

Biomarker candidates of neurodegeneration in Parkinson's disease for the evaluation of disease-modifying therapeutics

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Abstract Reliable biomarkers that can be used for early diagnosis and tracking disease progression are the cornerstone of the development of disease-modifying treatments for Parkinson's disease (PD). The German Society of Experimental and Clinical Neurotherapeutics (GESENT) has convened a Working Group to review the current status of proposed biomarkers of neurodegeneration according to the following criteria and to develop a consensus statement on biomarker candidates for evaluation of disease-modifying therapeutics in PD. The criteria proposed are that the biomarker should be linked to fundamental features of PD neuropathology and mechanisms underlying neurodegeneration in PD, should be correlated to disease progression assessed by clinical rating scales, should monitor the actual

disease status, should be pre-clinically validated, and confirmed by at least two independent studies conducted by qualified investigators with the results published in peer-reviewed journals. To date, available data have not yet revealed one reliable biomarker to detect early neurodegeneration in PD and to detect and monitor effects of drug candidates on the disease process, but some promising biomarker candidates, such as antibodies against neuromelanin, pathological forms of α -synuclein, DJ-1, and patterns of gene expression, metabolomic and protein profiling exist. Almost all of the biomarker candidates were not investigated in relation to effects of treatment, validated in experimental models of PD and confirmed in independent studies.

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Introduction and aims of the present review

A biomarker (or biological marker) is defined as a characteristic that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention (Biomarkers Definitions Working Group 2001). According to the type of information they provide, biomarkers for central nervous system (CNS) diseases can be classified as clinical, neuroimaging, biochemical, genetic or proteomic biomarkers. Biomarkers serve a wide range of purposes, including confirmation of diagnosis, epidemiological screening, predictive testing, monitoring of disease progression after diagnosis, drug development and response to treatment, and studies of brain–behaviour relationship.

There is a growing need for biomarkers of Parkinson's disease (PD, synonyms: idiopathic Parkinson syndrome, paralysis agitans) pathology to improve drug development related to the disease (Eller and Williams 2009; Gerlach et al. 2008; Halperin et al. 2009; Maetzler et al. 2009a; Marek et al. 2008; Michell et al. 2004; Morgan et al. 2010). Current therapeutic strategies for PD focus primarily on reducing the severity of its symptoms using dopaminergic medications. Although these strategies significantly improve motor symptoms and the quality of life for patients suffering from this neurodegenerative disease, treatment does not slow or halt the underlying pathologic processes. The goal of finding such a therapy (i.e., a neuroprotective or disease-modifying therapy) or one that could reverse pathologic damage (i.e., a neurorestorative therapy) is a major drive for preclinical research in PD. Despite 25 years of work dedicated to this goal, success has remained elusive. Several promising candidates for a disease-modifying therapy have failed in human studies, although they showed neuroprotective effects in experimental models of PD. Problems with establishing a disease-modifying therapy arise from the complexity of the disease process as well as the limitations of clinical tools available to monitor the progression of the disease and to observe the effects of an intervention. Major issues of the complexity of the disease, which become frequently evident in clinical studies are the long duration and slow progressive course of the disease, the variability and heterogeneity of symptoms and signs, cyclic episodes in severity of the symptoms during the day related to the time of medication (wearing-off and on/off fluctuations) and

polypharmacy. In addition, misdiagnosis, co-morbidity and co-medication add to the heterogeneity of the patient population.

Since a disease-modifying therapy is likely to be most effective early in the course of disease, early diagnosis is highly desirable before neurodegeneration becomes severe and widespread. Thus, there is a great need for biomarkers that can be used for early diagnosis and tracking disease progression to monitor a disease-modifying therapy.

The German Society of Experimental and Clinical Neurotherapeutics (GESENT) has convened a Working Group to develop a position paper and, if possible, a consensus statement on biomarker candidates of neurodegeneration in PD for evaluation of disease-modifying therapeutics. In June 2010, the Working Group met to define the criteria for evaluation biomarkers of neurodegeneration in PD, to review the current status of all proposed biomarkers of neurodegeneration according to the defined criteria and to develop this consensus statement. This paper is planned as a basis for further discussion to finally reach the goal of a comprehensive evaluation of biomarkers for progression in PD.

Criteria for the development of biomarkers of neurodegeneration in PD for proof of disease-modifying therapeutics

Driven in part by Alzheimer's disease (AD) drug discovery research, AD is at the forefront of biomarker development for CNS diseases, and many current concepts about ideal biomarkers for PD have come from AD research (Frank et al. 2003; Hampel et al. 2004, 2010; Shaw et al. 2007; The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group 1998). We propose the following criteria for an ideal biomarker to be useful to assess neurodegeneration in PD and to evaluate disease-modifying therapeutics: The biomarker should be

- linked to fundamental features of PD neuropathology and mechanisms underlying neurodegeneration in PD,
- correlated to disease progression assessed by clinical rating scales,
- able to monitor the actual disease status,
- pre-clinically validated,
- confirmed by at least two independent studies conducted by qualified investigators with the results published in peer-reviewed journals.

