effect</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipper (1975)</td>
<td>97</td>
<td>Psychotic depression</td>
<td>Marked improvement</td>
<td>NA</td>
<td>Well tolerated</td>
<td>NA</td>
<td>NA</td>
<td>[69]</td>
</tr>
</tbody>
</table>

**AIMS**: Abnormal Involuntary Movement Scale; **BDI**: Beck Depression Inventory; **CORSD**: Cronhom & Ottosson Depression Rating Scale; **CRSD**: Carroll Rating Scale for Depression; **Duvoisin**: Columbia University Rating Scale for Depression; **ECT**: Electroconvulsive Therapy; **EPS**: Extrapyramidal Symptoms; **HAM-D**: Hamilton Rating Scale for Depression; **H&Y**: Hoehn and Yahr Scale; **MADRS**: Montgomery–Asberg Depression Rating Scale; **MDD**: Major Depressive Disorder; **MECT**: Maintenance Electroconvulsive Therapy; **MMSE**: Mini-Mental Status Exam; **NA**: Not applicable; **NOS**: Not otherwise specified; **OCD**: Obsessive-compulsive Disorder; **SCRS**: Short Clinical Rating Scale; **SDS**: Zung Self-Rating Depression Scale; **Simpson**: Simpson–Angus Scale; **UPDRS**: Unified Parkinson’s Disease Rating Scale; **UPDRS(III)**: Motor Section (Part 3) of the UPDRS.
such as pramipexole have also been found useful [11]. A small randomized pilot study demonstrated efficacy for depression in PD with Omega-3 fatty-acid supplementation [12]. The latest review of evidence from UpToDate recommends using SSRIs first, based on the lesser likelihood of adverse side effects with SSRIs than with TCAs, and using TCAs if SSRIs do not lead to improvement of depressive symptoms [13].

Nonmedication treatment options for depression in PD have been studied to an even lesser extent. Though a growing body of evidence for cognitive behavioral therapy exists, it is limited to only a few studies [14,15].

In cases of treatment refractory depression, repetitive transcranial magnetic stimulation (rTMS) and electroconvulsive therapy (ECT) have been shown to be effective, with ECT being more effective than rTMS [16]. Unfortunately, there is no specific data regarding treatment refractory depression among depressed patients with PD. A recent meta-analysis included eight small randomized trials showing that rTMS was superior to sham-rTMS, with similar efficacy to SSRIs in the treatment of depression for patients with PD [17]. This study also showed that rTMS potentially improved movement in these patients.

ECT has been used in PD for decades for a variety of psychiatric and motor symptoms. The latest comprehensive review of the effect of ECT on motor symptoms in PD was published in 2005. Authors concluded that ECT may exert a significant effect on motor function in PD patients, though the number of studies included in the analysis was small and these data could only be interpreted with caution [18]. A thorough review published in 1991 concluded that ECT improved motor symptoms in over half of PD patients, regardless of psychiatric comorbidity [19]. Another, similar in scope, review was published in 2003. Although the authors pointed out the literature limitations and biases, they concluded the existence of “considerable evidence...that ECT improves motor symptoms of Parkinson’s disease in patients with and without mood disorders.” [20].

Table 2. Depressive and motor symptoms’ response to electroconvulsive therapy.

<table>
<thead>
<tr>
<th>Depression measure ↓</th>
<th>Motor symptoms →</th>
<th>Improved</th>
<th>Not improved</th>
<th>Not reported</th>
<th>Worse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improved</td>
<td></td>
<td>39</td>
<td>7</td>
<td>62</td>
<td>1</td>
</tr>
<tr>
<td>Not improved</td>
<td></td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

ECT has also been used for treatment of psychosis and anxiety in PD, though the body of literature is smaller than that describing treatment of motor symptoms and depression. Only two patients with PD who benefitted from ECT for refractory anxiety have been reported [21]. Significant improvements in psychosis were seen in five elderly patients with PD and medically refractory psychosis [22] and eight patients with PD and quetiapine-resistant psychosis [23].

Proponents of ECT such as Dr Max Fink, Dr Richard Abrams and Dr Iannis Zervas had advocated for more widespread use of ECT in PD [24–26], with Dr Abrams calling for a therapeutic trial of ECT in all patients with intratable or drug-resistant PD [25]. Dr Cummings recommended TCAs or ECT for treatment of depression in PD in a review from 1992 [27]. However, these endorsements have not found their way into the most recent guidelines for treatment of PD. Further, there are no recent comprehensive reviews of ECT used specifically for depression in PD. Here, we will summarize the literature and provide recommendations based on the data available.

Methods

Search strategy

We systematically searched for all available published studies that focused on treatment of patients with documented PD and comorbid depression. The computerized databases of PubMed, EMBASE, Cochrane and Google Scholar were searched for studies published from earliest entry date in the database through September 2015. Further, we searched through the relevant journals in the field (Movement Disorders, Journal of ECT and Psychosomatics) over the course of 6 months prior to September 2015, for any publications that were not yet available in PubMed or EMBASE. We used the search strategy with the following parameters in each database: “depression and Parkinson’s and ECT,” and “depression and Parkinson’s and electroconvulsive therapy,” and hand-searched through the reference sections of relevant articles for studies we may have missed. The identified
studies were entered into Endnote to ensure as full as possible a collection of available literature and no duplication of studies.

- **Study eligibility**
  Inclusion criteria required that studies describe the use of ECT for treatment of depression in patients with PD, rather than any other psychiatric or neurological problem. We included any studies that reported patient data, such as case reports, case controlled studies, clinical trials, cohort studies and randomized controlled trials, but we excluded expert opinions. We excluded studies describing patients with no firm diagnosis of PD, studies in which patients had not been assessed for presence of depression before and after the treatment, and studies that did not report any data about changes or lack thereof in patients’ depressive illness.

- **Review process & data extraction**
  Two independent reviewers (A Borisovskaya and W Bryson) conducted the literature search and review of study eligibility. We examined the titles and abstracts of all studies identified through our search strategy, and those studies that were clearly not pertinent to our review were eliminated. We read the full text of articles that appeared pertinent based on their titles and abstracts, 58 in total, and excluded studies that did not meet our inclusion criteria, 15 in total. The reasons for exclusions were as follows: four studies described patients who were not depressed prior to ECT, seven studies did not provide pertinent information regarding the patients they treated, two studies described patients with no clear PD diagnosis, one study did not use ECT for treatment of the patient and one study turned out to be a literature review upon translation from French without any specific patient data. The studies published in languages other than English (Turkish, German, French and Spanish), but that met our inclusion criteria, were translated using the Google Translate website.

  Forty-three studies met our inclusion criteria. Of those, 27 were case reports describing single cases, 13 were case series describing anywhere from two to eleven patients, all of these reported retroactively, two were retrospective chart reviews, and one was a retrospective case control study. The latter described the largest number of patients treated with ECT for PD and depression, 19 in total [28]. The studies were published between the years of 1975–2015.

  From the included studies, we extracted the following data: the number of patients treated with ECT, the measure of PD symptoms at baseline and after treatment with ECT, the measure of depressive symptoms at baseline and after treatment with ECT, the measure of cognition at baseline and after treatment with ECT, description of any side effects, and duration of the treatment effect after completion of ECT. Separately, we also extracted the data regarding method of ECT administration such as type of device, right unilateral versus bilateral lead placement, pulse width, number of ECT procedures performed, and the total energy given during the procedures. For the purpose of this review, we accepted descriptions of treatment results such as ‘improvement’ or ‘remission’ or ‘response’ or ‘relief’ or ‘cure’ or ‘no effect’ if validated scales to assess depressive symptoms were not used or cited. From the data obtained, we were able to determine the percentage of reported cases where depressive symptoms responded to treatment with ECT, as well as the percentage of

<table>
<thead>
<tr>
<th>Side effect</th>
<th>Number (percentage) of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delirium</td>
<td>31 (26.7%)</td>
</tr>
<tr>
<td>‘Transient confusion’</td>
<td>10 (8.6%)</td>
</tr>
<tr>
<td>Agitation</td>
<td>1 (0.9%)</td>
</tr>
<tr>
<td>Stuttering and slurred speech</td>
<td>1 (0.9%)</td>
</tr>
<tr>
<td>PVCs during induction</td>
<td>1 (0.9%)</td>
</tr>
<tr>
<td>MI/death</td>
<td>1 (0.9%)</td>
</tr>
<tr>
<td>Urinary retention (two of these patients also had a UTI)</td>
<td>5 (4.3%)</td>
</tr>
<tr>
<td>Choreiform movements</td>
<td>1 (0.9%)</td>
</tr>
<tr>
<td>Falls</td>
<td>2 (1.7%)</td>
</tr>
<tr>
<td>Disorientation*</td>
<td>8 out of 27 patients, not all of whom had PD</td>
</tr>
</tbody>
</table>

The numbers regarding frequency of urinary retention, choreiform movements and falls may be overestimated.

*Data taken from [29].

MI: Myocardial infarction; PD: Parkinson’s disease; PVC: Premature ventricular contraction; UTI: Urinary tract infection.
cases where motor symptoms improved as well. We evaluated the percentage of patients who experienced significant side effects from ECT, particularly cognitive impairment.

Results
The included studies are organized by year of publication and presented in Table 1, with exception of Moellentine et al. [28] which is summarized in the paragraph below. A total of 116 patients, described in these studies, were included in our analysis. While the majority of patients were diagnosed with major depressive disorder (MDD), some studies used descriptions rather than a specific diagnosis of MDD, for example: ‘delusional depression’, ‘depression and psychosis’, ‘severe depression’. One patient was diagnosed with depression not otherwise specified. Two patients were diagnosed with bipolar depression. One patient was diagnosed with bipolar mania and was not depressed at the time of ECT administration [29], however, we could not parse out the results of this patient’s treatment from those of other patients in that study. Therefore, this patient was included in our final calculations. The age of patients described in the studies ranged from 33 to 83, though the 33-year-old patient was an outlier; the rest ranged in age from 52 to 83 years.

Moellentine and colleagues compared the outcomes of ECT for psychiatric indications between 25 patients with PD and 25 patients without PD. MDD with or without psychosis constituted the psychiatric indication for ECT in the majority (78%) of patients, totaling 19 in the treatment group. They found a “significant decrease in depression” as measured with the Hamilton Depression Rating Scale (HAM-D) in both groups following ECT. The duration of the effect was not reported. Among the patients with PD, 56% reported subjective improvement in motor function, 40% reported no change and 4% reported deterioration. Average Mini Mental Status Exam scores improved after ECT in both groups, although the finding was not statistically significant. A higher proportion of patients with PD experienced ECT side effects compared with those without PD (56 vs 12%). The most common side effect was “transient intertreatment delirium,” which necessitated postponement or termination of ECT in 52% of patients with PD. Unfortunately, for any of the effects described above, it was not noted in the study which specific patients experienced them [28].

The results regarding ECT efficacy for treatment of depression and for motor symptoms of PD are summarized in Table 2. A total of 93.1% of all patients experienced improvement in their depression. Of those cases where a change (or lack thereof) in motor symptoms was recorded, 83% of patients reported improvement in their symptoms alongside improvement of depression, 15% had no improvement in motor symptoms despite improvement of depression and 2% of patients experienced worsening of motor symptoms despite improvement of depression. Approximately 6.9% of patients had no improvement in depression, though we do not have information on whether their motor symptoms had changed for better or for worse.

The duration of treatment effect was not noted for 46 of the cases described. In cases where it was recorded, the duration of treatment effect ranged in magnitude from weeks to years.

