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Abstract

BACKGROUND: The etiology and natural history of Parkinson's disease (PD) are not well understood. Some non-motor symptoms such as hyposmia, rapid eye movement sleep behavior disorder, and constipation may develop during the prodromal stage of PD and precede PD diagnosis by years.

OBJECTIVES: To discuss the promise and pitfalls of research on pre-motor symptoms of PD and to develop priorities and strategies to understand their clinical and etiological implications.

METHODS: This review was based on a workshop held on June 7-8, 2012 at the National Institute of Environmental Health Sciences.

DISCUSSION: Research on pre-motor symptoms of PD may offer an excellent opportunity to characterize higher-risk populations and to better understand PD etiology. Such research may lead to evaluation of novel etiological hypotheses such as the possibility that environmental toxicants or viruses may initiate PD pathogenesis in the gastrointestinal tract or olfactory bulb. At present, our understanding of pre-motor symptoms of PD is in its infancy and faces many obstacles. These symptoms are often not specific to PD and have low positive predictive value for early PD diagnosis. Further, the pathological bases and biological mechanisms of these pre-motor symptoms and their relevance to PD pathogenesis are poorly understood.

CONCLUSION: This is an emerging research area with important data gaps to be filled. Future research is needed to understand the prevalence of multiple pre-motor symptoms and their etiological relevance to PD. Animal experiments and mechanistic studies will help understand the biology of these non-motor symptoms and test novel etiological hypothesis.

Introduction

Parkinson's disease (PD) is the second most prevalent neurodegenerative disease and severely affects quality of life. Over one million older US adults live with PD and the number will double by the year 2030 (Bach et al. 2011). Clinical diagnosis of PD is currently based on the presence of motor dysfunction including rest tremor, bradykinesia, and rigidity. PD patients also suffer from a wide range of non-motor symptoms including hyposmia (poor sense of smell), gastrointestinal dysfunction, psychiatric features (e.g. depression, anxiety, and psychosis), sleep disorders, and mild to severe cognitive impairment, many of which are disabling and can be difficult to treat (Coelho and Ferreira 2012; Fernandez 2012) and greatly jeopardize the quality of life of PD patients (Storch et al. 2013). Pathologically, PD has been characterized by the loss of dopamine neurons in the substantia nigra pars compacta, which underlies motor dysfunction, and by the presence of Lewy bodies in selected regions of the brain.

The cardinal motor signs of PD become clinically evident when approximately 50% of the dopaminergic neurons in the substantia nigra are lost (Fearnley and Lees 1991). Despite symptomatic therapies for dopamine deficiency-related motor features, the disease continues to progress and often leads to severe mental and physical disabilities (Coelho and Ferreira 2012; Shulman et al. 2008) and increased mortality (Chen et al. 2006; Willis et al. 2012). To date, none of the available treatments can halt or reverse the pathological and clinical progression of PD, and novel strategies are needed. Research on disease-modifying strategies would be greatly assisted by the identification of high-risk populations.

Recent interest has focused on the non-motor symptoms of PD, some of which may predate motor signs and clinical diagnosis by years (hereafter referred as the “pre-motor symptoms” of PD). Accumulating epidemiological and clinical evidence suggests that hyposmia (Ross et al. 2008), constipation (Abbott et al. 2007; Gao X et al. 2011; Savica et al. 2009), depression (Bower et al. 2010; Fang et al. 2010; Ishihara-Paul et al. 2008; Shiba et al. 2000), anxiety (Bower et al. 2010; Ishihara-Paul et al. 2008; Shiba et al. 2000; Weisskopf et al. 2003), rapid eye movement sleep behavior disorder (RBD) (Claassen et al. 2010; Iranzo et al. 2006; Postuma et al. 2009; Schenck et al. 1996), excessive daytime sleepiness (EDS) (Abbott et al. 2005; Gao J et al. 2011), and autonomic dysfunction (Goldstein 2010) may occur prior to the appearance of the classic motor dysfunction of PD. Evidence comes primarily from large prospective population-based cohort studies that were initially established for research on cancer and cardiovascular disease such as the Honolulu Asia Aging study (HAAS) (Ross et al. 2008) and the Health Professionals Follow-up study (Gao X et al. 2011), and from retrospective examinations of archived medical records of PD cases and controls such as the Rochester Epidemiology Project (Savica et al. 2009). These findings are summarized in Table 1. A recent meta-analysis also confirmed that constipation and mood disorders were associated with higher risk of PD (Noyce et al. 2012). Hyposmia, RBD, and EDS were not included in this meta-analysis because risk estimates either were not available or were available only from one study.

The hypothesis that pre-motor symptoms precede the motor signs of PD is broadly compatible with neuropathological findings reported by Braak and colleagues (Braak et al. 2003). This work, although controversial (Burke et al. 2008), suggests that deposition of α -synuclein in the form of Lewy bodies and Lewy neurites develops in the PD brain in six sequential stages. α -Synuclein pathology begins in the dorsal motor nucleus of the vagus and glossopharyngeal nerves and the

anterior olfactory nucleus in stage 1, extends to the locus ceruleus and caudal raphe nuclei in the pons (stage 2), then to the substantia nigra (stage 3), to the temporal mesocortex (stage 4), and finally to the neocortex (stages 5-6). A later extension of this hypothesis further posits that the synucleinopathy may even first develop in the enteric nerves in the gut and later spread along the vagus nerve into the brain (Hawkes et al. 2007, 2009). Importantly, according to the Braak hypothesis, the irreversible loss of dopamine neurons in the substantia nigra and associated progressive motor dysfunction may not be evident until Braak stages 3 and 4. Although the Braak hypothesis is not universally supported (Burke et al. 2008; Dickson et al. 2010), it presents the intriguing possibility that the extra-nigra, nondopaminergic pathologies are intrinsic to early PD pathogenesis and pre-motor symptoms could well be part of the disease's natural history (Hawkes et al. 2010).

