



Premotor Diagnosis of Parkinson's Disease

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Abstract Typical Parkinsonian symptoms consist of bradykinesia plus rigidity and/or resting tremor. Some time later postural instability occurs. Pre-motor symptoms such as hyposmia, constipation, REM sleep behavior disorder and depression may antecede these motor symptoms for years. It would be ideal, if we had a biomarker which would allow to predict who with one or two of these pre-motor symptoms will develop the movement disorder Parkinson's disease (PD). Thus, it is interesting to learn that biopsies of the submandibular gland or colon biopsies may be a means to predict PD, if there is a high amount of abnormally folded alpha-synuclein and phosphorylated alpha-synuclein. This would be of relevance if we would have available means to stop the propagation of abnormal alpha-synuclein which is otherwise one of the reasons of this spreading disease PD.

Keywords Parkinson's disease · Gut-brain-axis · Premotor symptoms · Hyposmia · Constipation · REM sleep behavior disorder

Introduction

In recent years, the field of neuropathology has made important contributions to the understanding of the pathogenesis of Parkinson's disease (PD), the second-most common neurodegenerative disease. For decades it has been known that the neuropathological hallmark of PD is the so-

called Lewy body [1], which was named after the German neuropathologist Friedrich Heinrich Lewy. While for many years it was unclear whether Lewy bodies are a positive or a negative finding, nowadays it seems that both viewpoints have some basis. Polymeropoulos and co-workers [2] made a seminal contribution to the understanding of PD with the detection of a mutation in the alpha-synuclein gene in a kindred of Italian and US-American patients who all suffered from PD. Until then, most neurologists were not aware of this protein, and even less aware of its function. Now, alpha-synuclein is considered to be a chaperone and also to be important for membrane fluidity [3]. After the description of this gene defect, a number of studies were performed in non-genetically defined PD patients, and Jellinger [4] and Braak and co-workers [5] were able to demonstrate that abnormally folded and phosphorylated alpha-synuclein can also be found in patients with so-called idiopathic PD. A huge amount of this protein is found in Lewy bodies, so that this formation seems to be an attempt by the affected neuron to sequester the abnormal alpha-synuclein. On the other hand, it can be assumed that Lewy bodies do block cell trafficking and thus have a negative as well as a positive effect on the progression of this disease. In this context, the next step, to which we also contributed, was the finding that this abnormally folded alpha-synuclein spreads from cell to cell in the majority of patients. Braak *et al.* [5] claimed that the earliest Lewy bodies are found in the olfactory bulb, the dorsal motor vagal nerve, and the enteric nervous system of the gut. Thus, it is intriguing to speculate that the disease may be initiated by a substance originating in the environment. This substance may be sniffed and/or swallowed. In this way, it causes the typical pathology in the olfactory bulb, the submandibular gland, and the enteric nervous system. From there, it spreads *via* the vagal nerve to the central nervous system, i.e. the brain. This speculation was substantiated by

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an animal model created by Pan-Montojo and colleagues in our group [6, 7] in which we administered tiny doses of the complex I inhibitor rotenone into the stomach of mice. We showed that this led to the formation of abnormally aggregated alpha-synuclein in the enteric nervous system, which then travelled *via* the vagus nerve all the way to the substantia nigra [6]. In a second set of experiments, we performed the same studies in which one vagus nerve or parts of the sympathetic system were cut, which resulted in an absence of pathology on the side of the vagal dissection [7]. Recently, it was discovered that patients who had undergone a total vagotomy due to peptic ulcers had a lower incidence of PD than either people who had not undergone this procedure or who had a selective partial vagotomy [8, 9]. In view of these animal experiments and the work of Braak and others, it is understandable that the mesencephalic sleep centers and the locus coeruleus are involved early in the pathological process [4]. This all happens probably years before the dopaminergic neurons of the substantia nigra are impaired, resulting in the movement disorder called PD. Based upon all these findings, it should be feasible to develop substances that either cleave the abnormally folded protein aggregates, or stop the propagation of the pathology from cell to cell, or bind to the abnormal alpha-synuclein and destroy it. If such treatment does become available, it may well be that the movement disorder PD ceases to exist. This can only be achieved if we develop a sensitive and specific alpha-synuclein therapy which will block the spread of PD. On the basis of this reasoning, it is very important to discuss the premotor signs of PD.