In addition, an ideal biomarker of neurodegeneration should be inexpensive, non-invasive, simple to use, and technically validated (e.g., reliable, sensitive to change).

Clinical biomarker candidates

Symptoms associated with motor function

The most widely used scale currently available for the clinical evaluation of motor dysfunction in PD is the Unified Parkinson's Disease Rating Scale III (UPDRS-III) (Goetz et al. 2007). This scale is subjective, has suboptimal sensitivity, and it is widely accepted that more objective and shorter assessments are needed. A promising tool is the timed motor test, and, in particular, the pegboard test (Haaxma et al. 2008). At group level, a trial using "change from baseline" as endpoint and applying these tests would require only 57–75% of the patients needed with the UPDRS-III (Haaxma et al. 2008).

In addition, there is a relevant and growing body of literature which reports about objective, quantitative and mobile assessments of movement disturbances in PD using technical devices, such as accelerometers and gyroscopes. A definite advantage of such methods is the possibility to focus on cardinal motor disturbances, i.e. bradykinesia, rigidity, tremor and postural instability, but also on problems of sensorimotor integration which is also a key symptom associated with PD. So far, studies carried out have mainly focused on the usefulness of the parameters in differentiating PD from controls, but not on correlation aspects, e.g. with disease duration. In addition, most of them have not been put into context to clinical scales and are thus not validated with regard to measuring disease progression. Nevertheless, as these methods are generally easy to perform (e.g. in an ambulatory setting), cheap, unobtrusive, focus on mechanisms underlying the neurodegeneration in PD (e.g. cardinal motor symptoms), and based on a well-investigated pathophysiological background (many of the investigated symptoms have been—directly or indirectly—validated in pre-clinical models in an extensive way), they should be seriously considered when defining e.g. an assessment panel for future progression studies in PD.

Mobile quantitative assessment of bradykinesia

According to the definition of Berardelli et al. (2001), "bradykinesia" encompasses problems of slowness or absence of movement (including increased gait variability and freezing): Promising quantitative markers are sit-to-stand and stand-to-sit procedures (Bloem et al. 1997; Hausdorff 2008; Najafi et al. 2002; Weiss et al. 2010), anticipatory postural adjustment (i.e. the attempt to voluntarily initiate the first step to begin walking) (Carpinella et al. 2007; Mancini et al. 2009), gait variability (Plotnik et al. 2007, 2009), and peak arm swing velocity (Zampieri et al. 2010).

Mobile quantitative assessment of rigidity

Rigidity is defined as an increase in muscle tone leading to a resistance to passive movement throughout the range of motion. Promising quantitative markers are turning procedures when walking or receiving rotational perturbations (Carpenter et al. 2004; Carpinella et al. 2007; Huxham et al. 2008; Visser et al. 2007; Zampieri et al. 2010) and straight walking (pelvic oscillations) (Huxham et al. 2008).

Mobile quantitative assessment of tremor

Although tremor is an obvious sign of PD, and clinically easily to diagnose, the quantification of this symptom remains a technical challenge. In a study with PD patients using electromyography, tremor amplitude and burst duration increased, whereas frequency decreased with longer disease duration (Milanov 2002). The first results with acceptable accuracy in detecting the severity of resting tremor using tri-axial accelerometers have been published (Mamorita et al. 2009; Rigas et al. 2009; Schlesinger et al. 2009).

Mobile quantitative assessment of postural instability

Accurate assessment of postural instability in PD remains difficult with currently available clinical measurement tools, but may be quantifiable with ambulatory devices which focus on anterior–posterior and medial–lateral angular velocity deviations, e.g. at the trunk (Adkin et al. 2005). In addition, prospective detection of frequency of near-falls and falls may be a promising approach to detect PD progression.

Mobile quantitative assessment of sensorimotor integration deficits

There is an increasing awareness of sensorimotor integration deficits in PD patients, and it is highly probable that this feature also declines with increasing disease duration. Promising markers may be the switch from kinaesthetic-dependent to vision-dependent balance control (De Nunzio et al. 2007), and overestimation of (balance) limits (Kamanli et al. 2008).

Biomarker candidates of cognition and neuropsychiatric symptoms

Cognitive symptoms

There is an increasing awareness of the high prevalence of cognitive dysfunction in the course of PD. Independent studies found a higher incidence of dementia in PD patients

as compared to healthy persons of the same age (Aarsland et al. 2001; de Lau et al. 2004). There is compelling evidence that dementia prevalence increases with disease duration (Maetzler et al. 2009a). Deterioration of cognitive decline was most often assessed with the MMSE and the cognitive section of the Cambridge Examination for Mental Disorders (CAMCOG), two assessment tools validated for AD (Aarsland et al. 2004; Athey and Walker 2006). As cognitive symptoms in PD clearly differ from AD symptoms, it was more and more realised that AD-related assessment tools have relevant flaws in determining cognitive dysfunction in PD, and effort has been put into the development of sensitive and reliable PD-relevant measurement tools. One of the most promising tools is the “Scales for outcomes in Parkinson’s disease-cognition (SCOPA-COG)” having advantages as compared to the MMSE, the most important having a greater discriminative capacity (Serrano-Duenas et al. 2010); however, longitudinal studies are not yet available.