Side effects of ECT treatment are summarized in Table 3. Though we chose to list the relevant data from study by Moellentine et al. [28], it was unclear which of the patients had urinary retention, choreiform movements or falls, and it is possible that these side effects occurred in 6 of 25 patients who were not included in our study. No side effects were noted for 43 of the studied cases, though we cannot conclude from this absence of data whether the patients did not, in effect, have any side effects. The main side effects of treatment were delirium and ‘transient confusion’; several studies mentioned that these side effects necessitated postponement or discontinuation of ECT. ‘Disorientation’ arose in 8 out of 27 patients in the study by Zwil et al. [29] but is reported separately since it is unclear whether these patients had PD.

We paid particular attention to the reports of patients’ cognition and whether it was affected by ECT. Unfortunately, in 75 of the reported cases, there was no description of whether the patients’ cognition worsened, improved or remained unchanged – or no conclusions could be drawn based on the reported data. Improvement in cognition was described in three (9%) of the reported cases. Worsening cognition was described in two (6%) cases. Two of the patients could not complete cognitive screening prior to ECT, but completed the testing thereafter. Unfortunately, no definitive conclusions about changes in their cognition could be made [22]. Twenty-eight (85%) of the patients had no significant change in their cognition.
<table>
<thead>
<tr>
<th>Study (year)</th>
<th>ECT Parameters</th>
<th>Number of ECT sessions</th>
<th>Cognition measure at baseline</th>
<th>Cognition measure after ECT</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nishioka (2014)</td>
<td>Thymatron; BL; 'half-age method'</td>
<td>7</td>
<td>HDS-R or MMSE 14</td>
<td>HDS-R or MMSE 26</td>
<td>[31]</td>
</tr>
<tr>
<td>Gadit (2012)</td>
<td>None reported</td>
<td>9</td>
<td>NA</td>
<td>NA</td>
<td>[32]</td>
</tr>
<tr>
<td>Berg (2011)</td>
<td>Thymatron; RUL; 70-100%; 0.5 ms</td>
<td>12</td>
<td>MMSE 'normal'</td>
<td>NA</td>
<td>[33]</td>
</tr>
<tr>
<td>Bui (2011)</td>
<td>BL</td>
<td>12</td>
<td>NA</td>
<td>NA</td>
<td>[34]</td>
</tr>
<tr>
<td>Ducharme (2011)</td>
<td>MECTA; BL; 40 Hz; 0.8 mA; 1 ms</td>
<td>8</td>
<td>NA</td>
<td>NA</td>
<td>[35]</td>
</tr>
<tr>
<td>Nasr (2011)</td>
<td>MECTA; BL; 62-80 J; 576.4 mC</td>
<td>7</td>
<td>MMSE 21</td>
<td>MMSE 30</td>
<td>[36]</td>
</tr>
<tr>
<td>Ueda (2010)</td>
<td>CS-1; BL; 95-105 V; 50 Hz</td>
<td>5</td>
<td>Neither could complete baseline MMSE due to PD symptoms</td>
<td>MMSE 24</td>
<td>[22]</td>
</tr>
<tr>
<td>Virit (2010)</td>
<td>BL</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>[37]</td>
</tr>
<tr>
<td>Bailine (2008)</td>
<td>Thymatron; BL; 40%</td>
<td>7</td>
<td>NA</td>
<td>NA</td>
<td>[38]</td>
</tr>
<tr>
<td>Belzeaux (2007)</td>
<td>None reported</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>[39]</td>
</tr>
<tr>
<td>Chou (2005)</td>
<td>Thymatron; BL frontal; 45–75%</td>
<td>9</td>
<td>NA</td>
<td>NA</td>
<td>[40]</td>
</tr>
<tr>
<td>Ozer (2005)</td>
<td>MECTA; BL; 39.4 J; 50 Hz; 1.4 ms (same protocol used for all 3 courses)</td>
<td>5 in first course</td>
<td>NA</td>
<td>NA</td>
<td>[41]</td>
</tr>
<tr>
<td>Shulman (2003)</td>
<td>BL</td>
<td>4</td>
<td>NA</td>
<td>NA</td>
<td>[42]</td>
</tr>
<tr>
<td>Benito (2002)</td>
<td>Thymatron</td>
<td>10</td>
<td>NA</td>
<td>NA</td>
<td>[43]</td>
</tr>
<tr>
<td>Kramer (1999)</td>
<td>MECTA SR-1; 'most patients' BL</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>[47]</td>
</tr>
<tr>
<td>Avila (1997)</td>
<td>BL; 36.7 J</td>
<td>9</td>
<td>NA</td>
<td>NA</td>
<td>[48]</td>
</tr>
<tr>
<td>Nymeyer (1997)</td>
<td>Thymatron; RUL; 70%</td>
<td>NA</td>
<td>MMSE 26</td>
<td>NA</td>
<td>[49]</td>
</tr>
<tr>
<td>Factor (1995)</td>
<td>BL</td>
<td>6</td>
<td>NA</td>
<td>NA</td>
<td>[50]</td>
</tr>
<tr>
<td>Sandyk (1993)</td>
<td>None reported</td>
<td>‘Several’ in first course</td>
<td>NA</td>
<td>NA</td>
<td>[51]</td>
</tr>
<tr>
<td>Friedman (1992)</td>
<td>RUL; ‘brief pulse’</td>
<td>12</td>
<td>NA</td>
<td>NA</td>
<td>[52]</td>
</tr>
<tr>
<td>Holzer (1992)</td>
<td>MECTA SR-1; UL; 80 Hz; 1.4 ms</td>
<td>8</td>
<td>NA</td>
<td>NA</td>
<td>[53]</td>
</tr>
</tbody>
</table>

BL: Bilateral; ECT: Electroconvulsive therapy; HDS-R: Hierarchic dementia scale, revised; mC: milliCoulomb; MECTA/MECTA SR-1: ECT device; MMSE: Mini-mental state examination; ms: millisecond; NA: Not applicable; RUL: Right unilateral; Thymatron: ECT device; UL: Unilateral; V: Volt.
## Table 4. Electroconvulsive therapy parameters, number of electroconvulsive therapy procedures and cognition (cont.)

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>ECT Parameters</th>
<th>Number of ECT sessions</th>
<th>Cognition measure at baseline</th>
<th>Cognition measure after ECT</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oh (1992)</td>
<td>Thymatron; UL for 9 patients; BL for 1 patient; UL -&gt; BL for 1 patient</td>
<td>Range 3–9 (mean 5.9)</td>
<td>NA</td>
<td>NA</td>
<td>[54]</td>
</tr>
<tr>
<td>Zwil (1992)</td>
<td>For all 8 patients: MECTA Model D; 800 mA current; variable frequency, pulse width, and stimulus duration; started UL and proceeded to BL if needed</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>[29]</td>
</tr>
<tr>
<td>Figiel (1991)</td>
<td>MECTA SR-1; RUL</td>
<td>3</td>
<td>MMSE 29</td>
<td>MMSE 28</td>
<td>[55]</td>
</tr>
<tr>
<td></td>
<td>MECTA SR-1; RUL</td>
<td>6</td>
<td>MMSE 26</td>
<td>MMSE 23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MECTA SR-1; BL</td>
<td>10</td>
<td>MMSE 29</td>
<td>MMSE 28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MECTA SR-1; BL</td>
<td>6</td>
<td>MMSE 28</td>
<td>MMSE 29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MECTA SR-1; RUL</td>
<td>4</td>
<td>MMSE 26</td>
<td>MMSE 25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MECTA SR-1; 3 RUL -&gt;5 BL</td>
<td>8</td>
<td>MMSE 26</td>
<td>MMSE 25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MECTA SR-1; 8 RUL -&gt;3 BL</td>
<td>11</td>
<td>MMSE 28</td>
<td>MMSE 26</td>
<td></td>
</tr>
<tr>
<td>Stern (1991)</td>
<td>None reported</td>
<td>8</td>
<td>NA</td>
<td>NA</td>
<td>[56]</td>
</tr>
<tr>
<td>Liberzon (1990)</td>
<td>UL</td>
<td>7</td>
<td>NA</td>
<td>NA</td>
<td>[57]</td>
</tr>
<tr>
<td>Douyon (1989)</td>
<td>MECTA SR-1 and BL for all patients</td>
<td>Average number of ECT treatments per patient is reported to be 7</td>
<td></td>
<td></td>
<td>[58]</td>
</tr>
<tr>
<td></td>
<td>Initial parameters: 56 ± 9 Hz; 1.5 ms</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Final parameters: 76 ± 9 Hz; 1.6 ± 0.2 ms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burke (1988)</td>
<td>UL</td>
<td>5</td>
<td>NA</td>
<td>NA</td>
<td>[59]</td>
</tr>
<tr>
<td></td>
<td>UL</td>
<td>6</td>
<td>NA</td>
<td>NA</td>
<td></td>
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<tr>
<td></td>
<td>RUL</td>
<td>8</td>
<td>NA</td>
<td>NA</td>
<td></td>
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<td>Young (1985)</td>
<td>'Modified' UL</td>
<td>7</td>
<td>MMSE 12</td>
<td>MMSE 13</td>
<td>[60]</td>
</tr>
<tr>
<td>Holcomb (1983)</td>
<td>BL</td>
<td>14</td>
<td>NA</td>
<td>NA</td>
<td>[61]</td>
</tr>
<tr>
<td>Levy (1983)</td>
<td>None reported</td>
<td>10</td>
<td>NA</td>
<td>NA</td>
<td>[62]</td>
</tr>
<tr>
<td>Balldin (1980)</td>
<td>All patients: Siemens convulsator 628; BL; 50 Hz; peak current 0.8 A</td>
<td>8</td>
<td>NA</td>
<td>NA</td>
<td>[63]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Yudofsky (1979)</td>
<td>None reported</td>
<td>10</td>
<td>NA</td>
<td>NA</td>
<td>[64]</td>
</tr>
<tr>
<td>Barcia (1978)</td>
<td>None reported</td>
<td>3</td>
<td>NA</td>
<td>NA</td>
<td>[65]</td>
</tr>
<tr>
<td>Asnis (1977)</td>
<td>BL</td>
<td>6</td>
<td>NA</td>
<td>NA</td>
<td>[66]</td>
</tr>
<tr>
<td>Dysken (1976)</td>
<td>BL; 120 V</td>
<td>12</td>
<td>NA</td>
<td>NA</td>
<td>[67]</td>
</tr>
<tr>
<td>Lebensohn (1975)</td>
<td>None reported</td>
<td>4</td>
<td>NA</td>
<td>NA</td>
<td>[68]</td>
</tr>
<tr>
<td></td>
<td>None reported</td>
<td>4</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Lipper (1975)</td>
<td>140 V</td>
<td>7</td>
<td>NA</td>
<td>NA</td>
<td>[69]</td>
</tr>
</tbody>
</table>

BL: Bilateral; ECT: Electroconvulsive therapy; HDS-R: Hierarchical dementia scale, revised; mC: milliCoulomb; MECTA/MECTA SR-1: ECT device; MMSE: Mini-mental state examination; ms: millisecond; NA: Not applicable; RUL: Right unilateral; Thymatron: ECT device; UL: Unilateral; V: Volt.