Olfaction

Since >90% of PD patients present with hyposmia [10], we have argued for years that in addition to tremor, rigidity, bradykinesia, and postural instability, hyposmia should be considered a cardinal symptom of PD. In the most recent description of the course of the disease for the Movement Disorder Society (MDS) [11], hyposmia was for the first time included as a cardinal symptom. In contrast to the motor symptoms, however, hyposmia occurs earlier in the disease, can be considered a premotor symptom, and thus may indeed turn out to be one of the most sensitive features in the early diagnosis of PD. Studies indicate that many patients develop hyposmia >5 years prior to the onset of the movement disorder. This also means that the majority of patients with an idiopathic hyposmia who do not develop PD 5 years after the advent of hyposmia may be safe [12].

The first hint that hyposmia is associated with PD was provided by Ansari and Johnson [13] who described this phenomenon in 22 patients. In the 1990's several groups obtained convincing data about olfactory malfunction in PD

patients using the "University of Pennsylvania Smell Identification Test" [14, 15]. Using this test, Doty *et al.* [17] reported that the ability to identify smells was diminished in 90% of the 81 PD patients studied. In addition, the odor threshold was analyzed in 38 PD patients and was found to be significantly more impaired than in an age- and sex-matched control group. This was independent of age, gender, duration, state of the disease, clinical symptoms, cognitive impairment, and medication. In contrast to the American groups, we used "sniffing sticks" [16, 18, 19] and found that odorous substances such as apple, turpentine, licorice, aniseed, and cinnamon were particularly difficult to identify, while orange and garlic were identified correctly by 14 and 18, respectively, of the 20 patients tested.

For our scientific work, we use a rather robust test battery which consists of 48 individual tests. In general, we use three subtests: determination of odor threshold, discrimination, and identification. The results are summarized as an SDI (Schwelle = threshold, differentiation, identification) value. The threshold is analyzed with different concentrations of odoriferous substances. Differentiation is assessed with a triplet of sticks where one stick contains an odoriferous substance different from the other two. Identification is tested by offering a choice of 4 odors to the patient. We suggest that using all 3 components results in reliable findings [20].

In addition to the sniffin' stick tests we also used olfactory-evoked potentials [21] which were significantly delayed in PD patients compared to age- and sex-matched controls. Stimulation of the trigeminal system did not show any deficits, which confirms the selective impairment of the olfactory system in PD. Another intriguing finding was the good correlation between the prolongation of olfactory-evoked potentials and disease progression [22]. In another study, we analyzed 27 consecutive patients (22 male and 5 female). The patients were 27–64 years old and the disease duration ranged 0–19 years. All patients were examined at least twice during a period of 3–6 years. We investigated the patients again after 4.4 years [23]. In this study, we showed that most patients remained hyposmic but that only some deteriorated, whilst others actually improved and switched from anosmic to hyposmic. Using quantitative methods such as sniffin' sticks confirms that only a limited number of PD patients develop anosmia and that no patient is normosmic. This work raises the question as to why patients hardly ever deteriorate and why some even improve. A possible answer can be found in a paper by Huisman and colleagues [24]. They showed, using tyrosine hydroxylase staining, that the number of dopaminergic cells in the olfactory bulb is twice as high as normal in PD patients. In the olfactory bulb glomeruli, dopamine inhibits signal transduction, the first and most important step in odor perception. The authors speculate that the increased

number of dopaminergic neurons in the olfactory bulb could explain the loss of olfaction [25]. This hypothesis also explains why dopaminergic medication is unlikely to improve olfaction in PD patients [26]. If some patients lose dopaminergic neurons in the olfactory bulb, this might lead to improved olfaction, which may explain the improved olfaction that we found in some patients.

In two small observational studies we demonstrated that the monoamine oxidase B (MAO-B) inhibitor, rasagiline, improves the SDI test results and improves olfaction in PD patients; this was also the case in rats treated with rasagiline [27, 28]. The mechanism by which this is achieved is completely speculative and could even stem from an improvement in cognition.