Hallucinations and depression

Based on the retrospective pathologically confirmed (Williams and Lees 2005) and prospective studies (Forsaa et al. 2008; Goetz et al. 2005) visual hallucinations are regularly observed in PD patients in particular at later disease stages, and frequency increases with longer disease duration.

Depression has been shown to occur with higher incidence in PD as compared to the general population, but incidence and severity of symptoms may not relevantly change during disease course (Karlsen et al. 1999; Rojo et al. 2003; Schrag et al. 2007).

Sleep disturbances

Among a number of sleep disturbances which are associated with PD rapid eye movement (REM) sleep behaviour disorder (RBD) may be the most promising biomarker candidate for detecting disease progression. A prospective longitudinal study investigating patients with questionnaire and polysomnography found an increase of RBD-associated features from baseline (6–11% after 3, 24% after 6, and 39 percent after 8 years) (Onofrj et al. 2002). This increase in occurrence could be confirmed in an evidence level II study with mid- to late-stage PD patients using a semi-structured interview and a sleep questionnaire (Gjerstad et al. 2007).

Biomarker candidates of autonomic and sensory dysfunction

Based on a controlled prospective study of 3 years duration (Mesec et al. 1999) and a cross-sectional study (Linden et al. 1997) heart rate variability decreases, and orthostatic

dysfunction probably increases with longer disease duration. A reduction in sympathetic skin response with increasing disease duration has been demonstrated in two cross-sectional studies (Orimo et al. 1999; Schestatsky et al. 2006). Prevalence and severity of urinary and gastrointestinal symptoms most probably also increase during PD course (Wullner et al. 2007); however, it may be difficult to quantify these changes adequately.

There is compelling evidence from prospective longitudinal studies (Diederich et al. 2002; Katsarou et al. 1998) that visuospatial and colour discrimination deteriorate with longer PD duration. These symptoms may be influenced by medication status (Onofrj et al. 2002). There is no evidence that olfactory dysfunction progresses significantly during PD course (Maetzler et al. 2009a).

Myocardial [^{123}I]metaiodobenzylguanidine (MIBG) scintigraphy and [^{18}F]fluorodopamine positron emission tomography (PET) are used to detect local sympathetic nerve damage in the heart, which regularly occurs in PD, but rarely in healthy older people and in other forms of parkinsonism. However, it is unlikely that cardiac sympathetic innervation decreases with PD duration in the clinical phase (Orimo et al. 1999; Shibata et al. 2009; Suzuki et al. 2007).

Biomarker candidates of brain imaging

Presynaptic imaging of dopaminergic neurons is part of clinical diagnostics of PD and appears to be a useful progression marker (Table 1). Disadvantages of this approach are that the subjects are exposed to radioactivity, that the costs are relatively high, and that the method is only available at specialised centres. The imaging of the dopaminergic system is possible by measuring aromatic amino acid decarboxylase activity (e.g. with [^{18}F]-DOPA) or by visualisation of synaptic membrane dopamine transporter (e.g. [^{123}I] β -CIT, [^{123}I]FP-CIT, [^{123}I]IPT, [^{18}F]CFT). In longitudinal studies of PD progression, PET and single photon emission computed tomography (SPECT) studies using these tracers have shown an annualised striatal rate of reduction in tracer uptake of about 4–13% in PD patients versus 0–2.5% change in healthy controls (Marek et al. 2008; Nurmi et al. 2001). This decline may rather be exponentially (Hilker et al. 2005). With regard to using these functional imaging techniques for measuring disease progression, it needs to be considered that correlations of changes in imaging and clinical findings are rather inconsistent, probably, because different aspects of the disease are reflected (Marek et al. 2008).

[^{18}F]-2-F-Deoxyglucose-PET may have some potential in detecting metabolic changes associated with motor (Huang et al. 2007) and cognitive decline (Huang et al.