We also collected the sparse data regarding the method of ECT administration and the total number of sessions done for each patient. Eleven cases came with no information on ECT stimulus, five cases came with no information on the number of ECT sessions. Disparate and limited information was provided in the majority of the papers, with no reporting, variably, regarding ECT device, lead placement, stimulus intensity, pulse width or stimulus duration. Available
Follow DSM-V criteria to diagnose the depressive syndrome in question. If depression is mild to moderate, consider referral to psychotherapy (interpersonal therapy, cognitive behavioral therapy, psychodynamic therapy) and starting an antidepressant. If depression is severe (refusal to eat, significant dysfunction in the social or occupational role, catatonic symptoms, psychotic symptoms, suicidal ideation with intent or plan), consider hospitalization and ECT sooner rather than later. When choosing an antidepressant, start preferentially with an SSRI (evaluate risk vs benefit for each given case since side effects may be potentially significant). If no response after an adequate trial, consider a switch to a TCA. Bupropion \[83\] may also be appropriate. If an adequate trial of antidepressants (as above) results in no response or partial response only, consider augmentation with lithium, quetiapine (at a low dose), or a switch to a monoamine oxidase inhibitor since these are the most effective antidepressants at our disposal (after an evaluation of risks vs benefits for each given case) \[84\]. If a combined treatment with validated psychotherapy and medication regimen results in nonresponse, consider a referral to TMS in cases of mild to moderate depression, where available. If depression is severe or worsening, consider referral to ECT.

**Box 1. Algorithm for treatment of depression in Parkinson’s disease.**

- Follow DSM-V criteria to diagnose the depressive syndrome in question
- Use validated scale to measure depressive symptoms
- If there is a suspicion for contribution of medical illness (besides PD) and/or substance as etiologies of depression, perform workup and treat as indicated
- If depression is severe (refusal to eat, significant dysfunction in the social or occupational role, catatonic symptoms, psychotic symptoms, suicidal ideation with intent or plan), consider hospitalization and ECT sooner rather than later
- If there is evidence of cognitive impairment, particularly executive dysfunction, consider referral to problem solving therapy
- If depression is mild to moderate, consider referral to psychotherapy (interpersonal therapy, cognitive behavioral therapy, psychodynamic therapy) and starting an antidepressant
- When choosing an antidepressant, start preferentially with an SSRI (evaluate risk vs benefit for each given case since side effects may be potentially significant). If no response after an adequate trial, consider a switch to a TCA. Bupropion \[83\] may also be appropriate
- If an adequate trial of antidepressants (as above) results in no response or partial response only, consider augmentation with lithium, quetiapine (at a low dose), or a switch to a monoamine oxidase inhibitor since these are the most effective antidepressants at our disposal (after an evaluation of risks vs benefits for each given case) \[84\]
- If a combined treatment with validated psychotherapy and medication regimen results in nonresponse, consider a referral to TMS in cases of mild to moderate depression, where available. If depression is severe or worsening, consider referral to ECT

ECT: Electroconvulsive therapy; PD: Parkinson’s disease; TCA: Tricyclic antidepressants; TMS: Transcranial magnetic stimulation.
Cognitive deficits often resolve with improvement in cognition after ECT were noted; some patients’ cognition improved. This finding is not unusual. Our study demonstrated lack of cognitive decline and sometimes improvement in depressed elderly patients after ECT [86].

There is a high prevalence of dementia in PD patients – 24–31% [73]. Even in those without dementia syndrome, deficits in executive and visuospatial functioning and mild cognitive impairment are not uncommon. Our study was reassuring in that no significant changes in visuospatial functioning and mild cognitive impairment are not uncommon. Our study was reassuring in that no significant changes in cognition after ECT were noted; some patients’ cognition improved. This finding is not unusual. Cognitive deficits often resolve with improvement in depression [74]. A recent prospective study demonstrated lack of cognitive decline and sometimes improvement in depressed elderly patients after ECT [75].

Still, ECT was not without side effects in this population. Reports of delirium and ‘transient confusion’ were frequent. Autonomic dysregulation and falls are common in PD, and ECT may have increased the patients’ susceptibility to urinary retention and falls.

Patients and clinicians should be assured that ECT side effects are temporary. Fears about the extent of cognitive losses may be one of the main reasons why so few PD patients get referred to ECT, along with the impermanence of positive effect on motor symptoms and difficulty predicting how long the effect of treatment would last [76,77]. Stigma still surrounds ECT, and the general public often gets a maligned view of ECT from the media [78].

Little is known specifically about maintenance ECT for PD. Three reports are included in this review [42,46–47]. The results are not always positive. Two studies, not included in this review, suggest significant benefit from maintenance ECT for motor symptoms in PD [79] and schizophrenia comorbid with PD [80]; another study cites benefit in two patients, but also describes two patients who developed significant cognitive impairment and delusions during maintenance ECT [81]. Nonetheless, some authors cite inevitable relapse within 6 months upon completion of index ECT course [82]. More severe and chronic forms of depression, as well as history of resistance to medications are indicators that patients may need maintenance ECT to prevent relapse.

We suggest an algorithm for treatment of depression in patients with PD, largely based on what we know regarding the treatment of depression in the elderly and treatment-resistant depression (Box 1). Further, we make recommendations to minimize cognitive losses during ECT for patients with PD (Box 2).

**Conclusion**

ECT is a beneficial treatment for patients suffering from PD and depression, often effective for mood and motor symptoms. Common side effects include delirium, transient confusion, falls, urinary retention and rarely exacerbation of abnormal movements and motor symptoms. Cognitive losses are not universal during a course of ECT. To date, little is known about the utility of maintenance ECT specifically in patients with PD and depression.

**Future perspective**

While we realize that randomized controlled trials of ECT and medications in this population would be costly and impractical, future studies should focus on comparison of similar cohorts of patients’ response to medications, ECT and TMS. Each study should thoroughly

---

**Box 2. Strategies to prevent or decrease cognitive losses during electroconvulsive therapy.**

- Perform cognitive testing prior to ECT and during ECT to determine the patient’s baseline functioning and the impact that ECT might have had. If significant changes occur, consider taking a break from treatment or decreasing amount of energy used during the procedure.
- Start with right unilateral stimulus administration
- Use ultrabrief pulse width (0.3 ms) [85]. If using bilateral ECT, use pulse width of 0.5 ms rather than 1 ms
- Decrease frequency of treatments (perform procedures twice a week rather than three-times a week)
- Minimize concurrent medications that may compound the cognitive losses (anticholinergics, benzodiazepines)
- Keep in mind that the higher the total amount of energy is administered, the more significant the potential memory losses. Utilize the energy setting that improves depressive symptoms but minimizes side effects
- For those who have been diagnosed with dementia, start and continue a cholinesterase inhibitor, which may be protective against cognitive losses during ECT [86]

ECT: Electroconvulsive therapy
document the patients’ motor symptom severity with a validated scale such as UPDRS, severity of depressive symptoms with a validated scale such as HAM-D or PHQ-9, and cognitive status with a validated scale such as Montreal Cognitive Assessment (MOCA). The ECT treatment course should be documented according to recommendations in the literature [87], so as to include the stimulus waveform characteristics, mode of stimulus delivery, stimulus intensity parameters, electrode placement and seizure monitoring. Each patient being referred for ECT should have a thorough documentation of all prior treatment trials and response to them, as well as their response to ECT. Even then, the patients’ medications are likely to change during the course of ECT, both for motor symptoms of PD and for depression, making comparisons among treatment modalities more challenging.

A weakness of current literature is that long-term follow-up with such patients has not been sufficiently described. It is necessary to follow patients who respond to ECT over time, if at all possible, and document whether such a response has been sustained, what kind of treatments the patients have done well with, and which treatments have failed.

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**References**

Papers of special note have been highlighted as: • of interest; •• of considerable interest


• Excellent evidence based review of available literature looking at various psychiatric manifestations of Parkinson’s disease (PD).

•• Of considerable interest


• A compelling study which adds information to the literature supporting the use of paroxetine and venlafaxine for depression in PD.


• A summary of available evidence regarding use of repetitive transcranial magnetic stimulation for depression in PD.

Electroconvulsive therapy for depression in Parkinson’s disease  

**SYSTEMATIC REVIEW**


• Strong advocacy for use of electroconvulsive therapy in PD.


58 Douyon R, Serby M, Klutchko B, Rotrosen J. ECT and Parkinson's disease revisited:


Borisovsky, Bryson, Buchholz, Samii & Borson

Very relevant study demonstrating poor efficacy of switching an antidepressant in case of nonresponse.


Borisovsky, Bryson, Buchholz, Samii & Borson

**One of the most important studies regarding prevention of relapse of depressive symptoms after a successful electroconvulsive therapy course.**


Mild cognitive impairment: an update in Parkinson’s disease and lessons learned from Alzheimer’s disease

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2Rush University Medical Center, Department of Neurological Sciences & Rush Alzheimer’s Disease Center, 600 South Paulina, Suite 1038, Chicago, IL 60612, USA
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Practice points

- Cognitive deficits are frequent in Parkinson’s disease (PD) and encompass a broad spectrum of clinical features and severity. Patients may have difficulty with attention, working memory, executive function, psychomotor speed, visuospatial abilities, language and memory domains, individually or in combination.
- It is important for clinicians to inquire about cognitive changes or problems, even early in the course of PD and even when symptoms are at mild stages.
- Mild cognitive impairment (MCI) has gained recognition as a construct, an early stage of cognitive decline and a risk factor for developing dementia in PD.
- Recent advances in our understanding of PD-MCI, its variable clinical presentations and differences in progression to dementia, however, suggest that PD-MCI may not be a single, uniform entity. Differences in underlying neurobiological substrates, neuropathology, genetics, among other factors, may contribute to the clinical variability of PD-MCI.
- Research studies have investigated biomarkers such as cerebrospinal fluid markers, neuroimaging studies and genetics that may be associated with PD cognitive impairment and could potentially be used for diagnosis, prognosis or early detection of cognitive decline.
- Compared to the field of MCI and Alzheimer’s disease (AD), PD-MCI is a ‘relative newcomer’ with more recent advances in diagnostic criteria, biomarker studies and therapeutic trials.
- Many lessons can be learned from the MCI-AD field including the evolution of MCI definitions over the years, clinical trials that now incorporate biomarkers and genetics in the study design and emerging therapeutic strategies targeting specific biological mechanisms, novel compounds and delivery systems, and earlier stages of cognitive impairment with potential disease-modifying or prevention trials.

Cognitive dysfunction is an important focus of research in Parkinson’s disease (PD) and Alzheimer’s disease (AD). While the concept of amnestic mild cognitive impairment (MCI) as a prodrome to AD has been recognized for many years, the construct of MCI in PD is a relative newcomer with recent development of diagnostic criteria, biomarker research programs and treatment trials. Controversies and challenges, however, regarding PD-MCI’s definition, application, heterogeneity and different trajectories have arisen. This review will highlight current research advances and challenges in PD-MCI. Furthermore, lessons from the AD field, which has witnessed an evolution in MCI/AD definitions, relevant advances in biomarker research and development of disease-modifying and targeted therapeutic trials will be discussed.

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2Rush University Medical Center, Department of Neurological Sciences & Rush Alzheimer’s Disease Center, 600 South Paulina, Suite 1038, Chicago, IL 60612, USA
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While the presence of cognitive deficits in Parkinson’s disease (PD) has been recognized for many years, it is only more recently that mild cognitive impairment in PD (PD-MCI) has emerged as a concept and distinct entity, with epidemiological studies, proposed diagnostic criteria and symptomatic treatment trials. PD-MCI may represent an early stage of cognitive decline and a risk factor for developing dementia [1,2], and thus, an intermediate state between normal cognition and dementia, similar to amnestic MCI in the context of developing Alzheimer’s disease (AD). Recent advances in our understanding of PD-MCI, however, suggest that PD-MCI is rather heterogeneous with different clinical phenotypes, rates of progression and perhaps underlying mechanisms. In 2012, a Movement Disorder Society (MDS) Task Force proposed diagnostic criteria for PD-MCI in order to harmonize disparate definitions of PD-MCI across multiple clinical and research sites and to identify PD-MCI cohorts for future therapeutic trials (Figure 1) [3]. The MDS PD-MCI criteria have now been applied in clinical and research settings, including international validation efforts and recent treatment trials, but unresolved issues and areas for further study remain [4]. This review will discuss recent findings related to PD-MCI, highlighting its heterogeneity and challenges, discussing several debates and unmet needs regarding PD-MCI, and exploring lessons that can be learned from the MCI-AD field.