In post-mortem analyses, other investigators [29] demonstrated a loss of neurons in the olfactory bulb in 7 PD patients, which correlated well to disease duration. Lewy bodies were found in all patients both in the olfactory bulb and tract. More recent studies performed by Braak and colleagues [30] are discussed in detail later. This group demonstrated typical α -synuclein deposits in neurons, but not in the glomeruli.

Using MRI volumetry we did not detect a significant decrease in the volume of the olfactory bulb [31]. This study included 11 PD patients (6 with functional anosmia and 5 hyposmic patients) and 9 healthy normosmic controls. It is difficult to speculate why olfactory neurons are so vulnerable, but this could reflect damage or the uptake of a pathogen early in life with consequent neurodegeneration. Selective vulnerability could stem from the fact that the ciliary surface is not protected by the blood-brain barrier. Finally, neuropathological studies indicate a progressive loss of neurons in the amygdala that contributes to the secondary olfactory system [32].

Cognitive impairment hampers studies on olfaction, because these patients have problems to validate and recognize odoriferous substances [33]. As noted above, we have no evidence that medication improves olfaction. This is in contrast with our finding that deep brain stimulation (DBS) improves olfaction in PD [34], which could be due to cognitive improvement or improvement of alertness following DBS.

Impairment of Olfaction as a Differential Diagnostic Tool

Wenning and colleagues [35] were the first to demonstrate a difference in the degree of olfactory impairment between patients with idiopathic PD, multisystem atrophy (MSA), progressive supranuclear palsy (PSP), and corticobasal degeneration (CBD). They reported that patients with MSA showed less pronounced olfactory loss and no impairment

of the sense of smell was found in patients with PSP and CBD. We supported these findings using sniffin' sticks. MSA patients showed less hyposmia and PSP patients no olfactory impairment at all, while PD patients demonstrated hyposmia or functional anosmia in 86% of cases [36, 37]. Patients with Alzheimer's dementia are also hyposmic, and this again confirms the link between cognitive impairment and impaired olfaction [38]. We recently showed that olfaction also deteriorates in PSP patients as the disease progresses (which may be due to their cognitive impairment).

Loss of Olfaction as an Early Marker of Parkinson Disease

There is good evidence that most PD patients develop impairment of olfaction 4–6 years before they start to present with motor impairment [39]. This is supported by recent work from the Braak group [30] who demonstrated the presence of Lewy bodies and Lewy neurites in the olfactory bulb even when the patients were still in the premotor phase of PD. There is a good correlation between alpha-synuclein pathology and the clinical symptom of hyposmia.

Several studies have been performed to test the hypothesis that hyposmia may be a valuable tool for the detection of early pre-motor PD. Our group investigated 30 patients with idiopathic hyposmia (19 male, 11 female; average age 59 years, average duration of hyposmia 5.8 years) [40]. None of these patients had pre-existing neurological disease. In addition, we performed a careful neurological examination including the Unified Parkinson Disease Rating Scale (UPDRS) parts II and III and sonography of the substantia nigra in all patients. Sonography of the substantia nigra was abnormal in six cases [41]. These patients presented with hyperechogenicity of the substantia nigra probably due to the incorporation of iron ions [42]. A ^{123}I -FP-CIT (dopamine transporter scan) was performed in all patients with abnormalities in the UPDRS or in parenchymal sonography. One patient showed typical clinical features of PD and two had suspected PD. Parenchymal sonography was abnormal in 11 patients. In 10 of these 11 patients a dopamine transporter scan was performed; it was abnormal in five and equivocal in two cases, while only three showed normal activity. Two years later, another two patients developed motor symptoms of PD [43]. Thus, we concluded from these studies that a combination of hyposmia and other substantial abnormalities in parenchymal sonography and a dopamine transporter scan is associated with an extremely high probability of developing PD.