Table 1 Qualification of some biomarker candidates for the use in clinical trials of disease-modifying therapeutics in Parkinson's disease (PD)

Analyte/method	Link to neuropathology/pathomechanisms	Track of disease progression	Monitoring the actual disease status	Validation in experimental models of PD	Confirmation by others
<i>Clinical biomarker candidates</i>					
Mobile quantitative assessment of bradykinesia	Yes (cardinal symptom)	Not investigated	No	(Yes: models for bradykinesia)	Yes
Mobile quantitative assessment of rigidity	Yes (cardinal symptom)	Not investigated	No	(Yes: models for rigidity)	Yes
Mobile quantitative assessment of tremor	Yes (cardinal symptom)	Not investigated	No	(Yes: models for tremor)	Yes
Mobile quantitative assessment of postural instability	Yes (cardinal symptom)	Not investigated	No	(Yes: models for postural instability)	No
Mobile quantitative assessment of sensorimotor integration deficits	No	Not investigated	?	No	No
Sleep disturbances	?	Yes	Not investigated	Not investigated	Yes
Reduction in sympathetic skin response	No	Yes	Not investigated	Not investigated	Yes
Visuospatial and colour discrimination	Yes	Yes	No	Not investigated	Yes
Olfactory dysfunction	Yes	No	Not investigated	Not investigated	Yes
Cardiac sympathetic innervation	Yes	No	Not investigated	Not investigated	Yes
<i>Neuroimaging biomarker candidates</i>					
[¹⁸ F]-DOPA-PET	Yes	Yes	No	Yes	Yes
β -CIT-SPECT	Yes	Yes	No	Yes	Yes
Magnet resonance imaging (T2 relaxation time)	Yes	No	Not investigated	Yes	Yes
<i>Biochemical biomarker candidates</i>					
Antibody response against neuromelanin	Yes	Not affected by disease severity assessed by Hoehn and Yahr staging and the UPDRS Negative correlation with disease duration	It appears to be, but has to be confirmed in larger samples	Not investigated	No
α -Synuclein concentrations in the CSF	Yes	No association with the severity of PD	It appears to be, but has to be confirmed in larger samples	Not investigated	No, there are inconsistent results obtained
Complex I and IV activity in platelet mitochondria	Yes	Negative correlation between activity and disease duration	Not investigated	Not investigated	No
8-Hydroxydeoxyguanosine concentrations in urine and blood	Yes	Stage-dependent increase in one study	Surprisingly no effect of L-DOPA therapy in one study	Not investigated	Yes
DJ-1 concentrations in the CSF	Yes	No association to severity of PD	It appears to be, but has to be confirmed in larger samples	Not investigated	No, there are inconsistent results obtained
Reduced glutathione (GSH) in CSF	Yes	No association to severity of PD	It appears to be, but has to be confirmed in larger samples	Not investigated	No

Table 1 continued

Analyte/method	Link to neuropathology/pathomechanisms	Track of disease progression	Monitoring the actual disease status	Validation in experimental models of PD	Confirmation by others
Osteopontin in CSF	Yes	Positive (weak) correlation with disease duration	It appears to be, but has to be confirmed in larger samples	Yes	No
Total homocysteine in plasma	No	Correlation with disease duration and duration of L-DOPA treatment	Probably not	Not investigated	No, there are inconsistent results obtained

CSF cerebrospinal fluid, L-DOPA L-3,4-dihydroxyphenylalanine, PET positron emission tomography, SPECT single photon emission computed tomography

2007; Liepelt et al. 2009), but these preliminary data should be confirmed in prospective longitudinal studies.

There is only limited evidence that magnetic resonance imaging is of added value in detecting disease progression in PD: Two cross-sectional studies with advanced PD patients showed a positive correlation between T2 relaxation time in the putamen and disease duration which indicates a progressive loss of iron (Graham et al. 2000; Ryvlin et al. 1995). However, a recent study in PD patients and controls measuring quantitative magnetic resonance parameters sensitive to complementary tissue characteristics (i.e. volume atrophy, iron deposition and microstructural damage) in six subcortical structures including the SN and the putamen showed no relation of the relaxation rates such as R_2^* as an indirect measure of the iron level to disease progression (Peran et al. 2010).

Genetic and biochemical biomarker candidates

Genetic markers

PD-associated DNA variants (including mutations and polymorphisms) are by definition predictive markers and are not suitable for measuring progression. However, gene expression profiling may be a promising approach for defining valuable progression markers as human SN pars compacta of PD patients showed down-regulation of 68, and up-regulation of 69 genes, as compared to control SN (Grunblatt et al. 2004). Based on the recent publications particularly interesting targets are pyridoxal kinase and pyruvate metabolism (Ahmed et al. 2009; Elstner et al. 2009).

A recent study (Grunblatt et al. 2010) examined the profiling of 12 transcripts via quantitative RT-PCR in RNA originating from peripheral blood samples that were chosen

based on the previous postmortem brain profiling (Grunblatt et al. 2004). Multiple analyses resulted in four significant genes: proteasome (prosome, macropain) subunit- α type-2 (PSMA2), laminin, β -2 (laminin S) (LAMB2), aldehyde dehydrogenase 1 family-member A1 (ALDH1A1), and histone cluster-1 H3e (HIST1H3E) differentiating between medicated PD subjects versus controls. Using the combination of these four gene profiles for PD diagnosis, a sensitivity and specificity of more than 80% was achieved. In AD subjects, no significant results were observed. Therefore, the authors concluded that this combination is specific for PD.

