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Invited review

Biomarkers in Parkinson's disease (recent update)

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ABSTRACT

Parkinson's disease (PD) is the second most common neurodegenerative disorder mostly affecting the aging population over sixty. Cardinal symptoms including, tremors, muscle rigidity, drooping posture, drooling, walking difficulty, and autonomic symptoms appear when a significant number of nigrostriatal dopaminergic neurons are already destroyed. Hence we need early, sensitive, specific, and economical peripheral and/or central biomarker(s) for the differential diagnosis, prognosis, and treatment of PD. These can be classified as clinical, biochemical, genetic, proteomic, and neuroimaging biomarkers. Novel discoveries of genetic as well as nongenetic biomarkers may be utilized for the personalized treatment of PD during preclinical (premotor) and clinical (motor) stages. Premotor biomarkers including hyper-echogenicity of substantia nigra, olfactory and autonomic dysfunction, depression, hyposmia, deafness, REM sleep disorder, and impulsive behavior may be noticed during preclinical stage. Neuroimaging biomarkers (PET, SPECT, MRI), and neuropsychological deficits can facilitate differential diagnosis. Single-cell profiling of dopaminergic neurons has identified pyridoxal kinase and lysosomal ATPase as biomarker genes for PD prognosis. Promising biomarkers include: fluid biomarkers, neuromelanin antibodies, pathological forms of α-Syn, DJ-1, amyloid β and tau in the CSF, patterns of gene expression, metabolomics, urate, as well as protein profiling in the blood and CSF samples. Reduced brain regional N-acetyl-aspartate is a biomarker for the in vivo assessment of neuronal loss using magnetic resonance spectroscopy and T_2 relaxation time with MRI. To confirm PD diagnosis, the PET biomarkers include [^{18}F]-DOPA for estimating dopaminergic neurotransmission, [^{18}F]dG for mitochondrial bioenergetics, [^{18}F]BMS for mitochondrial complex-1, [^{11}C](R)-PK11195 for microglial activation, SPECT imaging with ^{123}Iflupane and βCIT for dopamine transporter, and urinary salsolinol and 8-hydroxy, 2-deoxyguanosine for neuronal loss. This brief review describes the merits and limitations of recently discovered biomarkers and proposes coenzyme Q_{10}, mitochondrial ubiquinone-NADH oxidoreductase, melatonin, α-synculein index, Charnoly body, and metallothioneins as novel biomarkers to confirm PD diagnosis for early and effective treatment of PD.

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Abbreviations: AD, Alzheimer's disease; Alpha-Syn$_{ko}$, syn knock out; ALS, amyotrophic lateral sclerosis; ANS, autonomic nervous system; CB, Charnoly body; DAT, dopamine transporter; DLB, dementia with Lewy body; HD, Huntington's disease; LBs, Lewy body; KRS, Kufor-Rakeb syndrome; MPTP, 1-methyl, 2-phenyl, 1, 2, 3, 6-tetrahydropyridine; MRS, magnetic resonance spectroscopy; MS, multiple sclerosis; MSA, multiple system atrophy; MTs, metallothioneins; MT$_{dko}$, metallothionein double gene knockout; MT$_{trans}$, metallothionein transgenic; PD, Parkinson's disease; PNS, peripheral nervous system; PET, positron emission tomography; MRI, magnetic resonance imaging; α-Syn, α-synculein; SI, α-synculein index; SPECT, single photon emission tomography.

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1. Introduction

Neurodegenerative diseases such as Parkinson's disease (PD) are incurable debilitating disorders that affect approximately 30 million people worldwide (Federico et al., 2012). Parkinson's disease (PD) is the second most common age-related neurodegenerative disorder with movement disorders and is clinically characterized by parkinsonism and wide spread Lewy body pathology in the central nervous system (CNS), peripheral nervous system (PNS), and autonomic nervous system (ANS) (Gasser et al., 2011; Jellinger, 2012). PD affects ~1% of people over the age of 65 and approximately 4% of the population aged over 80 years. Almost 6 million people worldwide are suffering from PD with a slow progression of movement disorder symptoms. Over a million people in the United States alone are suffering from PD. It is characterized by rigidity, bradykinesia, resting tremor, and postural instability (Martin and Teismann, 2009). Its prevalence is expected to double in the most populated areas within the next two decades in parallel with an increasing aging population. Since PD is becoming a serious concern for many countries, early therapy and disease prevention is essential – this can only be accomplished with early diagnosis. Neuropathological hallmarks of PD are loss of dopaminergic neurons in the substantia nigra and the formation of intraneuronal protein inclusions termed Lewy bodies, composed primarily of α-Syn (Stefanis, 2012; Yasuda et al., 2013). Currently PD treatment is symptomatic as clinical symptoms appear late after degeneration of a significant number of dopaminergic (DA-ergic) neurons and potential disease-modifying/neuroprotective therapies would have no effect. As a result, the identification and development of disease-modifying therapies is difficult, making PD a socio-economic burden and a serious challenge for the public health system. Thus novel discoveries of sensitive and specific biomarkers for PD are needed to facilitate diagnosis at early stages, monitor disease progression, and assess response to existing and future treatments (Schlossmacher and Mollenhauer, 2010). The development of suitable experimental models to investigate the mechanisms of progression and the discovery of selective and specific molecular biomarkers for early and differential diagnosis are important goals for better clinical management of PD. Currently, the diagnosis is made by the presence of cardinal motor features and associated non-motor symptoms (Goldstein et al., 2011, 2012a, 2012b, 2012c). More than 70% of patients with PD have tremor as the presenting feature. This tremor is typically asymmetric, occurs at rest, and becomes less prominent with voluntary movement. The diagnosis of tremor is based on clinical information obtained from a thorough history and physical examination. For more challenging cases, single photon emission computerized tomography (SPECT) imaging to visualize the integrity of the dopaminergic pathways in the brain may be useful to diagnose PD (Crawford and Zimmerman, 2011). However, at this point, the underlying

neuropathological changes are already underway, and efforts in basic and clinical research have converged to suggest that PD actually begins well before onset of symptoms. In a recent review, Akhtar and Stern (2012) have highlighted early symptomatology in Parkinson's at-risk syndrome and how this conceptual framework can be useful to study early disease biomarkers and putative disease-modifying therapeutics. They have discussed clinical assessments, radiological studies, and molecular assays that may be useful in the early detection of PD. Clinically, PD is characterized by bradykinesia, postural irregularity, resting tremor, and rigidity. Molecular profiling promises to help in identifying of at-risk individuals, detecting the disease at early stages, improving diagnostic accuracy, aiding prognosis, as well as establishing biomarkers of therapeutic significance. Despite extensive efforts to define the molecular mechanisms underlying neurodegeneration in PD, many aspects of these pathologies remain unknown.

There is no doubt that early PD detection will pave the way for major advances in disease modifying therapies. Hence various diagnostic modalities hold promise for the early and preclinical diagnosis of PD. It is very likely that the future diagnosis of PD will rely on the combination of clinical, laboratory, imaging, and molecular genetic data. Chahine and Stern (2011) have reviewed recent developments in the early diagnosis of PD, with an emphasis on the detection of preclinical PD. Several clinical, laboratory, and imaging procedures are now being developed as early diagnostic biomarkers of PD. These include nonmotor features that occur before motor manifestations of PD, including sleep abnormalities, neurobehavioral symptoms, and olfactory dysfunction. Tests of the autonomic nervous system, such as cardiac functional imaging, allow measurement of cardiac sympathetic denervation in PD, discovered for the first time by Goldstein more than 15 years ago. PD patients have a diffuse left ventricular myocardial sympathetic denervation, a neurological condition present only in a small number of affected subjects. MIBG cardiac imaging is universally performed to estimate cardiac sympathetic innervations (Cascini et al., 2013). Furthermore, CSF and serum estimations of α-Syn and DJ-1 have been developed. Various imaging modalities have contributed to the diagnostic and theranostic applications, including transcranial Doppler ultrasonography, radiolabeled PET tracer imaging, MRI, and nanomedicine. Recently single-cell expression profiling of dopaminergic neurons combined with association analysis has identified pyridoxal kinase as a biomarker gene for PD prognosis (Vilariño-Güell et al., 2010; Gerlach et al., 2012). Besides genetic predisposition, biomarkers have been developed to determine vulnerability to PD several years prior to disease manifestation. These include premotor manifestations like hyper-echogenicity of the substantia nigra, olfactory and autonomic dysfunction, depression, REM sleep disorder, and neuropsychological impairments. Initial symptoms of involvement of the substantia nigra like PET and SPECT abnormalities and motor symptoms may be detected before a definite diagnosis can be established. However further studies are needed to establish the diagnostic accuracy of these biomarkers to determine the population at risk for PD, elucidate the etiology and pathophysiology, and to develop neuroprotective strategies (Berg, 2008). In a recent study, Pankratz et al. (2012) performed a meta-analysis to identify a novel RIT2 gene locus, responsible for genetic susceptibility to PD.

In general, the genetic biomarkers include mutational analysis of α-Syn, Parkin, and ubiquitin; electrophysiological biomarker for the estimation of motor action potential, ultrasonography for determining iron overloading in the substantia nigra; magnetic resonance imaging and spectroscopy to estimate brain regional N-acetyl aspartate; PET imaging with ^{18}F-DOPA to quantitatively estimate nigrostriatal dopaminergic neurodegeneration, ^{18}FdG to determine mitochondrial bioenergetics; and ^{123}Iflupane and β-CIT SPECT imaging to determine dopamine transporter (DAT) activity for early and effective clinical diagnosis, prognosis, and treatment of PD. Although none of the aforementioned interventions are yet to be established as PD biomarkers, brain imaging using the ^{123}I-ioflupane ligand with SPECT has been recently approved in the US to aid in PD diagnosis, and research on other imaging modalities is in progress.

In the present review we have systematically described various biomarkers (including premotor vs. motor, in vitro vs. in vivo, specific vs. nonspecific) for the early differential diagnosis and effective clinical management of PD. Furthermore, we have introduced α-synculein index, Charnoly body, coenzyme Q_{10}, and metallothioneins (MTs) as novel diagnostic biomarkers and discussed the merits and limitations of these and other recently discovered biomarkers for the safe and effective treatment of PD.

2. Rationale for biomarkers research

As described earlier, in idiopathic PD (IPD) many nigrostriatal (NS) dopaminergic neurons are already destroyed before a neurologist can confirm the diagnosis. Neuroprotective therapy starting at such an advanced stage may be unsuccessful to attenuate progressive neurodegeneration. Therefore, the identification of patients at risk at earlier stages is exceedingly important to accomplish therapeutic success. Moreover, early diagnosis of PD is important to slowdown disease progression at its initial stages.

3. Definitions of a biomarker

A biomarker is an indicator of a particular disease state or a particular state of an organism. It is a parameter that can be used to evaluate the progress of disease or the effects of treatment. The parameter can be chemical, physical or biological. According to NIH study group a biomarker is "*a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention*." In molecular terms biomarker refers to subset of markers that can be discovered by employing genomics, proteomics or imaging technologies. According to the Biomarkers Definitions Working Group, a biomarker is a characteristic that can be measured and evaluated as an indicator of normal biological and pathogenic processes or pharmacologic responses to a therapeutic intervention. Biomarkers facilitate early diagnosis, disease prevention, drug target identification, and drug response. Biomarkers can be classified into molecular biomarkers and imaging biomarkers (CT, PET, SPECT, and MRI). In general, molecular biomarkers refer to nonimaging biomarkers that have biophysical properties, which permit their measurement in biological samples (e.g., plasma, serum, CSF, bronchoalveolar lavage, biopsy). Molecular biomarkers include nucleic acids-based biomarkers such as gene mutations or polymorphisms, gene expression analysis, peptides, proteins, lipid metabolites, and other small molecules. Molecular biomarkers have been defined as biomarkers that can be discovered using basic genomics and proteomics strategies (Loukopoulos et al., 2007). Although significant discoveries have been made in biomarker research, a PD biomarker, that is simple, validated, and inexpensive, is yet to be made available. Depending on the kind of information they provide, biomarkers can be classified as clinical, neuroimaging, biochemical, genetic or proteomic. Indeed biomarkers serve a variety of functions, including confirmation of diagnosis, epidemiological screening, predictive testing, monitoring disease progression following diagnosis, drug development, response to treatment, and learning brain-behavior. Modern therapeutic

strategies for PD focus primarily on reducing the severity of symptoms using dopaminergic medications. However this approach has limited therapeutic potential for the clinical management of PD. Hence several investigators have now realized that there is a dire need of early, sensitive, and specific biomarker(s) for PD to improve drug development (Michell et al., 2004; Marek et al., 2008; Gerlach et al., 2008; Eller and Williams, 2009; Halperin et al., 2009; Maetzler et al., 2009; Morgan et al., 2010). Recently Gerlach et al. (2012) have suggested that the biomarker should be (a) linked to fundamental features of PD neuropathology and mechanisms underlying neurodegeneration, (b) correlated to disease progression as assessed by clinical rating scales, (c) able to monitor the exact disease status, (d) pre-clinically validated, (e) and confirmed by at least two independent investigators and results published in peer-reviewed journals. Moreover, a biomarker should be inexpensive, non-invasive, simple to use, and technically validated. Presently available literature has not yet qualified even one reliable biomarker to detect precisely early neurodegeneration in PD and monitor effects of drug candidates on the disease process, but some biomarker candidates including neuromelanin antibodies, pathological forms of α-Syn, DJ-1, gene expression, metabolomics, and protein profiling seem quite promising (Jellinger, 2012). Obviously the most reliable biomarkers could be used for early diagnosis, tracking disease progression, and development of effective treatments of PD. However most of the disease-associated changes are relatively small and overlap between patients and controls. Ray et al. (2010) have proposed that all of the aforementioned or new biomarker candidates will require detailed investigation in relation to validation in experimental models of PD, clinical prognosis, and confirmation in independent clinical trials. Currently, PD cannot be prevented or cured as no single diagnostic biomarker is available. Quite often it is difficult to make accurate diagnosis and distinguish PD from other diseases with overlapping symptoms of parkinsonism, particularly during the early stages of the disease. However, recent advances in diagnostic interventions, including molecular biomarkers, genetic testing, and imaging techniques permit earlier diagnosis, and improve clinical management of PD. Waragai et al. (2013) have reported that in addition to clinical symptoms and neuroimaging, a number of genetic and biological markers from CSF may hold promise for the early detection of PD. Biomarkers which are specific for PD, in combination with clinical symptoms may facilitate the early diagnosis and improve clinical management of PD. A suitable biomarker(s) for PD could facilitate early diagnosis, manage, and track the disease progression. Thus integrated analysis using various types of biomarkers may allow the detection of preclinical PD, which may facilitate prevention of disease onset with disease-modifying drugs.

Roongroj and Reichmann (2013) have described different diagnostic criteria for PD, highlighting specific limitations and pitfalls. With significant progress in the understanding of PD, particularly in a view of diverse clinical symptomatology and its evolution, it becomes difficult to establish a single criterion that is capable of capturing all cases at different disease stages. Despite a refined set of criteria that may facilitate recognition of PD, the diagnostic accuracy still depends on the clinical skills and knowledge of the physician. In addition to clinical symptoms and neuroimaging, a number of genetic and biological markers from blood and CSF may hold promise for the early diagnosis of PD.

4. Parkinson progression marker initiative (PPMI)

A clinical study sponsored by the Michael J. Fox Foundation, the Parkinson Progression Marker Initiative (PPMI), is an international, multi-center study designed to identify PD progression biomarkers to improve understanding of disease etiology and course to provide means to enhance the likelihood of success of PD modifying therapeutic trials (Marek et al., 2011). PPMI is expected to discover novel biomarkers of PD and will be carried out over five years at 20 centers in the US and Europe. The early diagnostic inaccuracy of PD is a major incentive behind these studies. The initiative, expected to cost US$ 40 million, will enrol 400 newly diagnosed patients of at least 30 years of age who are not yet on medication and who have evidence of dopamine transporter (DAT) loss on SPECT and 200 healthy age-matched controls. The PPMI will enhance further academic and industry-initiated studies and innovations and discover promising biomarker candidates. Eventually, well-defined biomarkers that are consistent in several labs will be established. The brain-derived CSF proteins are potential biomarkers considering the predominant role they play in PD pathogenesis. Gene expression analysis technologies will be employed to investigate altered pathways during degeneration and to identify potential biomarkers and drug targets. This study will perform clinical tests on blood, urine, CSF samples, and perform neuroimaging, to draw scientific conclusions, develop better strategies to determine the progression of PD, and will be made available through agencies interested in drug development. The ultimate goal of the PPMI is to discover one or more biomarkers for early and accurate diagnosis of PD. The discovery of a biomarker of PD is critical to the development of new and better treatment options that could slow or halt the disease progression. The initial emphasis is on fluid biomarkers including α-Syn, DJ-1, amyloid β, and tau in the CSF, and urate in the blood. The most promising biomarker candidates will be tested using neuroimaging, blood, urine, CSF, and DNA sampling, motor, neuropsychiatric, and cognitive assessments. All data and biological specimens, stored in a central repository will be available for the research community. Thus PPMI will provide a valuable resource to trigger further academic and industry-initiated investigations and innovations, and promising biomarker candidates will be validated against the large prospective PPMI dataset. Detailed analyses of correlations between different biomarkers might help to generate useful screening algorithms. This study might also enable the identification of different pathophysiological subgroups of patients who respond differently to certain drugs, thereby allowing treatment to be tailored to individuals. The primary objective is to standardize processes across participating centers, and quality-control mechanisms to ensure data are acceptable before they are made available to public. Standardization is quite feasible for CSF and blood biomarker data, but combining imaging data with different devices is likely to be more complicated. Moreover, for biomarkers to be useful, they would need to be economical and easy to apply on a large scale, hence fluid-based biomarkers might be more applicable than those based on sophisticated imaging modalities. While this initiative might give rise to biomarkers that would be useful at a group level, the substantial variability in disease manifestations means that their applicability to individual patients – and the hope for personalized medicine – might be difficult. Despite the challenges, PPMI is expected to provide a comprehensive, longitudinal and prospective dataset, with open access it will undoubtedly complement and stimulate traditional investigator-initiated biomarker research in PD. In brief, participants will undergo diagnostic tests including motor, neuropsychiatric and cognitive examinations; brain imaging with DAT-SCAN and MRI; and blood, CSF, urine and DNA analyses. All the data will be integrated in the PPMI database and will be available through the PPMI web site www.ppmi-info.org. Blood, CSF, and urine samples will be available to the scientific community through PPMI specimen review committee and through the PPMI web site. Obviously, PPMI will rely on a partnership between government, PD foundations, industry and academic institutions. This approach is crucial to enhance the successful development

of PD biomarkers which will accelerate research in disease modifying therapeutics. The University of California, San Diego School of Medicine is one of 20 official study sites for the PPMI, which will use a combination of advanced imaging, biologics sampling and behavioral assessments to identify biomarkers for PD progression. Enrollment of 20 patients and 10 controls is expected to continue for two years. PPMI holds potential, not only to accelerate the development of future PD treatments but also to improve the diagnosis and treatment of modern generation of PD patients. Similarly, the Arizona PD center will discover biomarkers that have high predictive values for the diagnosis of both PD and PD with dementia (PD-D). The clinical data will be validated by autopsy for diagnosing PD. Multiple projects have been included that will explore potential biomarkers in the CSF and brain tissue for PD and PD-D. This program will also utilize the antemortem data to predict the likelihood of individuals to progress to PD or PD-D. The clinical core will evaluate subjects in the brain donor program categorizing them as being controls, PD, and PD-D. The primary goal of this study is to discover biomarkers that will be used to predict who will develop PD and PD-D. In addition, the clinical core will provide a detailed description of the autopsy material. The neuropathology core will perform autopsies, provide data on Lewy bodies and other biomarkers to correlate with the clinical data, brain tissue and CSF analyses. The first project will investigate whether a loss of brain-derived neurotrophic factor (BDNF) in the cortex of PD-D may lead to neuronal susceptibility to cell death. The second project will determine whether mitochondria from patients of PD-D have different levels of the TNF type 1 death receptor (TNFR1) and whether this influences changes in a-Syn and DJ-1. The third project will utilize microarray analyses to identify the genetic pathways leading to α-synulein/DJ-1 aggregation and cell death. The fourth project will utilize CSF proteomics to investigate biomarkers that distinguish PD without dementia from PD-D. All these projects will propose randomized controlled trials of treatments that will be designed to stop or slow down the progression of PD or PD-D. PPMI will employ several genomic and proteomics procedures and recently discovered metabolomics, lipidomics, and glycomics in their identification. Genomic approaches including (a) Northern blot (b) gene expression (c) SAGE (iv) DNA microarray; and proteomic approach including (a) 2D-PAGE (b) LS/MS (c) SELDI-TOF (d) antibody microarray (e).tissue microarray. They will provide a wealth of basic information to validate early, sensitive, and specific biomarkers of PD. The information gathered from these procedures is critical to the future development of new and better treatments of PD. (*More detailed information regarding other study centers can be obtained from the PPMI website www.ppmi-info.org*)

5. Biomarker classification

In general various biomarkers for PD can be divided into two major catagories; (i) premotor (preclinical) biomarkers, and (ii) motor (clinical) biomarkers. These biomarkers can be further subdivided into two categories (i) in vitro biomarkers and (ii) in vivo biomarkers. Based on the currently available information and ease of description, we have now divided these biomarkers as (a) clinical (b) biochemical (c) biophysical (d) physiological (e) genetic (f) morphological (g) immunological, and (h) pathological categories as illustrated in Fig. 1.

