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REVIEW

Can Biomarkers Help the Early Diagnosis of Parkinson's Disease?

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Abstract Parkinson's disease (PD) is a complex neurodegenerative disease with progressive loss of dopamine neurons. PD patients usually manifest a series of motor and non-motor symptoms. In order to provide better early diagnosis and subsequent disease-modifying therapies for PD patients, there is an urgent need to identify sensitive and specific biomarkers. Biomarkers can be divided into four categories: clinical, imaging, biochemical, and genetic. Ideal biomarkers not only improve our understanding of PD pathogenesis and progression, but also provide benefits for early risk evaluation and clinical diagnosis of PD. Although many efforts have been made and several biomarkers have been extensively investigated, few if any have been found useful for early diagnosis. Here, we summarize recent developments in the discovered biomarkers of PD and discuss their merits and limitations for the early diagnosis of PD.

Keywords Parkinson's disease · Biomarker · Early diagnosis · Molecular imaging · Biochemical markers

Introduction

Parkinson's disease (PD) is normally diagnosed based on the clinical history and motor symptoms. When the motor symptoms occur, it is believed that ~60% of dopaminergic neurons in the substantia nigra (SN) pars compacta have been lost and the biology of the disease is already at the middle or late stage [1]. Furthermore, it frequently takes patients a couple of years after the onset of motor symptoms to consult neurology specialists for diagnosis. In clinical practice, the early diagnosis of PD before motor symptoms is difficult because of the lack of distinguishable clinical and laboratory markers to identify pre-motor PD. This delayed diagnosis limits the therapeutic benefits of disease-modifying therapies to reverse or delay the progression of the disease, which makes PD treatment still symptomatic. According to The International Parkinson and Movement Disorder Society (MDS) Task Force on the Definition of PD, early PD can be divided into two phases: a preclinical phase (neurodegeneration has occurred, but without clinical symptoms) and a prodromal phase (clinical symptoms are present, but have not yet reached the criteria for a PD diagnosis) [2]. Therefore, the early diagnosis of PD in the prodromal or even preclinical phase is critical, and if successful, will greatly help define the therapeutic time-window and subsequently may lead to a better clinical outcome [3]. Unfortunately, although clinicians and scientists are aware of the importance and necessity of an early

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diagnosis, to date, no confirmed biomarkers can be applied clinically for the accurate prediction of PD onset at the prodromal or preclinical phase. Therefore, there is an unmet need to exert more effort on the discovery and identification of specific biomarkers for the early diagnosis of PD.

A biomarker is objectively measured and evaluated as an indicator of a normal biological process, a pathogenic progress, or a pharmacological response to a therapeutic intervention. Biomarkers have been used clinically for different purposes. (1) Differential diagnosis, e.g. between PD and other neurological disorders, for example, progressive supranuclear palsy [4]. Even when differences are found, they are between groups and none is sensitive enough to help in the diagnosis of individual patients. Moreover, typically comparisons are done between patient groups with a secure diagnosis, while we need biomarkers that can be helpful in establishing a diagnosis of early or problematic cases. (2) Identifying disease at a preclinical stage is particularly relevant for neurodegenerative diseases, in many of which biological changes occur many years prior to diagnosis. While at present no disease-modifying intervention is available, as soon as any is discovered, biomarkers of early changes could be critically important. (3) Biomarkers can be used as surrogate markers when following up patients, particularly in drug studies. An example is multiple sclerosis, where magnetic resonance imaging (MRI) data have been used as primary endpoints in drug studies. (4) A very important feature of biomarkers is to help in understanding the underlying processes leading to their alteration, and thus allowing intervention in the process.

From a methodological point of view, biomarkers can also be categorized as clinical, imaging, biochemical, and genetic [5]. Chemical biomarkers can be any molecule from genes, RNAs, microRNAs, proteins, and peptides, to neurotransmitters. Biomarker discovery for PD has appealed to many researchers for decades [6–8] and thus numerous trials and analyses have been conducted (see Table 1 for a representative sample of larger studies) [9–16]. In this review, we focus on the recent developments of biomarker discovery for early PD.