A transcriptome-wide scan using RNA microarrays in whole blood of patients with early-stage PD (Scherzer et al. 2007) identified a molecular multigene marker that is associated with risk of PD in 66 samples of the training set comprising healthy and disease controls. This was further validated in 39 independent test samples. Insights into disease-linked processes detectable in peripheral blood are offered by 22 unique genes differentially expressed in patients with PD versus healthy individuals (Scherzer et al. 2007). These include the co-chaperone ST13, which stabilises heat-shock protein 70, a modifier of α -synuclein misfolding and toxicity. ST13 messenger RNA copies are lower in patients with PD than in controls in two independent populations.

Biochemical markers

A summary of the most thoroughly investigated biochemical biomarker candidates that may be used in the diagnostics of PD was published previously (Fasano et al. 2008; Halperin et al. 2009; Morgan et al. 2010; Nyhlen et al. 2010). Here we focus on biomarker candidates with a particular reference to their potential for monitoring neurodegeneration and disease-modifying therapeutics.

PD is neuropathologically characterised at the cellular level by a relative selective destruction of neuromelanin (NM)-containing dopaminergic cells in the SN pars compacta (Hirsch et al. 1988). When melaninised dopaminergic neurons die, NM is released from the degenerating cell body and removed from the brain by the cells of the immune system (Beach et al. 2007; Depboylu et al. 2011; Orr et al. 2005). It was hypothesised that the removal of NM from the brain by immune cells might stimulate an antibody response that could be measured in blood. Indeed, a novel enzyme-linked immunosorbent assay (ELISA) to measure levels of antibodies against NM in human blood sera (NM-ELISA) demonstrated an increased antibody response in the sera of PD patients when compared with age-matched controls (Double et al. 2009). The immune response was not affected by disease severity assessed by Hoehn and Yahr staging and the UPDRS. However, there was a negative correlation with disease duration.

α -Synuclein is the major component of Lewy bodies, one of the pathological hallmarks of PD, and mutations and duplications of the α -synuclein-encoding gene, SNCA, have been found to cause familial forms of PD. Aberrant metabolism of the protein has been suggested as a possible driving force in the degenerative process of PD in a manner similar to $A\beta_{1-42}$ in AD. Cerebrospinal fluid (CSF) $A\beta_{1-42}$ levels in PD tend to be lower with longer disease duration and cognitive decline (Maetzler et al. 2009b; Mollenhauer et al. 2006). A recent study demonstrated that the CSF fractalkine (an inflammatory marker)/ $A\beta_{1-42}$ ratio was positively correlated with PD severity in cross-sectional samples as well as with PD progression in longitudinal samples (Shi et al. 2011).

Increased concentrations of soluble α -synuclein oligomers in plasma appear to have good specificity (85%) for detecting PD when compared with controls in some studies (El-Agnaf et al. 2006). The most consistent finding is decreased α -synuclein concentrations in the CSF from PD when compared with controls (see for a review, Morgan et al. 2010; Nyhlen et al. 2010), but there is still no convincing evidence that these levels change over disease course (Hong et al. 2010). There is increasing evidence that α -synuclein can be used to distinguish PD and related synucleinopathies (dementia with Lewy bodies and multiple system atrophy) from other movement disorders and dementia (Mollenhauer et al. 2011); however, discriminatory power is limited. In addition, the current α -synuclein assays are limited because they do not attempt to discriminate between normal and pathological (phosphorylated and/or aggregated) forms of this protein.

The major hypotheses believed to contribute to the eventual demise of nigral dopamine producing cells include protein aggregation, oxidative stress, mitochondrial dysfunction, dysfunction of proteasomal pathways and

neuroinflammation (Alvarez-Erviti et al. 2010; Chu et al. 2009; Double et al. 2010; Gerlach et al. 2006; Hatano et al. 2009; Schiesling et al. 2008; Yang et al. 2009). Complex I and IV mitochondrial activity has been shown to be lower in PD patients than in controls, and at very early disease stages a negative correlation between complex I and IV activity in platelet mitochondria, and disease duration has been demonstrated (Benecke et al. 1993).

Markers of oxidative stress showed that in the blood of PD patients there is either an increased production of free radicals, reactive oxygen and nitrogen species or a disturbed defence mechanisms against oxidative damage (Gerlach et al. 2008; Morgan et al. 2010; Younes-Mheni et al. 2007). However, these markers are not specific for PD because similar results in other neurodegenerative diseases, including AD were found. In addition, a variety of conditions alter oxidative stress in a given patient (for example normal ageing, smoking, vigorous exercise, antioxidants, food, drugs, cancer, and chemotherapy), and these may be hard to control for. Interestingly, some markers of oxidative stress appear to be useful for tracking disease progression in PD. For example, concentrations of 8-hydroxydeoxyguanosine, a product of oxidised DNA, were shown to be stage-dependently increased in the urine of PD patients (Sato et al. 2005). Surprisingly, this increase was not influenced by L-DOPA (3,4-dihydroxyphenylalanine, levodopa) therapy. Multiple large epidemiological studies have demonstrated a reduced risk of developing PD with higher concentrations of uric acid (a potent antioxidant and free radical scavenger in the blood) in serum (Schlesinger and Schlesinger 2008), but recent evidence also indicates a potential for slower progression of PD with higher uric acid levels (Schwarzschild et al. 2008). Clinical use for uric acid as a biomarker is not supported by existing knowledge, since the studies conclude that it is a risk marker rather than a diagnostic marker. In addition, to date there are no data available which make hope that uric acid is a potential progression marker.