6. Premotor/preclinical (nonmotor) biomarkers

It is now well recognized that a long preclinical or asymptomatic period may occur in PD. Hence the presence of early risk factors in PD is consistent with a long prodromal period. As

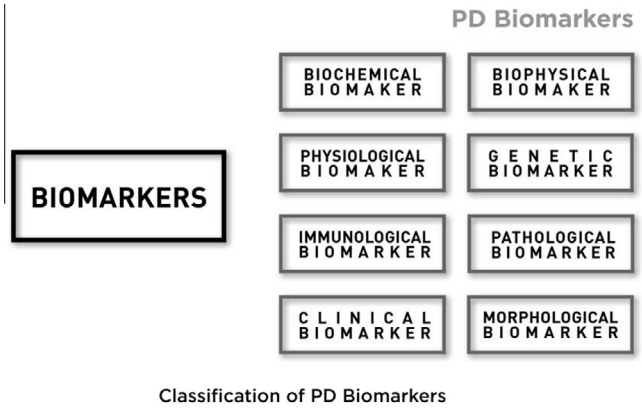

Fig. 1. A flow diagram illustrating various major classes of PD biomarkers. PD biomarkers may be divided into following broad classification based on the biomedical field they belong such as (i) biochemical (ii) biophysical, (iii) physiological, (iv) genetic (v) morphological, (vi) pathological, (vii) immunological, and clinical biomarkers as illustrated in this diagram.

described above, marked degeneration of the substantia nigra and loss of striatal dopamine occur before clinical symptoms become obvious. Moreover Lewy bodies, the histological hallmark of PD, occur even in 10% of normal individuals over 50 years of age. Clinical symptoms develop slowly and are often intermittent in early PD and nonmotor signs (depression or sensory changes) often precede motor signs after several years. Although reduction in striatal dopamine can be detected with PET in "at-risk" asymptomatic individuals, this diagnostic approach is costly and available only in advanced clinical centers. Individual sensitivity to drug-induced parkinsonism may also suggest a preclinical state. Hence economical and novel biomarkers may detect preclinical PD (Koller, 1992). As both premotor and risk biomarkers play a crucial role in the pre-diagnostic phase of PD, the ultimate aim is to determine the relationship between the risk factors, hyperechogenicity of the substantia nigra (SN+) and/or positive family history of PD (faPD+), and putative premotor biomarkers of PD. In a PRIPS cohort, Liepelt-Scarfone et al. (2011) studied 1149 volunteers older than 50 years free of PD. In addition to the risk factors SN+ and faPD+, olfactory dysfunction was tested using the Sniffin' sticks and motor examination. History of depression and constipation was evaluated by a semi-structured interview. Of all individuals, 880 had none of the risk biomarkers (76.6%), 143 (12.4%) had SN+, 84 (7.3%) were classified as faPD+ and 42 (3.7%) had both risk factors. Volunteers with SN+ demonstrated olfactory dysfunction and mild motor impairment. Depression was more prominent in individuals having two risk factors. Accumulation of premotor biomarkers was observed in the SN+ group with or without faPD+, but not in patients with faPD+ only. Thus the profile of premotor biomarkers seemed to differ in patients having SN+ and/or faPD+, with SN+ exhibiting the highest association with premotor biomarkers, suggesting that SN+ could be a reliable biomarker of dopaminergic neurodegeneration in PD.

7. Presymptomatic biomarkers

As a chronic progressive disease, PD has a presymptomatic period during which the pathological process begins, but motor signs required for the clinical diagnosis are absent. The ability to identify the preclinical stage is critical for the development and application of neuroprotective therapy in PD. In the early clinical phase, a number of motor and non-motor signs can be identified several years before the diagnosis is made, particularly olfactory dysfunction, depression, or motor signs such as changes in handwriting,

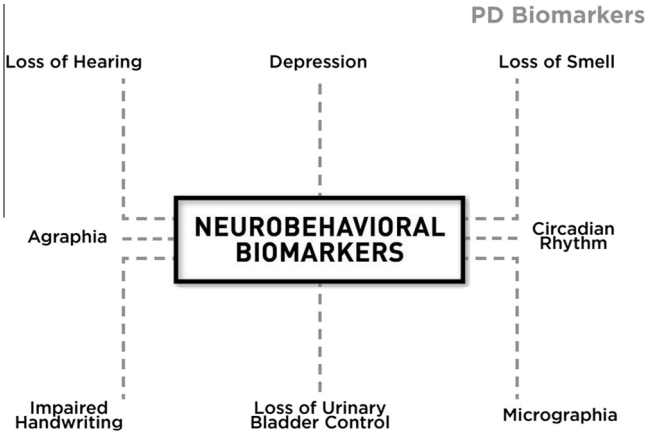

Fig. 2. A flow diagram illustrating various neurobehavioral biomarkers of PD. Neurobehavioral biomarkers can provide a wealth of basic information regarding PD progression and alleviation of typical symptoms which could induce deleterious consequences in a PD patient. For instance >40% patients of PD exhibit typical symptoms of depression due to depletion of not only dopamine but also serotonin. Persistent depression may lead to loss of cells in the hippocampal CA3 and dentate gyrus. The loss of neurons in these regions is the early cause of dementia and morbidity in these patients. Antidepressants including specific serotonin reuptake inhibitors including Fluoxetine have shown promise in PD patients. Recent studies have demonstrated that Fluoxetine exhibited reduction of apoptosis in the hippocampal region in gene manipulated mice exhibiting loss of dopaminergic and hippocampal neurons. In addition PD patients exhibit impairments in the sleep wakeful cycle due to abnormal circadian rhythm, remain awake during night, and sleep a lot during the day time. Recent studies have shown that although PD patients did not exhibit any reduction in the circulating melatonin levels, their somnograms exhibits significant impairment in the REM phase sleep. Furthermore, PD patients had increased incidence of nocturia without any evidence of benign prostate hypertrophy which also hampers in the quality of their sleep. Furthermore, these patients exhibit loss of olfaction and hearing deficit (deafness) in addition to impaired handwriting which is represented by micrographia, bizzar handwriting, and eventually agraphia, when these patients become completely bed-ridden. At this point PD patients become speechless, motionless with swallowing, digestion, defecation, micturition, ejaculation difficulties before mortality.

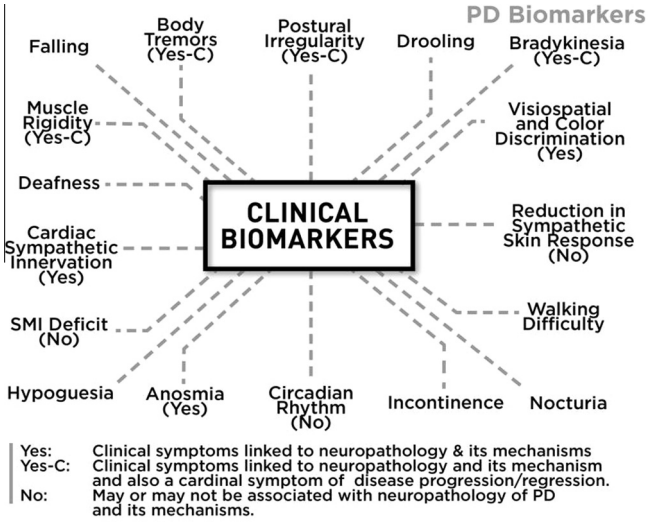

Fig. 3. A flow diagram illustrating general types of PD biomarkers. In general, PD biomarkers can be divided into two major types such as in vitro and in vitro Biomarkers. The in vitro and in vivo biomarkers could be specific or nonspecific. However there are two major clinical stages in the progression of PD; Premotor, and motor period. Although 60–65% nigrostriatal dopaminergic neurons are degenerated during premotor phase the PD patient remains free from typical symptoms including body tremors, muscular rigidity, and postural irregularity. These cardinal symptoms are noticed only during motoric impairment phase. Hence we can divide premotor vs. motor biomarkers based on the clinical diagnosis. Various symptoms such as body tremors, postural irregularities, drooling, walking difficulty falling, nocturia, impaired circadian rhythm, loss of smell, loss of hearing, muscle rigidity, and loss of urinary bladder control may be noticed in PD patients, however these symptoms may or may not confirm PD diagnosis. Hence reduction in the ^{18}F-DOPA uptake with PET imaging, and Lewy body pathology following the death of a patient only can confirm the PD diagnosis. Hence these are only two consistent diagnostic biomarkers which authenticate the Disease. In fact ^{18}F-DOPA uptake may be used as criteria of effective treatment clinical prognosis of PD as the primary objective of effective treatment is to halt the progressive nigrostriatal dopaminergic neurodegeneration in PD. Usually dementia is not noticed during premotor period of PD patients. Parkinsonian dementia occurs very late during the progression of disease.

speech, or reduced ambulatory arm motion. These signs can be detected by easy and inexpensive tests. As one single diagnostic modality may not be adequate, a battery of tests has to be performed to estimate the parameters of the incipient disease. Patients with abnormal findings may eventually be subjected to nuclear medicine evaluation to determine the extent of dopaminergic loss for effective treatment of PD (Becker et al., 2002). Recently proposed staging systems of PD have suggested that neurodegeneration may occur initially in areas outside the substantia nigra, suggesting that non-motor manifestations may be markers of presymptomatic PD (Postuma et al. (2010). Decreased olfaction has been demonstrated to predict PD in pathological studies, although the positive predictive value is low. Idiopathic RBD has a high predictive value, with approximately 50% of affected individuals developing PD or dementia within 10 years, suggesting that idiopathic RBD patients are highly suitable to test preclinical biomarkers. However, the specificity of symptoms for RBD is not yet established. Moreover not all persons with PD develop RBD, and there are only limited interventions available to predict which RBD patients will develop PD. Hence other tests based upon autonomic symptoms; depression, and personality changes, quantitative motor testing, and sleep disorders may be useful biomarkers, but have not been extensively studied. More expensive interventions such as autonomic testing, cardiac MIBG-scintigraphy, dopaminergic imaging, and transcranial ultrasound may be useful in defining disease risk in those identified through primary screening. Neurobehavioral biomarkers of PD include (i) depression, (ii) hyposmia (loss of smell), (iii) altered circadian rhythm (sleep-wakeful cycle), (iv) micrographia (small words), (v) incontinence (loss of urinary bladder control), (vi) deafness (loss of hearing), (viii) agraphia (impaired handwriting), and (ix) acalculia (difficulty solving simple calculations due to dementia) as illustrated in Fig. 2.

8. Clinical biomarkers

There are several clinical biomarkers including body tremors; postural irregularity; muscular rigidity, bradykinesia, walking difficulty, incontinence, nocturia, altered circadian rhythm, hyposmia, hypoguesia, impaired visio-spatial and color discrimination, cardiac sympathetic innervation, deafness, muscle rigidity, falling, body tremors, postural irregularity, drooling, reduction in sympathetic skin response, and neurobehavioral deficits (depression, dementia, olfaction, nocturia, impaired circadian rhythm, narcoplesy,and impulsive behavior).as illustrated in Fig. 3.

9. Impulse control disorder in PD

Recently Vilas et al. (2012) have reported that the dopaminergic therapy to relieve motor manifestations of idiopathic PD may cause inappropriate hypersexuality. However, the pathophysiological basis of hypesexuality in PD patients on L-DOPA therapy remains enigmatic. A pathological reward system may underlie addiction, although the precise role of dopaminergic pathways remains controversial (Berridge, 2007). Recently Politis et al. (2013) provided an important mechanism in elucidating the pathophysiology

of impulse control disorders in PD whereas Ushe and Perlmutter (2013) provided guidelines for the prevention and treatment of impulsive behavior among PD patients. Usually impulse control disorders including gambling, eating, and hypersexuality seem to occur more commonly with dopamine receptor agonist therapy than L-DOPA and can lead to substantial morbidity with social, psychological, and legal consequences (Vilas et al., 2012). The unregulated dopamine receptor activation may provide potential clues to the underlying mechanisms. Interestingly, dopaminergic pathways may also play a key role in neuronal networks involved in the reward system, and pathological responses in this system may underlie addiction (Volkow et al., 2012). Similarities between addictive behavior and drug-induced hypersexuality in PD suggest that they may share common neuropathological mechanisms. Thus investigations of brain regions and neurocircuitry related to dopamine-induced hypersexuality in PD could shed some light on these potentially common underlying mechanisms and provide clues for rationale treatment. Moreover, dopaminergic neurotransmission has been linked to drug abuse (Blum et al., 2012). In fact, the dopamine dysregulation syndrome in parkinsonian patients with L-DOPA craving, shares several clinical features with drug addiction (Katzenschlager, 2011). Recently Berridge (2012) proposed the theory of incentive salience and sensitization as the mechanism by which dopamine acts in reward pathways. Incentive salience means that the 'wanting' of stimulus has higher valence than achieving the reward from the stimulus, and this may be mediated by mesolimbic dopaminergic pathways. Politis et al. (2013) have investigated in 12 patients with hypersexuality in association with PD and 12 without hypersexuality. Ratings of sexual desire (the 'wanting') after sexual image exposure correlated with BOLD responses in the posterior cingulate and ventral striatum (L-DOPA OFF) and anterior cingulate and medial orbitofrontal cortex (L-DOPA ON) in PD with hypersexuality group, whereas the degree to which each participant liked the images did not correlate with responses in any brain region suggesting that dopamine acts on reward pathways through salience of the stimulus (Berridge, 2007). Furthermore, dopamine may interfere with control circuits involved in reducing desire in patients of PD with hypersexuality. Interestingly, sexual visual cues did not induce any response in the ventral striatum of the PD group without hypersexuality, whereas other investigators have demonstrated this phenomenon even in subjects without PD (Kringelbach and Berridge, 2009; Oei et al., 2012). However Politis et al. (2013) did not include a control group without PD in their study. The major limitation in their study was that the patients with hypersexuality were taking more dopamine agonists than the PD control group. Thus dopaminergic agonists may have longer lasting effects than L-DOPA that could bias the BOLD responses as well as the neurobehavioral findings. Dopaminergic agonists may also have regional and pathway specificity that differs from L-DOPA. Furthermore, since dopaminergic agonists are more likely to produce impulse control disorders in patients with PD than L-DOPA, the group differences could be a brain response to drug treatment rather than a manifestation of the disease (Weintraub et al., 2010). However, this does not undermine the importance of investigating neurocircuits that are implicated in hypersexual behavior among PD patients on L-DOPA therapy. There has been increasing concern regarding the potential effects of movement in BOLD functional MRI studies. Power et al. (2012) performed analysis to eliminate those scans with body movements by excluding volumes that had >2 mm movement and checked that movement metrics did not differ across groups. However it is more likely to introduce noise thereby reducing sensitivity for the identification of other BOLD signals. These studies have enhanced our basic understanding of hypersexuality associated with PD and generated interest on the analysis of impulse control disorders in patients with PD on dopaminergic medications.

10. Sex hormones

Extensive literature has been reported on the neuroprotective effect of estrogens and the ovarian hormone progesterone as well as androgens in clinical and experimental animal models of PD (D'Astous et al., 2005; Bourque et al., 2009; Sánchez et al., 2010). Particularly dehydroepiandrosterone, the precursor of estrogens and androgens, exhibits significant influence on brain regional dopaminergic neurotransmission. Clinical and experimental evidence have supported the role of steroid-dopamine interactions in the pathophysiology of PD and other related disorders. Although dopaminergic cell loss is well documented in PD, dopamine hypofunction is proposed in certain depressive states, whereas dopamine hyperactivity is implicated in schizophrenia. The sex differences in these diseases remains the focal point of many studies and could be explained not only by genetic differences but also by an effect of steroids in the brain. Sánchez et al. (2010) demonstrated in animal models the effects of estrogens, progesterone, and androgens on biomarkers of dopaminergic neurotransmission. They investigated the effects of selective estrogen receptor modulators (SERMs), estrogen receptors, and their specific drugs, as well as progesterone drugs, and suggested that specific steroidal receptor agonists and SERMs prescribed for endocrine and cancer treatments may find applications in PD and other neurological disorders.

11. Molecular pathology biomarkers

In the past few years, there have been advances in understanding regarding intra-and extracellular proteins that may display impaired function or expression in AD, PD, and other neurodegenerative disorders, associated with amyloid beta (Aβ), α-Syn, tau protein, and neuroinflammatory markers. It is now known that at least 10% of diagnoses of PD that are made while the patient is alive are not confirmed at autopsy. To help address this issue the European Federation of Neurology and the Movement Disorder Society European Section have provided evidence-based guidelines and recommendations for the clinical diagnosis of PD (Martí and Tolosa, 2013). This is a synthesis of current materials and techniques used to not only explore but also to validate potentially new biomarkers of dementias associated with PD, AD, and Lewy body pathology. The molecular Pathology biomarkers including α-Syn index, Lewy body formation, nigrostriatal dopaminergic loss, and the striatal iron accumulation are illustrated in Fig. 4(*panel A*). A histological picture of a typical α-Syn-positive Lewy body is presented in (*panel B*). (*Courtesy: Dr. Denis Dickson, Mayo Clinic Jacksonville, Florida*).

12. Serotonin as a biomarker of PD

Early post-mortem data suggest that damage to brain serotonergic neurons might play a significant role in depression of PD (Karen et al., 2011). However, it remains unknown whether such damage is a characteristic feature of patients with PD or whether the changes are widespread. To address this question, Guttman et al. (2007) performed PET imaging to estimate the brain serotonin transporter (SERT) activity, a marker for serotonergic neurons, as inferred from binding of [^{11}C]-3-amino-4-(2-dimethylaminomethyl-phenylsulfanyl)-benzonitrile (DASB), a second generation SERT radioligand, in subcortical and cerebral cortical brain areas of clinically advanced non-depressed patients with PD. The SERT binding in PD were lower than those in the controls, with

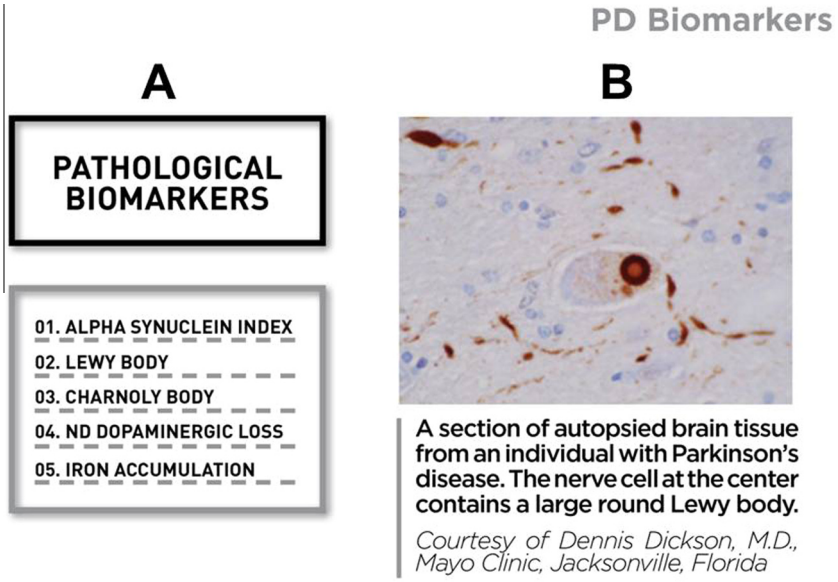

Fig. 4. A flow diagram illustrating various molecular and pathological biomarkers of PD. The pathological biomarkers are the hallmarks of PD diagnosis and its final confirmation. However these biomarkers either biopsy or autopsy samples to confirm the final diagnosis. These samples provide information regarding the presence of filamentous Lewy bodies. Lewy bodies are around the dense core of degenerated dopamineregic neurons and exhibit α-Syn immunoreactivity. Lewy bodies exhibit Parkin and ubiquitin positivity but not a prominent as compared to α-Syn. In addition the striatal region exhibit lot of iron deposits which can be confirmed histochemically or by estimating its concentrations employing sensitive atomic absorption spectrophotometry using a specific spectral wavelength lamp or inductively coupled plasma mass spectrometry (ICP-MS) which can provide information about as many as 34 metal ions in 10 μL sample within 20 min. However Charnoly bodies (CBs) are formed at an earlier stage as a consequence of mitochondrial degeneration. These electron-dense penta or heptalamellar structures are formed as a consequence of oxidative and nitrative stress leading to α-Syn aggregation and eventually progressive loss of dopaminergic neurons. Although CB formation is sensitive and can be detected early compared to Lewy body formation it is not a specific diagnostic biomarker of PD prognosis and diagnosis. It can only confirm that neurodegeneration has initiated in any neurodegenerative disease including PD. We have reported that MTs inhibit CBs formation in the degenerating neurons by acting as free radical scavengers and hence Lewy body pathgenesis (Sharma et al., 2013).

significant changes in the orbitofrontal cortex, caudate, putamen, and midbrain. However, only a slight reduction was noticed in the dorsolateral pre-frontal cortex (*an area implicated in major depression*), suggesting that loss of brain regional serotonergic innervation might be a common feature of advanced PD. Further investigations are needed to establish whether SERT binding is decreased in patients with PD who also have major depressive disorder (MDD). Andreasen and Blennow (2005) evaluated three CSF biomarkers (the 42 amino acid form of β-amyloid (Aβ), total tau, and phospho tau). These biomarkers have high sensitivity to differentiate early PD and AD from normal aging, depression, and alcohol dementia, but lower specificity against other dementias biomarkers. Blennow (2004a) has pointed out that If these biomarkers are used along with medical history, clinical examination, laboratory tests, and molecular neuroimaging, the PD diagnostic accuracy can be further enhanced. Some candidate biomarkers including ubiquitin, neurofilament proteins, growth-associated protein-43 (neuromodulin), and neuronal thread protein (AD7c) have also shown promising results but require further investigations.