Berendse *et al.* used another approach [44, 45]. In a prospective study, they examined 361 asymptomatic first-

grade relatives of PD patients (parents, children, and siblings) aged 50–75 years. Based upon quantitative olfactory tests they identified 40 persons with an impaired sense of smell. These probands were compared with 38 probands with normal olfaction. Both groups received a dopamine transporter scan at baseline and a second after two years. The other 283 relatives were followed up with a validated questionnaire aimed at specific features of idiopathic PD. Two years after the baseline examination, 10 of the relatives with initial hyposmia showed motor symptoms indicative of PD. None of the 38 normosmic relatives had developed PD symptoms. These results suggest that idiopathic hyposmia may lead to PD in 10% of cases. It is clear that if we could predict the development of PD in patients with hyposmia, we could evaluate whether existing drugs, such as coenzyme Q, dopamine agonists, MAO-B inhibitors, and amantadine, are able to postpone or even halt the process of dopaminergic degeneration. In my view, a drug that stops the propagation of alpha-synuclein from cell to cell may be even more promising.

Constipation

Gastrointestinal disturbances are common in PD and may precede the occurrence of motor symptoms. Such disturbances involve the whole gastrointestinal tract (GIT) and can be present in all stages of PD. It is noteworthy that they worsen during the course of the disease and malabsorption is often the reason that dopaminergic replacement therapy does not work. Malabsorption can be caused by delayed gastric emptying and/or prolonged transport times. Some patients even present with the pills in their throat. Using scales for quality of life, it has been shown that these disturbances are quite unpleasant for PD patients. Dysphagia and weight loss due to malabsorption may result in life-threatening situations. In a seminal paper, Abbott *et al.* [46], in the Honolulu Study, reported the frequency of bowel movements in 6790 men between 1971 and 1974 and followed these patients for up to 24 years. Out of these patients, 69 developed PD with an average time to onset of 12 years. Nineteen out of 10,000 persons with less than one bowel movement per day developed PD, whereas only about 4 out of 10,000 persons with more than 2 bowel movements per day developed PD. From these figures they concluded that constipation may be a marker of early PD or a susceptibility marker for environmental factors that may cause PD. It is also noteworthy that PD patients present more often with constipation than their age- and sex-matched peers [47, 48]. PD patients suffer from constipation about twice as often as controls. In a recent paper from Italy [49], it was stated that constipation was always present in 60% of their PD patients and even more importantly, that 87% started with constipation before they

developed bradykinesia, tremor, and rigidity. Again, a reasonable explanation for these observations comes from Lewy body and alpha-synuclein analyses in post-mortem tissue by Braak's group [50]. They detected gastric alpha-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for PD-related brain pathology. Even earlier, Wakabayashi *et al.* [51] had reported the presence of Lewy bodies in the esophagus, small intestine, and the colon, more specifically in the Auerbach and Meissner plexuses. They claimed that there might be a predilection of Lewy bodies in the upper GIT. More recently, Shannon and colleagues [52] investigated biopsies from 10 untreated PD patients during sigmoidoscopy, and all of them were positive for alpha-synuclein. In a follow-up paper [53] they analyzed 3 biopsies from patients 2–5 years before the onset of PD and again they detected abnormal alpha-synuclein aggregation. Taken together, these papers indicated that a colon biopsy may be a biomarker and diagnostic tool for patients with an uncertain clinical picture or may even be suitable as a predictor for the later development of the motor features of PD. Thus, some argue that PD may be a disease of the GIT, even more since biopsies of the submandibular gland have also shown abnormal alpha-synuclein [54]. The initial euphoria after these findings vanished when new findings showed conflicting results, especially when biopsies were taken from the rectal part of the gut [55]. These authors demonstrated that biopsies should be taken from the colon and not the rectum. In a very comprehensive review, Ruffmann and Parkkinen demonstrated that certain prerequisites are necessary for the reliable and reproducible detection of alpha-synuclein [56]: at least two antibodies reactive for different epitopes and/or variants of alpha-synuclein, one of which should stain phosphorylated alpha-synuclein, and antibodies which recognize oligomeric alpha-synuclein should be used. Flexible sigmoidoscopy should be used and biopsies should be preferentially taken from parts innervated by the vagus nerve. Staining intensity should be evaluated by software-based image analysis. They further claim that one marker of nervous tissue should always be used. We speculated that if it is true that the olfactory bulb and the enteric nervous system of the gut are the first sites to show alpha-synuclein aggregation and Lewy bodies, it may well be that we sniff something from the environment and swallow it. This may lead to the disturbances in the olfactory and gastrointestinal systems [57]. To support this view, we created the Dresden mouse model [6]. In this model we administered rotenone intragastrically to one-year-old mice *via* a gastric tube. Rotenone was given in extremely low doses to harm only the gut and not the whole animal. This was underlined by the fact that we could not measure any rotenone in blood, brain, and muscle tissue using high-performance liquid