DJ-1 is a part of the cellular defence against oxidative stress (Kahle et al. 2009), and mutations in its gene are responsible for some forms of familial PD (Klein et al. 2009). A study has also found elevated DJ-1 levels in CSF from patients with multiple sclerosis (Hirotani et al. 2008), suggesting a link between secreted DJ-1, neuroinflammation and oxidative stress. Studies using CSF of PD patients have demonstrated both increased (Waragai et al. 2006) and decreased values compared with controls (Hong et al. 2010), thus warranting further investigations. In the study by Hong et al. (2010), no association between DJ-1 and the severity of PD was demonstrated. The results obtained from studies using serum of PD patients are also inconsistent, showing no change (Maita et al. 2008) or elevated concentrations compared with controls (Waragai et al. 2006).

The complement system is part of the non-specific immune system. Using 2D-gel-electrophoresis, Goldknopf et al. (2006) found differences in serum levels of nine complement factors between PD and controls. Osteopontin is a molecule with multiple functions, including modulation of inflammatory response of microglia, and shows much higher levels in CSF than in serum. Higher CSF and serum levels are detectable in PD as compared to controls, and there is some evidence that CSF osteopontin levels increase with disease duration (Maetzler et al. 2007).

Several studies suggest that elevated plasma total homocysteine, an endogenous product of methionine metabolism, is a risk factor for cognitive impairment and AD (Clarke et al. 1998; McCaddon et al. 2003). However, other studies did not detect significant associations with AD or cognitive status (Miller et al. 2002). In agreement with these studies, it was recently reported that plasma total homocysteine concentrations did not differ across AD, mild cognitive impairment, cerebral amyloid angiopathy, and non-demented control subjects, but were increased in the PD group (Irizarry et al. 2005). The elevated levels within the PD group were the result of high concentrations of plasma total homocysteine in PD patients treated with L-DOPA. Two cross-sectional studies found also increased homocysteine plasma levels in PD compared with controls, these levels correlated positively with disease duration (Dos Santos et al. 2009; Hassin-Baer et al. 2006). In one study, in addition, homocysteine levels were associated with L-DOPA treatment duration, but not with L-DOPA dose (Hassin-Baer et al. 2006). In the other study (Dos Santos et al. 2009), L-DOPA treatment did not significantly correlate with plasma homocysteine levels.

There are some interesting first results which may potentially reflect very early disease activity. Serum insulin-like growth factor 1 (IGF-1) levels have been shown to be higher in PD patients compared with controls, with high levels in particular at early PD disease stages, and a negative correlation between serum IGF-1 levels and disease duration (Godau et al. 2010). In addition, using rapidly processed CSF samples, we recently found lowered levels of reduced glutathione in the CSF of Lewy body disease patients as compared to controls (Maetzler et al. 2011)—which basically confirms neuropathological findings in the brainstem of PD patients (Sian et al. 1994)—and these levels were negatively associated with age but not with disease-associated parameters. Thus, it is tempting to speculate that changes of the glutathione system, similar to IGF-1, may be an early event in the disease course.

Several recent studies have used hypothesis-unrelated, explorative proteomic and metabolomic techniques to find novel biomarker candidates for PD in brain tissue and CSF

(see for a review Fasano et al. 2008; Morgan et al. 2010; Nyhlen et al. 2010). Generally, these studies may be considered promising. However, these techniques require considerable technical expertise and have not been well tested for PD versus other neurodegenerative diseases and the link to disease progression. In addition, the biomarker candidates found in these studies need to be validated in a greater and statistically significant universe of individual samples employing distinct methodologies, such as Western blot, ELISA or single and multiple reaction monitoring (Martins-de-Souza 2010).

A study using a multiplex quantitative proteomics method for detecting biomarkers in the CSF of patients with neurodegenerative diseases, including AD, dementia with Lewy body and PD, suggests as potential candidates for the clinical diagnosis of PD and monitoring disease progression chromogranins, amyloid precursor protein-like protein 1 and the prion protein (Abdi et al. 2006), but more studies are needed to confirm or refute the findings and to assess the specificity of the protein profiles against other neurodegenerative diseases (Zetterberg et al. 2008). Recent research has identified an eight-protein CSF multi-analyte profile using proteomics that fully differentiate PD patients from controls, with the profile designation agreeing with an expert clinical diagnosis of PD 95% of the time (Zhang et al. 2008).