13. Nonmotor biomarkers

Although motor dysfunction represents the best characterized symptoms, the non-motor symptoms (NMS) can be equally debilitating for PD patients. Cognitive impairment and dementia are among the most common nonmotor changes in PD. Although our understanding of PD-associated nonmotor symptoms (NMS) has increased considerably there is still a lack of awareness of the importance of NMS for patients with PD. A comprehensive survey of members of the charity Parkinson's UK took place in 2008. This resulted in returns from 10,101 patients with PD. The self-completed non-motor questionnaire (NMSQuest) and quality of life scale (PDQ-8) were evaluated in this survey. The results showed that the percentage of people with PD experiencing NMS increased with the duration of the disease. However, people who had the younger onset reported a greater impact of NMS, in memory, depression, and sleep pattern. There was an inverse correlation between NMS and PDQ-8 scale. Recently Breen and Drutyte (2013) reported that a significant number of people with PD experience problems with olfaction, taste, nocturia, and constipation prior to diagnosis, which may help to serve as biomarker(s) of PD. Hence further studies are needed to identify the best treatment options that should be implemented to address NMS. Balzer-Geldsetzer et al. (2011) reported on the diagnostic utility of neuropsychological tests obtained from a large cohort of nondemented and demented patients with PD. They introduced factor and cluster analyses that differentiate between different subtypes of PD patients, detect similarities between test results, and reduce large number of biomarkers to increase the quality of clinical data to improve understanding of PD and related disorders.

14. Sleep disturbance

Out of many early biomarkers of PD, is the abnormal rapid eye movement sleep behavior disorder (RBD) (Boeve, 2010). REM sleep abnormalities (RBD) are present in approximately 50% of the patients, suggesting a heterogeneous pathophysiology in PD. In addition to RBD; olfaction, constipation, and depression may be present during the prodromal phase of PD. Particularly idiopathic RBD is characterized by loss of atonia, resulting in motor activity during dreams. In addition, visual changes, autonomic symptoms, and subtle cognitive changes may also be present at prodromal stages. Postuma et al. (2012) provided evidence for the utility of idiopathic RBD, olfaction, autonomic biomarkers, visual changes, mood disorders, and cognitive loss as biomarkers of prodromal PD and the

sensitivity and specificity of these biomarkers. A critical issue in utility of these biomarkers is the assessment of sensitivity, specificity, and positive and negative predictive values. Although these features are yet to be fully explored, olfactory deficits, visual changes, and autonomic symptoms occur in the majority of PD patients. However, with the exception of RBD and some specific autonomic measures, specificity and predictive value of these biomarkers may be insufficient to be used alone as biomarkers of prodromal disease. In their earlier studies Postuma et al. (2006) compared 25 patients with polysomnography-confirmed RBD without PD with age-and sex-matched controls. They also evaluated color vision, olfaction, quantitative motor testing, and indices of depression, personality, and autonomic function in this study. PD patients demonstrated significant impairment in color discrimination and olfactory function, and had abnormalities in quantitative testing of motor and gait speed. Autonomic symptoms were more common in PD patients than controls. Moreover abnormalities were heterogeneous, with some patients scoring normally in all domains whereas others were severely impaired in multiple domains. Patients who performed poorly on one test tended to perform poorly on the others as well. Usually excessive daytime sleepiness is common in PD and has been associated with PD-related dementia. Narcoleptic features are observed in PD patients with excessive daytime sleepiness and hypocretin cell loss has been observed in the hypothalamus of PD patients. Mignot et al. (2002) conducted a study to delineate hypocretin deficiency syndrome and to establish CSF hypocretin-1 as a diagnostic tool for narcolepsy, HLA-DQ, clinical data, the multiple sleep latency test (MSLT), and CSF hypocretin-1 were studied in PD patients with sleep disorders. Signal detection analysis was used to determine the CSF hypocretin-1 levels predictive for International Classification of Sleep Disorders (ICSD)-defined narcolepsy. Hypocretin-1 levels <110 pg/mL were used as a diagnostic criteria for narcolepsy. Values above >200 pg/mL were considered normal. Most subjects with low levels were HLA-DQB-positive narcolepsy-cataplexy patients. Based on these findings they concluded that CSF hypocretin-1 may be used as a definitive diagnostic test provided it is interpreted within the clinical context particularly in cases with cataplexy when the MSLT is difficult to interpret in subjects already treated with psychoactive drugs or with other sleep disorders. Compta et al. (2009) estimated CSF hypocretin-1 in PD patients with and without dementia to study its relationship to dementia and excessive daytime sleepiness. Twenty-one PD patients without dementia and 20 PD patients with dementia, along with 22 controls without sleep complaints, participated in this study. Both the Epworth sleepiness scale and the mini-mental state examination were recorded. Eight PD patients without dementia and seven PD patients with dementia underwent the video-polysomnogram and multiple sleep latencies test. The Epworth sleepiness scale scores were higher in PD patients without dementia and PD patients with dementia than controls and >10 scores were more frequent in PD patients with dementia than in PD patients without dementia. Lumbar CSF hypocretin-1 levels were similar among groups, and unrelated to either the Epworth sleepiness scale or the mini-mental state examination. Dominant occipital awake frequency was slower in PD patients with dementia than in PD patients without dementia. Presence of slow dominant occipital frequency and/or loss of normal NREM sleep architecture were frequent among PD patients with dementia. Thus, excessive daytime sleepiness was more frequent in PD patients with dementia than PD patients without dementia. However lumbar CSF hypocretin-1 levels were normal and unrelated to severity of sleepiness or the cognitive status. Thus the lumbar CSF did not accurately reflect the hypocretin cell loss in the hypothalamus of advanced PD, suggesting mechanisms other than hypocretin dysfunction might be responsible for excessive daytime sleepiness and altered sleep behavior in PD patients (Compta et al., 2009). Although several studies on the efficacy and the toxicity of exogenous melatonin in PD patients have been carried out, there are no systematic data on melatonin secretion in these patients. A small number of controlled trials indicated that melatonin is useful in treating disturbed sleep in PD, in particular RBD. Hence melatonin and melatonergic agents (Ramelteon, Tasimelteon, Agomelatine) may have therapeutic potential in PD (Srinivasan et al., 2011).

15. Hyposmia

Hyposmia, psychiatric disorders, and cognitive problems are common nonmotor manifestations of PD, but how they are inter-related remains enigmatic. Recently Morley et al. (2011) have studied a relationship between olfactory dysfunction and neuropsychiatric manifestations by performing a cross-sectional study of 248 patients. Psychiatric measures were the Geriatric Depression Scale-15, Inventory of Depressive Symptomatology, State Anxiety Inventory, Apathy Scale, and Parkinson's Psychosis Rating Scale. Cognitive measures were the Mini-Mental State Examination, Hopkins Verbal Learning Test-Revised, Digit Span, Tower of London-Drexel, and the Stroop Color Word Test. Olfaction was tested with the University of Pennsylvania Smell Identification Test. There was no significant association between olfaction and mood measures, but psychotic symptoms were more common in patients with olfaction scores below the median. Worse olfaction was associated with impaired memory (Hopkins Verbal Learning Test-Revised delayed recall items: and executive performance). Odor-identification score was a significant predictor of abnormal performance on the cognitive tests after adjustment for age, sex, and disease characteristics. Thus the relationship between hyposmia, psychosis, and specific cognitive impairments may reflect the distribution of Lewy body pathology and suggests that olfactory dysfunction could be a biomarker of PD. Future studies are needed to assess whether hyposmia, an early feature of PD, might be used to predict the appearance of other nonmotor symptoms.

16. Depression

Depression is the most common neuropsychiatric co-morbidity in PD. Depression in PD is distinguished from other depressive disorders by anxiety and less self-punitive ideation. Depressive symptoms affect 40–50% of PD patients and can adversely impact their quality of life. Approximately half of the depressed patients with PD meet criteria for MDD and half have dysthymia. Depression in PD is associated with bradykinesia and gait instability unlike in tremor-dominant syndromes. Depressed patients with PD have greater frontal lobe dysfunction and involvement of dopaminergic and noradrenergic systems than nondepressed PD patients. The underlying mechanism of depression in PD is complex and involves biological, psychosocial, and therapeutic factors. The biological mechanism may involve changes in monoaminergic systems, particularly the serotonergic system since a decrease of 5-HT in the synaptic cleft is considered the cause of depression. Recently Tan et al. (2011) reported that mesencephalic dopaminergic neurons (mDA) and serotonergic (5-HT) neurons are involved in depression. Degeneration of mDA is associated with PD; and defects in the serotonergic system are related to depression, obsessive–compulsive disorder, and schizophrenia. Although these neuronal subpopulations reveal positional and developmental relationships, the physiological events that govern specification and differentiation of mDA or 5-HT neurons revealing missing determinants are not yet understood precisely. However the serotonergic system is markedly affected in the parkinsonian brain with evidence of loss of axons as well as cell bodies in the dorsal and median raphe

nuclei of the midbrain. However, it remains unresolved whether alteration of the serotonergic system alone is sufficient to confer vulnerability to depression. Low 5-HT combined with altered network activity within the basal ganglia is involved in depression among PD patients. The latter hypothesis is derived from recent findings that highlight the interaction between the basal ganglia and the serotonergic system, not only in motor functions but also limbic functions. These findings provide further evidence that depression is a side effect of deep brain stimulation (DBS) of the subthalamic nucleus (STN), a treatment option in advanced PD. Furthermore, it has been shown that DBS in animal models inhibits serotonergic neurotransmission and that this change may induce depressive side effects. We have reported that a tryptophan-rich diet can alleviate symptoms of depression as it is a precursor for the synthesis of brain regional 5-HT (Shabbir et al., 2013). The reuptake of 5-HT released into the synaptic cleft is mediated by the 5-HT transporter (5-HTT). Hence studies have been focused on the relationship between the 5-HTT-linked polymorphic region (5-HTTLPR) and depression. Zhang et al. (2009) have investigated an association between the polymorphisms in the promoter region of the 5-HTT gene (including 5-HTTLPR and rs25531), which determine either a higher or lower 5-HT uptake, and risk of depression in PD patients. They randomly recruited 306 idiopathic PD patients for epidemiological studies depression scale (CES-D) analysis for the clinical diagnosis and rating scale of depression. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used for genotyping the PD patients. No evidence of association between variants of 5-HTTLPR and rs25531 alleles, and depressive symptoms in PD patients was noticed. In general, MAO inhibitors have been used for the treatment of PD, dementia, and depression. Hwang and Kim (2004) studied the effects of M. alba extracts on the changes of the MAO activities during and after physical exercise in rats. Activity was measured by 5-HT and benzylamine as substrates. MAO-A activity was decreased with stress of physical activities compared to the normal group, whereas MAO-B activity was increased for 60 min after exercise. These indices were recovered to normal by oral administration of M. alba extract suggesting that it may modulate the MAO activity and promote physical endurance and exhibit anti-stress effect. Another study was undertaken to assess whether general risk factors for depression are also markers of depression in patients with PD and to identify additional disease-specific biomarkers. A two-step logistic regression was performed on data from 161 PD patients, 40 of whom suffered from major depressive disorder. A first logistic model was created with five general risk factors for depression. Five potential disease-specific biomarkers were used to explore whether this would improve the model. The logistic model of general risk factors for depression also predicted depression in PD patients. A family history of depression was the most important biomarker but right-sided onset was the only disease-specific biomarker that improved the model. However established risk factors for depression in the general population are also biomarkers of depression in PD. Lower CSF levels of 5-hydroxyindoleacetic acid, (5-HIAA), a past history of depression, and greater functional disability are associated with a greater risk of depression in PD. Female gender, early age at onset of PD, and greater left brain involvement may also be risk factors. Consequently the importance of correcting for general risk factors for depression in PD has been emphasized by Leentjens et al. (2002). Moreover mood changes in PD respond to treatment with conventional tricyclic antidepressants (TCAs) or electroconvulsive therapy (ECT). Depression in PD may be mediated by dysfunction in mesocortical/prefrontal reward, motivation, and stress-response systems. Neuropsychological, metabolic, clinical, pharmacological, and anatomical studies support the involvement of frontal dopaminergic projections in patients with PD and depression. Overexpression of the wild type or mutant α-Syn affects the generation of new neurons in the hippocampal dentate gyrus in experimental models of PD. Hippocampal dysfunction with reduced neurogenesis plays a significant role in the pathogenesis of depression in PD. Recently Kohl et al. (2012) conducted a study to explore whether impaired hippocampal neurogenesis in the A53T transgenic animal model of PD may be restored by oral administration of the SSRI, fluoxetine. Fluoxetine increased neurogenesis in the hippocampus threefold in treated A53T mice compared to controls. The pro-neurogenic effect of fluoxetine was related to an increased proliferation of neural precursor cells in the DG, and to a lesser extent by induction of differentiation into mature neurons. Fluoxetine induced brain and glial cell-derived neurotrophic factor suggesting the potential utilization of SSRI-dependent mechanisms to promote hippocampal neurogenesis in experimental models of α-Syn. Further studies in this direction may lead to better understanding of neuropsychiatric symptoms and their alleviation in PD.

17. Electrophysiological biomarkers

Earlier studies on the Saudi population have shown significant differences in somatosensory evoked response and brain stem auditory evoked response as compared to age-matched controls, however, the clinical characteristics of PD were not significantly different from those reported for patients elsewhere (Al-Bunyan, 2000). Recently, Pedrosa and Timmermann (2013) reviewed new therapeutic interventions such as continuous pump therapies with apomorphine or parenteral L-DOPA, or the implantation of electrodes for deep brain stimulation for the treatment of PD with limited success. Hohlefeld et al. (2012) reported that neuronal activity in the subthalamic nucleus (STN) of patients with PD is characterized by excessive neuronal synchronization, particularly in the β frequency range. However, little is known about the temporal dynamics of neuronal oscillations in PD. Long-range temporal correlations (LRTC) can quantify the neuronal dynamics on different timescales and have been shown to be relevant for optimal information processing in the brain. Although the presence of LRTC has been demonstrated in cortical data, their existence in deep brain structures remains an open question. These investigators have examined (i) whether LRTC are present in local field potentials (LFP) recorded bilaterally from the STN at wakeful rest in ten patients with PD after overnight withdrawal of L-DOPA (OFF) and (ii) whether LRTC can be modulated by L-DOPA treatment (ON). Detrended fluctuation analysis was utilized to quantify the temporal dynamics in the amplitude fluctuations of LFP oscillations. They demonstrated the presence of LRTC extending up to 50 s in the STN. The ON state was characterized by stronger LRTC than the OFF state, both in β (13–35 Hz) and high-frequency (>200 Hz) oscillations. The existence of LRTC in subcortical structures such as STN provided further evidence for their ubiquitous nature in the brain. The weaker LRTC in the OFF state might indicate limited information processing in the dopamine-depleted basal ganglia. These results suggest LRTC as a potential biomarker of pathological neuronal processes in PD. Further studies are needed in this direction.

18. Lewy bodies

It is well established that the neuropathological hallmark of PD is the existence of proteinaceous inclusions of α-Syn, known as Lewy bodies and Lewy neurites in some of the remaining dopaminergic neurons (Covy and Giasson 2011). The existence of Lewy bodies in the CNS, PNS, and ANS confirms the late diagnosis of PD as was discussed earlier.

19. CSF biomarkers

CSF is regarded as an excellent source for identifying biomarkers for neurological diseases affecting the CNS as it remains in

direct contact with the CNS and reflects the neurochemical state under different physiological and pathological conditions. One finds brain-specific proteins in the CSF that are prioritized from blood-derived proteins. Hence levels of CSF proteins could be promising biomarkers for PD. Quantitative proteomics and computational software to analyze the protein content of CSF has been considered as an attractive approach to discover novel biomarkers for PD. Recently Kroksveen et al. (2011) focused on some of the potential pitfalls in biomarker studies using CSF, and have summarized the status of CSF proteomics in general. They discussed some of the most promising proteomics biomarkers in PD. Mollenhauer and Trenkwalder (2009) reviewed CSF biomarkers in movement disorders and discussed reports on the neurochemical diagnosis of neurodegenerative disorders (including CSF α-Syn). These studies have demonstrated reduced CSF α-Syn in patients with advanced PD. Recently the neurochemical analysis of proteins in CSF has been accepted for the diagnosis of PD and neurodegenerative dementia diseases such as AD and Creutzfeldt–Jakob disease. Flood et al. (2011) assessed the CSF biomarker in PD and other related brain disorders. In addition to biomarkers from CSF, blood, serum, plasma, and urine can be analyzed by estimating the following: DJ-1 concentration in the CSF, neuromelanin antibody, platelet complex-1 activity, urinary 8-OH-2dG, reduced CSF glutathione-SH, α-synuculein, parkin, ubiquitin, brain-derived neuronal factor (BDNF), cytokines, monoamines, tetrahydroisoquinolines (TIQs: salsolinol), and plasma homocysteine,osteopontin, and Monoamine oxidase-B (MAOB) in the plasma as in vitro biomarkers as illustrated in Fig. 5.