Symptomatic Biomarkers

Motor symptoms of PD do not appear suddenly; it is widely accepted that before the classical motor symptoms occur, subtle motor dysfunction can occur. In most cases, the standard motor measures such as the Unified Parkinson's Disease Rating Scales (UPDRS) are used to evaluate the progression of PD, and UPDRS is able to predict the risk of PD in prodromal PD patients as well [9, 12]. It is known that rapid eye movement sleep behavior disorder (RBD) is a common symptom for predicting PD or other synucleinopathies in the prodromal stage [17]. Among patients with RBD, a UPDRS score >4 usually indicates prodromal parkinsonism with high sensitivity and specificity at 2 years before diagnosis, and higher UPDRS scores are always correlated with a higher risk of parkinsonism [18]. Recently, Anette *et al*. analyzed the prevalence of a range of early symptoms of PD before diagnosis and found that the most common motor symptom is tremor at 2 years before diagnosis, and the frequency is still high at 5 and 10 years [19]. Therefore, although motor symptoms often follow non-motor symptoms, they have a higher predictive value than most non-motor symptoms.

Compared with the motor symptoms of PD, the non-motor symptoms are often neglected but may be of great benefit for early diagnosis. Besides RBD, olfactory dysfunction, autonomic dysfunction, and mood disorders are common non-motor symptoms and are referred as hopeful biomarkers [20]. Evidence has demonstrated RBD to be a robust biomarker of prodromal or early-stage PD and other synucleinopathies. RBD is strongly associated with PD with a 45% risk of developing neurodegeneration at 5 years and 76% risk at 10 years according to a follow-up study [17]. In early PD, the occurrence of RBD is increased from 25% to 43% in PD patients within 2 years and RBD can be detected in more than half of *de novo* PD patients [21, 22]. Olfactory dysfunction occurs in >85% PD patients and hyposmia has been reported to be associated with other non-motor symptoms [23, 24]. The specificity of olfactory dysfunction is however lower than RBD and motor markers [25–27]. Some previous studies have shown that impaired olfaction is not a good predictor due to the time limitation. Olfactory loss is apparent only during 3–5 years before motor symptoms occurrence, but other reports provide a longer prediction time [25, 27].

Autonomic dysfunction such as constipation, orthostatic hypotension, somnolence, and urinary and sexual dysfunction also shows different degrees of specificity and predictive value for the early diagnosis of PD [25, 28]. Constipation has a high incidence even 10 years before PD diagnosis, and is the most common non-motor symptom within 2 years before diagnosis [19]. Patients, especially male patients, with constipation have a sustained increased risk of PD [29]. Besides, people with orthostatic hypotension have a 2.1-fold increased risk of PD, but the sensitivity is relatively low because the occurrence of symptomatic orthostatic hypotension is not common in PD [19, 30]. Other autonomic symptoms such as erectile and urinary dysfunction also have predictive value, but much lower than constipation [19]. Depression can occur even 15–25 years before PD diagnosis [31]. The risk of developing PD declines with time [31]. However, the relative

Table 1 Clinical studies of biomarkers for early diagnosis of PD.

Study name	Full name	Participants	Outcomes	References
PRIPS	Prospective evaluation of Risk factors for Idiopathic Parkinson's Syndrome	1847 subjects aged $\geq$ 50 years without diagnosis of PD	Age, hyposmia, enlarged SN hyperechogenicity, UPDRS may be potential biomarkers to predict PD	[9]
TREND	Tübinger Evaluation of Risk Factors for Early Detection of Neurodegeneration	715 subjects aged 50–80 years reporting prodromal markers	Individuals with prodromal markers for PD report a higher prevalence of other prodromal PD symptoms	[10]
PARS	Parkinson Associated Risk Syndrome Study	4999 subjects aged $\geq$ 50 years with no diagnosis of PD, and no known reason for abnormal olfaction	Hyposmia, male sex, and constipation are predictive of DAT deficit	[11]
PPMI	Parkinson Progression Marker Initiative	400 recently diagnosed PD and 200 healthy subjects	RBD, depression, hyposmia, biomarkers in body fluid, UPDRS and some imaging tests may predict the early stage of PD	[12–14]
PREDICT-PD	PREDICT-PD	1323 subjects aged 60–80 years with no diagnosis of PD	Patients with poorer sense of smell, increased rates of RBD and slower finger-tapping speed have higher risk of PD	[15, 16]

DAT dopamine transporter, *PD* Parkinson's disease, *RBD* rapid eye movement sleep behavior disorder, *SN* substantia nigra, *UPDRS* Unified Parkinson's Disease Rating Scales.

risk of depression and anxiety is only $\sim$1.5 [19]. These autonomic dysfunctions and mood disorders have a common characteristic in that they are easy to screen and the testing cost is low. Remarkably, the non-motor features are a mixture of biomarkers and risk factors. To some extent, they highlight evaluation of the risk of developing PD. Most of those non-motor symptoms are not specific to PD, such as constipation, depression, and olfactory dysfunction [3]. Constructing a series of objective measures or scales and auxiliary methods in combination with the results of different non-motor signs would have higher predictive value and be more practical in the primary care of PD patients.