The idea that the whole metabolism, regulated by genes, exogenous substances and proteins, might be affected in diseased patients, and that these affected molecules could together, form a distinct profile, underlies metabolomics (Kaddurah-Daouk and Krishnan 2005). This approach has been tested in PD and some results appear promising, such as the confirmatory finding of reduced concentrations of uric acid in plasma of idiopathic PD patients and PD patients with *LRRK2* mutations (Johansen et al. 2009). However, both idiopathic and *LRRK2* PD subjects involved in this study were taking anti-parkinsonian medications, and no samples from the un-medicated patients were available. Therefore, it is possible that the observed separation could be related to drug effects, which could involve unknown drug metabolites and drug-induced changes in metabolism.

Bogdanov et al. (2008) were able to accurately categorise 25 controls and 66 un-medicated PD patients based only on their metabolic profiles in blood, obtaining complete separation between the two groups. Interestingly, concentrations of 8-hydroxydeoxyguanosine, a marker of oxidative DNA damage, were significantly increased in PD patients (confirming results obtained in urine), but overlapped controls. In addition, concentrations of two other markers of oxidative stress, uric acid and glutathione were significantly reduced and significantly increased in PD, respectively.

Review of some putative biomarkers for the use in clinical trials of disease-modifying therapeutics

Table 1 summarises the qualification of biomarker candidates for the use in studies to proof disease-modifying therapeutics by considering the criteria for the development of biomarkers of neurodegeneration in PD as defined above. Qualification is used to mean the establishment of the credibility of a biomarker assay in its application to questions relevant to drug treatment (Hampel et al. 2010). Validation is usually applied to mean the determination of the performance characteristics of an assay such as for example sensitivity and specificity in measuring a specific analyte. Qualification requires specific patient populations and a specific therapeutic intervention. For example, a validated assay may be qualified as a PD biomarker to detect and monitor effects of drug candidates on the disease process by intervention in the α -synuclein aggregation, but not in non- α -synuclein mechanisms. It could be said therefore that the assay which was validated for quantification of α -synuclein fibrillisation in the brain or CSF is “qualified for use” as a biomarker in α -synucleinopathies such PD and Lewy body dementia for drugs that inhibit the aggregation of α -synuclein. The ultimate use of a biomarker is a surrogate end point, which requires that a biomarker has been qualified to substitute for a well-established clinical endpoint such that the biomarker reasonably predicts the clinical outcome and therefore can serve as a surrogate (Hampel et al. 2010).

The first criterion means that the biomarker is linked to the neuropathology of PD and/or mirror fundamental pathogenetic events in PD. The validity of a biomarker with respect to a supposed pathogenetic mechanism will be relevant for the evaluation of disease-modifying treatments. Pathologically, PD is characterised by a preferential loss of NM-containing dopamine neurons in the pars compacta of the SN, with intracellular proteinaceous inclusions named Lewy bodies in the SN and other brain regions, and a reduction in striatal dopamine (Bernheimer et al. 1973; Braak et al. 1995; Jellinger 1991). This ongoing loss of nigral dopaminergic neurons mainly leads to clinical diagnosis due to occurrence of motor symptoms such as rigidity, tremor and bradykinesia, which results from a reduction of about 70% of striatal dopamine (Bernheimer et al. 1973; Riederer and Wuketich 1976).

To mirror a pathological feature it would be helpful to know the cause of the disease. However, despite numerous attempts, the cause of PD remains unclear. It is hypothesised that the cause of neurodegeneration in PD is multifactorial in terms of both aetiology and pathogenesis. Genetic factors are known to cause PD in small numbers of patients with a familial form of the disorder. Mutations in different genes (for example, SNCA-synuclein, LRRK2,

parkin, DJ1 and PINK1) have been identified, and PD subtypes have been linked in addition to different chromosomal loci (for example, Hatano et al. 2009; Schiesling et al. 2008; Yang et al. 2009). These Mendelian forms of PD are relatively rare. However, high-throughput genotyping and sequencing technologies have more recently provided evidence that low-penetrance variants in some of these and other genes may also contribute significantly to the aetiology of the common sporadic disease. Moreover, rare variants in further genes, such as the glucocerebrosidase A gene associated with Gaucher's disease, have been found to be important risk factors in a subgroup of patients (Gasser 2010). Therefore, an increasingly complex interplay of different genes seems to contribute in distinct ways to disease risk and progression. Hence, current thinking favours the hypothesis that most sporadic cases are caused by a complex interplay between different genetic and environmental factors. This interplay may result in alterations of biochemical cascades. Altered biochemical pathways involved in the pathogenetic cascade of events leading to cell dysfunction and neuronal cell death in PD result among others in measurable mitochondrial complex I deficiency, a disturbed iron metabolism, free radicals, excitotoxicity, disturbed calcium homeostasis, microglia activation and protein aggregation (Alvarez-Erviti et al. 2010; Chu et al. 2009; Double et al. 2010; Gerlach et al. 2006; Hatano et al. 2009; Schiesling et al. 2008; Yang et al. 2009).