chromatography. In addition, we did not show any inhibition of complex I of the mitochondrial respiratory chain in muscle and brain from these animals. The rotarod test showed a significant decrease in the rodents' ability to remain on the rod after three months of rotenone treatment. Alpha-synuclein aggregation was detected only in rotenone-treated animals. This aggregation was detectable in the enteric nervous system, and after longer periods in the intermediolateral nucleus in the spinal cord and the dorsal motor nucleus of the vagus. After three months of treatment, alpha-synuclein abnormalities were detectable in the substantia nigra pars compacta combined with a 15% decrease in the number of tyrosine hydroxylase-positive neurons. In another series of animals, we performed hemivagotomy and partial sympathectomy in addition to the rotenone exposure. Both interventions delayed the disease progression in our mouse model. Because of these findings, I encouraged colleagues from Scandinavia to test for the prevalence of PD in patients who had undergone vagotomy due to peptic ulcers. This work could not be done in Germany since we have no such register. Svensson *et al.* [8] showed that patients with a truncal vagotomy developed PD less often than their peers, while the incidence of PD in patients with superselective vagotomy was similar to that in the general population. Tysnes *et al.* [9] investigated patients with superselective vagotomy and also did not demonstrate an increased prevalence of PD. Taken together, these clinical observations and basic research are intriguing, and suggest that the disease comes from the environment and ascends to the brain *via* the vagus nerve. Interestingly, several conditions are already known that support such speculations. Several papers show that in rural areas of the USA such as in Iowa or California, farmers use a lot of pesticides and herbicides that contain paraquat and rotenone and drink well-water from their own land [58]. There are also individuals who attempted suicide with carbon monoxide, and finally miners from Chile who suffered manganese intoxication: all these patients presented with a Parkinsonian syndrome. Another example involving the toxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) may be the most important model of PD. When a group of chemistry students in California attempted to synthesize heroin they made a mistake and instead created MPTP, which is converted to MPP⁺ (1-methyl-4-phenylpyridinium) in astrocytes. This metabolite is actively transported into dopaminergic neurons where it inhibits complex I of the mitochondrial respiratory chain and ultimately leads to a Parkinsonian syndrome [59]. In Guadeloupe, many inhabitants also suffered from a Parkinsonian syndrome, which was eventually found to be caused by chewing *Annona muricata*. Infections, such as the influenza pandemic (which was also called von Economo encephalitis) in Vienna and New York

City have also been associated with symptomatic PD. Thus, many causative factors may attack the patient *via* the environment. A limitation of such an assumption is certainly the fact that not all patients have such an obvious exposure, and that not all people so exposed develop PD. To overcome this objection it is helpful to know that more and more susceptibility gene patterns have been described. Research involving the analysis of the gut microbiota is related to this topic, and already some studies have shown stress abnormalities in PD patients but not in healthy controls [60]. Finally, it is noteworthy that the observation that smoking and coffee-drinking are protective against PD may also have its cause in the gut microbiota. Both smoking and coffee drinking may alter pro-inflammatory cytokines in the gut towards anti-inflammatory microbiota, which prohibit the propagation of abnormal alpha-synuclein to the brain *via* the vagus nerve [61].

Rapid Eye Movement (REM) Sleep Behavior Disorder (RBD)

One of the most frequent and important prodromal symptoms of PD is RBD [62, 63], which is characterized by sometimes aggressive and violent behavior and the demonstration of REM sleep without atonia on polysomnography. The Barcelona group was one of the first to address the question of whether such behavior may be a prodromal sign for PD [64]. They showed that out of 44 consecutive patients with RBD, 20 developed a neurodegenerative disorder after a mean of 11.5 years after the reported onset of RBD. Specifically, most patients developed PD, while the remainder developed MSA or dementia with Lewy bodies. Most groups agree that 50% of patients with spontaneous RBD convert to PD within a decade, and that ultimately 90% develop some kind of neurodegenerative disease [62]. Thus, it is clear that RBD may open a window of opportunity for disease-modifying treatment [65]. In addition, such patients also allow us to search for other biomarkers and investigate the course of PD.