Recently Alberio et al. (2013) developed an automated literature analysis procedure to retrieve all the background information available in public databases. This bioinformatic platform allowed the analysis of >51,000 scientific papers dealing with PD, containing information on 4121 proteins. Out of these, 35 PD-related proteins could be tracked in at least two published 2-DE maps of human plasma. Then, 9 different proteins (haptoglobin, transthyretin, apolipoprotein A-1, serum amyloid P component, apolipoprotein E, complement factor H, fibrinogen γ, thrombin, and complement C3) split into 32 spots were identified as potential diagnostic biomarkers. They compared the literature data to gels from 90 subjects (45 PD patients, 45 non-neurodegenerative control subjects) to verify their potential as plasma biomarkers of PD. By performing 2-D gel electrophoresis they have identified proteome alterations in T-lymphocytes of 17 control subjects and 15 PD patients. These changes were used to build predictive models that were verified by the leave-one-out cross-validation. Using Western blotting, they identited spots corresponding to β-fibrinogen and transaldolase, two recurrent proteins in six out of 20 spots. β-Fibrinogen levels were lowered in PD patients, whereas transaldolase isoforms were more abundant. Eventually, they identified 7 proteins with different levels in early-onset and late-onset PD patients (Alberio et al. 2012). Furthermore, Zhao et al. (2010) identified protein changes in the sera from Chinese PD patients, with the goal of finding biomarkers for PD diagnosis, and to elucidate the events occurring at the onset of PD. Using differential display to identify proteins with altered expression in PD patients, they obtained 15 protein spots corresponding to 13 different gene products that were involved in PD. Two-D gel electrophoresis and mass spectrometry identified 7 proteins which were not previously associated with PD patients. These proteins are likely to be involved in antioxidation, lipid metabolism, intracellular transport, cell proliferation and immunoregulation. The altered levels of these proteins may be related to the pathophysiological mechanisms of PD. Hence some of these proteins could be considered as candidate biomarkers of PD. Furthermore, Hall et al. (2012) performed a cross-sectional, clinic-based study to assess the ability of five CSF biomarkers to differentiate between common dementia and parkinsonian disorders. CSF samples were obtained

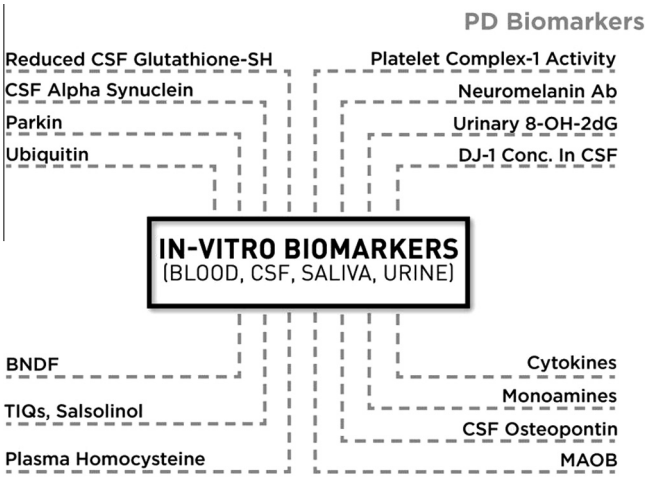

Fig. 5. A flow diagram illustrating in vitro biomarker of PD. Although in vitro diagnostic biomarkers can be estimated from blood, CSF, saliva, and urine samples of a PD patient, these biomarkers are nonspecific, as these biomarkers can confirm the diagnosis. However these diagnostic biomarkers facilitate in the identification and differential diagnosis of PD. These in vitro diagnostic biomarkers can be detected with very high sensitivity at nano and picomolar concentrations. Yet none of these biomarkers can confirm that a patient is suffering from PD. These days sensitive ELISA is performed to detect their concentrations from biological fluids and even tissue samples. These biomarkers also provide some basic information regarding the molecular biology and molecular genetic of PD. These biomarkers are brain-derived growth factor (BNDF), α-Syn, Parkin, Ubiquitin, Cytokines (NFκβ, TNFα, IL1β), catecholamines and indolamines. Their metabolites (DOPAC, DOPAL, HVA, 5-HIAA), endogenously synthesized tetrahydroisoquinolines (TIQs), Salsolinol, and monoamine oxidase-B activity. Salsolinol levels are significantly increased in the CSF and urine samples of PD patients. Direct exposure of CSF from PD patients to cultured dopaminergic neurons causes apoptosis due to the presence of Salsolinol.

from healthy individuals serving as controls and from patients with PD, PD with dementia(PDD), dementia with Lewy bodies (DLB), Alzheimer disease (AD), progressive supranuclear palsy (PSP), multiple system atrophy (MSA), or corticobasal degeneration (CBD). α-Syn was decreased in patients with PD, PDD, DLB, and MSA but increased in patients with AD. α-Amyloid 1-42 was decreased in DLB and further decreased in AD. Total tau and hyperphosphorylated tau were increased in AD. Multivariate analysis revealed that these biomarkers could differentiate AD from DLB and PDD with α-Syn and total tau contributing most significant values. The levels of neurofilament light chain were increased in atypical parkinsonian disorders (i.e., PSP, MSA, and CBD), and multivariate analysis revealed that the level of neurofilament light chain alone could differentiate PD from atypical parkinsonian disorders. Ascertainment of the α-Syn level in CSF improved the differential diagnosis of AD vs. DLB and PDD when combined with established AD biomarkers. The level of neurofilament light chain alone could differentiate PD from atypical parkinsonian disorders.

It is now known that DJ-1 is a multifunctional protein that plays an important role in oxidative stress, cell death, and Synucleinopathies, including PD. Previous studies have demonstrated that DJ-1 levels decrease in the CSF, but do not change significantly in plasma from patients with PD when compared with controls. Lin et al. (2012) measured total DJ-1 and its isoforms in the whole blood of patients with PD at various stages of AD, and healthy controls to identify potential peripheral biomarkers of PD. In an initial study of 119 subjects, 7 DJ-1 isoforms were detected, and blood levels of those with 4-hydroxy-2-nonenal modifications were altered in late-stage PD. These results were further confirmed in other 114 participants, suggesting that, unlike total DJ-1, post-translationally-modified isoforms of DJ-1 from whole blood may be considered as candidate biomarkers of late-stage PD. Hence identifying biomarkers that distinguish PD from normal control (NC)

individuals has the potential to increase diagnostic sensitivity for the detection of early-stage PD. A previous proteomic study identified potential biomarkers in postmortem ventricular CSF from PD subjects lacking AD neuropathology. The researchers assessed these biomarkers as well as p-tau (181), Aβ42, and S100B by ELISA in PD and NC cases. The p-tau (181)/Aβ42 ratio and ApoA-1 showed significant differences between groups. Regression analysis demonstrated that p-tau(181)/Aβ42 had a significant odds ratio suggesting coexistence of AD CSF biomarkers within the PD group (Maarouf et al., 2013). Furthermore, Molochnikov et al. (2012) assessed whether a gene signature could be detected in blood for the diagnosis of early PD, focusing on genes found particularly altered in the substantia nigra of sporadic PD. The transcriptional expression of 7 selected genes was examined in blood samples from 62 early stage PD patients and 64 healthy age-matched controls. Regression analysis identified 5 genes as predictors of PD: p19 S-phase kinase-associated protein 1A, huntingtin interacting protein-2, aldehyde dehydrogenase family 1 subfamily A1, 19 S proteasomal protein PSMC4 and heat shock 70-kDa protein 8. At a 0.5 cut-off the gene panel yielded a sensitivity and specificity in detecting PD of 90.3 and 89.1 respectively. The performance of the 5-gene classifier on the de novo PD individuals alone composing the early PD cohort resulted in a similar ROC with an AUC of 0.95, indicating the stability of the model and that patient medication had no significant effect on the predictive probability (PP) of the classifier for PD risk. The predictive ability of the model was validated in a cohort of 30 patients at advanced stage of PD, classifying correctly all cases as PD. The nominal average value of the PP for PD was higher than that of the early PD group suggesting a potential for the model to assess disease severity. Lastly, the gene panel fully discriminated between PD and AD. These findings provided evidence on the ability of a 5-gene panel to diagnose early/mild PD, with a possible diagnostic value for the detection of asymptomatic PD before overt clinical symptoms. Shi et al. (2011) and others have demonstrated that a decrease in DJ-1 and/or α-Syn in the CSF is a potential biomarker for PD diagnosis, but not for PD severity. Using quantitative Luminex assays, they measured total tau, phosphorylated tau, amyloid β peptide 1-42 (Aβ (1-42)), Flt3 ligand, and fractalkine levels in CSF in PD patients as well as in healthy and diseased controls. The utility of the 5 biomarkers was evaluated for PD diagnosis and severity/progression correlation alone and in combination with DJ-1 and α-Syn. The results demonstrated that combinations of these biomarkers could differentiate PD patients not only from normal controls but also from patients with AD and multiple system atrophy (MSA). Particularly, with CSF Flt3 ligand, PD could be clearly differentiated from MSA, a disease that overlaps with PD clinically. In addition, they identified that CSF fractalkine/Aβ (1-42) correlates positively with PD severity in cross-sectional samples as well as with PD progression in longitudinal samples indicating that these 7 CSF proteins could aid in PD diagnosis, differential diagnosis, and correlation with disease severity and progression. Most recently Mollenhauer et al. (2013) have estimated CSF α-Syn in untreated PD patients and 48 HC subjects. α-Syn was estimated using independently operated immunoassays, i.e., one academia-based and previously validated (ELISA-1) and industry-based commercially available (ELISA-2). Mean values for CSF α-Syn were significantly lower in de novo PD patients when compared to HC subjects, as demonstrated by both assays. Using the ELISA-2, CSF α-Syn concentrations of 1884.31 pg/mL or less showed a sensitivity of 0.91 and a specificity of 0.25 for the diagnosis of PD. Total CSF α-Syn was reduced early in the course of PD, as measured by two ELISA platforms and this reduction appeared independent from drug treatment. Recently, Goldstein et al. (2012a) reported that the central catecholamine deficiency characterizes α-Synopathies such as PD. They hypothesized that metabolites of catecholamines can provide neurochemical biomarkers of PD. To test this hypothesis CSF dopamine, norepinephrine and their main respective neuronal metabolites dihydroxyphenylacetic acid (DOPAC) and dihydroxyphenylglycol in PD and two other Synopathies, MSA and pure autonomic failure were estimated. CSF catechols were assayed in 146 subjects-108 Synucleinopathy patients (34 PD, 54 multiple system atrophy, 20 pure autonomic failure) and 38 controls. In 14 patients CSF was obtained before or within 2 years after the onset of parkinsonism. In the three Synucleinopathy including PD, MSA, and pure autonomic failure, reduced DOPAC were detected. Dihydroxyphenylglycol was higher in PD than in pure autonomic failure. DOPAC was 100% sensitive and 89% specific in distinguishing patients with recent onset of parkinsonism, but could not differentiate PD from MSA. Synucleinopathy feature CSF neurochemical evidence for central dopamine and NE deficiency. PD and pure autonomic failure involve differential dopaminergic vs. NE lesions. Furthermore, Goldstein et al. (2012b) examined whether intracellular DOPAL contributes to apoptosis and, whether α-Syn oligomers may be pathogenetic in PD. Catechols were assayed in PC12 cells after reserpine to block vesicular uptake, with or without inhibition of enzymes metabolizing DOPAL-daidzein for aldehyde dehydrogenase and AL1576 for aldehyde reductase. Vesicular uptake was quantified by a method based on $^{18}F^{-}$ or ^{13}C-dopamine incubation; DOPAL toxicity by apoptosis responses to exogenous dopamine, with or without daidzein+AL1576; and DOPAL–induced Syn oligomerization by Syn dimer production during DOPA incubation, with or without inhibition of L-aromatic-amino-acid decarboxylase or monoamine oxidase. Reserpine inhibited vesicular uptake by 95–97% and increased DOPAL. Daidzein+AL1576 augmented DOPAL responses to reserpine. Intracellular DOPAL contributed to dopamine-induced apoptosis and DOPA-induced α-Syn dimerization. We have also reported that direct exposure to DOPAL induces apoptosis in human dopaminergic (SK-N-SH) cell lines (Sharma and Ebadi, 2008a, 2011a). All these findings support the catecholaldehyde hypothesis which states that decreased vesicular sequestration of cytosolic catecholamines and impaired catecholaldehyde detoxification contribute to the catecholaminergic denervation in PD.

20. Advanced end glycation products (AGE)

Several proteins implicated in neurodegenerative diseases such as α-Syn, amyloid β, tau, and prions are glycated and the extent of glycation is correlated with the pathologies of the patients suggesting the involvement of AGEs in the progression of neurodegeneration. The age-related neurodegenerative disorders such as PD, AD, and HD are characterized by the abnormal accumulation or aggregation of proteins (Rachael et al., 2009). Recently Li et al. (2012) reported that AGEs are proteins or lipids that become glycated after exposure to sugars. The formation of AGEs promotes the deposition of proteins due to the protease resistant crosslinking between the peptides and proteins. AGEs modification triggers the abnormal deposition and accumulation of the modified proteins, which sustain the local oxidative stress and inflammatory response, eventually leading to neurodegenerative diseases. Madampage et al. (2012) recently described the nanopore analysis technique that enables conformational analysis of a single peptide or protein molecule. A pore is introduced into a membrane under voltage clamp conditions. When a molecule interacts with the pore there is a change in the current, *I*, for a time, *T*. Small unfolded molecules can translocate the pore whereas folded or large molecules tend to bump into the pore and diffuse away. The parameters, *I* and *T*, depend on the conformation of the molecule, which facilitates the detection of multiple conformations simultaneously. The analysis can be performed from dilute samples such as CSF to

determine folding or dimerization of a peptide, which is difficult to study for proteins that are prone to aggregate. The α-Syn (implicated in PD), Aβ peptides (deposited as amyloid plaques in AD); and prion proteins (whose misfolding is evident in transmissable spongiform encephalopathies) have been used to conduct nanopore analysis. This conformational information may help in understanding the early steps in the misfolding pathways of these proteins in progressive neurodegenerative disorders including PD.

21. Genetic biomarkers

Increasing evidence suggests that both genetic and environmental factors contribute to the etiology of PD. For example, genetic mutations (duplications, triplications or missense mutations) in the α-Syn gene can lead to PD, but even in these patients, age-dependent physiological changes or environmental exposures may be involved in disease progression. The identification of single genes and their functional characterization has enhanced our understanding of the pathogenesis of parkinsonism, improved the diagnosis for genetic parkinsonism, and allowed discoveries of novel therapeutic targets (Klein and Lohmann-Hedrich, 2007). Several additional modifications in many other genes increase the risk of PD. The discovery of genetic mutations raised the possibility that these or other biomarkers may help to identify persons at risk for PD (Bogaerts et al., 2008). Hardy (2010) has argued as to whether we should consider PD as one or more than one entity and discuss genetic findings from Mendelian and whole-genome association analysis for evaluating the genetic and epidemiologic risk factors for this disease. Recently Varçin et al. (2012) reported autosomal dominant missense mutations in the gene for leucine-rich repeat kinase 2 (LRRK2/PARK8) that have been recognized as the cause of PD. G2019S, the disease-causing mutant of LRRK2, has significant impact on the kinase activity of LRRK2:. The wild-type LRRK2 activity is inhibited by manganese, whereas G2019S mutation abrogates this inhibition. Based on the kinetic properties of LRRK2, it has been proposed that LRRK2 may serve as a sensor of cytoplasmic manganese levels and that the G2019S mutant has lost this function, suggesting that dysregulation of neuronal manganese homeostasis can play a crucial role in the etiology of PD. Mutations in five genes [α-Syn (SNCA), Parkin, PTEN-induced kinase 1 (PINK1), DJ-1, Leucine-rich repeat kinase 2 (LRRK2)] account for 2–3% of all cases with parkinsonism, and are clinically indistinguishable from idiopathic PD (Klein and Lohmann-Hedrich, 2007). In addition, Melrose (2008) has established a functional role of PINK1 and LRRK2 as kinases and linked mutations in the ATP13A2 gene to Kufor-Rakeb syndrome, (a form of parkinsonism). ATP13A2 encodes lysosomal ATPase and exhibits increased expression of PARK9 in the brains of sporadic cases, suggesting a potential role in idiopathic PD. Mark et al. (2007) reported that these genetic discoveries are quite promising for the early clinical diagnosis, prognosis, and treatment of PD. Osterberg et al. (2011) investigated the impact of the transcription factor Sim1 in the differentiation of mDA and rostral serotonergic (5-HT) neurons in vivo using $Sim1^{-/-}$ mouse embryos and newborn pups, and in vitro by gain- and loss-of-function approaches. They observed a selective reduction in the number of dorsal raphe nucleus (DRN) serotonergic neurons in $Sim1^{-/-}$ newborn mice. However serotonergic neurons of the raphe nuclei as well as dopaminergic neurons were not affected. Analysis of the underlying molecular mechanism revealed that tryptophan hydroxylase 2 (Tph2) and the transcription factor Pet1 are regulated by Sim1. Moreover, the transcription factor, Lhx8 and the modulator of 5-HT_{1A}-mediated neurotransmitter release, Rgs4, exhibit increased expression in ventral hindbrain compared to midbrain and are target genes of Sim1. Thus these results have demonstrated a selective transcription factor dependence of the serotonergic cells and Sim1 as a regulator of DRN acting upstream of Pet1 and Tph2 and have suggested that Sim1 may modulate serotonin release via regulating RGS4 transcription factor in PD. Houlden and Singleton (2012) recently summarized the genetic, clinical and pathological findings of autosomal dominant disease linked to mutations in SNCA, LRRK2, ATXN2, ATXN3, MAPT, GCH1, DCTN1 and VPS35 genes. The mutations in PARK2, PARK7, PINK1, ATP13A2, FBXO7, PANK2 and PLA2G6 genes have been identified in addition to the monogenic forms of PD. They indicated that although CSF biomarkers may be utilized for early diagnosis of PD, their use in clinical diagnosis is difficult due to increased variability observed between centers in the concentrations of PD biomarker (α-Syn) as well as AD biomarkers (Aβ42, total tau and phosphorylated tau). This variability is attributed to different analytical procedures suggesting the need to establish standard operating procedures (SOPs). Hence del Campo et al. (2012) merged two previous protocols to accomplish updated guidelines for PD diagnosis. The proposed SOPs are applicable not only to CSF biomarkers in PD but also to other neurodegenerative disorders. Advances in PD genetics have revealed a prominent role for mitochondrial dysfunction in the pathogenesis of PD, and the products of several associated genes, including *SNCA*, *Parkin*, *PINK1*, *DJ-1*, *LRRK2* and *HTR2A*, exhibit intramitochondrial localization under pathological conditions. Notably, Waragai et al. (2010) suggested that two familial PD-linked molecules, a-Syn and DJ-1, are present in CSF and that their levels may be altered during PD progression. The levels of both molecules are significantly decreased in the CSF in patients with PD compared with age-matched controls. Furthermore, detection and quantification of neurotoxic oligometric forms of α-Syn in the blood using ELISA is anticipated to be promising peripheral biomarkers for PD. Currently, neither α-Syn nor DJ-1 is satisfactory as a single diagnostic biomarker. However combined evaluation of these biomarkers and imaging techniques may provide reliable information for PD diagnosis. Recently several in vitro and in vivo studies have been conducted to detect early, sensitive, and specific serum, CSF, and cellular protein biomarkers employing proteomics and genomics approaches (Li et al., 2011; Chen et al., 2011; Xie et al., 2011; Alberio and Fasano, 2011; Mellick et al. 2010; Shi et al., 2010; Guo et al., 2009; Liu et al., 2008; Pan et al., 2008; Chin et al., 2008; Leverenz et al., 2007; Abdi et al., 2006; Noureddine et al., 2005). These investigators have speculated on the utility of novel biomarkers for evaluating PD progression, staging the disease, treatment efficacy, and identifying individuals at risk for developing PD and related disorders. Emphasis has been on the potential role of tau, amyloid precursor protein (APP), and α-Syn-associated fragments. They have focused primarily on the potential of quantifying small brain protein degradation fragments in blood and CSF which are generated by brain-derived proteases, to diagnose and follow neurodegenerative processes. Deleidi and Maetzler (2012) emphasized that disturbances in protein clearance mechanisms contribute to neurodegenerative dementias associated with amyloid β and Lewy body pathology. Considerable evidence has been accumulated pointing to defective protein clearance mechanisms involved in the initiation and progression of sporadic PD and AD. An extensive overview dealing with protein clearance in Lewy body-associated dementias, with a focus on intraneuronal and extraneuronal clearance mechanisms of α-Syn and amyloid β is now available. Furthermore, Kasuga et al. (2012) highlighted the importance of α-Syn, and the detection of its monomeric and oligomeric species in CSF and blood of patients with dementia with Lewy bodies. Consequently the determination of altered α-Syn species (such as truncated, phosphorylated, and oligomeric forms) may differentiate between healthy and disease state. Thomas et al. (2013) has provided a comprehensive overview of promising biomarkers as well as state-of-the-art techniques for their evaluation. Caranci et al. (2013) recently compared total

plasma α-Syn concentrations in 69 patients with PD and 110 age-matched healthy control subjects by ELISA. α-Syn was decreased in the more advanced parkinsonian disease stages in men but not in women. The reduction in α-Syn was associated with cognitive impairments, hallucinations, and sleep disorders, suggesting gender-related differences and plasma α-Syn expression as a potential biomarker for PD progression in male patients. What is less clear is how this neuronal phenotype might shape the susceptibility to proteostatic dysfunction or to the spread of α-Syn fibrils deposited in the extracellular space. Recently Surmeier and Sulzer (2013) provided a review that explores these issues and their translational implications. Benetti et al. (2012) proposed that microarray technology must be associated with functional proteomics and physiology in an effort to identify specific and selective biomarkers and drug targets, thereby facilitating the successful discovery of disease-modifying therapeutic treatments. Furthermore, Alberio and Fasano (2011) provided a review that is focused on cellular and animal models of PD.

22. Omics biomarkers

Recent studies on molecular-genetic and biochemical biomarkers of PD have not only targeted hypothesis-driven measures of specific substrates involved in processes such as protein misprocessing, but also the analyses of transcriptomic, proteomic, and metabolomic approaches. In general omics biomarkers include proteomic (ii) genomic (iii) lipidomic, metabolomic, glycomic, and miscellaneous biomarkers including microRNA as illustrated in Fig. 6. A brief overview of these biomarkers for the effective clinical management of PD is provided below. Caudle et al. (2010) have reported that although considerable effort has been made to uncover the complex molecular mechanisms involved in the pathogenesis of PD, a satisfactory explanation remains to be discovered by applying an omics approach. The emergence of several – omics techniques, including transcriptomics, proteomics and metabolomics, have been integral in confirming pathways that are associated with dopaminergic neurodegeneration and subsequently PD. This includes mitochondrial and proteasomal function and synaptic neurotransmission. Additionally, these unbiased techniques have greatly enhanced our ability to identify novel pathways, such as axon-guidance, that are potentially involved in PD pathogenesis. A comprehensive appraisal of the results obtained by different – omics has also reconfirmed the increase in oxidative stress as a common pathway in PD development/progression. It is envisaged that integration of this emerging technology will yield a more comprehensive understanding of PD etiology and the biological pathways that mediate neurodegeneration. Recent, large-scale omics studies have generated terabytes of data but not yet met the goal of developing biomarkers suitable for clinical use in PD. Mellick et al. (2010) evaluated the recent literature to identify the key roadblocks and opportunities in utilizing molecular profiling to improve the diagnosis and treatment of PD. To date, clinical assessment remains the gold standard in the diagnosis of PD and rating scales are well established for tracking PD progression.