Imaging Biomarkers

Although not universally specific, some imaging tests such as single-photon emission computed tomography (SPECT), photon emission tomography, and MRI have been applied to the differential diagnosis between PD and other parkinsonisms. Although current investigations mainly focus on the progression patterns in diagnosed PD, many studies have explored imaging biomarkers for PD in the preclinical or prodromal stage, and some are already showing promise.

The dopamine transporter (DAT) has been reported to have 98% sensitivity and specificity to detect dopaminergic neuronal loss in PD [32]. Besides, a DAT deficit at baseline is associated with the appearance of motor and non-motor features of PD over 22 months [33]. According to a follow-up study, $\sim$40% of patients with idiopathic RBD have dopaminergic SPECT abnormalities, and are more likely to develop synucleinopathies [34]. Another popular targeted molecule is I-123-metaiodobenzylguanidine (MIBG), which is an indicator of sympathetic uptake. Cardiac sympathetic innervation is lost in PD patients, and this test can be used to differentiate PD from multiple system atrophy [35]. Moreover, MIBG uptake is significantly reduced in RBD patients. So these could be markers of prodromal PD and have potential diagnostic value for Lewy body disease [36–38].

Several anatomical and functional MRI studies have provided evidence that the nigrostriatal and nigrocortical connectivity and parameters of brain tissue or neural fiber integrity can characterize RBD [39, 40]. Given that RBD is a risk biomarker of prodromal PD, these MRI findings may support diagnosis before the onset of PD [39, 40]. Other novel MRI techniques, such as quantitative susceptibility mapping, diffusion tensor imaging, and neuromelanin-sensitive MRI, have been suggested to be able to quantify the iron level in multiple grey matter nuclei or the SN region in early PD, showing a higher iron content in PD patients. This is considered as a possible early diagnostic biomarker for PD [41–43].

Transcranial sonography (TCS) is recommended as an effective auxiliary examination for the differential diagnosis of PD. According to a follow-up study, TCS has 87.5% sensitivity and 96.2% specificity compared to clinical diagnosis of PD by Hoehn and Yahr (H&Y) score [44]. The Prospective evaluation of Risk factors for Idiopathic Parkinson's Syndrome (PRIPS) study reported that enhanced SN hyperechogenicity is one of the best approaches to predict incident PD [9]. However, another study found no change during the progression of PD, and

the baseline SN hyperechogenicity does not have a high sensitivity and specificity for predicting RBD [45]. The predictive value of TCS for early diagnosis thus remains unclear.

Biochemical Biomarkers

Based on understanding the pathogenesis of PD, biomarkers in body fluids and tissues provide an effective route to detect proteins and other molecules correlated with the early diagnosis and progression of PD [7, 8]. Several blood and cerebrospinal fluid (CSF) biomarkers such as α-synuclein and DJ-1 (Parkinson disease protein 7) have been tested for the diagnosis of PD or distinguishing it from other parkinsonisms, but the outcomes have been inconsistent, possibly due to the highly heterogeneous study population, sample contamination, and non-standardized sampling processes [12, 46–49]. Given that the feasibility of CSF testing is limited by safety considerations and cost, it is more realistic to identify biomarkers from blood, saliva, and tissue biopsy.

In addition to α-synuclein and DJ-1, evidence has demonstrated that urate in serum might be correlated with PD risk [50, 51]. Besides, the serum protein apolipoprotein A1 is the major component of high-density lipoprotein. Lower plasma apolipoprotein A1 levels have been associated with an earlier onset of PD and greater motor severity [52]. Furthermore, lower plasma apolipoprotein A1 has been correlated with dopaminergic system vulnerability and a high risk of PD, as reflecting in a greater putamenal DAT deficit in DAT SPECT estimation [53]. Nicotinamide phosphoribosyltransferase (NAMPT), an adipokine regulating lipid and glucose metabolism, is thought to be significantly increased in early-stage and drug-naïve PD patients, but this needs to be confirmed in larger prospective longitudinal studies [54].