The second criterion for the development of a biomarker of neurodegeneration and to detect and monitor effects of drug candidates on the disease process is that the biomarker must track disease progression. We defined a progression marker as a disease-associated feature that changes in the frequency of occurrence or severity, or both, over time. The definitions used in our evaluation of progression of features have been published previously (Maetzler et al. 2009a). For the qualification of a biomarker as a surrogate endpoint, there should be a link between a treatment-induced change in the biomarker and the desired clinical outcome measure, as well as a link between the treatment-induced change in the biomarker and change of disease process (Hampel et al. 2010).

Although imaging techniques measuring the presynaptic nigrostriatal system with, for example [^{18}F]-DOPA or [^{123}I] β -CIT, can readily distinguish subjects with early PD from controls, and abnormalities can be observed even before motor symptoms and signs are apparent, these studies have been failed in monitoring both disease-modifying (Ponsen et al. 2009) and neurorestorative therapies (Freed et al. 2001; Whone et al. 2003). This was discussed to be due, in part, to potential pharmacological modulation or regulation of presynaptic proteins that may not relate to the actual disease status. Therefore, we postulated as a third

criterion that the biomarker is monitoring the disease status. This would require that the drug aimed to proof a disease-modifying effect does not pharmacologically influence the biomarker. To date, trials to proof disease-modifying drugs have largely evaluated subjects in the early clinical stages of the disease (generally untreated), using clinical endpoints that involve either the change in a classical clinical measure of the disease over time or progression to the point of reaching a disease milestone (for example, need for a dopaminergic therapy). The greatest concern in these studies has been the potential for the study intervention to cause symptomatic benefit that precludes the determination of disease-modifying effects. Approaches developed to overcome this problem, such as the washout and delayed-start design, however, have either failed to adequately overcome this problem or is not without its own potential problems (Lang, 2010). Therefore, clinical biomarkers that are changed by symptomatic drug therapy cannot be considered sufficient surrogate biomarkers for the evaluation of disease-modifying therapeutics.

The fourth criterion for the development of a biomarker of neurodegeneration for proof of disease-modifying therapeutics in PD is that the biomarker candidate is validated in experimental models of PD. This means that in an *in vivo* model of PD there should be a correlation between the degree of neurodegeneration and the change of the biomarker candidate. In addition, based on the assumed mechanism of action of a given compound, the proposed mechanism underlying neurodegeneration should be modified. This validation will be relevant for predicting the response of PD patients to putative disease-modifying therapies.

The fifth criterion, validation of the biomarker in independent studies is essential but also trivial. Only a progression marker that mirrors progression independent from the investigator can be used as a biomarker for disease-modifying therapeutics.

Conclusions and future perspectives

Reliable biomarkers that can be used for early diagnosis and tracking disease progression are the cornerstone of the development of disease-modifying treatments for PD. To date, available data have not yet revealed one reliable biomarker to detect early neurodegeneration in PD and to detect and monitor effects of drug candidates on the disease process, but some promising biomarker candidates, such as antibodies against NM, pathological forms of α -synuclein, DJ-1, and patterns of gene expression, metabolomic and protein profiling exist (Table 1). Most of the reported disease-associated changes are relatively small, with a

clinically problematic overlap between patients and controls. Almost all of the biomarker candidates were not investigated in relation to effects of treatment, validated in experimental models of PD and confirmed in independent studies.

To solve some of the problems associated with the development of biomarkers that can be used for early diagnosis and tracking disease progression, the Parkinson's Progression Markers Initiative (PPMI) was founded (<http://www.ppmi-info.org>). This public-private partnership led by the Michael J. Fox Foundation aims to identify clinical, imaging, and biological markers of disease progression. The emphasis will initially be on fluid markers including α -synuclein, DJ-1, amyloid β , and tau in CSF and urate in blood. The initiative will enrol 400 newly diagnosed patients who are not yet on medication and who have evidence of dopamine transporter loss on dopamine transporter imaging with SPECT and 200 healthy age-matched controls. As with the AD Neuroimaging Initiative (ADNI), a crucial aspect of the PPMI is that all data and biological specimens, stored in a central repository, will be available for the research community. The PPMI will thus provide a valuable resource to fuel further academic and industry-initiated studies and innovations, and promising biomarker candidates identified through such efforts could be validated and qualified against the large, prospective PPMI dataset.

However, to fulfil the promise of the PPMI for delivering objective biomarkers that can be used for early diagnosis and tracking disease progression, specimen collection, processing, and storage methods have to be standardised. Further, quality-control mechanisms should be in place to ensure data are acceptable before they are made publicly available. Finally, well-defined quantitative biomarker outcomes that are consistent among many research sites and laboratories should be established.

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