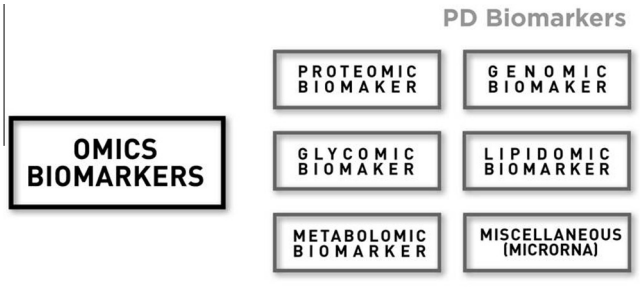

Fig. 6. A flow diagram illustrating various omic biomarkers for PD diagnosis, prognosis, and treatment. The Omics analysis provides lot of data which can further establish the diagnosis and basic information regarding the genetic and environmental insult to a PD patient. These sophisticated techniques employ bioinformatic approach to analyze thousands of functional molecules including proteins (proteomics), DNA/RNA (Genomics), Lipids (Lipidomics), and metabolites (Metabolomics), and carbohydrates (Glycomics). The detailed analysis of genes including pattern recognition can provide more precise information regarding the disease process and possible therapeutic strategies. Very limited information is as yet available in this direction as omics analysis requires lot of time, money and energy to accomplish the specific aims and objectives. However there is considerable promise for the future pharmacotherapy of PD in this area.

The term metabolomics has been introduced to address the global analysis of all metabolites in a biological sample. Metabolomics has also been introduced to refer specifically to the analysis of metabolic responses to drugs or diseases. Metabolomics is becoming an important area of research as it is the complex study to identify the biomarker for PD and various other diseases. Lipidomics refers to the analysis of lipids. Recent improvements in new analytical procedures have made it possible to identify and to quantify most of lipid metabolites from a single sample. Three key technologies employed for lipid profiling include mass spectrometry, chromatography, and nuclear magnetic resonance spectroscopy. Mass spectrometry has been used to determine the relative concentration and composition of high-density lipoproteins (HDL) particles from lipid extracts isolated from tissues of patients and healthy volunteers. Morgan et al. (2010) identified numerous potential biomarkers that may aid in the differential diagnosis of PD and/or tracking disease progression. Hence clinical, genetic, blood and CSF (proteomics, transcriptomics, metabolomics), and neuroimaging biomarkers may be useful in the diagnosis of PD and in measuring disease progression and response to therapies. Some potential biomarkers are inexpensive and do not require much technical expertise, whereas others are expensive or require specialized equipment and technical skills. Many potential biomarkers in PD show great promise; however, they need to be assessed for their sensitivity and specificity in large and varied samples of patients with and without PD (Robinson, 2010). Furthermore, van Dijk et al. (2010) discussed the current hypotheses regarding the pathogenesis of PD and identified the most promising candidate biomarkers among the CSF proteins. The potential markers include proteins involved in various pathogenetic processes, such as oxidative stress and protein aggregation. A single biomarker may not be sufficient to reach high sensitivity and specificity, as PD is pathogenetically heterogeneous and shares etiological factors with other neurodegenerative diseases. Therefore, the candidate biomarkers must be validated before being implemented as diagnostic aids. The search for effective biomarkers for diagnosis and surveillance of PD is still in progress (Shtilbans and Henchcliffe, 2012). Most recent biomarker studies of PD have discussed future directions that might lead to development of specific PD biomarkers. Indeed studies of genetic and biochemical biomarkers of PD have not only targeted hypothesis-driven measures of specific substrates involved in protein misfolding, but also have made use of transcriptomic, proteomic, and metabolomic approaches. However none of these advanced strategies are as of yet established as authenticated PD biomarkers.

22.1. Genomic biomarkers

There is increasing evidence that molecular genetics, focused – omic (proteomic, metabolomic, and transcriptomic) assessment of blood and CSF, and in vivo neuroimaging will provide critical clues to assist in the diagnosis and clinical management of PD (Marek et al., 2008; Altar et al., 2009). Biomarkers offer the potential to provide a window onto disease mechanism, potentially generating therapeutic targets for disease. In particular, biomarkers enable

investigation of the premotor period of PD before typical symptoms are manifested, but after degeneration has already begun. Given the multiple genetic causes for PD, the variability in the loss of dopaminergic markers measured by imaging at motor symptom onset and the heterogeneity of clinical symptoms in PD onset and disease progression, many biomarkers with a focus ranging from clinical symptoms to PD pathobiology to molecular genetic mechanisms will be necessary to fully map PD risk and progression. Biomarkers are also critical in new drug development for PD, both in early validation studies to assess drug dosing and to determine drug penetrance into the brain, and in efficacy studies to complement PD prognosis. Recently Morgan et al. (2010) discussed clinical, genetic, blood and CSF (proteomics, transcriptomics, metabolomics), and neuroimaging biomarkers that may be useful in the differential diagnosis of PD and in assessing disease progression and response to therapies. The longitudinal and biomarker study in PD (LABS-PD) is a study designed to measure the evolution of motor and non-motor features of PD and sample promising biomarkers from early to late stage illness. LABS-PD is organized on the premise that cohorts from completed clinical trials can be re-recruited for long-term follow up. Ravina et al. (2009) examined biomarker sampling in the initial cohorts. The first PD cohort (PostCEPT) comes from the clinical trial of a mixed lineage kinase inhibitor (PRECEPT) has assessed the recruitment from PRECEPT to PostCEPT, the ability to link data from the two studies, and sample collection for a variety of biomarkers. A total of 537 of 709 eligible PRECEPT subjects (76%) enrolled in PostCEPT; 509 (95%) had repeat DAT imaging. PRECEPT clinical and imaging data were linked to PostCEPT to provide 3–4 year follow-up. A biomarker sub-study enrolled over 100 PD cases from PostCEPT and 100 controls to measure olfaction and blood markers of gene expression, α-Syn, and proteomic profiles. They have been successful in linking clinical and biomarker data to DNA samples. The PostCEPT cohort and associated studies have supported the feasibility of the LABS-PD clinical trial to collect longitudinal clinical and biomarker data.

Although the mechanisms underlying PD and Lewy body (LB) formation, a pathological hallmark of PD, are incompletely understood; mitochondrial dysfunction is likely to be at least partially responsible. To study the processes that might be related to nigral neurodegeneration and LB formation, Jin et al. (2005) employed quantitative proteomics with isotope-coded affinity tag (ICAT) to compare the mitochondrial protein profiles in the substantia nigra (SN) between controls and mice treated chronically with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a potent mitochondrial toxicant, and an adjuvant, probenecid (prob) for 5 weeks, which produces selective nigrostriatal neurodegeneration with formation of LB-like cytoplasmic inclusions in the nigral neurons. A total of >300 proteins were identified; of these, more than 100 displayed significant changes in relative abundance in the MPTP/prob-treated mice. One of these proteins, DJ-1 was validated and whose mutation has been implicated in familial PD, in relation to cytoplasmic inclusions in mice, as well as in classical LBs in PD patients. DJ-1 was not only colocalized with α-Syn in dopaminergic neurons but also to cytoplasmic inclusions. In addition, DJ-1 was localized in the halo but not in the core of LBs in patients with PD suggesting that DJ-1 plays an important role in mitochondrial dysfunction, as well as LB formation in PD. Mutations in α-Syn gene giving rise to the production of degradation-resistant mutant proteins or multiplication of wild-type α-Syn gene allele can cause rare inherited forms of PD. Therefore, the existence of and abnormally high amount of α-Syn is considered responsible for the dopaminergic neuronal death in PD. Normally, α-Syn localizes to presynaptic terminals of neuronal cells, regulating the neurotransmitter release through the modulation of soluble N-ethylmaleimide-sensitive factor attachment protein receptor complex. On the other hand pathological examinations on the recipient patients of fetal nigral transplants provided a prion-like cell-to-cell transmission hypothesis for abnormal α-Syn. The extracellular α-Syn fibrils can internalize and enhance intracellular formation of inclusions, thereby reducing cell viability suggesting that effective removal of abnormal α-Syn in the extracellular space as well as intracellular compartments can be of therapeutic relevance. Abnormally accumulating α-Syn-triggered neuronal death may provide possible disease-modifying therapeutic targeting.

Pathological data from autopsies genotyped for PD-related mutations in α-Syn, Parkin, PINK1, DJ1, LRRK2, and glucocerebrosidase have accumulated in recent years. Poulopoulos et al. (2012) recently provided a systematic review of all pathological mutation carriers, pathological patterns, and lacunae in the present knowledge. Nineteen autopsies of α-Syn mutation carriers, forty-nine of LRRK2 mutation carriers, nine of Parkin mutation carriers, one of a PINK1 mutation carrier, and eighty-six of glucocerebrosidase mutation carriers have been identified. Most autopsies of α-Syn, LRRK2 G2019S, and glucocerebrosidase mutation carriers demonstrated Lewy body pathology, as opposed to Parkin and LRRK2 non-G2019S mutation carriers. However, there was a significant variability in carriers of identical mutations.

Recent identification of mutations in genes linked to lysosomal function and neurodegeneration has offered a unique opportunity to directly examine the role of lysosomes in PD neuropathogenesis. Lysosomal dysfunction has been implicated in several neurodegenerative disorders including PD, and related Synucleinopathy that are characterized by accumulations of α-Syn in Lewy bodies. ATP13A2 is a lysosome-specific transmembrane ATPase protein of unknown function. This protein has been linked to Kufor-Rakeb syndrome (KRS) where it is absent or mutated. While previous data suggests a role of ATP13A2 in α-Syn misfolding and toxicity, the mechanistic link has not been established. Mutations in lysosomal membrane protein ATP13A2 (PARK9) cause familial Kufor-Rakeb syndrome (KRS) characterized by early-onset parkinsonism, pyramidal degeneration, and dementia. Point mutations in ATP13A2 have been linked to familial cases of PD. However the association between idiopathic PD and the ATP13A2 (PARK9) Ala746Thr variant, associated with KRS, is controversial. NCL and KRS may share etiological features and implicate the lysosomal pathway in PD. These results unravel an important role of ATP13A2 in lysosomal function and in cell viability and validate ATP13A2 as a therapeutic target against PD degeneration. Recently Bras et al. (2012) reported that the loss of ATP13A2 in human fibroblasts from patients with Kufor-Rakeb syndrome (KRS) or in mouse primary neurons leads to impaired lysosomal degradation. The lysosomal dysfunction results in accumulation of α-Syn and toxicity in primary cortical neurons. Importantly, silencing of endogenous α-Syn attenuated the toxicity in ATP13A2-depleted neurons, suggesting that loss of ATP13A2 mediates neurotoxicity via the accumulation of α-Syn. These findings implicate lysosomal dysfunction in the pathogenesis of KRS and suggest that upregulation of lysosomal function and downregulation of α-Syn represent important therapeutic strategies for this disorder. Neuronal ceroid lipofuscinoses (NCLs) comprise a heterogeneous group of metabolic storage diseases characterized by the accumulation of lipopigment, neurodegeneration and premature death. Nine genes have been identified as the cause of different types of NCL, with ages at onset ranging from around birth to adult, although the underlying etiology of the disease still remains unknown. A family with NCL pathology has been recognized in which exon sequencing has been performed to identify a single homozygous mutation in ATP13A2 that fully segregates with disease within the family. Gene-based candidates for PD include the ubiquitin–proteasome system, scavengers of reactive oxygen species (ROS), brain-derived neurotrophic factor (BDNF), its receptor, TrkB, and downstream target early growth response-1, Nurr-1, and signaling through protein kinase C and RAS

pathways. Chan et al. (2013) recently investigated this association in 69 patients with early onset PD (EOPD; ≦50 years of age), 192 patients with late onset PD (LOPD; >50 years of age), and 180 healthy controls in the Chinese population in Hong Kong. The presence of the Ala746Thr variant in the ATP13A2 locus was examined in these participants. A heterozygous Ala746Thr variant in one healthy control, one patient with EOPD, and one patient with LOPD was detected suggesting that the ATP13A2 Ala746Thr variant is not a risk factor for PD. Mutations in ATP13A2 (PARK9) cause an autosomal recessive form of early-onset parkinsonism with pyramidal degeneration and dementia called KRS. The ATP13A2 gene encodes a transmembrane lysosomal P5-type ATPase (ATP13A2) which has physiological functions in mammalian cells, and hence has a potential role in PD. KRS-linked mutations in ATP13A2 leads to lysosomal alterations in ATP13A2 KRS patient-derived fibroblasts, including impaired lysosomal acidification, decreased proteolytic processing of lysosomal enzymes, reduced degradation of lysosomal substrates and diminished lysosomal-mediated clearance of autophagosomes (AP). Thus mutations in ATP13A2 (PARK9), encoding a lysosomal P-type ATPase, are associated with both KRS and neuronal ceroid lipofuscinosis (NCL). KRS is a rare genetic form of PD, whereas NCL is a lysosomal storage disorder. Although the transport activity of ATP13A2 has not been defined, in vitro studies show that its loss compromises lysosomal dysfunction, which may cause neuronal degeneration. To understand the role of ATP13A2 dysfunction in KRS, Schultheis et al. (2013) disrupted its gene in mice. ATP13A2$^{-/-}$ and ATP13A2$^{+/+}$ mice were tested to assess sensorimotor and cognitive function at multiple ages. In the brain, lipofuscin accumulation, α-Syn aggregation, and dopaminergic pathology were measured. Behaviorally, ATP13A2$^{-/-}$ mice displayed late-onset sensorimotor deficits. Accelerated deposition of lipofuscin was observed in the cerebellum, in the hippocampal neurons and the cortex of ATP13A2$^{-/-}$ mice. Immunoblot analysis showed increased α-Syn in the hippocampus, but not in the cortex or cerebellum. There was no change in the number of dopaminergic neurons in the substantia nigra or in striatal dopamine levels in aged ATP13A2$^{-/-}$ mice signifying that the loss of ATP13A2 causes sensorimotor impairments, α-Syn aggregation as occurs in PD, Synopathies, and accumulation of lipofuscin deposits characteristic of NCL, thus providing the direct demonstration that mutations in ATP13A2 can cause pathological features of both diseases in the same organism. Most recently Lopes da Fonseca et al. (2013) described the zebrafish homologue of human ATP13A2, protein sequence homology, which supports its conserved biological role. They studied the spatial pattern of protein expression. The lethality of the knockdown of ATP13A2 suggests its crucial role during embryonic development. These findings have provided new insight into the biology of ATP13A2 and have opened novel opportunities using zebra fish as an animal model of PD. Furthermore, Usenovic et al. (2012) reported that autophagy-lysosomal pathway plays an important role in the clearance of proteins and dysfunctional organelles. Similar alterations are noticed in stable ATP13A2-knockdown dopaminergic cell lines, which are associated with cell death. Restoration of ATP13A2 levels in ATP13A2-mutant/depleted cells is able to restore lysosomal function and attenuate cell death. Dehay et al. (2012) determined that ATP13A2 levels are decreased in dopaminergic nigral neurons from sporadic PD patients. Interestingly, the main signal of ATP13A2 was detected in the Lewy bodies of these patients. Mendonça et al. (2012) recently investigated differentially-expressed proteins in CSF of ALS patients compared to control subjects, with the primary objective to identify biomarkers for the disease. The differentially expressed protein spots were subjected to 2D-electrophoresis and recognized with matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry. Parkin-like and several iron and zinc binding proteins were detected in the CSF. Parkin is a ligase involved in the ubiquitin–proteasome pathway and mutations in the parkin gene are the most common cause of recessive familial PD. Iron and zinc are involved in several metabolic processes and are related to neurodegenerative disease. Common features of neurodegenerative diseases comprise failure of the ubiquitin–proteasome system and increased levels of metal ions in the brain. Therefore, the identification of these proteins will be a significant step in the future novel discovery of PD biomarkers.

22.2. Neuroproteomics

Proteomic technologies are now widely used to understand the molecular mechanism of PD and to develop biomarkers for early diagnosis and treatment. Application of proteomics to the human brain, CSF, and plasma has significantly enhanced the unbiased and high-throughput searches for novel biomarkers. There are many critical steps to biomarker discovery for neurodegenerative diseases, including sample preparation, protein/peptide separation and identification, as well as independent confirmation and validation. The differential expression patterns of brain, CSF, and blood proteins of PD patients or chemically-induced animal models are now being used to identify protein fingerprints for developing diagnostic and therapeutic strategies for PD. Shi et al. (2009) summarized proteomics technologies in the discovery of biomarkers for neurodegenerative diseases, practical considerations and limitations of major aspects, as well as the status of candidate biomarkers by proteomics for PD. In addition, Srivastava et al. (2010) explained a number of differentially expressed proteins associated with energy metabolism, oxidative stress, signal transduction, electron transport, and detoxification pathways that have been identified using proteomic strategies. Indeed proteomics have immensely contributed to the detection of qualitative and quantitative changes of expressed proteins and their post-translational modifications. An update on proteomics-driven research for developing early biomarkers and understanding the molecular aspects of PD, along with their translational snags, challenges, and future possibilities have been highlighted in this review.van Dijk et al. (2010) have discussed the current hypotheses about the pathogenesis of PD and identified the most promising candidate biomarkers among the CSF proteins. The list of potential markers included proteins involved in various pathogenetic processes, such as oxidative stress and protein aggregation. They proposed that a single biomarker may not be sufficient to reach high sensitivity and specificity, because PD is pathogenetically heterogeneous and shares etiological factors with other neurodegenerative diseases. Moreover, identified candidate biomarkers will have to be validated before they could be implemented as diagnostic tools. The most valuable proteomic studies performed to date are those aimed at identifying endogenous binding partners, substrates, post-translational modifications, and cellular pathways affected by these proteins. Similar to global proteomic approaches, even these approaches have often been characterized by the production of several proteins. Consequently, the parallel development of more refined protein–protein interactions maps may increase the chances of identifying those protein complexes and/or cellular pathways, which, when disrupted, leads to the development of disease. They may include agents that modulate kinase activities (e.g., PINK1 and LRRK2), modulate the activity of the ubiquitin-protein ligase, Parkin, proteostasis agents to prevent α-Syn filament assembly and toxicity, or promote the refolding of mutant proteins, modulate α-Syn transfer between cells, reagents to regulate cargo dynamics along axonal microtubule networks, and stimulators of autophagy and/or modulate cellular stress pathways. The second major challenge will be to identify biomarkers to enable population screening to identify those with asymptomatic early-stage disease. The outcome will depend on adopting strict SOPs for the

collection, processing and storage of samples, combined with the need for the identification of the most robust methods of pre-fractionation of samples to remove the most abundant proteins prior to proteomic screening. Licker et al. (2009) proposed that PD may be an acquired or genetically-determined brain proteinopathy involving an abnormal processing of several, rather than individual neuronal proteins, and have discussed pre-analytical and analytical developments that may help in verifying this concept. Most recently Rango et al. (2013) described mutations in the PINK1 gene that are associated with early onset autosomal recessive parkinsonism (EOP), characterized by a phenotypic presentation that, although variable, generally overlaps with that of idiopathic PD. Furthermore, Cheng et al. (2010) proposed that degeneration of axons, not cell bodies, is the primary determinant of clinically apparent progression of disease, and that future experimental therapeutics intended to halt disease progression will benefit from the distinct mechanisms of axonal degeneration. Shtilbans and Henchcliffe (2012) conducted the most recent biomarker studies of PD, provided an update on biomarker research, and discussed future directions that might lead to the successful development of PD-specific biomarkers.

22.3. Lipidomics

The advent of electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) has made it possible to study various lipid structures in the brain. These include phospholipids, ceramides, sphingomyelin, cerebrosides, cholesterol and their oxidized derivatives. Thus lipid analyses have delineated metabolic defects in PD and other related disorders. Additionally, proteomic strategies for characterizing lipid-metabolizing proteins in the CSF have been identified. These proteins may be potential therapeutic targets since they transport lipids required for neuronal growth or convert lipids into molecules that control brain physiology. Thus combining lipidomics and proteomics will enhance our existing knowledge of PD pathology and increase the chances of discovering specific biomarkers and biochemical mechanisms of neurological diseases (Fonteh et al., 2006; Venugopal et al., 2009).