PD-MCI: a heterogeneous construct

Frequent & identifiable
MCI in nondemented PD is frequent, affecting 25–50% [5–10]. While estimates vary across studies depending on the PD population (e.g., clinic or community-based, incident or prevalent PD), presence/absence of co-morbid neuropsychiatric disorders (e.g., depression, anxiety, apathy, sleep), severity of motor problems, potential effects of PD-related and other medications and methodological issues (e.g., diagnostic criteria, cognitive assessments, definitions of impairment), which will be further discussed below, are fairly consistent across these studies and definitions. PD-MCI has gained attention as an identifiable cognitive category within the PD cognitive spectrum, a common problem and a state distinct from dementia. However, PD-MCI has emerged as a more heterogeneous entity in its clinical phenotype, timing, progression, and pathology, perhaps even beyond what might be expected by differences in PD-MCI definitions across studies.

Clinical phenotypes & definitions
Cognitive dysfunction in PD-MCI encompasses a broad spectrum of clinical deficits and severity with impairment in attention, working memory, executive function, psychomotor speed, visuospatial abilities, language and memory domains, individually or in combination. In older PD studies using modified Petersen’s MCI criteria or other definitions, cognitive phenotypes were frequently categorized as nonamnestic and amnestic cognitive domains affected and as single and multiple-domain impairment [11]. Instead of classifying PD-MCI as nonamnestic or amnestic type, the MDS PD-MCI criteria recommended specification of the affected domain(s) in order to examine potential differences among cognitive domain subtypes and since episodic memory function, albeit impaired at times in PD, was not the main hallmark as found in AD. Subtype designation of PD-MCI nonamnestic deficits thereby captures individual domains (e.g., attention/working memory vs executive function vs language vs visuospatial function). Moreover, these proposed subtype distinctions may facilitate investigations of whether different types of cognitive impairment differ in their progression rates and neurochemical or neuropathological substrates.

Clinical phenotypes of PD-MCI, in studies of incident and prevalence cohorts and pre- and post-MDS PD-MCI criteria, are highlighted below and in Table 1 [5,7,8,10,12–23]. Newly diagnosed PD patients across different studies demonstrate deficits in executive function, attention, psychomotor speed and visuospatial skills, as well as memory, in some studies [5,10,17,24]. In one study of incident PD cases, PD-MCI as defined by MDS criteria level II (comprehensive neuropsychological battery), occurred in 42.5% with memory deficits in 15.1% [17]. In studies of prevalent, nondemented PD cohorts prior to MDS PD-MCI criteria, similar cognitive profiles occur with greater nonamnestic subtypes, but predominantly as single domain impairment [7–9,14,15,25]. Recent studies applying MDS PD-MCI level II criteria demonstrate that PD-MCI remains frequent, ranging from 35 to 65% of PD cohorts [13,15,18,26–28]. Several studies categorize the PD-MCI cohorts as having single domain and multiple domain
impairment, but details regarding individual cognitive domain subtypes are limited. One consistent, notable finding across recent studies utilizing the MDS PD-MCI level II criteria is an increased frequency of multiple domain impairment. PD-MCI multiple domain impairment occurred in 90, 93, 91.2 and 65% of PD-MCI, compared with single domain impairment in 5, 7, 8.5 to 35%, respectively, a feature that may relate to criteria requirements of impairment in at least one test in two or more cognitive domains [13,15,18,26]. Another schema for categorizing PD cognitive impairment has emerged from the CamPaIGN study with two distinct phenotypes: frontostriatal/executive function deficits and posterior cortical dysfunction (i.e., impaired language/semantic fluency and visuospatial orientation/pentagon copying) [2,29]. Executive deficits may primarily relate to disrupted dopaminergic frontostriatal networks, whereas posterior cortical impairment reflects nondopaminergic dysfunction, cortical Lewy body deposition and/or AD-type pathology [30]. Different neurochemical and neuropathological predispositions may underlie not only the cognitive phenotype in early PD, but also their rates of progression and conversion to dementia. This cognitive categorization of ‘frontostriatal’

Figure 1. The interface of different Parkinson’s disease-mild cognitive impairment criteria. PD-MCI, diagnostic flowchart adapted from MDS task force criteria for diagnosis of PD-MCI and Petersen’s amnestic/nonamnestic mild cognitive impairment criteria MDS PD-MCI criteria features in solid dark gray; MCI criteria features (Petersen) in gray striped pattern; overlap of both of these criteria features in light gray. MCI: Mild cognitive impairment; MDS: Movement Disorder Society; NC: Normal cognition; PD: Parkinson’s disease.
<table>
<thead>
<tr>
<th>Population/sample size</th>
<th>MCI criteria</th>
<th>Domains and neuropsychological tests used</th>
<th>PD-MCI diagnosis</th>
<th>Single/multiple domains affected</th>
<th>Study (year)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalent, community, n = 103</td>
<td>≥2 SD below normative data on ≥1 test</td>
<td>General: MMSE, DRS; attention/executive function: Stroop Color Word Test; memory: BVRT; visuospatial/constructive skills: JLO</td>
<td>55%</td>
<td>57.1%/42.8%</td>
<td>Janvin et al. (2003)</td>
<td>[6]</td>
</tr>
<tr>
<td>Incident, community, n = 159</td>
<td>MMSE ≥24 and impairment on pattern recognition memory test or Tower of London task</td>
<td>General: MMSE, NART; frontal lobe: phonemic fluency, semantic fluency, CANTAB modified Tower of London; temporal lobe: CANTAB pattern recognition memory task; frontal/temporal: CANTAB spatial recognition memory task</td>
<td>36%</td>
<td>58%/42%</td>
<td>Foltynie et al. (2004) (CamPaIGN)</td>
<td>[5]</td>
</tr>
<tr>
<td>Incident, community, n = 115</td>
<td>≥2 SD below normative data on ≥3 tests</td>
<td>General: MMSE, DART; attention: Digit Span Forward and Backward, TMT-B, Stroop Color Word Test Part C; executive function: modified WCST, COWAT, semantic fluency, WAIS-III Similarities, Tower of London-Drexel Test; language: BNT; memory: RAVLT (delayed free recall), recognition, RBMT Logical Memory Test immediate (delayed recall); WMS-III Face recognition immediate (delayed recognition); Visual Association Test; Psychomotor speed: WAIS-R Digit Symbol test, TMT-A, Stroop Color Word Test (Parts A/B); visuospatial/constructive skills: JLO, Groningen Intelligence Test spatial test, Clock Drawing Test</td>
<td>23.5%</td>
<td>Not specified</td>
<td>Muslimovic et al. (2005)</td>
<td>[10]</td>
</tr>
<tr>
<td>Prevalent, clinic, n = 86</td>
<td>≥1.5 SD below normative data on ≥1 domain</td>
<td>Attention: digits forward and backward; executive function: TMT-B, Stroop; language: COWAT, semantic fluency; memory: RAVLT learning, delayed recall; visuomotor processing speed: TMT-A (TMT-B); visuospatial (motor/nonmotor): JLO, clock drawing test</td>
<td>21%</td>
<td>67%/33%</td>
<td>Caviness et al. (2007)</td>
<td>[8]</td>
</tr>
<tr>
<td>Incident, community, n = 196</td>
<td>&gt;1.5 SD below normative data in &gt;1 domain</td>
<td>General: MMSE, IQ-CODE; attention/executive function: serial 7s from MMSE, semantic fluency, Stroop; memory: CVLT-II Immediate recall, short- and long-delay recall; visuospatial: VOSP silhouettes</td>
<td>18.9%</td>
<td>86.5%/13.5%</td>
<td>Aarsland et al. (2009) (ParkWest)</td>
<td>[12]</td>
</tr>
</tbody>
</table>

BNT: Boston naming test; BVRT: Benton visual retention test; CANTAB: Cambridge neuropsychological test automated battery; CDR: Cognitive drug research; CERAD: Consortium to establish a registry for Alzheimer’s disease; COWAT: Control word association test; CVLT: California verbal learning test; DART: National adult reading test, Dutch version; D-KEFS: Delis-Kaplan executive function system; DRS: Dementia rating scale; FCSRT: Free and cued selective reminding test; HMT: Hopkins verbal learning test; IQ-CODE: Informant questionnaire on cognitive decline in the elderly; JLO: Judgment of line orientation test; MCI: Mild cognitive impairment; MMSE: Mini-mental state exam; MoCA: Montreal cognitive assessment; NAI: Nueenberger alternans test; NART: National adult reading test; NIB: Neurobehavioral signs and symptoms Abbreviated Inventory; PD: Parkinson’s disease; PD-CRS: Parkinson’s disease cognitive rating scale; RAVLT: Rey auditory verbal learning test; RBMT: Rivermead behavioral memory test; RCF: Rey complex figure test; SD: Standard deviation; SRT: Selective reminding test; TAP: Test for attentional performance; VOSP: Visual object space perception test; WAIS: Wechsler adult intelligence scale; WCST: Wisconsin card sorting test; WMS: Wechsler memory scale; WTAR: Wechsler test of adult reading.
Table 1. Cross-sectional studies of mild cognitive impairment in Parkinson’s disease cohorts (cont.).

<table>
<thead>
<tr>
<th>Population/sample size</th>
<th>MCI criteria</th>
<th>Domains and neuropsychological tests used</th>
<th>PD-MCI diagnosis</th>
<th>Single/multiple domains affected</th>
<th>Study (year)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incident and prevalent, community and clinic, multi-center, n = 1346</td>
<td>≥1.5 SD below norms on ≥1 domain</td>
<td>Attention/executive function: DRS attention/initiation, Stroop Color Word Test, phonemic fluency, semantic fluency, Tower of London, PD-CRS subtests (attention)/Serial 7s from MMSE, CDR Digit Vigilance and simple/choice reaction time, Digit Span, Cancellation test, Similarities, Corsi block span, TMT-A (executive function); memory: CVLT-II (immediate recall short- and long-delay recall), DRS memory, CDR delayed word recognition, SRT (immediate delayed recall recognition), HVLT (immediate delayed recall), PD-CRS (immediate and delayed recall), RAVLT (immediate and delayed recall, verbal)/BVRT, CANTAB pattern and spatial recognition memory, CDR delayed picture recognition, RCF recall (visual); visuospatial: Benton test matching, DRS construction, Intersecting Pentagons, JLO, RCF, PD-CRS Clock Copy, VOSP cube and silhouettes</td>
<td>25.8%</td>
<td>76.1%/23.9%</td>
<td>Aarsland et al. (2010)</td>
<td>[7]</td>
</tr>
<tr>
<td>Prevalent, retrospective clinic, n = 72</td>
<td>Petersen criteria, SD cutoff not specified, deficits on ≥2 tests/domain</td>
<td>Attention: Digit Span Forwards, TMT-A; executive function: TMT-B, ’WORLD’ backwards from MMSE; language: BNT, phonemic fluency, semantic fluency; memory: CERAD or HVLT-R, 3-item recall from MMSE; visuospatial: Intersecting Pentagons, JLO</td>
<td>52.8%</td>
<td>60.5%/39.5%</td>
<td>Sollinger et al. (2010)</td>
<td>[25]</td>
</tr>
<tr>
<td>Prevalent, clinic, n = 143 (n = 119, non-demented PD)</td>
<td>≥1.5 SD below normative data on 2 tests in ≥1 one domain, or ≥1.5 SD or ≥2 SD below normative data for 1 test (multiple cutoffs and combinations explored)</td>
<td>Attention: Stroop Color Word Test, Digit span Forward and Backward, Digit Ordering, Map Search, TMT-A; executive function: action verb fluency, verbal fluency (letter, category), category switch from D-KEFS, Stroop Interference, TMT-B; memory: CVLT-II acquisition, short delay, long delay, RCF short delay, long delay; visuospatial: RCF copy, JLO, Fragmented Letters</td>
<td>30% (for ≥1.5 SD below normative data on 2 tests/domain)</td>
<td>53%/47%</td>
<td>Dalrymple-Alford et al. (2011)</td>
<td>[19]</td>
</tr>
</tbody>
</table>