Given that α-synuclein is not only present in brain, but also in peripheral tissues such as skin, enteric mucosa, and submandibular salivary glands, tissue biopsy has been assessed for potential biomarker in the last few years [55]. It has been suggested that the accumulation of α-synuclein in the gastrointestinal tract of patients with PD can be detected even 7 to 8 years prior to the onset of motor symptoms [56, 57]. However, some discrepant findings have revealed that colonic deposits of α-synuclein lack specificity and are similar in PD and in multiple system atrophy [58, 59]. Skin nerve biopsy seems to hold promise in the early diagnosis of PD, while phosphorylated α-synuclein in peripheral nerves has potential as a highly-sensitive biomarker to distinguish PD from other parkinsonisms [60, 61]. Submandibular tissue biopsy of α-synuclein is a safe procedure and has relatively high specificity for the diagnosis of PD at prodromal stage. Recent studies have demonstrated that α-synuclein deposits can be detected in the submandibular gland in 89% of patients with RBD, and 74% of early PD patients [62, 63]. Overall, biopsy seems to be more reliable and has a higher predictive value than biomarkers in body fluids, and may become an accepted part of the diagnosis of prodromal PD.

Recently, it has been reported that there is a gut microbiome–brain axis *via* the enteric nervous system and vagus nerve [64]. Comparing the fecal microbiome in PD patients and controls, Filip *et al*. found that the gut microbiome is associated with the severity of constipation and motor phenotype [65]. High levels of Prevotellaceae seem to be a biomarker to exclude PD [65]. Given that bowel dysfunction occurs in the prodromal stage of PD, it is worthwhile investigating whether the gut microbiome can serve as a biomarker of early PD.

In addition, microRNAs have been considered as potential biomarkers for PD due to their capacity to cross the blood–brain barrier, and therefore could theoretically be detected in the circulation. Studies have shown that microRNA19b is downregulated in patients with RBD. Moreover, this change can be detected even 4.5 years before PD diagnosis [66]. Recently, more and more microRNAs have been found and identified to distinguish PD and other parkinsonian diseases [67, 68]. MicroRNAs and other small molecules could be potential biomarkers for the early diagnosis of PD in the near future, but more work is needed.

Genetic Biomarkers

Approximately 15% of PD patients have history of PD among their first-degree relatives [69]. Many genes have been linked with PD, such as α-synuclein (*SNCA*), leucine-rich repeat kinase 2 (*LRRK2*), tensin homolog-induced kinase 1 (*PINK1*), and β-glucocerebrosidase (*GBA*) [70]. Although these gene defects might just represent a subgroup of PD patients, exploring more common genetic biomarkers could be important to identify populations at risk and improve exploration of the pathogenesis of PD [7]. Importantly, genetic susceptibility has also been implicated in idiopathic PD patients, whose pathogenesis is attributed to the dual action of environmental factors and genetics [71]. Recently, Beavan *et al*. have longitudinally evaluated a *GBA* mutation-positive cohort with regard to their prodromal features. They have found worsened (increased) scores in BDI, RBDQ, and UPDRS part II in patients with *GBA* mutations [72]. Indeed at least 7% of PD patients have a *GBA* mutation, and those with a homozygous or heterozygous mutation have a 20–30-fold higher risk for PD [73, 74]. Another study investigated a group of

Table 2 Potential biomarkers for early diagnosis of PD.

Biomarkers	Strength level[a]	Sensitivity[b]	Specificity[b]	Approximate relative risk	Testing cost[c]	References
Symptomatic biomarkers						
Motor testing	Moderate	High (UPDRS > 4 identified prodromal parkinsonism with 88% sensitivity)	High (UPDRS > 4 identified prodromal parkinsonism with 94% specificity)	3–4	Moderate	[18, 19]
RBD	High	Low (~50% of PD patients occur RBD in 2 years)	High (76% risk of PD at 10 years)	50	High	[17, 21, 22]
Olfactory dysfunction	High	High (>80% of early PD)	Low	5	Low	[25–27]
Constipation	High	Moderate	Low (15%–20% prevalence in general population)	2–2.5	Low	[19, 29]
Depression	Moderate	Low (30%–40% of PD)	Low	1.8	Low	[19, 31]
Other autonomic symptoms	Low	Low	Low	1.5–2.5	Low	[19]
Imaging biomarkers						
Dopaminergic PET/SPECT	Moderate	High (98% to detect nigrostriatal cell loss)	High	20	High	[32–34]
MRI	Low	Not estimated	Not estimated	Not estimated	High	[39, 40]
TCS	Moderate	Inconsistent	Inconsistent	15	Moderate	[44, 45]
MIBG scintigraphy	Moderate	High (88%)	High (85%)	Not estimated	High	[35–38]
Biochemical biomarkers						
Plasma urate	Low	Not estimated	Not estimated	Hazard ratio 0.7–0.8	Moderate	[50, 51]
Plasma apolipoprotein A1	Low	Not estimated	Not estimated	Hazard ratio 0.742	Moderate	[52, 53]
Plasma NAMPT	Low	High	High	Not estimated	Moderate	[54]
α-synuclein gastrointestinal biopsy	Low	Inconsistent	Inconsistent	Not estimated	High	[56–59]
α-synuclein skin biopsy	Low	High (80% sensitivity in early PD)	High (nearly 100%)	Not estimated	High	[60, 61]
α-synuclein submandibular biopsy	Low	High	High	Not estimated	High	[62, 63]