22.4. Metabolomics

The repertoire of biochemicals present in cells, tissue, and body fluids, is known as the metabolome. It is known that CNS disorders are linked to disturbances in metabolic pathways related to neurotransmitter systems (dopamine, serotonin, GABA, and glutamate); fatty acids such as arachidonic acid-cascade; oxidative stress, and mitochondrial function. Quinones and Kaddurah-Daouk (2009) emphasized the use of metabolomics and its promise for biomarker discovery for the early diagnosis of PD and other related disorders. Currently clinicians utilize only a part of the information existing in the metabolome, as revealed by the quantification of a limited set of analytes to obtain information on human health. Metabolomics has the potential to have significant impact on clinical practice by providing relevant biochemical data. Moreover the overall health status of an individual can be evaluated by the metabolic state which is encoded by the genome and modified by environmental factors. Consequently metabolomics promises to improve current, single metabolites-based clinical assessments by identifying biomarkers that embody global biochemical changes in disease, predict prognosis, or side effects of medication (pharmachometabolomics). Hence metabolomic platforms and bioinformatics tools are now being developed to map potential biomarkers for PD. Furthermore, metabolomic tools are enabling to map perturbations in various biochemical pathways and functional relationships among these pathways. The information gained through metabolomics is extremely important for the development of novel biomarkers that are disease-specific.

23. Mitochondrial dysfuncttion in PD

Henchcliffe and Beal (2008) have examined evidence for the roles of mitochondrial dysfunction and increased oxidative stress in the neuronal loss that leads to PD and have discussed how this information might improve patient management and the development of mitochondrial therapy for PD. Impaired mitochondrial function is likely to increase oxidative stress; rendering cells vulnerable to PD and other related disorders including excitotoxicity. The mitochondria, therefore, represent a promising target for the development of PD biomarkers by employing genetic, biochemical, and neuroimaging approaches. Novel therapeutic interventions that modify mitochondrial function are under development, and phase-3 clinical trial is underway to determine whether high-dose coenzyme Q_{10} could slow down PD progression. The spatially distributed, at-risk population of nigrostriatal neurons shares a number of features, including autonomously generated activity, broad action potentials, low intrinsic calcium buffering capacity and long, poorly myelinated, highly branched axons. Several of these traits add to the metabolic burden in these neurons, suggesting that mitochondrial deficits could drive pathogenesis in PD.

24. MicroRNA analysis

Besides the classic mutations in coding regions of genes, the crucial role of gene expression regulators in disease states is being recognized. Mouradian (2012) have reported that the network of small non-coding microRNAs is crucial for the normal development and survival of distinct neuronal populations that are vulnerable in various neurodegenerative disorders. In midbrain dopaminergic neurons, which degenerate in PD causing motor signs and symptoms, disruption of this network results in the progressive loss associated with impaired motor activity in Drosophila and mouse models. Studies of families with dominantly inherited PD linked to multiplication of the α-Syn gene locus indicate that the amount of α-Syn in neurons is an important determinant of its tendency to aggregate and increase neuronal susceptibility. Recent studies have demonstrated that the α-Syn mRNA is under negative control by at least two microRNAs, miR-7 and miR-153. In addition to studying the regulation of candidate genes by specific microRNA species, profiling approaches are revealing variations in the abundance of certain microRNAs that may prove relevant to the disease. For example, miR-133b is deficient in the PD midbrain as well as in mouse models, and miR-34b/34c is decreased in affected brain regions in PD and Lewy body disease. Polymorphisms in the 3′-untranslated region of microRNA target mRNAs in the gene encoding α-Syn found in Genome Wide Association studies, are another reason for variations in the rate of protein synthesis and thus disease risk. In addition the impact of a disease-specific gene product, and in particular LRRK2, on the microRNA network increases the complexity of the microRNA system and pathogenic proteins. The knowledge gained from these studies promise to translate into therapeutic strategies for PD.

25. In vivo molecular imaging biomarkers

In vivo biomarkers including estimations of T_2 relaxation times by magnetic resonance imaging (MRI), SPECT biomarkers (^{123}Ifflupane and β-CIT), PET imaging with ^{18}F-DOPA and ^{18}FdG, sleep EEG (somnography), ultrasonography for striatal iron estimation, and muscle action potential, are illustrated in Fig. 7. The underlying pathological mechanisms leading to tremor, coexistent

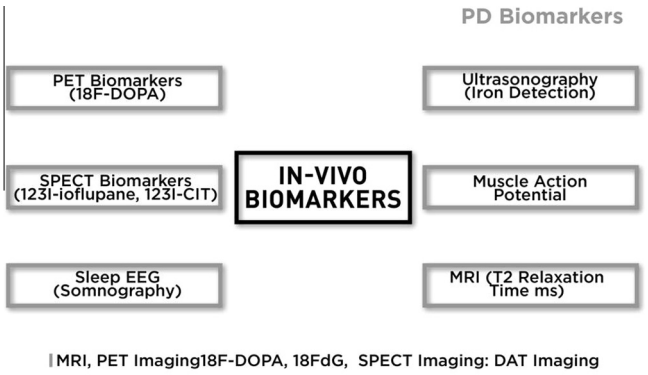

Fig. 7. A flow diagram illustrating in vivo molecular biomarkers of PD. In vivo molecular imaging has shown a great promise in the armamentarium of early clinical diagnosis, prognosis, and treatment of PD. For instance the striatal iron content is almost 2.5 times increased in PD patients, which can be detected by employing ultrasonic diagnostic procedure. The echo of ultrasonic waves is significantly increased as the concentration of iron in the striatal region is increased in PD patients as function of progression of disease. MRI estimates the proton density per unit area in the brain and can estimate the extent of loss of dopaminergic neurons as the proton density of degenerated dopaminergic neurons is significantly altered which facilitates the early prognosis of PD progression. However this procedure is not specific. In vivo PET imaging employing specific ^{18}F-DOPA and ^{18}FdG can provide very precisely brain regional dopaminergic and mitochondrial bioenergetics in the PD brain. Hence this noninvasive dynamic functional provide the exact estimate of the disease progression and confirms the clinical diagnosis of PD. However PET imaging is not easily available and requires onsite cyclotron for the synthesis of PET biomarkers and nuclear medicine facility attached to the clinic, which extremely at present. On the other hand SPECT imaging is more common and can be effectively used for the estimation of cerebral blood flow, dopamine transporter imaging and other noninvasive in vivo investigations for longitudinal analysis of PD and other neurological patients. In addition there are two electrophysiological biomarkers including muscle action potential and sleep EEG (to generate somnograms) which can provide basic information regarding the quality of sleep and muscle tremors which are impaired in PD patients. Although less specific, these electrophysiological diagnostic biomarkers further facilitate in the confirmation and deciding the therapeutic regimen of PD patients.

dementia and depression in PD and the role of imaging as a biomarker for testing neuroprotective agents have been emphasized by Brooks (2007). Particularly MRI has opened a new window into the brain. Measuring hippocampal volume with MRI has provided clinically important information about several neuropsychiatric and neurodegenerative disorders including PD. Smaller hippocampal volumes have been reported in epilepsy, AD, dementia, mild cognitive impairment, the aged, traumatic brain injury, cardiac arrest, PD, HD, Cushing's disease, herpes simplex encephalitis, Turner's syndrome, Down's syndrome, survivors of low birth weight, schizophrenia, major depression, posttraumatic stress disorder, chronic alcoholism, personality disorder, obsessive–compulsive disorder, and antisocial personality disorder. On the other hand, significantly larger hippocampal volumes have been correlated with autism and children with fragile X syndrome. Preservation of hippocampal volume has been reported in congenital hyperplasia, children with fetal alcohol syndrome, anorexia nervosa, attention-deficit hyperactivity disorder (ADHD), bipolar disorder, and panic disorder (Geuze et al., 2005). Standard univariate analysis of neuroimaging data has revealed neuroanatomical and functional differences between healthy individuals and patients suffering from neurological and psychiatric disorders. Recently Orrù et al. (2012) provided a method for the investigation of PD and other neurodegenerative disorders employing Support-Vector-Machine (SVM). Attention has been focused on the alternative forms of analysis, employing SVM which allows categorization of an individual's previously unseen data into a predefined group using a classification algorithm, developed on a training data set. SVM has been applied in the diagnosis, treatment, and prognosis, using both structural and functional neuroimaging data.

26. Neuroimaging in PD diagnosis

Recent developments in the neuroimaging employing MRI, PET and SPECT allow the non-invasive tracking of molecular targets of relevance to neurodegeneration in vivo. The analysis of use of PET and SPECT imaging in the diagnosis, differential diagnosis and evaluation of treatment efficacy of CNS diseases has been reported by Granov et al. (2012). In addition the possibility of radionuclide imaging techniques in different variants of dementia, PD brain tumors has been demonstrated. Neuroimaging is now used to support the clinical diagnosis of PD patients with cognitive impairment.

26.1. MRI in PD

To test the hypothesis that degeneration of the substantia nigra pars compacta (SNc) precedes that of the cholinergic basal forebrain (BF) in PD, Ziegler et al. (2013) utilized multispectral structural MRI to measure the volumes of the SNc and BF. Participants included 29 patients with PD (Hoehn and Yahr [H&Y] stages 1–3) and 27 matched healthy controls. Multiecho T_1-weighted, multiecho proton density, T_2-weighted, and T_2-weighted fluid-attenuated inversion recovery (FLAIR) sequences from each participant were acquired. A weighted mean of the multiple echoes, yielding a single volume with a high ratio of contrast to noise was created for SNc. The BF was visualized using T_2-weighted FLAIR images. Relative to the controls, 13 patients with H&Y stage 1 PD had significantly decreased SNc volumes. Sixteen patients with H&Y stage 2 or 3 PD showed additional volume loss. In contrast, the BF volume loss occurred later in the disease, with a significant decrease in patients having H&Y stage 2 or 3 PD compared with the controls and the patients having H&Y stage-1 PD. The latter group did not differ significantly from the controls. These findings support the proposed neuropathological trajectory and established novel multispectral methods as MRI biomarkers for tracking the degeneration of the SNc and BF in PD.

Dementia is a frequent and disabling complication of PD. We lack a biomarker capable of tracking the structural and functional changes that underlie the evolution of cognitive dysfunction in PD. Dementia in PD is associated with extensive cortical atrophy, which may be quantified with structural MRI. More promisingly, patterns of atrophy may be present in those who have PD with MCI (PD-MCI). Subcortical white matter tract degeneration is detectable early in the disease with diffusion tensor imaging and may precede changes observed on structural MRI. Although less well studied, other techniques such as functional MRI, MR perfusion imaging with arterial spin labeling, and MR spectroscopy, have demonstrated differences in activation and metabolism between PD and PDD. The ability to compare studies has been limited by the heterogeneity of patients, cognitive testing, and imaging protocols, indicating that future work ought to adopt scan protocols, should be adequately powered, and patients should be carefully phenotyped to maximize the contribution of MRI as a biomarker for PDD (Duncan et al., 2013). Gomperts et al. (2013) recently designed a study to determine whether amyloid burden, as indexed by Pittsburgh compound B (PiB) retention, identifies patients with PD with mild cognitive impairment (PD-MCI) compared to those with normal cognition (PD-nl). The aim of this study was to determine whether amyloid burden predicts cognitive decline in subjects with PD without dementia. 46 subjects with PD without dementia, of whom 35 had normal cognition and 11 met criteria for PD-MCI. All subjects underwent neurologic and

neuropsychological examinations and PiB-PET at baseline, and clinical examinations were conducted annually for up to 5 years. At baseline, PiB retention did not distinguish PD-MCI from PD-nl subjects with PD-MCI declined more rapidly than PD-nl subjects in cognitive tests of memory, executive function, and activation retrieval. Of the 35 PD-nl subjects, 8 progressed to PD-MCI and 1 to dementia; of the 11 PD-MCI subjects, 5 converted to dementia. Both higher PiB retention and a diagnosis of PD-MCI predicted a greater hazard of conversion to a more severe diagnosis. Baseline PiB retention predicted worsening in executive function over time. The *APOE* ε4 allele also related to worsening in executive function, as well as visuospatial function, activation retrieval, and performance on the mini-mental state examination. In contrast to its relation to cognitive decline, PiB retention did not affect progression of motor impairment. The amyloid burden did not distinguish between cognitively impaired and unimpaired subjects with PD without dementia however these data suggest that amyloid contributes to cognitive, but not motor decline in PD patients. The anterior cingulate (AC) gyrus and the presupplementary motor area (pre-SMA) exhibit pathological changes in PD. Pérez-Gómez et al. (2000) reported that magnetic resonance spectroscopy (MRS) is a non-invasive technique for the neurochemical study of the brain in vivo with the nuclei, phosphorus (^{31}P) and hydrogen (^{1}H). The low N-acetyl-aspartate in the CNS is a biomarker of neuronal loss and its determination is complementary to the structural studies with MRI. Thus MRI/MRS has increased our knowledge about the physiopathology of normal aging, degenerative processes, demyelinating, psychiatric diseases, and can contribute to differential diagnose of PD. It has also opened new avenues in the pharmacotherapy of discrete cerebral regions. PD patients exhibit MRS changes in N-acetyl acetic acid/creatine (NAA/Cr) in the AC, pre-SMA, or posterior cingulate (PC). Camicioli et al. (2007) performed a study on forty-four (27 male, 17 female) healthy nondemented PD patients and 38 controls (18 male, 20 female) 65 years of age and older were examined using the Unified PD Rating Scale (UPDRS), Mini-Mental State Examination, Frontal Assessment Battery, and Geriatric Depression Scale. MRS was performed at 1.5 T. Gray matter and white matter volumes were measured within voxels using SPM2. Spectra were analyzed using LC model to yield NAA/Cr and Cho/Cr ratios. Pre-SMA NAA/Cr was decreased in PD, consistent with neuronal dysfunction. In addition, Rossi et al. (2013) studied nonheme iron in PD using MRI in 36 patients and 21 healthy volunteers. The subjects underwent clinical investigation, including 3-T MRI. Disease-related changes were present not only in the substantia nigra but also in the globus pallidus. These changes were associated with neurodegeneration, reflecting the severity of motor impairment.

26.2. SPECT and PET biomarkers

Recent developments in the imaging techniques of SPECT and PET allow the non-invasive tracking of such molecular targets of known relevance to neurodegenerative disorders in vivo. Moreover neurophysiological tests are being adapted for biomarker research. With the development of specific radioligands as SPECT and PET biomarkers it will be possible to accurately diagnose and effectively manage PD in the future. Moreover high-resolution SPECT imagng reduces the cost and number of gene-manipulated animals used to perform longitudinal studies of PD and other related neurodegenerative disorders as we have reported earlier (Sharma and Ebadi, 2008a). While loss of putamen dopaminergic function leads to motor disability, Lewy bodies not only target dopamine neurons but have also been observed in serotoninergic, noradrenergic, and cholinergic neurons. Accumulating data have authenticated the concomitant degeneration of nigrostriatal and other dopaminergic pathways and of the serotonergic, cholinergic and noradrenergic neurotransmitter system in PD. In addition, the pathologic process is not only restricted in the brain, since the spinal cord and the peripheral autonomic nervous system are also affected. Hence the use of SPECT and PET imaging may contribute to the understanding of these aspects of the disease. As a consequence, nondopaminergic neurotransmission is also impaired resulting in non-motor symptoms including sleep disturbance, fatigue, depression, dementia, and autonomic dysfunction. SPECT and PET ligands evaluate the function of monoaminergic and cholinergic neurons. The correlation of striatal DAT imaging with anxiety and depression symptoms in PD has been determined. Several radiotracers are now used as biomarkers of dopamine storage capacity, vesicular monoamine, and DAT availability. DAT imaging, such as DAT-SCAN SPECT, tests the integrity of the nigrostriatal pathway, whereas ^{18}FdG-PET identifies typical patterns of cortical and subcortical hypometabolism. Benadiba et al. (2012) summarized recent findings of SPECT and PET studies using these methods, and discussed their potential role in drug development for neurodegeneration as well as clinical applications in the differential diagnosis of neurodegenerative disorders and monitoring the disease progression. Thus DAT and regional glucose metabolism imaging may be utilized effectively for the differential diagnosis of PD. In a study 27 subjects were investigated for neurodegenerative dementia associated with parkinsonism by DAT-SCAN SPECT and ^{18}FdG-PET imaging. They were grouped according to the clinically established diagnosis, including probable AD (5 subjects), corticobasal degeneration (6 subjects), Lewy body dementia (8 subjects), frontotemporal dementia (4 subjects), and PD with dementia (4 subjects). This approach demonstrated that the information provided by normalized ^{18}FdG uptake and DAT is highly significant for the differential diagnosis of dementia and that both normalized ^{18}FdG uptake and DAT uptake allows a better classification of individual patients supporting the usefulness of both modalities in the clinical management of PD (Garibotto et al., 2013). Recently Giza et al. (2012) discussed the role of PET and SPECT in imaging the extrastriatal dopaminergic system and other neurotransmitter systems as well as the imaging of microglial activation and cardiac sympathetic denervation in PD. They proposed several PET and SPECT ligands to detect changes in extrastriatal dopaminergic system as well as in the serotonergic, cholinergic and noradrenergic systems in PD and also explored its correlation with motor and nonmotor symptoms. Indeed the use of PET scintigraphy allows the detection of microglial activation in PD, while ^{123}I-MIBG scintigraphy with SPECT demonstrates cardiac sympathetic denervation in PD and is a useful neuroimaging modality for differentiating PD from other types of parkinsonism (Cascini et al., 2013). Cortical and limbic Lewy body disease is seen in more advanced PD and can be detected with ^{18}FdG-PET as an abnormality between levels of resting brain metabolism in these regions. Additionally, microglial activation can be detected in PD with PET imaging (Brooks and Pavese, 2011). ^{18}F-DOPA and ^{18}FdG have been used extensively as sensitive noninvasive and early diagnostic PET biomarkers of progressive neurodegenerative disorders such as PD, AD, drug addiction, and Schizophrenia (Sharma and Ebadi, 2005; Ben-Shachar et al., 2007). This sophisticated approach can detect the progression of PD and other neurodegenerative disorders at an early stage of disease development. In an earlier study, Goldstein et al. (2008) demonstrated that PD and MSA feature low putamen: occipital cortex (PUT:OCC) ratios of 6-[^{18}F]fluorodopa-derived radioactivity and low CSF DOPAC and DOPA concentrations, cross-validating the neuroimaging and neurochemical approaches but not distinguishing the diseases. PUT:SN and PUT:OCC ratios of 6-[^{18}F]fluorodopa-derived radioactivity, cardiac 6-[^{18}F]fluorodopamine-derived radioactivity, and olfactory testing separate PD from MSA. Although evidence of cardiac sympathetic denervation is associated with other non-motor manifestations