Several studies have investigated the idea that the occurrence of other premotor signs such as hyposmia may potentiate the predictive value of RBD in the conversion to PD. Stiasny-Kolster and colleagues studied 30 RBD patients with a mean age of 48 ± 14 years and compared them to age- and gender-matched controls [66]. They analyzed the sense of smell in both cohorts using sniffin' sticks. The RBD patients had a significantly higher olfactory threshold, lower discrimination score, and lower identification score. Thus, up to 97% of their patients had an abnormal olfactory test. On neurological examination, signs of Parkinsonism were newly found in five RBD patients without narcolepsy. Eleven RBD patients agreed to

a dopamine transporter scan, and 3 of them presented with an abnormal scan. The authors suggest that combined analyses of pre-motor features may increase the chance of correctly predicting which patients will develop PD. In a more recent study [67], Mahlknecht *et al.* analyzed the predictive value of olfactory dysfunction for the early development of a synuclein-mediated neurodegenerative disease in individuals with RBD. They used a sniffin' sticks test battery in 34 patients with polysomnography-confirmed RBD and followed them up for 4.9 ± 0.3 years. After 2.4 ± 1.7 years, 9 patients (26.5%) with idiopathic RBD developed a Lewy body disease (6 PD and 3 dementia with Lewy bodies). They state that the sniffin' stick test had a diagnostic accuracy of 82.4% in predicting conversion. Another approach may be to use parenchymal sonography of the substantia nigra in patients with RBD [68]. It is known that hyperechogenicity of the brainstem, most probably of the substantia nigra, is present in the vast majority of PD patients. In this prospective study, 55 idiopathic RBD patients underwent transcranial parenchymal sonography at baseline and over a follow-up period of 5 years. Twenty-one (38.2%) were diagnosed with a synucleinopathy (PD in 11, dementia with Lewy bodies in 9, and MSA in 1). The sensitivity of baseline substantia nigra hyperechogenicity for the development of a synucleinopathy was 42.1%, specificity 67.7%, and the positive predictive value 44.4%. There was no change in the degree of hyperechogenicity in consecutive analyzes. Thus, in idiopathic RBD, transcranial sonography is not a good tool to identify individuals at risk of developing a synucleinopathy. A broader approach was used by Aguirre-Mardones *et al.* [69], who analyzed the prevalence and perceived timeline of non-motor symptoms using scales and olfactory tests in 44 idiopathic RBD and 40 matched controls. Hyposmia and constipation were more frequent in patients than in controls, and memory problems were more often reported by patients. The first symptoms perceived were RBD in 38.6%, hyposmia in 15.9%, constipation in 11.4%, and depression in 6.8%. In addition, this study shows that the perceived timeline of non-motor symptoms in RBD is highly variable. Heller *et al.* [70] claim that we are at the advent of new imaging tools that may help to predict phenotypic conversion in RBD patients. As discussed above, the presence of abnormal alpha-synuclein in colon biopsies from PD patients is generally accepted. For this reason, Sprenger *et al.* [71] prospectively analyzed alpha-synuclein in colon biopsies taken from 17 RBD patients, 19 PD patients, and 14 controls. They used two different antibodies for staining. The results obtained using the antibody against serine 129-phosphorylated alpha-synuclein in submucosal nerve fibers or ganglia differed between the groups: none of the controls, but 4 out of 17 RBD patients and 1 out of 19 PD patients showed

abnormalities. No differences between the groups were found using the other antibody. The authors will analyze whether the PBD patients with pathological colon biopsies convert to PD and the others do not in a follow-up study. Our own group [72] examined skin punches from the distal leg of 18 idiopathic RBD patients and 22 age- and sex-matched controls using immunohistochemistry and microscopy. Intradermal nerve fiber density was reduced in RBD patients. Patients with RBD reported non-motor symptoms, especially hyposmia and daytime sleepiness more frequently than controls, whereas cognition did not differ between the groups. Taken together, this study showed small fiber neuropathy in idiopathic RBD patients. However, the prognostic value needs to be further investigated in longitudinal studies. The studies suggest that a combination of tests and the occurrence of a variety of pre-motor symptoms may be the best way to predict whether a patient with RBD may develop PD.