BNT: Boston naming test; BVRT: Benton visual retention test; CVLT: California verbal learning test; CANTAB: Cambridge neuropsychological test automated battery; CDR: Cognitive drug research; CERAD: Consortium to establish a registry for Alzheimer’s disease; COWAT: Control word association test; CVLT: California verbal learning test; DART: National adult reading test, Dutch version; D-KEFS: Delis-Kaplan executive function system; DRS: Dementia rating scale; FCSRT: Free and cued selective reminding test; HVLT: Hopkins verbal learning test; IQ-CODE: Informant questionnaire on cognitive decline in the elderly; JLO: Judgment of line orientation test; MCI: Mild cognitive impairment; MMSE: Mini-mental state exam; MoCA: Montreal cognitive assessment; NAI: Nuembreger altersinventar; NART: National adult reading test; NBI: Neurobehavioral signs and symptoms Abbreviated Inventory; PD: Parkinson’s disease; PD-CRS, Parkinson’s disease cognitive rating scale; RAVLT: Rey auditory verbal learning test; RCF: Rey complex figure test; RBMT: Rivermead behavioral memory test; SRT: Selective reminding test; SD: Standard deviation; TAP: Test for attentional performance; VOSP: Visual object space perception test; WAIS: Wechsler adult intelligence scale; WMS: Wechsler memory scale; WPA: Wechsler test of adult reading; WCST: Wisconsin card sorting test.
### Table 1. Cross-sectional studies of mild cognitive impairment in Parkinson’s disease cohorts (cont.)

<table>
<thead>
<tr>
<th>Population/sample size</th>
<th>MCI criteria</th>
<th>Domains and neuropsychological tests used</th>
<th>PD-MCI diagnosis</th>
<th>Single/multiple domains affected</th>
<th>Study (year)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalent, clinic, n = 107</td>
<td>≥1 SD, ≥1.5 SD, or ≥2 SD below normative data on one test/domain or ≥1 SD, ≥1.5 SD, or ≥2 SD below normative data on ≥2 tests/domain (multiple cutoffs and combinations explored)</td>
<td>Attention: TAP (Alertness, Go-Nogo subtests); executive function: Tower of London, TMT-B, NAI, Digit Span Forward and Backward; memory: CERAD word list memory, delayed recall, recognition, Logical Memory I and II; psychomotor speed and naming ability: TMT-A, BNT, CERAD semantic fluency; praxis and visual function: CERAD line drawings, object decision of VOSP</td>
<td>9.9–92.1% (depending on definition used)</td>
<td>25.8–100%/0–74.2% (depending on definition used)</td>
<td>Liepelt-Scarfone et al. (2011)</td>
<td>[23]</td>
</tr>
<tr>
<td>Prevalent, clinic, n = 61</td>
<td>≥1.5 SD below normative data in ≥1 one domain</td>
<td>General: MMSE, NART; executive function: TMT-B; language: semantic fluency, phonemic fluency; memory: Logical Memory; psychomotor speed: TMT-A; working memory: Digit Span total</td>
<td>62%</td>
<td>37.7%/24.6%</td>
<td>Naismith et al. (2011)</td>
<td>[20,21]</td>
</tr>
<tr>
<td>Prevalent, clinic, n = 40</td>
<td>≥1.5 SD below standardized mean (or scaled score ≤6 or percentile range ≤10) on two tests in the same domain</td>
<td>General: DRS–2, MMSE; attention/executive function: Stroop Color Word Test, TMT-B, semantic fluency, phonemic fluency; memory: RAVLT lists, immediate and delayed recall, recognition; visuospatial: RCF copy, Block design (WAIS-III), Bell test</td>
<td>45%</td>
<td>61.1%/38.9%</td>
<td>Villeneuve et al. (2011)</td>
<td>[21]</td>
</tr>
<tr>
<td>Prevalent, clinic, n = 350</td>
<td>≥1.5 SD below normative data in ≥1 one domain</td>
<td>General: MMSE; attention/executive function: Digit Span Forward and Backward, Symbol Digit Modalities Test, semantic fluency; language: BNT, Similarities; memory: CERAD word list learning and delayed recall; visuospatial: intersecting pentagons, JLO</td>
<td>36.6%</td>
<td>67%/33%</td>
<td>Goldman et al. (2012)</td>
<td>[14]</td>
</tr>
<tr>
<td>Prevalent, clinic, n = 80</td>
<td>≥1.5 SD below normative data in ≥1 one domain</td>
<td>General: MMSE; attention: Digit Span; executive function: Stroop Color Word Test; memory: RAVLT immediate recall, delayed recall; visuospatial: Clock Drawing Test</td>
<td>60%</td>
<td>58.3%/41.7%</td>
<td>Wu et al. (2012)</td>
<td>[22]</td>
</tr>
</tbody>
</table>

### PD cohorts with MDS PD-MCI Level II criteria (with modifications as noted)

<table>
<thead>
<tr>
<th>Population/sample size</th>
<th>MCI criteria</th>
<th>Domains and neuropsychological tests used</th>
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<th>Single/multiple domains affected</th>
<th>Study (year)</th>
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<tr>
<td>Prevalent, clinic, n = 104</td>
<td>≥1.5 SD below normative data</td>
<td>General: MMSE; attention/working memory: TMT, Digit cancellation, Digit Span Forwards and Backwards, Stroop, Corsi test; executive function: Phonemic fluency, FAB, Clock Drawing Test; language: Similarities, semantic fluency; memory: RAVLT immediate and delayed recall, RCF immediate recall; visuospatial: Clock Drawing Copy Test, RCF copy</td>
<td>33%</td>
<td>Not specified</td>
<td>Biundo et al. (2013)</td>
<td>[24]</td>
</tr>
</tbody>
</table>

BNT: Boston naming test; BVRT: Benton visual retention test; CVLT: California verbal learning test; CANTAB: Cambridge neuropsychological test automated battery; CDR: Cognitive drug research; CERAD: Consortium to establish a registry for Alzheimer’s disease; CONAT: Control word association test; CVL: California verbal learning test; DART: National adult reading test, Dutch version; D-KPS: Delis-Kaplan executive function system; DRS: Dementia rating scale; FCSRT: Free and cued selective reminding test; HVLT: Hopkins verbal learning test; IQ-OCDQ: Informant questionnaire on cognitive decline in the elderly; JLO: Judgment of line orientation test; MCI: Mild cognitive impairment; MMSE: Mini-mental state exam; MoCA: Montreal cognitive assessment; NAI: Nunenberger alteration inventory; NART: National adult reading test; NBI: Neurobehavioral signs and symptoms Abbreviated Inventory; PD: Parkinson’s disease; PD-CRS, Parkinson’s disease cognitive rating scale; RAVLT: Rey auditory verbal learning test; RCF: Rey complex figure test; RBMT: Rivermead behavioral memory test; SRT: Selective reminding test; SD: Standard deviation; TAP: Test for attentional performance; VOSP: Visual object space perception test; WAIS: Wechsler adult intelligence scale; WMS: Wechsler memory scale; WTAR: Wechsler test of adult reading; WCST: Wisconsin card sorting test.
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<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incident, clinic, n = 123</td>
<td>≥1.5 SD below normative data</td>
<td>General: MMSE, DART; attention: Digit Symbol Test, TMT-A; executive function: Modified WCST, COWAT; language: BNT, WAIS-III Similarities; memory: RAVLT, RBMT Logical Memory subtest; visuospatial: Clock Drawing Test, JLO</td>
<td>35%</td>
<td>35%/65%</td>
<td>Broeders et al. (2013)</td>
<td>[26]</td>
</tr>
<tr>
<td>Prevalent, clinic, n = 76</td>
<td>≥2 SD (also 1–2.5) below normative data</td>
<td>General: MMSE; attention/working memory: Digit Span Forwards, LNS, SDMT, TMT-A; executive function: Clock Drawing Test, COWAT, Digit Span Backwards, Progressive Matrices, TMT-B; language: BNT, semantic fluency, WAIS-III Similarities; memory: CERAD word list learning, delayed recall, and recognition, Logical Memory I and II, FCSRT, Figural Memory; visuospatial: Clock Copying Test, Intersecting Pentagons, JLO</td>
<td>62% (for ≥2 SD below normative data)</td>
<td>8.5%/91.5%</td>
<td>Goldman et al. (2013)</td>
<td>[13]</td>
</tr>
<tr>
<td>Prevalent, clinic, multicenter, n = 139</td>
<td>≥1.5 SD below normative data</td>
<td>General: MMSE, MoCA, NBI, WTAR; attention: LNS, DKEFS Color Word Interference Color Naming test; executive function: Visual Verbal Test, TMT B-A; language: BNT, D-KEFS Verbal Fluency, Category Fluency test; memory: RCFT Delayed Recall, CVLT-II LongDelay Free Recall test, visuospatial: JLO, RCF copy</td>
<td>33%</td>
<td>7%/93%</td>
<td>Marras et al. (2013)</td>
<td>[15]</td>
</tr>
<tr>
<td>Incident, community, n = 219</td>
<td>≥1.5 SD (also 1–2) below norm in ≥1 domain</td>
<td>General: MMSE, MoCA; attention: CDR Power of Attention score; executive function: Modified Tower of London task, phonemic fluency, semantic fluency; language: Naming, MoCA sentence subsets; memory: CANTAB Pattern Recognition Memory, Spatial Recognition Memory, Paired Associates Learning; visuospatial: Intersecting Pentagons</td>
<td>42.5%</td>
<td>Not specified</td>
<td>Yarnall et al. (2013) (ICICLE)</td>
<td>[17]</td>
</tr>
<tr>
<td>Prevalent, multicenter, clinic, n = 142</td>
<td>≥1.5 SD below norm in ≥1 test</td>
<td>General: MMSE, MoCA, DR5–2, Shipley–2; attention/working memory: Digit Symbol subtest, LNS, Digit Span, TMT; executive function: Clock Drawing Test, phonemic fluency; language: semantic fluency, BNT; memory: HVLT-R, Logical Memory; visuospatial: JLO, Cube Copy</td>
<td>67%</td>
<td>5%/95%</td>
<td>Cholerton et al. (2014)</td>
<td>[14]</td>
</tr>
</tbody>
</table>

**Table 1. Cross-sectional studies of mild cognitive impairment in Parkinson’s disease cohorts (cont.).**

BNT: Boston naming test; BVRT: Benton visual retention test; CVLT: California verbal learning test; CANTAB: Cambridge neuropsychological test automated battery; CDR: Cognitive drug research; CERAD: Consortium to establish a registry for Alzheimer’s disease; COWAT: Control word association test; CVLT California verbal learning test; DART: National adult reading test, Dutch version; D-KEFS: Delis-Kaplan executive function system; DRS: Dementia rating scale; FCSRT: Free and cued selective reminding test; HVLT: Hopkins verbal learning test; IQ-CODE: Informant questionnaire on cognitive decline in the elderly; JLO: Judgment of line orientation test; MCI: Mild cognitive impairment; MMSE: Mini-mental state exam; MoCA: Montreal cognitive assessment; NAI: Nuernberger altersinventar; NART: National adult reading test; NB: Neurobehavioral signs and symptoms Abbreviated Inventory; PD: Parkinson’s disease; PD-CRS, Parkinson’s disease cognitive rating scale; RAVLT: Rey auditory verbal learning test; RCF: Rey complex figure test; RBMT: Rivermead behavioral memory test; SRT: Selective reminding test; SD: Standard deviation; TAP: Test for attentional performance; VOSP: Visual objects space perception test; WAIS: Wechsler adult intelligence scale; WMS: Wechsler memory scale; WJAR: Wechsler test of adult reading; WCST: Wisconsin card sorting test.
versus ‘posterior cortical’ deficits is reminiscent to some degree of the nonamnestic and amnestic categorization. Along with the aforementioned challenges in parsing out individual subtypes of single domain PD-MCI and identifying sufficient subject numbers per subtype, further research is needed regarding optimal definitions of PD-MCI subtypes and whether subtyping by individual cognitive domains will be a fruitful concept.