MIBG, I-123-metaiodobenzylguanidine; MRI, magnetic resonance imaging; NAMPT, nicotinamide phosphoribosyltransferase; PD, Parkinson's disease; PET, positron emission tomography; RBD, rapid eye movement sleep behavior disorder; SPECT, single photon emission tomography; TCS, transcranial sonography; UPDRS, Unified Parkinson's Disease Rating Scales.

[a] Strength level, predictive value of supportive evidence ['low', single study/indirect evidence; 'moderate', >1 prospective study (patients assessed before PD developed); 'high', > 3 prospective studies.

[b] Sensitivity and specificity levels ('low', <40%; 'moderate', 40%–70%; 'high', >70%).

[c] Testing cost ('low', evaluation by questionnaire; 'moderate', inexpensive; 'high', expensive (>$200).

asymptomatic carriers of the R1441G *LRRK2* mutation. Compared with non-carriers, they show nigrostriatal dopaminergic denervation and a decline in a series of motor tests [75]. PREDICT-PD, a large-scale longitudinal study, has shown that the addition of *GBA* variants and G2019S *LRRK2* mutations in the algorithm could improve the strength of association between baseline risk and incident PD [16]. The nuclear receptor related 1 (*NURR1)* gene plays a critical role in the survival and development of dopaminergic neurons. Our previous studies have shown that the expression of this gene is significantly decreased in PD patients and closely correlated with age and gender [76]. Besides, pituitary homeobox 3 (*PITX3*), a gene playing roles similar to *NURR1*, confers susceptibility to PD and is associated with its early onset [77]. Some of these genes may be associated with a high incidence of

several prodromal symptoms. Detection of these genes would help with the primary evaluation of the risk of developing PD, especially before the classical symptoms develop. For those patients with a positive PD family history or having non-motor symptoms at a young age, genetic biomarker screening may be the best choice. Future research should focus on the predictive value of genetic markers, especially combined tests for the early or prodromal stage of PD.

Genome-wide association studies have focused on the genetic basis, including the identification and replication of risk loci for common diseases [78, 79], of which 28 loci have been identified as independent risk variants for PD [79]. Besides, the discovery of risk loci may assist the risk evaluation of PD, and more importantly, improve the exploration of PD pathogenesis.

Conclusions

Current biomarkers mainly focus on the symptomatic evaluation of PD, neuroimaging specific changes, and biochemical measurements of body fluids and tissues (see Table 2). These biomarkers have diverse predictive value for PD diagnosis. Many are likely to help with early diagnosis, and show high specificity or sensitivity, such as RBD, olfactory dysfunction, and dopaminergic imaging tests [17, 25, 32]. However, because of the lack of quality-control for sample collection, storage, possible contamination, and selective deviation of patients and controls, inconsistent results have been found for some biomarkers, especially in previous investigations of α-synuclein in CSF and plasma [12, 46–49]. Other biomarkers such as α-synuclein biopsy and biochemical marker detection in body fluids still need large-scale prospective studies to accurately estimate their predictive and practical value.

The next step is to establish unified criteria to estimate the likelihood ratio for early PD. Several studies have been undertaken. PRIPS, PREDICT-PD, and other studies have proposed models of combining age, gender, family history, and some motor and non-motor biomarkers for the early diagnosis of PD [2, 9, 15, 80, 81]. These models evaluate the likelihood ratio of developing PD and dramatically increase the sensitivity and specificity of diagnosis. In 2015, diagnostic criteria for prodromal PD were generated by MDS and have been validated in a retrospective study [80, 81]. These studies provide promising tools for identifying PD, but still need more clinical studies to evaluate their predictive value. Updated biomarkers may be included in the near future. Besides, the testing cost and lead-time should also be considered when applying them in clinical practice. We believe that biomarker exploration will continue and the outcomes are optimistic in aiding the early diagnosis of PD, as well as helping the development of disease-modifying therapies for PD.

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