such as anosmia, REM behavior disorder, dementia, baroreflex failure, and orthostatic hypotension, across individual patients the severities of orthostatic hypotension and of the cardiac sympathetic lesion (as indicated by thoracic 6-[^{18}F]fluorodopamine PET scanning) are unrelated to the severity of the putamen dopaminergic lesion. Moreover, whereas cases have been reported with neuroimaging evidence of cardiac sympathetic denervation several years before motor onset of PD, in other cases loss of cardiac sympathetic innervation progresses approximately concurrently with the movement disorder or can even occur as a late finding. The bases for independent sympathetic noradrenergic and striatal dopaminergic lesions in Lewy body diseases remain poorly understood. In elderly patients with unexplained orthostatic hypotension or other evidence of autonomic failure, it is reasonable to look for subtle signs of parkinsonism, such as masked facies, cogwheel rigidity, and shuffling gate (Goldstein et al., 2011). Braak's staging concept of Lewy body disease pathogenesis is based on a spatiotemporal sequence of α-Syn deposition, with autonomic nervous system involvement before Synucleinopathy in substantia nigra neurons. A patient with chronic autonomic failure underwent brain ^{18}F-DOPA and myocardial ^{18}F-dopamine imaging over 4 years. Low myocardial radioactivity indicated cardiac noradrenergic denervation. Striatal ^{18}F-DOPA-derived radioactivity initially was normal, 2 years later was decreased subtly, and by 4 years was clearly decreased, accompanied by dementia and parkinsonism. The neuroimaging evidence of cardiac noradrenergic denervation and subsequent progressive striatal dopaminergic denervation in this patient fit with Braak staging (Goldstein et al., 2012c). Recently, we have utilized ^{18}F-DOPA and ^{18}FdG as sensitive and early biomarkers of microPET imaging of genetically-engineered α-Syn, MTs, and MTs over-expressing wv/wv mice with a primary objective to establish that MTs are neuroprotective agents as anti-inflammatory, antiapoptotic, and antioxidant proteins (Sharma and Ebadi 2005, 2008a; Sharma et al., 2006, 2014). We have discovered that cocaine and methamphetamine induce significant reduction in the striatal ^{18}F-DOPA uptake in C57BL6J mice. The uptake of ^{18}F-DOPA was further reduced when ethanol was administered simultaneously with cocaine and methamphetamine-abused mice, suggesting that ethanol augments cocaine and methamphetamine neurotoxicity (Sharma and Ebadi, 2008a). The distribution kinetics of ^{18}F-DOPA was significantly impaired in wv/wv mice (Sharma and Ebadi, 2005). A progressive reduction in the striatal ^{18}F-DOPA was observed in wv/wv mice as a function of aging (Sharma et al., 2006). Since wv/wv mice exhibit progressive loss of striatal dopaminergic neurons, whereas MTs transgenic (MT_{trans}) mice live long; we proposed to transplant mesencephalic fetal stem cells derived from MT_{trans} embryos in wv/wv mice striatal regions and evaluate their therapeutic potential by performing ^{18}F-DOPA microPET neuroimaging (Ebadi et al., 2006). We have established that progressive reduction in ^{18}F-DOPA occurs as a consequence of oxidative and nitrative stress in wv/wv mice (Ebadi and Sharma, 2006). Since brain regional MTs and CoQ_{10} were significantly reduced in wv/wv mice, we developed sensitive colorimetric enzyme-linked immunosorbant assay (ELISA) for the estimation of brain regional MTs and HPLC-UV method for the estimation of CoQ_{10} from genetically-engineered rare biological samples of α-Syn-metallothioneins triple gene knock out (α-Syn-MT_{tko}) mice (Sharma and Ebadi, 2004). Crossbreeding of wv/wv mice with MT_{trans} mice developed a progeny of MTs over-expressing wv/wv with significantly improved striatal ^{18}FdG as well as ^{18}D-DOPA in MTs-over-expressing (wv/wv-MTs) mice, authenticating the hypothesis that MTs provide CoQ_{10}-mediated neuroprotection by rejuvenating the down-regulated mitochondrial (ubiquinone-NADH-oxidoreductase) complex-1, a rate limiting enzyme complex involved in oxidative phosphorylation and ATP synthesis for maintaining the mitochondrial bioenergetics (Sharma and Ebadi, 2014). Fukumoto et al. (2012) recently imaged the acute ischemic neuronal damage in rat brain caused by photochemically-induced thrombosis (PIT) using [^{18}F]BMS-747158-02 ([^{18}F]BMS) for mitochondrial complex-1 (MC-1) and [^{11}C](R)-PK11195 ([^{11}C](R)-PK) for peripheral benzodiazepine receptor [PBR; translocator protein] at preischemic (day 1), and 7 days after ischemic insult with a primary objective to assess microglial activation in PD. [^{18}F]BMS was taken up by the rat brain, with a homogeneous distribution, and the uptake was suppressed by rotenone, a specific MC-1 inhibitor. The specificity of [^{18}F] BMS binding to MC-1 was also confirmed by brain slice imaging. At day 1, [^{18}F] BMS uptake was low in infarct and peri-infarct regions where neuronal damage was detected by 2, 3, 5-triphenyltetrazolium chloride (TTC) staining. At day 7, the damaged areas determined using [^{18}F] BMS revealed some discrepancy from those detected by TTC staining, suggesting that TTC stains not only surviving cells but also activated microglia in the peri-infarct region which was confirmed by [^{11}C] (R)-PK imaging and immunohistochemical assessment with Iba1 antibody.

Weintraub et al. (2005) performed SPECT neuroimaging with ^{99m}Tc-TRODAT-1, that selectively binds to the DAT in patients with idiopathic PD and healthy volunteers. TRODAT-1 distribution volume ratios, a reflection of DAT availability, were calculated for six regions of interest (ROIs) in the caudate and putamen. The association between neuropsychiatric symptoms (anxiety, depression, and fatigue) and DAT availability was explored for both groups and the impact of disease severity was examined in the PD group. PD patients showed lower DAT activity than did healthy volunteers. In PD patients, anxiety and depression were associated with diminished DAT availability in the left anterior putamen. The association between total scores and DAT availability was present only in the subset of patients with less severe PD, but subjects with the highest DAT availability did not show high total affect scores. No association between neuropsychiatric measures and DAT activity was observed in the controls suggesting that decreased DAT availability seem necessary for but may not be associated with the development of affective symptoms in PD (Speigel, 2011).

27. Transcranial sonography and molecular imaging

Transcranial ultrasound has shown susceptibility factors for PD related to iron overload in the substantia nigra. Midbrain/nigral structural abnormalities can be demonstrated in vivo with both transcranial sonography (TCS) and diffusion tensor MRI (DTI) while PET and SPECT ligands can be employed to demonstrate dysfunction in the dopaminergic neurotransmission.

28. α-Syn induced microglial activation

Indeed genetic studies have revolutionized our understanding of PD. α-Syn was the first gene to be linked to PD, and is the most important as it forms a principal constituent of Lewy bodies and variation at its locus is the major genetic risk factor for sporadic disease (Devine et al., 2011). Recently, Devine et al. (2011) have suggested that α-Syn-directed microglial activation results in increased expression of TNF-α, NOS, IL-1β, MMP-9, ROS and morphological changes consistent with enhanced inflammation within 24 h. It has been shown that α-Syn incites microglial activation via toll-like receptors (TLRs) activation. An hypothesis has been proposed that α-Syn released from neurons and/or presynaptic terminals activates microglia (Beraud and Maguire-Zeiss, 2012). α-Syn-mediated activation of microglia results in increased expression of TLRs. TLRs can mediate downstream pathways that result in the translocation of NFκB to the nucleus, which causes increased expression of proinflammatory cytokines that are

detrimental to dopaminergic neurons suggesting "*Dopamine Neuron Vulnerability*". Microglial activation is often accompanied by a change in morphology from highly branched cells to rounded cells with branching. If this pro-inflammatory milieu continues, progressive loss of dopaminergic neurons releasing α-Syn would ensue followed by activation of microglia. However, since TLRs can also promote cell growth and survival, it will be important to establish the specific TLRs that are altered by α-Syn conformers and to determine the downstream TLR pathways (MyD88 and/or PI3K) so that targeted therapies for PD could be developed. Duplications and triplications of the locus, as well as point mutations, cause familial disease. Hence alterations in α-Syn expression can manifest with a phenotype. Furthermore, Béraud et al. (2013) reported that α-Syn-directed glial response is involved in protein misfolding, oxidative stress, and inflammation in PD and represents a potential locus for the development of novel therapeutics focused on induction of the Nrf2-directed antioxidant pathway and inhibition of protein misfolding. Recently Pfefferkorn et al. (2012) have summarized biophysical approaches for the study of membrane proteins and their application for the characterization of the interactions of the natively unfolded and PD related α-Syn. These studies have led to the understanding of membrane-bound α-Syn structure and specific properties that affect α-Syn-membrane binding.

29. Cytokines as biomarkers

Cytokines, which are immunological messengers facilitating both intra- and inter-system communication, are considered key players in the neuroinflammatory cascades associated with the neurodegenerative process in PD. Recently Litteljohn and Hayley (2012) have reported that cytokines may hold great promise as serological biomarkers in PD, with potential applications ranging from early diagnosis and disease staging, to prognosis, drug discovery, and better prognosis. The cytokines have also been implicated in depression and other cognitive (memory impairment, dementia) and affective disturbances (anxiety) as a co-morbidity with PD (Leonard and Myint, 2006). Sub-classification or risk stratification in PD could be based on reliably determined cytokines of particular co-morbidities or at-risk groups (PD alone, PD with depression and/or dementia). Researchers and clinicians seeking to describe cytokine variations in health vs. disease will benefit from advanced technologies that allow a high degree of multiplexing and thus permit the simultaneous determination of several cytokines in small-volume samples. Litteljohn and Hayley (2012) have highlighted recent advances in the cytokine multiplex assay for biomarker discovery of PD and its depressive and cognitive co-morbidities. Subclassification or risk stratification in PD could be based on reliably determined cytokine panel profiles or "signatures" of particular co-morbid disease states or at-risk groups (PD alone, PD with depression and/or dementia). The need for such highly paralleled assays is extremely important because cytokines do not act in isolation but rather against a backdrop of complementary and antagonistic effects; ascribing valence to the actions of any one cytokine thus requires specific knowledge about the larger cytokine milieu.

30. Neuroinflammatory biomarkers

According to the neuroinflammatory hypothesis, drugs with an anti-inflammatory properties should slow the progression of PD. Advances in anti-inflammatory drugs those are becoming a new therapeutic strategies for PD have been reviewed by Klegeris et al. (2007) and Tansey and Goldberg (2010). The involvement of inflammatory mechanisms in PD has been revealed through in vitro and in vivo experimental studies supported by pathological and epidemiological findings. Several of the inflammatory mechanisms are shared by other neurodegenerative disorders but some PD-specific mechanisms have also emerged. These include inflammatory stimulation by interaction of α-Syn with microglia and astrocytes and a suppressive action by nonsteroidal anti-inflammatory drugs (NSAIDs) on dopamine quinone formation. Hence more detailed understanding of neuroinflammatory mechanisms in PD will lead to new cellular and molecular targets, which may, in turn, permit novel design of PD modifying drugs. Thus future treatment may involve combination therapies with drugs directed at both inflammatory and non-inflammatory mechanisms. The health and socioeconomic impacts of dementia with Lewy bodies and dementia with PD has already been recognized (Burn, 2006). Epidemiological studies have explored basic differences between dementia with Lewy bodies and PD. The pathology underlying dementia with Lewy bodies and PD is heterogeneous, and is neither stereotyped in its topography nor its composition. Although cholinesterase inhibitors improve cognition and neuropsychiatric symptoms, the clinical response is unpredictable in PD patients. In addition abnormal protein aggregation has been postulated as a molecular basis for many neurodegenerative diseases, including PD, AD, and prion diseases, as well as trinucleotide repeat disorders (Richard et al., 2008; Roodveldt et al., 2011). The findings that mutations in α-Syn lead to autosomal-dominant, early-onset PD in some families and that α-Syn is found in Lewy bodies of all PD patients has strengthened the hypothesis that the pathophysiology of all PD patients starts with an abnormal folding of α-Syn, producing aggregates that overwhelms the anti-aggregation mechanisms of the cell. We have reported that pro-inflammatory cytokine NFkβ is significantly increased where as mitochondrial ubquinone-NADH oxidoreductase (complex-1) is significantly down-regulated in homozygous weaver mutant (wv/wv) mice exhibiting progressive nigrostriatal dopaminergic neurodegeneration, early morbidity, and mortality as noticed in PD patients (Ebadi et al., 2004a). CoQ_{10} treatment significantly reduced NFkβ induction and rejuvenated complex-1 in these genotypes suggesting anti-inflammatory potential of CoQ_{10} in PD (Sharma and Ebadi, 2008a, 2011a, 2011b, 2014; Sharma et al., 2013).

31. Brain-derived neurotrophic factor (BNDF)

BDNF is one of the key molecules modulating neuroplasticity and it affects cognitive deficit associated with aging and neurodegenerative disease. Plasma BDNF is also a biomarker of impaired memory and cognitive function in aging. Gonadal steroids are involved in the regulation of several CNS processes, specifically mood, affective and cognitive functions during reproductive aging. These observations lead to investigate a putative co-regulation between BDNF and gonadal and/or adrenal steroids and their relationship with gender difference in the incidence of neurological diseases. BDNF is a neurotrophin expressed in several areas of the CNS and is known to induce a long-lasting potentiation of synaptic efficacy, to enhance specific learning and memory processes (Lu, 2003). BDNF is the most widely distributed neurotrophin in the CNS, where it plays crucial roles in synaptic plasticity and neuronal survival (Teixeira et al., 2010). Hence, BDNF has become a biomarker of interest in the physiopathology of PD. Several studies have shown an altered BDNF production and secretion in PD and AD but also in mood disorders like depression, eating disorders, and schizophrenia (Tamatam et al., 2012). Updated knowledge on the correlation between BDNF expression/function and both gonadal (progesterone, estrogens, and testosterone) and adrenal hormones (mainly cortisol and dehydroepiandrosterone (DHEA)) with relevance to clinical application has been highlighted in a recent review by Pluchino et al. (2013). Altered levels of BDNF have been reported in the circulation (serum or plasma) of patients with PD,

major depression, bipolar disorder, AD, and HD. Correlations between serum BDNF levels and affective, cognitive and motor symptoms have also been reported. However BDNF seems to be a nonspecific biomarker of neurodegenerative diseases including PD (Teixeira et al., 2010).

32. Multimodality biomarkers

Indeed early PD detection will pave the way for major advances in disease modifying therapies. Chahine and Stern (2011) have emphasized the importance of developing PD biomarkers at preclinical stage. Various diagnostic modalities hold promise for the preclinical diagnosis of PD. Recently tests of the autonomic nervous system, such as cardiac functional imaging to measure cardiac sympathetic denervation, CSF and serum tests, including a-Syn and DJ-1, have been developed and improved (Ho et al., 2011). Hence the future diagnosis of PD will rely on a combination of clinical, laboratory, imaging, and genetic data. Various imaging modalities have contributed to the diagnostic armamentarium in PD, including transcranial Doppler ultrasonography, radiolabeled tracer imaging, and MRI. These include nonmotor features that predate the motor manifestations of PD, including sleep abnormalities, neurobehavioral symptoms, and olfactory dysfunction. Poewe et al. (2008) have highlighted clinical and neurobiological aspects of dementia in PD and proposed criteria for a clinical diagnosis of dementia in PD. A very high cumulative prevalence of dementia in PD has been shown in two independent long-term cohorts. Mild cognitive impairment occurs even in early PD and is associated with a shorter time to dementia. Emerging evidence from pathology, as well as in vivo studies using novel procedures within genetics, imaging, and CSF investigations, indicates that α-Syn aggregation and disturbances of other candidate proteins are associated with dementia in PD. Miscellaneous biomarkers of PD including α-Syn index, Charnoly body formation, Lewy body formation, increased striatal iron, reduced ferritin, and reduced pyridoxal kinase activity are presented in Fig. 8.

33. Pharmacological biomarkers

It is well established that folate and vitamin B_{12} are essential cofactors for the methionine/homocysteine cycle in the brain (Herrmann and Obeid, 2007). These vitamins mediate the remethylation of homocysteine, which affects the production of methyl donor, S-adenosylmethionine (SAM) in the brain. Increased plasma homocyeteine is associated with cerebrovascular disease and can compromise the blood–brain barrier. Homocycteine in brain and CSF are increased in several psychiatric and neurological disorders including PD. Disturbances in the transmethylation pathway indicated by abnormal SAM, S-adenosylhomocysteine or their ratio have been reported in several neurodegenerative diseases, such as dementia, depression, and PD. Similarly cobalamine is essential for neuronal generation and its deficiency can cause neurodegeneration. Deficiency of folate and vitamin B_{12} can lead to elevated concentrations of homocycteine and disturbed methylation in the brain. Therefore, acquired or inherited disorders in these metabolic pathways are associated with brain abnormalities and neurological symptoms that are irreversible, even after providing the missing cofactors. Consequently the relationship between brain and blood levels of key vitamins and metabolites related to one carbon metabolism is extremely important for normal brain function. Patients exhibiting features of parkinsonism including – tremors, rigidity, postural instability, slowed movements, sleep disturbances, and depression may also display severe cognitive disturbances (Wurtman, 2013). All of these motoric and behavioral symptoms may arise from PD, but can also characterize Lewy Body

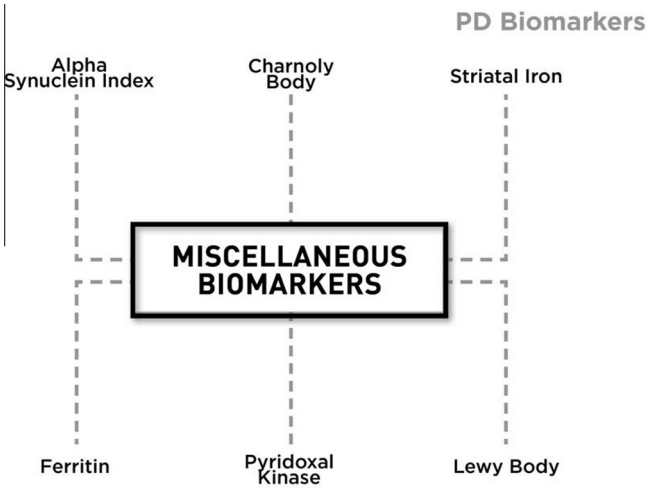

Fig. 8. A flow diagram illustrating newly discovered biomarkers of PD. We have reported these novel diagnostic biomarker which can be evaluated by quantitatively estimating the nitrated α-Syn as well as native α-Syn using double radioimmunoprecipitation and specific monoclonal and polyclonal antibodies to α-Syn. The α-Syn index is the ratio of nitrative α-Syn vs. native α-Syn and provides sensitive and accurate estimate of nitrative as well as oxidative stress to α-Syn implicated in Lewy body pathogenesis. By employing MTs transgenic (MT_{trans}) mice we have confirmed that MTs attenuate MPP^+, rotenone, and 3-morpholinosydnonimine (SIN-1)-mediated nitration of α-Syn and hence could prevent neurodegenerative alpha-Synucleinopathies as seen in PD and AD (Sharma and Ebadi, 2011a, 2011b, 2013).

dementia [LBD] or concurrent PD and AD. Abnormalities of movement and cognition are observed in several other neurologic diseases, for example Huntington's disease (HD) and the frontotemporal dementia. Hence differential diagnosis of these diseases is important in personalizing the mode of treatment, since an agent that is often effective in one of the diagnoses might be ineffective (L-DOPA or muscarinic receptor antagonists in PD) or even damaging to the other neurological disorder. Such personalized medicine, based on genetic, biochemical, and imaging-based biomarkers, is achievable as numerous genetic abnormalities have already been discovered and by the variety of regional and temporal patterns employing different imaging modalities in PD.

34. Molecular biomarkers in drug development

In general molecular biomarkers are used in early drug development studies. Some of the main areas where molecular biomarkers are used are: early drug development studies, safety studies, proof of concept studies, and molecular profiling (Li et al., 2011). Particularly the molecular biomarkers are used in phase I studies to establish doses and dosing regimen for future phase II studies. PD biomarkers are commonly observed to respond proportionally with dose. These data, in conjunction with safety data, help determine doses for phase II studies. In addition, safety molecular biomarkers are being developed and utilized both in preclinical and clinical research. Since biomarker evaluation has now become a mainstream approach, they have become fully automated for both animal and human drug evaluation.