- **Timing**
  Besides its clinical spectrum, PD-MCI also can be thought of in terms of its time course and relationship to motor symptom onset and PD diagnosis. Cognitive impairment in PD is no longer just a late-stage phenomenon but rather it can occur in incident PD with reports of PD-MCI in 20–40% \([5,10,12,17]\). Although these studies vary in definitions of PD cognitive impairment or PD-MCI used, it is apparent that cognitive dysfunction can be a symptom in PD early on and even prior to initiation of dopaminergic therapy. Furthermore, these studies support the importance of asking PD patients and caregivers about cognitive symptoms even at this early disease stage.

  The presence of cognitive deficits in *de novo*, untreated PD patients leads to several questions including: how early in the course of PD can cognitive deficits occur, are they present in premotor PD, is their presence related to dopaminergic deficiency (and perhaps improved by dopaminergic treatments) or related to other neurochemical, neuropathological or clinical issues (e.g., depression, anxiety, sleep disturbances), and is there a distinction between early cognitive deficits in PD or in dementia with Lewy bodies (DLB)? Indeed, there is increasing evidence for cognitive deficits in ‘premotor’ PD (e.g., persons who do not have motor features characteristic of diagnosable PD but who may have nonmotor features affecting smell, bowel function, mood or sleep), ‘preclinical’ PD (e.g., persons who may not have any clinical features but have abnormalities on neuroimaging measures such as *[18F]-fluorodopa PET* or dopamine transporter [DAT] SPECT imaging), or in cohorts ‘at risk’ or relatives of PD patients, who also may be at genetic risk \([31]\). Rapid eye movement behavior disorder (RBD) is associated with cognitive deficits in executive function, memory and visuospatial abilities and the development of synucleinopathies such as PD or DLB by 5 or more years \([34]\, and about half of ‘idiopathic’ RBD patients will develop a synucleinopathy after 12 years \([35]\). The Parkinson Associated Risk Study found that healthy relatives of PD patients with hyposmia and decreased DAT uptake on imaging scans had worse scores on verbal fluency, attention/executive function and processing speed \([36]\). While a primary inclusion criterion of the MDS PD-MCI is the presence of clinically diagnosed PD, there is a current movement in the PD field to redefine the criteria for PD \([37]\). Indeed, studies of these premotor or ‘at-risk’ cohorts challenge our notions of when PD actually begins and at what stage MCI may occur within the PD diagnostic spectrum.

  Another challenge in defining PD-MCI is determining how this construct fits in with DLB. Whether PD dementia and DLB are the same disorder has been debated over the years \([37,38]\). In the development of the MDS PD-MCI criteria, the task force recognized this issue, particularly since the onset of cognitive symptoms relative to motor symptoms can be historically vague, and in some cases, occur concurrently. The PD-MCI criteria focus on clinically diagnosed PD but acknowledge the challenge of differentiating PD-MCI from incipient DLB. Indeed, the concept of MCI as prodromal DLB has gained attention and support from studies documenting the progression of nonamnestic MCI to DLB and other non-AD dementias as well as clinicopathological correlates of MCI in longitudinally followed cohorts \([11,39]\). Nonamnestic MCI subjects, compared with those with amnestic MCI, had a 10-fold greater likelihood to develop probable DLB; these subjects initially manifested greater attention and/or visuospatial impairment (88%) than memory deficits (25%) as well as RBD, daytime sleepiness and fluctuations \([40]\), clinical features found in other MCI cases later confirmed by autopsy to have DLB \([41]\). Further studies regarding MCI as prodromal DLB, including clinical features, biomarkers and pathological correlates, may be needed to determine the timing, phenotype, definitions and context of MCI in parkinsonian/synuclein disorders.

- **Progression, stability or reversion**
  Emerging data from longitudinal studies of PD-MCI shed light on the progression of PD-MCI and its conversion to PD dementia, but also raise questions regarding whether PD-MCI subtypes differ in their course and whether all
PD-MCI progresses to dementia. In a study of prevalent PD subjects, 18/29 (62%) of those with PD-MCI who completed follow-up at 4 years converted to PD dementia, whereas dementia ensued in only 6/30 (20%) with intact cognition at baseline; although a small sample with a limited neuropsychological battery, the study suggested that single domain nonamnestic MCI, along with higher depression scores, were associated with dementia conversion, whereas predominant amnestic deficits and multiple domains were not [1]. The CamPaIGN study provides over 10-year follow-up of incident PD persons and suggests divergent patterns of PD-MCI [2,29,29,2]. At 3–5 years follow-up, 13/126 (10%) converted to demented and an additional 57% had cognitive impairment, mainly frontostriatal deficits [2]. Multiple factors predicted global cognitive decline at 5 years including; age ≥72 years (Odds ratio [OR]: 4.81; 95% CI: 1.14–20.23), decreased semantic fluency (OR: 6.89; 95% CI: 1.30–36.55), impaired copy of intersecting pentagons (OR: 2.78; 95% CI: 1.001–7.73), non-tremor dominant motor phenotype (OR: 3.93; 95% CI: 0.79–19.57) and a genetic variant in the MAPT gene (H1/H1 genotype) (OR: 12.14; 95% CI: 1.26 = 117.36). Older age along with the impaired posterior cortical cognitive function (i.e., semantic fluency and intersecting pentagons) had a combined OR of 88 for developing dementia within the first 5 years of PD diagnosis [29]. This study suggests that not all cognitive impaired PD patients will necessarily develop dementia and proposes that PD patients with greater posterior cortical phenotypes, but not those with greater frontostriatal-based/executive dysfunction, develop dementia at follow-up. Moreover, a functional polymorphism in the dopamine-regulating enzyme COMT was associated with executive dysfunction but not dementia, whereas the MAPT and APOE4 polymorphisms were strongly associated with earlier dementia in this cohort [29,29,42].

Other longitudinal studies of PD cohorts, particularly those using MDS PD-MCI diagnostic criteria, are in early stages but provide some estimates of PD-MCI progression. In a community-based incident PD cohort in Sweden, 37/134 (27%) of PD patients developed dementia over 5 years of follow-up [43]. Of the 49 PD patients diagnosed as MCI, 25/49 (50%) developed dementia in this timeframe. Presence of MCI and older age predicted dementia, and baseline scores on episodic memory, semantic fluency, mental flexibility and visuospatial function tests were worse in those PD-MCI who converted to dementia, compared with those who did not. A follow-up study of the incident Norwegian ParkWest PD cohort at 1 year and 3 year supports that PD patients with MCI at baseline were more likely to progress to dementia, with 27% of the initial group subsequently diagnosed with PD dementia [44]; similar findings were found in a Netherlands study with increasing rates of PD-MCI and of those with baseline PD-MCI, dementia at 3-year and 5-year follow-up [26]. These studies, however, also demonstrate a high rate of attrition at follow-up and thereby, an important challenge of conducting longitudinal studies.

PD-MCI may also be an unstable state with reversion to normal cognitive status at follow-up in some studies. In the Swedish study, 6 (11%) patients with PD-MCI at baseline reverted to normal cognition, and 10 patients fluctuated between MCI and normal cognition [43]. Both the Dutch and Norwegian studies also demonstrate that some PD-MCI patients at follow-up revert to normal cognition, though with longer follow-up, may ultimately have MCI or dementia. In the ParkWest study, at 1-year follow-up approximately 20% of PD-MCI had normal cognition; however, among those patients with MCI at baseline and 1-year follow-up, only 9% reverted to normal cognition at 3 years. Thus, PD-MCI status may fluctuate, and there may be other contributing factors to consider, such as cognitive test performance, co-morbid nonmotor features, medication use, underlying neuropathology or other biomarkers.

**Biomarkers & neuropathology**

There is a growing interest in identifying biomarkers such as cerebrospinal fluid (CSF), genetics, neuroimaging, among others to characterize PD-MCI and its underlying neuroanatomical, neurochemical or neuropathological substrates and that may predict conversion to dementia. While the MDS PD-MCI criteria do not incorporate biomarkers into current definitions, there may be lessons to be learned from the MCI/AD field (as discussed below) with the inclusion of biomarkers in recent revisions of MCI research criteria and their use in AD prevention trials [45].

Proposed CSF biomarkers for PD cognitive decline include several previously associated with AD pathology but also others. Decreased CSF-αβ 1–42 levels are thought to reflect...
amyloid deposition in the brain, and increased tau or phosphorylated tau (p-tau) CSF levels, increased neuronal death. Several PD studies reveal decreased CSF-αβ 1–42 in cognitively impaired PD patients compared with healthy controls [46–48]. Lower αβ 1–42 levels correlated with semantic fluency [47] and a more rapid cognitive decline from baseline to 1-year follow-up [48]. Levels of tau and p-tau have been variable with some, but not all, studies reporting increased CSF levels in PD dementia; in one study, elevated tau also correlated with impaired naming and memory performance [47,48]. Newly diagnosed PD patients had decreased CSF-αβ levels, though not as reduced as in AD, and levels were significantly associated with memory impairment but not with attention/executive or visuospatial dysfunction; CSF total tau or p-tau levels neither differed between PD patients and controls, nor correlated with cognitive measures [49]. In another incident PD study, CSF-αβ correlated with pattern recognition memory and Montreal Cognitive Assessment (MoCA) scores, with lower levels in PD-MCI patients [17]. These CSF markers may reflect pathological processes of PD cognitive impairment, including possible co-morbid AD and thereby, generate new avenues for diagnostic and prognostic biomarkers and intervention targets.

Several genetic biomarkers have been explored in PD cognitive impairment. As previously mentioned, data from the CamPaIGN study suggest a genotype-phenotype dissociation regarding risk of PD dementia, increased with tau-related MAPT gene polymorphisms and posterior cortical deficits, but not COMT polymorphisms and frontal-executive type deficits [2,30]. Others have described similar associations between the MAPT H1 polymorphism and PD dementia [50] as well as an effect on parietal activation in spatial rotation tasks in early PD [51]. Although APOE ε4 is a strong risk factor for AD, conflicting results have been found in PD dementia [52]. Other genetic mutations associated with PD dementia, more rapid cognitive decline and greater neuropsychiatric features include those related to alpha-synuclein triplication and carriers of mutations in the GBA gene, encoding the lysosomal enzyme glucocerebrosidase [42,53]. In the CamPaIGN cohort, GBA mutations occurred in 3.5%, and GBA carriers exhibited greater risk for progression to dementia (hazard ratio 5.7) and worse motor function (hazard ratio 4.2). The relationship between cognitive dysfunction and mutations in LRRK2, a common genetic and sporadic cause of PD, has been variable with mixed study results, some revealing lower executive function or Mini-Mental State Examination scores [54–56]. Future studies including well-defined PD-MCI cohorts and longitudinal follow-up will be needed to establish links between genotype and dementia risk as well as the possibility of incorporating genotype in clinical trials and study design.