There is also some information about the development of RBD in patients that already suffer from PD [73]. In this study, it was shown that a significant increase in RBD from 50% to 63% is seen within 2 years of the diagnosis of PD. Such an increase and deterioration of RBD-associated symptoms were also shown in the DeNoPa Study [74]. A more recent study also showed that the coexistence of RBD and hyposmia is associated with a lower Mini-Mental State Examination score [75]. A Chinese consortium addressed the question of whether olfaction is more impaired in PD or in idiopathic RBD. They also used sniffin' sticks and demonstrated that PD patients have a greater impairment in their sense of smell than the idiopathic RBD patients [76].

RBD is generally treated with clonazepam and a prospective study on its efficacy was reported by Li *et al.* [77]. This group analyzed 39 RBD patients and used on average 1 mg clonazepam. At follow-up, 67% of all patients had a complete elimination of sleep-related injuries. Patients who had additional obstructive sleep apnea had a worse outcome. Thus, despite treatment, residual RBD symptoms are common. It is certainly also of interest that Wang *et al.* [78] demonstrated that rotigotine improved the frequency and severity of abnormal RBD-related motor behaviors.

Finally, Arnaldi *et al.* [79] discussed whether patients with RBD should be told that they have a high risk of developing a neurodegenerative disorder over the following years. It is known that coffee and smoking do not protect against the phenotype conversion and that patients with old age, hyposmia, impaired color vision, abnormal dopaminergic imaging, mild cognitive impairment, and possible sleepiness are more prone to develop conversion. Until we can offer a disease-modifying treatment, the general consensus is not to tell the patients that they have a high risk of developing a neurodegenerative disease but to monitor these patients very carefully.

Depression

We demonstrated that, independent of age, 30% of all PD patients suffer from depression [80]. In this context it is, however, even more important to state that depression is one of the earliest non-motor symptoms in PD. It is three times more common in patients who develop PD than in the general population [81]. Since abnormalities in the glucocerebrosidase gene may be a risk factor for developing PD, it is noteworthy that depression and mood disturbances are also found at a high degree in these patients [82]. In addition, there are also reports that the incidence of depression is increased in patients with a *LRRK2* point mutation or *Parkin* deficiency [83]. In our view, this makes perfect sense since the pathological abnormalities (i.e. accumulation of phosphorylated alpha-synuclein) may also occur in the raphe nuclei or the locus coeruleus as outlined by Braak and others [5], which lead to depression. Thus, it is not that depression causes PD, but rather that a common pathology leads not only to impairment and malfunction of the dopaminergic system but also of the noradrenergic and serotonergic systems [4]. Thus, depression may occur as a pre-motor symptom or at any stage of the disease. A retrospective cohort analysis of 32,415 individuals in the Netherlands revealed that the first depressive symptoms occurred 1 to 36 years before the onset of motor symptoms in PD. The average time before motor symptoms was 10 years [84]. Similar results were obtained in a Californian cohort [85], in which it was found that most patients developed depression about 5 years before motor symptoms. Schrag *et al.* [86] also convincingly demonstrated that anxiety along with depression often occurred before a definitive diagnosis of PD was reached. In this context, it has to be stated that depression is certainly hard to measure and its retrospective assumption is mainly based on interviews and subjective estimates of individuals. Interestingly, the depression in PD shows some variation from that in major depression. Suicidal acts and the feeling of guilt are less common in PD depression [87], whereas concentration is less well preserved in PD depression. Up to 22% of patients analyzed by a British consortium [88] also presented with major anxiety.

Since our goal is to find out which individuals with depression may develop PD in the next 5–10 years it is important to learn about sub-clinical depression which stems from early impairment of the serotonergic and/or adrenergic system.

Taken together, there is good evidence that we may be close to using pre-motor symptoms for the early detection of PD and as soon as alpha-synuclein-modifying therapy is available we will have a new window of opportunity to treat PD patients.

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