35. Most recent PD biomarkers

Recently Sierra et al. (2013) performed a study to ascertain whether substantia nigra (SN) echogenicity, olfaction, and dopamine transporter (DAT)-SPECT are reliable premotor biomarkers in asymptomatic carriers of the LRRK2 G2019S mutation (AsG2019S+). These biomarkers were evaluated in 49 AsG2019S+

patients. Olfaction and SN echogenicity was studied in 29 patients with G2019S-associated PD (PD-G2019S), 47 relatives who were noncarriers of the LRRK2 G2019S mutation (AsG2019S−), 50 patients with idiopathic PD, and 50 community controls. Eighty-five percent of unaffected mutation carriers (AsG2019S+) showed pathologic SN hyperechogenicity, with a similar proportion among both PD-G2019S and iPD cases, and 41% of AsG2019S− also showed increased SN echogenicity. The proportion of hyposmic individuals was not significantly different in patients with PD-G2019S (50%) and iPD (82%), but hyposmia was less common in both AsG2019S+ (26%) and AsG2019S− (28%). In AsG2019S+ cases, reduced striatal uptake in DAT-SPECT was observed in 43.7% cases. Irrespective of the age, the most frequently altered premotor biomarker in LRRK2 G2019S-associated PD was SN hyperechogenicity, whereas abnormal DAT-SPECT was more prominent in older unaffected mutation carriers. Furthermore, Butterfield and Dalle-Donne (2012) reported that proteins are major targets of reactive oxygen and nitrogen species (ROS/RNS) and numerous post-translational modifications, hence reversible or irreversible modifications have been characterized, which may lead to a change in the structure and/or function of the oxidized protein. Moreover redox proteomics is an emerging branch of proteomics aimed at identifying and quantifying changes within the proteome both in redox signaling and under oxidative stress conditions. Currently correlation between protein oxidation and human disease is widely accepted, although elucidating cause and effect remains unknown. Biomedical data have provided evidences for the involvement of perturbations in redox homeostasis in a large number of pathophysiological conditions including PD. Better understanding of the molecular mechanisms of PD together with the identification of specific targets of oxidative damage is urgently required. In the last few years, combined proteomics, mass spectrometry (MS), and affinity chemistry-based methodologies have contributed significantly to provide a better understanding of protein oxidative modifications under different physiological and pathological conditions. Several reports beyond the scope of this communication have been presented on various subjects ranging from redox proteomics studies of oxidatively modified brain proteins in PD to potential new biomarker discovery paradigm for human disease, to protein nitration in aging and age-related neurodegenerative disorders including PD, electrophile-responsive proteomes of diseases involving mitochondrial alterations, to cardiovascular physiology and pathology. Since ROS are known to contribute to the pathophysiology of PD, clinical trials of antioxidants are currently underway in PD patients. However, antioxidant research has been hindered by a lack of peripheral biomarkers. Mischley et al. (2012) selected 22 patients with PD to have a novel antioxidant assessment. Each PD case was compared to four age- and gender-matched controls in four separate, random trials using laboratory data. Logistic regression was used to determine the odds of functional deficiency in antioxidant nutrients (glutathione, CoQ_{10}, selenium, vitamin E and α-lipoic acid). The proportion of cases with functional deficiency was also compared to that for controls by Ψ^2 test. PD patients had greater odds of deficiency in CoQ_{10} status based on FIA results, but not of vitamin E, selenium, lipoic acid, or glutathione. CoQ_{10} deficiency was also significantly greater in PD patients than in controls suggesting that CoQ_{10} should be explored as a potential peripheral biomarker of antioxidant status in PD. Hall et al. (2012) recently assessed the ability of five CSF biomarkers to differentiate between common dementia and parkinsonian disorders. CSF samples were obtained from healthy individuals serving as controls and from patients with PD, PD with dementia (PDD), dementia with Lewy bodies (DLB), AD, progressive supranuclear palsy (PSP), multiple system atrophy (MSA), or corticobasal degeneration (CBD). CSF α-Syn was decreased in patients with PD, PDD, DLB, and MSA but increased in patients with AD. CSF α-amyloid 1-42 were decreased in DLB and even further decreased in AD. However total tau and hyperphosphorylated tau were increased in AD. Multivariate analysis revealed that these biomarkers could differentiate PD from, AD, DLB and PDD, with α-Syn and total tau. Furthermore, CSF neurofilament light chain was significantly increased in atypical parkinsonian disorders (PSP, MSA, and CBD), and multivariate analysis revealed that the neurofilament light chain alone could differentiate PD from atypical parkinsonian disorders. Thus estimation of the α-Syn in CSF improves the differential diagnosis of AD vs. DLB and PDD when combined with established AD biomarkers, whereas the level of neurofilament light chain alone may differentiate PD from atypical parkinsonian disorders. Beach et al. (2013) recently reported that the clinical diagnosis of PD is incorrect in 30% or more of subjects particularly at the time of symptom onset. Because Lewy-type alpha-Synucleinopathies is present in the submandibular glands of PD patients, these investigators assessed the feasibility of submandibular gland biopsy for diagnosing PD. Frozen submandibular glands taken with 18-gauge needles were positive for Lewy-type alpha-synucleinopathies in 17 of 19 PD patients suggesting that biopsy of the submandibular gland may be a feasible means of improving diagnostic accuracy of PD. This would be advantageous for subject selection in early-stage clinical trials for invasive therapies or for verifying other biomarker studies. Furthermore, Besong-Agbo et al. (2013) detected autoantibodies against α-Syn (α-Syn-nAbs) in the serum of patients with PD and developed an ELISA to quantify α-Syn-nAbs in serum samples as a .diagnostic marker for PD. Serum levels of α-Syn-nAbs were significantly lower in patients with PD compared to HC or patients with AD. The α-Syn-nAbs levels did not correlate with age, Hoehn and Yahr status, or duration of disease. Endogenous α-Syn had no influence on α-Syn-nAbs levels in sera. However the assay did not achieve criteria for use as a routine diagnostic tool to reliably distinguish PD from HC. Further studies are needed to assess α-Syn-nAbs as a biomarker in PD. Bidinosti et al. (2012) recently described two sensitive, time-resolved Förster's resonance energy transfer (TR-FRET)-based immunoassays for total and oligomeric α-Syn quantification. CSF analysis showed strong concordance for total α-Syn between two TR-FRET assays in agreement with a previously characterized ELISA, demonstrated lower α-Syn in PD donors. Critically, the assay suitability for high-throughput screening of siRNA constructs and small molecules aimed at reducing endogenous α-Syn levels was established. Furthermore, a reverse genetic screen of a kinase-directed siRNA library identified seven genes that modulated α-Syn levels. This provides critical new biological insight into cellular pathways regulating α-Syn steady-state expression that may help drug discovery efforts. They have also described an inherent limitation in current α-Syn oligomer detection methodology, a finding that will lead to the direct improvement of future assay design. This single-step TR-FRET-based platform for α-Syn quantification provides a novel approach with superior performance for the rapid screening of large biomarker cohorts and of compound and genetic libraries, both of which are essential to the development of PD biomarkers and the future therapies.

36. PD susceptibility genes

Recently Pankratz et al. (2012) identified a novel PD susceptibility locus, RIT2, replicated several previously identified loci, and more than 1 risk allele within SNCA and GBA. Individual level genotypic data from 5 recent PD GWAS (Discovery Sample: 4238 PD cases and 4239 controls) were combined. Following imputation, a logistic regression model was employed in each dataset to test for association with PD susceptibility and results from each dataset were meta-analyzed. Then 768 single-nucleotide polymorphisms

(SNPs) were genotyped in a Replication Sample (3738 cases and 2111 controls). Genome-wide significance was reached for SNPs in SNCA. Conditional analyses within each of the replicated regions identified distinct SNP associations within GBA and SNCA, suggesting that there may be multiple risk alleles within these genes. Further studies are needed in this direction to determine other genes responsible for PD susceptibility.

37. α-Syn index

We have introduced α-Syn index (a ratio of nitrated α-Syn vs. native α-Syn) as a sensitive diagnostic biomarker for the early clinical management of PD (Sharma et al., 2003, 2004, 2013; Sharma and Ebadi, 2011a, 2011b). Furthermore we have shown that Selegiline, a monoamine oxidase-B inhibitor provides neuroprotection in the cultured dopaminergic (SK-N-SH) neurons by augmenting MTs expression, CoQ_{10} synthesis and complex-1 (ubiquinone-NADH oxidoreductase) rejuvenation (Sharma et al., 2004). This was authenticated by employing MTs gene-manipulated and homozygous weaver mutant (wv/wv) mice exhibiting progressive nigrostriatal dopaminergic neurodegeneration and typical symptoms of PD and chronic drug addiction (Sharma and Ebadi, 2005, 2008a, 2011a, 2011b; Sharma et al., 2013). Furthermore, we have reported that MTs can be used as early and effective redox-sensitive diagnostic biomarkers for the clinical management of PD and drug addiction (Sharma and Ebadi, 2011a, 2011b).

38. MTs and Charnoly body (CBs)

Recently we have reported that MTs provide neuropotection by inhibiting nitration of α-Syn involved in neurodegenerative α-Synucleinopathies (Sharma and Ebadi, 2011a, 2011b, 2014). Hence in addition to MTs, α-Syn index may be utilized for the clinical diagnosis and prognosis of PD as illustrated in Fig. 8. We have authenticated these findings by developing α-Syn-MTs triple knock out (α-Syn-MT_{tko}) mice and MTs over-expressing wv/wv mice (Sharma and Ebadi, 2013). Furthermore, we have introduced Charnoly body (CB) as diagnostic biomarker that is formed as a deleterious consequence of oxidative and nitrative stress in the CNS due to degeneration of mitochondrial membranes. CBs are composed of aggregated electron-dense mitochondrial membranes stacks which form penta or heptalamellar structures in the degenerating neurons due to free radical overproduction compromising mitochondrial bioenergetics. CBs formation is augmented in nutritional stress or in response to environmental or parkinsonian neurotoxic insult, whereas MTs inhibit CBs formation and neurodegenerative Synopathies by inhibiting α-Syn index and by acting as free radical scavengers to provide mitochondrial neuroprotection (Sharma and Ebadi, 2014). We have also reported that calcium binding proteins, TRPCs are significantly down-regulated in human cultured dopaminergic (SH-SY5Y) neurons when exposed to 1-methyl, 4-phenyl, pyridinium (MPP^+). Therefore TRPCs may be considered as potential early and sensitive biomarkers for the in vitro assessment of various environmental neurotoxins implicated in the Lewy body pathogenesis and PD (Bollimuntha et al., 2005). Recent studies suggest that lithium has neuroprotective properties and may be useful in the treatment of PD and various other chronic neurodegenerative diseases such as AD, PD, HD and ALS (Luo, 2010). One of the most important neuroprotective properties of lithium is its anti-apoptotic action. In vivo and in vitro studies have indicated that lithium can ameliorate ethanol-induced neuroapoptosis. Lithium is an inhibitor of glycogen synthase kinase 3 (GSK3) which has been identified as a mediator of ethanol neurotoxicity. Lithium's neuroprotection may be mediated by inhibition of GSK3. In addition, lithium can affect many other signaling proteins and pathways that regulate neuronal growth, survival, and differentiation. Further studies have shown that Complexins play a critical role in the control of fast synchronous neurotransmitter release. Complexins operate by binding to trimeric SNARE complexes consisting of the vesicle protein Synaptobrevin and the plasma membrane proteins Syntaxin and SNAP-25, which are key executors of membrane fusion reactions. SNARE complex binding by Complexins stabilizes and clamps the SNARE complex in a highly fusogenic state, thereby providing synaptic vesicles that can be released quickly in response to an action potential and the concomitant increase in intra-synaptic Ca^{2+} release. Genetic elimination of Complexins from neurons causes reduction in neurotransmitter release. Altered Complexin expression with consequent deficits in synaptic transmission may contribute to the etiopathogenesis of PD and various neurodegenerative disorders (Brose, 2008). The concentration of plasma copper, ceruloplasmin (CRP), non-ceruloplasmin-bound Cu (NCBC), and MTs have been studied as putative biomarkers for PD patients and in their first-degree relatives. Increased Cu has been noticed in the plasma of PD, AD, and vascular dementia (VD) patients. CRP is also elevated in response to the inflammatory component of the diseases. The extent of MTs induction was proportional to the evolution of VD. Thus Cu/CRP and Cu/MTs ratios are both indicative of disease progression for AD patients but not for those with PD or VD. Moreover, there is a correlation between the NCBC levels and the cognitive impairment estimated through the Minimental State Examination (MMSE) scale. This dependence was linear for PD and AD patients and non-linear for the VD ones (Arnal et al., 2010). Furthermore, transplantation of genetically modified cells into the brain represents a promising strategy for the delivery and expression of specific neurotrophic factors, neurotransmitter-synthesizing enzymes, and cellular regulatory proteins for intervention in neurodegenerative diseases. The use of specific promoters (including MT-1) may also provide potential control of gene expression required for dose-specific or time-specific therapeutic strategies (Sharma and Ebadi, 2014). Despite the crucial role of oxidative stress in PD, the exact mechanism of Zn-induced dopaminergic neurodegeneration is not fully understood. An association between zinc accumulation in the brain and incidences of PD has been demonstrated in epidemiological and experimental studies (Kumar et al., 2012). The involvement of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and glutathione (GSH) in the pathogenesis of PD has also been proposed. A study was aimed to investigate the involvement of NADPH oxidase and GSH in Zn-induced dopaminergic neurodegeneration and also to assess its similarity with paraquat (PQ)-induced rat model of PD. Male Wistar rats were treated either with Zn or PQ in the presence and absence of NADPH oxidase inhibitor, apocynin and a GSH precursor, N-acetyl cysteine either alone or in combination along with the respective controls. Apocynin and/or NAC pre-treatment alleviated zinc and PQ-induced changes in neurobehavioral deficits, number of dopaminergic neurons, and the striatal dopamine and its metabolites. Apocynin and/or NAC also mitigated Zn- and PQ-induced alterations in oxidative stress, NADPH oxidase activation and cytochrome c release, caspases-9 and 3 activation and CD11b expression suggesting that Zn induces oxidative stress via the activation of NADPH oxidase and depletion of GSH, which in turn triggers apoptosis leading to dopaminergic neurodegeneration similar to PD. Traditionally, it has been thought that decrease in brain regional GSH is the consequence of increased oxidative stress, a process heavily implicated in PD pathogenesis. However, emerging evidence suggests that GSH depletion may itself play crucial role role in PD pathogenesis (Martin and Teismann, 2009).

39. Neuroprotective biomarkers

Presently-used anti-parkinsonian drugs neither stop nor slow-down the progression of the disease. Hence neuroprotective therapy is a primary aim in the treatment of PD. Despite neuroprotection being a research priority in PD, no effective strategies have been discovered so far. Some experts believe that we are already exerting a disease modifying effect in PD. Thus the concept of neuroprotection should be broadened and animal models should be improved. Linazasoro et al. (2009) proposed that a reliable biomarker is required to initiate neuroprotective therapies long before the appearance of motor symptoms to evaluate the neuroprotective effect. It is, therefore, essential to establish sensitive and specific biomarkers to identify subjects at risk before motor manifestations. A number of such "premotor" signs have been discovered and are being investigated. The final phase of PD is characterized by the presence of symptoms and signs resistant to dopaminergic agents, such as depression, dementia, freezing, and falls. Therefore, it is important to develop therapies that are able to modify this outcome at an earlier stage. A group of experts in PD prepared a questionnaire to explore the most important topics related to neuroprotection. A consensus about the current situation of neuroprotection in PD was established and future directions of development were suggested. Most of the answers emphasized the need for new concepts, the limitations of animal models, and difficulties in demonstrating neuroprotective effects owing to a lack of specific biomarkers. Such biomarkers may include a genetic vulnerability, hyperechogenicity of the substantia nigra, olfactory and autonomic dysfunction, depression, REM sleep behavior disorder, and visual and neuropsychological impairments (Berg, 2006). Moreover, the first signs of SN abnormalities and motor symptoms may be detected before a definite diagnosis can be made by performing PET and SPECT imaging. Although most of these signs and symptoms are nonspecific if singularly evaluated, a combination of these features may be valuable to detect populations at risk for PD. Recently we have reported a detailed information of antioxidants as neuroprotective biomarkers for the treatment of progressive neurodegenerative disorders including PD (Sharma and Ebadi, 2014). We have reported that various antioxidants provide neuroprotection in PD by acting as free radical scavengers including α-Syn index (nitrated α-Syn/native α-Syn), (b) melatonin (c) superoxide dismutase (d) catalase (e) metallothioneins (MT1, MT2, and MT3), (f) glutathione, (g) ubiquinone-NADH-oxidoreductase (complex-1), (h) coenzyme Q_{10}, and (i) neuromelanin (Sharma et al., 2013; Sharma and Ebadi, 2014) as illustrated in (Fig. 9). Future studies are necessary to establish the predictive value of these biomarkers singularly and/or in combination for the effective treatment of PD.

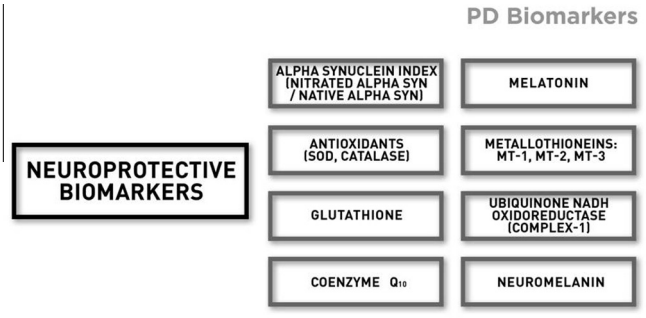

Fig. 9. A flow diagram illustrating neuroprotective biomarkers of PD. The primary objective PD treatment is to provide neuroprotection in the nigrostriatal dopaminergic neurons which can be accomplished by employing pharmacotherapy or by the use of antioxidants which can act as free radical scavengers. Free radicals are generated as a byproduct of mitochondrial oxidative phosphorylation during ATP synthesis. Primarily $^{\cdot}OH$ radicals and NO are produced which when interact with iron synthesize devastating peroxnitrite ions. Peroxynitrite ions can induce oxidative as well as nitrative stress to cause progressive neurodegeneration, early graft rejection, and failure of stem cell therapy in PD patients. Various antioxidants such as coenzyme Q_{10}, melatonin, neuromelanin, ferritin, and glutathione act as free radical scavengers and rejuvenate mitochondrial complex-1 (ubiquinone-NADH) oxidoreductase, a rate limiting enzyme complex involved in ATP synthesis. These neuroprotective biomarkers though nonspecific will continue to provide neuroprotection in PD and other neurodegenerative disorders of unknown etiopathogenesis. Hence antioxidants will prove effective therapeutic agents for the effective and safe clinical management of PD.

40. Conclusion

PD is one of the most common neurodegenerative disorders, involving progressive loss of the nigro-striatal dopaminergic neurons. Although neuronal loss may occur early, cardinal symptoms including body tremors, muscle rigidity, drooping body posture, walking difficulty, and other autonomic symptoms appear late during the progression of the disease after a significant number of dopaminergic neurons are already destroyed. It is therefore extremely important to discover early, sensitive, and specific, biomarker(s) for the differential diagnosis, prognosis, and effective treatment of PD. Novel discovery of biomarkers can play a significant role in the effective treatment of PD with minimum or no side effects. In the present report we have discussed the merits and limitations of recently discovered biomarkers which might help in the early diagnosis and effective treatment of PD. Recently significant progress has been made in the discovery of genetic as well as non-genetic biomarkers that could be utilized for the effective personalized treatment of PD during the preclinical (premotor) and clinical (motor) stage when body tremors, muscular rigidity, and postural irregularity become fully evident. Generally biomarkers for PD can be classified as clinical, neuroimaging, biochemical, genetic or proteomic. Premotor biomarkers like hyperechogenicity of the substantia nigra, olfactory and autonomic dysfunction, depression, REM sleep behavior disorder (RBD), and neuropsychological impairment become evident in PD patients during the preclinical stage. Both in vitro as well as in vivo biomarkers have proved useful for the effective clinical management of PD. The low brain regional N-acetyl-aspartate is also a good biomarker of neuronal loss and can be detected in vivo by MRS along with MRI. Moreover, earlier signs of the involvement of substantia nigra employing PET and SPECT imaging and motor signs can be detected as they may be evident before a definite clinical diagnosis can be made. PD is characterized by a prolonged preclinical period, with biomarkers indicative of disease vulnerability including CSF, serum, and neuroimaging biomarkers, in addition to olfactory dysfunction, and neuropsychological deficits including depression and sleep disturbance. Further studies are necessary to establish the predictive value of these biomarkers individually and/or in combination to detect the population at risk for PD, to elucidate the etiology and pathophysiology, and to develop neuroprotective strategies. Unfortunately the estimation of these biomarkers on a routine basis is costly, cumbersome, and time-consuming. Some biomarker candidates, such neuromelanin antibodies, pathological forms of α-Syn, DJ-1, and patterns of gene expression, metabolomics, and protein profiling seem quite promising. The emphasis is initially on fluid markers including α-Syn, DJ-1, amyloid β, and tau in CSF and urate in blood. Preferably, a novel discovery of peripheral diagnostic biomarker will be highly suitable. A battery of biomarkers comprising different modalities might be required to address clinical needs in this complex disorder. Collaborative efforts including centralized tissue repository and clinical research infrastructure that are being organized will advance this field further. Hence in vivo and in vitro diagnostic biomarkers are currently being

discovered. Both genetic and nongenetic biomarkers may facilitate clinical management in the early pre-symptomatic stage and significantly reduce early morbidity and mortality in PD. We performed in vivo molecular imaging studies by microPET imaging using ^{18}F-DOPA and ^{18}FdG as sensitive biomarkers of dopaminergic neurotransmission and mitochondrial bioenergetics employing MTs, α-Syn gene manipulated, and weaver (wv/wv) mouse models of PD and drug addiction. Furthermore, we have introduced α-synculein index and Charnoly body which may be utilized as sensitive biomarkers of progressive neurodegenerative disorders including PD, AD, and drug addiction. Charnoly bodies are electron-dense multilamellar membraneous structures that are formed before Lewy body formation in the degenerating neuron as a consequence of severe undernutriation and/or neurotoxic exposure (Sharma et al., 2013). MTs inhibit Charnoly body formation by acting as free radical scavengers. Like several other biomarkers of PD, α-Synculein index and Charnoly body are also nonspecific yet could facilitate confirmation of early PD diagnosis. It is envisaged that further advances in molecular neurobiology, omics analyses, dynamic functional neuroimaging, and neurobehavioral analyses will provide better opportunities for the effective clinical management of PD patients with minimum adverse effects of presently available or future medication. There remains lot of room for research and an ample scope for the discovery of early, noninvasive, sensitive, specific, economical diagnostic biomarker(s) for the safe and effective treatment of PD. Further studies in the novel discovery of PD biomarkers will provide avenues to treat PD patients more effectively with few or no side effects.

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