Structural and metabolic neuroimaging offer other opportunities to study biomarker correlates of PD-MCI [57,58]. Gray matter atrophy on brain MRI has been variably found in PD-MCI, depending on the cohort studied (incident vs prevalent), PD-MCI definitions and MRI analyses (voxel-based morphometry [VBM], cortical thickness, others). PD-MCI patients, defined using Petersen criteria, had reduced gray matter in the left frontal and bilateral temporal lobe regions, compared with PD without MCI; however, these differences did not remain significant after corrections for multiple comparisons and patient groups were small in size [59]. Other studies reveal that PD-MCI patients exhibit anterior caudate atrophy and posterior ventricular enlargement on MRI [60], and compared with healthy controls, multiple areas of reduced gray matter such as frontal, temporal (including the hippocampus), parietal and pre/post central gyri; however, compared with cognitively normal PD patients, PD-MCI patients have not always demonstrated statistically significant differences in gray matter atrophy [61]. In a comparison of PD patients with amnestic MCI (n = 41) to amnestic MCI patients (without PD, n = 78), the PD group had decreased gray matter in the right temporal and anterior prefrontal areas compared with amnestic MCI (without PD); when multiple domains were affected in PD-MCI, regional atrophy was more extensive [62]. Several studies have focused on MRI correlates of PD-MCI in the incident PD cohorts. Two VBM studies of de novo PD patients, however, did not reveal differences in gray matter atrophy in PD-MCI patients compared with PD without MCI or healthy controls [17,63], though PD-MCI patients drawn from a large, de novo cohort revealed cortical thinning in temporal, parietal, frontal and occipital areas compared with healthy controls, and in the right inferior temporal region compared with cognitively normal PD patients [64]. Metabolic studies of PD-MCI reveal abnormalities in posterior cortical regions, similar to
regions frequently abnormal in PD dementia patients and AD. PD-MCI patients with multiple domains impaired had decreased glucose metabolism on 18F-fluorodeoxyglucose (FDG) PET scans in prefrontal and parietal regions, while PD-MCI patients with single domain impairment had a similar pattern but to a lesser degree [65]. A PD-MCI cohort (of whom 11 had an isolated memory deficit and 4, a mild deficit in verbal fluency) demonstrated parietal, temporal and occipital hypoperfusion compared with cognitively intact PD [66]. Interestingly, the PD-MCI had greater hypoperfusion in parieto-occipital regions compared with the amnestic MCI patients (without PD), whereas the amnestic MCI patients had greater hypoperfusion in medial temporal lobe regions, thereby, perhaps suggesting different underlying neural substrates and neuropathologies. These neuroimaging studies support regional gray matter atrophy patterns or altered metabolism in PD-MCI, with notable abnormalities in posterior cortical areas that may potentially reflect shared substrates with PD dementia and in some cases, AD.

To date, few studies describe the neuropathology of PD-MCI. Adler et al. report on 8 PD-MCI cases (of 80 PD cases), of whom 4 had amnestic single domain MCI, 3 nonamnestic single domain MCI (executive dysfunction) and 1 nonamnestic multiple domain MCI (executive/visuospatial dysfunction); the neuropathologies of the PD-MCI cases were heterogeneous with varying Lewy body distributions and in 50%, moderate neuritic plaque pathology (though only 2 met AD criteria), and cerebrovascular pathology in 3 cases [67]. Jellinger also reported a mix of Lewy bodies, AD pathology and cerebral amyloid angiopathy in 8 PD-MCI autopsy cases with amnestic and nonamnestic deficits [68]. Future clinico-pathological studies will be needed to examine the underlying neuropathology of PD-MCI and its relationship to MCI subtype.

PD-MCI: theory, practice & debated issues

- Conceptual usefulness

Whether PD-MCI represents a useful concept has been debated in the field [38,69]. Recognition of mild cognitive deficits in PD has brought increased awareness, education and research to an important and previously under-recognized area of PD patient care. The emergence of PD-MCI as a diagnostic entity provides a framework for investigating its clinical features and pathophysiology and for identifying patients for clinical research trials for symptomatic therapies, and ultimately, disease-modifying or preventive agents. Greater awareness of PD-MCI can lead to appropriate counseling for patients and caregivers, validation of symptoms that are sometimes ‘dismissed’ or attributed to aging, and discussions regarding safety, driving and care planning. However, there are several concerns with the concept and diagnosis of PD-MCI. As previously discussed, PD-MCI is a heterogeneous condition, with different phenotypes and progression, and not necessarily a prodrome to dementia. In some cases, PD-MCI may be a static entity without further decline, a ‘short-term’ event with reversion to normal cognition, or a marker of impending dementia. How this information is conveyed to patients and caregivers in clinical settings and applied in research settings with symptomatic and disease-modifying treatment trials and selected target patients will need to be sorted out for the concept of PD-MCI to be successfully utilized in the field.

- Diagnostic challenges

The diagnosis of PD-MCI rests upon the concept that MCI, in general and in PD, refers to a clinical syndrome of cognitive impairment in the absence of dementia. However, many different definitions have been used over the years and thereby, influence our understanding of PD-MCI. The MDS PD-MCI criteria provide an important initial step toward a uniform diagnosis across multiple sites. Even with these criteria as a framework, there is latitude in interpretation and application with different neuropsychological tests, cut off scores and levels of assessment used.

There are a number of challenges in diagnosing PD-MCI clinically. First, one needs to identify that a decline in cognitive abilities has occurred. Estimates of cognitive impairment by patients and their caregivers vary in their reliability, due to either over or under-reporting [8,20,70] or to difficulty separating cognitive from motor problems, and information from several sources (e.g., patient, caregiver and clinician) may be needed. PD-MCI studies vary in how preservation of activities of daily living is evaluated, and this issue is further compounded by difficulty in distinguishing cognitive and motor effects. Other motor and nonmotor features of PD may affect cognitive
function and thereby, the diagnosis of PD-MCI. Cognitive performance may differ in ‘on’ versus ‘off’ motor states [71,72], and neuropsychological tests with timed components or significant motor demands may be difficult for PD patients. Nonmotor features such as depression, anxiety, apathy, psychosis, fatigue and sleep disturbances are common in PD, particularly alongside impaired cognition or dementia [73,74]. Lastly, there are unresolved methodological issues regarding choices for global screening tests, neuropsychological test batteries and cutoffs of 1–2 standard deviation (SD) below normative data. Different research groups have interpreted these elements of the MDS PD-MCI criteria differently. To date, there is no agreement regarding

### Table 2. Revised Alzheimer’s disease and mild cognitive impairment criteria by clinical and biomarker evidence.

<table>
<thead>
<tr>
<th>Diagnostic category</th>
<th>Core clinical criteria met</th>
<th>Likelihood of biomarker probability of AD etiology</th>
<th>Likelihood of Aβ presence (PET or CSF)</th>
<th>Likelihood of neuronal injury evidence (CSF tau, FDG-PET, structural MRI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AD by clinical criteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical criteria</td>
<td>Yes</td>
<td>Uninformative</td>
<td>Unavailable, indeterminant or conflicting</td>
<td>Untested, indeterminant or conflicting</td>
</tr>
<tr>
<td>Evidence of AD pathophysiological process</td>
<td>Yes</td>
<td>Intermediate high</td>
<td>Unavailable/indeterminant positive positive</td>
<td>Positive unavailable/ indeterminant positive</td>
</tr>
<tr>
<td><strong>Possible AD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical criteria</td>
<td>Atypical course or etiologically mixed presentation</td>
<td>Uninformative</td>
<td>Unavailable, indeterminant or conflicting</td>
<td>Untested, indeterminant or conflicting</td>
</tr>
<tr>
<td>Evidence of AD pathophysiological process</td>
<td>No, but meets non-AD dementia criteria (e.g., DLB, FTD)</td>
<td>High but does not exclude alternative etiology</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td><strong>Unlikely AD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical criteria</td>
<td>No, or sufficient evidence for alternative diagnosis (e.g., HIV dementia, HD)</td>
<td>Low</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Evidence of AD pathophysiological process</td>
<td>No</td>
<td>Low</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td><strong>MCI by clinical criteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCI core clinical criteria: concern regarding a change in cognition, impairment in one or more cognitive domains, preservation of independence in functional abilities, not demented; objective evidence of cognitive decline, preferably on cognitive testing, scores typically 1–15 SD below norms; episodic memory impairment, though other domains may be impaired</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MCI due to AD by biomarker criteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High likelihood</td>
<td>Yes</td>
<td>High</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Intermediate likelihood</td>
<td>Yes</td>
<td>Intermediate</td>
<td>Positive or untested</td>
<td>Positive</td>
</tr>
<tr>
<td>No likelihood</td>
<td>Yes</td>
<td>Low</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Aβ: Amyloid-beta; AD: Alzheimer’s disease; CSF: Cerebrospinal fluid; DLB: Dementia with Lewy bodies; FDG: 18fluorodeoxyglucose; FTD: Frontotemporal dementia; HD: Huntington’s disease; MRI: Magnetic resonance imaging; PET: Positron emission tomography; PPA: Primary progressive aphasia.

Data taken from [45,89].
the ideal neuropsychological battery, given the plethora of tests available to evaluate global and individual cognitive functions, the best sources of normative data, handling of normative scores and best cutoff scores to use, though data are emerging [11,28,75]. Cutoff scores used to define impairment can greatly influence frequency estimates of PD-MCI. Sensitivity and specificity of PD-MCI by MDS PD-MCI level II criteria varied depending on whether 1, 1.5, 2 and 2.5 SD cutoffs below norms were used in one study, with the best sensitivity (85.4%) and specificity (78.6%) measures achieved using a cutoff of 2 SD below norms; other cutoff scores compromised either specificity (21.4% for 1 SD below norms and 60.7% for 1.5 SD below norms) or sensitivity (58.3% for 2.5 SD below norms) [13]. Validation of the MDS PD-MCI criteria including efforts of a large international consortium may help elucidate these operationalization issues in defining PD-MCI, particularly across cognitive test batteries and diverse PD populations.

Lessons from MCI & AD
Looking toward the research conducted in MCI and AD, particularly regarding criteria and their revisions, biomarker studies, and clinical trials for disease-modification and symptomatic therapies, may be especially relevant in informing research studies and clinical trials conducted in PD cohorts. In this section, we will review pertinent lessons from AD and MCI research.

- Defining MCI – a construct in evolution
The past decades have witnessed considerable debate in the definition, classification and conceptualization of the MCI-AD state, and these debates have provided lessons, and continue to provide lessons, for the PD-MCI field. MCI has evolved from a general concept of impaired cognition but with an intact ability to carry out daily living activities, to more specifically focused on memory complaints and impairment, to subtype categorization with amnestic or nonamnestic deficits and single or multiple cognitive domains affected [11,76,77]. Different MCI subtypes have

<table>
<thead>
<tr>
<th>Trial</th>
<th>Population</th>
<th>Biomarker</th>
<th>Intervention</th>
<th>Aim</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOE e4 treatment trial</td>
<td>Persons who are homozygous for APOE e4 alleles</td>
<td>APOE e4</td>
<td>Antiamyloid medication</td>
<td>Prevent or delay the emergence of AD symptoms in persons at high risk for developing AD</td>
<td>[108]</td>
</tr>
<tr>
<td>Alzheimer prevention initiative</td>
<td>Large extended Columbian family with rare presenilin (PS1) gene mutation</td>
<td>PS1 mutation</td>
<td>Crenezumab</td>
<td>Study whether a monoclonal antibody targeting Aβ precursor protein can delay the onset of AD</td>
<td>[109]</td>
</tr>
<tr>
<td>Dominantly inherited Alzheimer network</td>
<td>Persons who have a known genetic mutation that causes autosomal dominant AD or have parent, sibling with a known genetic mutation</td>
<td>PS1, PS2 or APP mutation</td>
<td>Solanezumab, gantenerumab, beta-secretase inhibitor</td>
<td>Examine and compare the safety, side effects and effect on imaging and biomarkers of three investigational drugs</td>
<td>[110]</td>
</tr>
<tr>
<td>Antiamyloid treatment of asymptomatic AD (A4 ADCS- NIA, Lilly)</td>
<td>Healthy population sample with positive amyloid imaging on PET scan</td>
<td>Positive amyloid imaging</td>
<td>Solanezumab</td>
<td>Evaluate whether early treatment will slow down memory loss and cognitive decline and delay the progression of AD-related brain injury on imaging</td>
<td>[111]</td>
</tr>
<tr>
<td>A4 sub-study: LEARN (ADCS- NIA Alzheimer's Association)</td>
<td>Older individuals who have negative amyloid imaging on PET scans performed in A4 study</td>
<td>Negative amyloid imaging</td>
<td>None</td>
<td>Longitudinal natural history study of cognitive function outcomes in amyloid PET negative individuals from A4 study, examining differences in rates of clinical decline</td>
<td>[112]</td>
</tr>
<tr>
<td>Takeda/Zinfandel Trial (TOMMOROW)</td>
<td>Healthy population sample genetic risk of developing AD</td>
<td>APOE and TOMM40</td>
<td>Pioglitazone</td>
<td>Study a new investigational risk algorithm to predict the genetic risk for developing MCI and test the safety and effectiveness of an investigational medication in delaying MCI due to AD</td>
<td>[113]</td>
</tr>
</tbody>
</table>

Aβ: Amyloid-beta; AD: Alzheimer’s disease; MCI: Mild cognitive impairment; MRI: Magnetic resonance imaging.
been found to progress to different types of dementia syndromes, with amnestic MCI representing a potential precursor to AD, while nonamnestic MCI subtypes may progress to other forms of dementia [24,78]. These MCI studies paved the way for many of the early studies of cognitive impairment in nondemented PD, application of MCI definitions in PD cohorts, and subsequently, the generation of MCI as a construct in PD.

The MCI-AD field has also faced its own issues regarding variable prevalence estimates, conversion rates and heterogeneity, and these shared challenges may offer insights and support to the PD field. Prevalence estimates of MCI and its subtypes vary with respect to which diagnostic criteria were employed or what type of patient population was examined, similar to our recent experiences in the field of PD-MCI [79,80]. In addition, reversion rates of MCI to normal cognitive functioning have been reported, varying widely from 15% over a 3.6-year follow-up to 34% with 1.5-year follow-up [81–83]. These observations underscore the challenges encountered in accurately characterizing and diagnosing MCI and support the view that clinical classification of MCI should be considered a heterogeneous and potentially unstable diagnostic entity, in both AD and PD [84].

At present, there are no widely accepted screening tests for MCI. In the MCI-AD field, there have been efforts to develop tests and batteries (e.g., MMSE enriched with delayed recall items, or the MoCA) that can be validated against the clinical diagnosis of MCI or predictively against the development of dementia [85,86] as well as computerized cognitive assessment systems (e.g., CogState) that can discriminate MCI from cognitively healthy individuals and can be used to screen community-dwelling individuals or implemented in large scale clinical trials for AD prevention [87]. In PD-MCI, similar challenges are faced, and efforts to determine the optimal cognitive batteries or tests that discriminate PD-MCI from PD patients with intact cognition or dementia and mode of administration for clinical trials are underway [28,75].

### Incorporating biomarkers & genetics into clinical criteria & research study design

With advances in clinical and pathophysiological relationships over the years, the diagnostic definitions of AD and MCI have been refined to incorporate biomarkers. In 2011, consensus reports from National Institute on Aging (NIA) and the Alzheimer’s Association (AA) working groups described MCI-AD as three contiguous disease phases: the ‘AD pathophysiological process’, ‘MCI due to AD’ and ‘clinical AD dementia’ in an effort not only to assist physicians in diagnoses, but also to provide a platform for developing primary prevention therapies (Table 2) [45,88,89]. The modified definitions include biomarkers that reflect the underlying neurodegenerative processes [90,91], spanning those with potential for identifying early and subtle but measureable signs (e.g., decreased CSF-αβ42, increased total tau or p-tau levels) and later stage evidence (e.g., MRI-derived hippocampal and entorhinal cortex atrophy, reduced glucose metabolism in temporoparietal and posterior cingulate cortices) [92,93].

Other studies focus on the role of genetics such as dominantly inherited mutations for early-onset AD (APP, PSEN1 and PSEN2) and susceptibility factors for late-onset AD (APOE gene polymorphism, APOE ε4) [94–96]. Current clinical trials have now incorporated family history and genetic criteria into the study design to enrich the studies with persons at risk for development of cognitive decline, MCI and AD. Thus, the application of genetics and biomarkers in therapeutic trials is a growing research area, which in due time and with research advances, may also emerge in the PD field.

### Emerging therapeutic strategies in MCI & AD research

Conventional therapies to treat AD, such as cholinesterase inhibitors and NMDA receptor antagonists, have not produced a disease-modifying effect or impacted the progression of AD over a prolonged period of time. Lessons to be learned from the MCI-AD field include the development of novel therapies targeting pathophysiological mechanisms (e.g., amyloid cascade, tau production and processing or specific biological mechanisms related to inflammation, insulin, cholesterol, etc.) [97,98]. The amyloid hypothesis also has evolved in AD, from its initial focus on contributions of plaques in disease development to using specific soluble plaque components (oligomers, monomers) as potential drug targets. The latest antiamyloid strategies focus on facilitating amyloid’s clearance, inhibiting its production, or preventing its aggregation [99]. Metabolic factors influencing brain glucose utilization and insulin-like growth factor resistance also may play a role in cognitive function [100–102], and clinical trials of novel compounds such as intranasal insulin in MCI and AD are underway. In addition, clinical
trials with immunotherapy (e.g., intravenous immunoglobulin-G) and anti-amyloid antibodies (e.g., bapineuzumab, solanezumab) have been, and continue to be cautiously tested in patients, with lessons to be learned regarding safety issues, heightened immune responses and optimal doses and delivery [103–107].

Recent clinical trials now focus on the preclinical stages of AD with the aim of preventing cognitive decline or AD and implementing aggressive treatment at earlier stages. Biomarker profiles play an important role in guiding study design, selecting target populations and identifying drug interventions for several large-scale trials in persons at risk for developing AD (Table 3) [108–113]. Furthermore, studying disease mechanisms, biomarkers and therapies longitudinally, across preclinical phases to dementia, can inform the timelines and benchmarks of progression needed for trials and identify those persons who are the most likely to decline and thereby, potentially benefit from early therapeutic intervention, prior to substantial synaptic loss and neurodegeneration. Thus, strategic use of biomarkers and sensitive cognitive tests in prevention trials, whether for cognitive decline in AD or PD, may help provide the necessary evidence of efficacy to support future drug approval. The MCI-AD field has set forth informative examples for the MCI-PD field regarding the direct application of biomarker and genetic information in clinical trial design; development of novel therapeutic targets based on advances in neuroscience, animal models, neuroimaging and molecular studies; and early identification of those at highest risk of cognitive decline.

**Conclusion & future perspective**

Whether in PD or AD, the construct of MCI has taken hold over the years and has been defined, and redefined, and will likely continue to evolve as research advances. In both fields, research devoted to identifying persons at the earliest stage of cognitive symptoms has gained attention. Improved therapeutic interventions are still needed for symptomatic benefit and disease-modification. Discovery of biomarkers that reflect disease progression and underlying pathologies associated with cognitive impairment may provide a path toward early detection of persons at high risk for cognitive decline and thereby, prevention and/or early intervention. However, these advances, whether for PD or AD, do not come without some risks and limitations as studies will need to reconcile the potentially negative aspects of early diagnosis, the risk–benefit ratios of various therapeutics, and accessibility of biomarker testing and clinical resources, counseling and therapies once available. While MCI in PD is a relatively newer concept compared with MCI due to AD, lessons highlighted in this review may be shared by both neurological disorders and individually or collectively, advance our understanding of neurodegenerative processes and treatment interventions for both.

**Author disclosures**

JG Goldman: Consultancies: Acadia, Advisory Boards: Acadia, Pfizer, Teva; Employment: Rush University Medical Center, Honoraria: Movement Disorders Society, American Academy of Neurology, Michael J. Fox Foundation, Grants: NIH, Michael J. Fox Foundation, Parkinson’s Disease Foundation, Rush University, Teva (study site-PI), Biotie (study site-PI). NT Aggarwal: Consultancies: Medical Consultant – Illinois Institute of Continuing Legal Education (IICLE), Advisory Boards: Lilly Alzheimer’s Disease Environment Evolution (ADEE) Working Group, MERCK, Employment: Rush University Medical Center, Honoraria: Preventative Cardiologist Nursing Association, Grants: NIH/NIA, PCORI, Eli Lilly (study site), Lundbeck (study site). CD Schroeder: Employment: Rush University Medical Center, Governors State University, Grants: NIH T32AG000269–15. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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• of interest; •• of considerable interest


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Review of studies of MCI studies in PD which led to the development of the PD-MCI diagnostic criteria.

- \textit{Review} Goldman, Aggarwal & Schroeder


- Review of studies of MCI studies in PD which led to the development of the PD-MCI diagnostic criteria.


• Longitudinal study of incident PD patients from the CamPaIGN study that includes 10-year follow-up data and demonstrates baseline clinical and genetic variables that may predict poor outcomes.


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Mild cognitive impairment: an update in Parkinson’s Disease & lessons learned from Alzheimer’s Disease

44 Review article that generates discussion on considering redefine PD along with various challenges and controversies.


40 Ferman TJ, Smith GE, Kantarci K et al. Review article that generates discussion on defining PD along with various challenges and controversies.


37 Ferman TJ, Smith GE, Kantarci K et al. Review article that generates discussion on defining PD along with various challenges and controversies.


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**Revised recommendations for diagnostic guidelines including use of clinical and biomarker information for AD.**


**Revised recommendations for diagnostic guidelines including use of clinical and biomarker information for AD.**


105 Dodel R, Balakrishnan K, Keyvani K et al. Naturally occurring autoantibodies against beta-amyloid: investigating their role in transgenic animal and *in vitro* models of...


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ClinicalTrials Database: NCT01760005. ClinicalTrials.Gov/Ct2/Show/

ClinicalTrials Database: NCT02008357. ClinicalTrials.Gov/Ct2/Show/Nct02008357

ClinicalTrials Database: NCT02488720 ClinicalTrials.Gov/Ct2/Show/Nct02488720

ClinicalTrials Database: NCT01931566 ClinicalTrials.Gov/Ct2/Show/Nct01931566