Growing evidence on the importance of pre-motor symptoms, coupled with the Braak hypothesis, has generated substantial interest in understanding the origins and consequences of these symptoms. Clinical research primarily has focused on evaluating pre-motor symptoms and other factors as markers for the future development of PD, a subject elegantly reviewed by Berg and colleagues (Berg et al. 2012). Another potential line of inquiry is based on the idea that the presence of multiple pre-motor symptoms in the same individual represents common underlying pathogenesises that may eventually lead to PD, and thus pre-motor symptoms may provide a unique opportunity to understand the etiology of PD (Hawkes et al. 2007, 2009). Despite this potential promise, little research has been carried out to understand the etiological implications of the pre-motor symptoms of PD.

A comprehensive review of the clinical and epidemiological evidence for the existence of these pre-motor symptoms in PD is outside the scope of this article. Instead, we focus on outlining the

promises and pitfalls of the concept of pre-motor symptoms and on developing research priorities and strategies for understanding the clinical and etiological implications of these symptoms. Although these symptoms can also develop after the clinical diagnosis of PD, for the purpose of this article, we focus on the period prior to the emergence of diagnostic motor abnormalities.

Identification of high-risk populations

Several important studies have been carried out to test the hypothesis that pre-motor symptoms, coupled with neuroimaging, may lead to early identification, or even diagnosis of PD (Lang 2011; Tolosa et al. 2009). Preliminary results have been published from the Prospective Evaluation of Risk Factors for Idiopathic Parkinson's Syndrome (PRIPS) study (Berg et al. 2013) and the Parkinson At-Risk Syndrome (PARS) study (Siderowf et al. 2012). PRIPS aimed to test a two-stage screening strategy for early identification of PD cases utilizing the following predictors: hyposmia, PD family history, subtle motor impairment, and substantia nigra hyperechogenicity (SN+). This general population-based cohort recruited 1,352 participants aged 50 or older (mean age of 59 years). Hyposmia was evaluated using the Sniffin' Stick test and SN+ by transcranial sonography. A total of 10 participants developed PD during approximately three years of follow-up. Hyposmia was strongly associated with the risk of developing PD with sensitivity and specificity both higher than 70%. The positive predictive value (PPV), however, was only 2%, in part due to the low PD incidence in this relatively young population. Combining hyposmia with other characteristics (e.g. family history or SN+) only slightly increased the PPV, but substantially decreased the sensitivity. Unlike the PRIPS study, PARS was conducted among a risk-enriched population in which 45% of the 4,999 study participants (average age 64) had a

family history of PD. The study was designed to evaluate a two-stage strategy of at-risk identification: olfactory testing using the University of Pennsylvania Smell Identification Test followed by dopamine-transporter (DAT) imaging. Although findings on DAT scan are yet to be published, preliminary analyses showed that participants with hyposmia were more likely to have other nonmotor features and to report changes in motor function (Siderowf et al. 2012).

Of the pre-motor symptoms, the PRIPS and PARS studies focused on hyposmia. The results from these studies, albeit preliminary, clearly show that an individual pre-motor symptom by itself is inadequate for early disease identification. This is actually what one would expect for a relatively rare disease such as PD because the PPV depends of the prevalence of pre-diagnostic cases in the target population.

The utility of combinations of pre-motor symptoms for early disease identification has been little explored and merits consideration. Although the underlying etiologies of pre-motor symptoms in the general population are likely diverse, the presence of multiple symptoms in individuals who later develop PD may reflect common or similar underlying pathologies, for example Lewy pathology in various sites of the brain, spinal cord, and autonomic nervous system. In support of this notion, in the HAAS, both the sense of smell (Ross et al. 2006) and bowel movement frequency (Abbott et al. 2007) were strongly related to incidental Lewy body disease among individuals without PD. Further, α -synuclein was identified from colon tissues of PD patients collected 2-5 years prior to PD motor onset (Shannon et al. 2012), but not in any of the controls.

One may further hypothesize that among individuals who will develop PD, multiple pre-motor symptoms develop over time as a result of common pathologies and eventually become a clinically recognizable syndrome several years prior to PD diagnosis (Figure 1). In contrast,

among individuals who will not develop PD, these symptoms may also exist, but they are more independent of each other and more randomly distributed over the entire life period. Therefore, the joint prevalence of multiple pre-motor symptoms in a low risk population will be low. These hypotheses are yet to be systematically examined, but there are preliminary supportive data. Based on hyposmia, infrequent bowel movement, slow reaction time, and excessive daytime sleepiness, the HAAS showed that 2 of the 24 individuals with ≥ 3 of these symptoms developed PD within 4.6 years of follow-up, as compared with 8 out of 852 for those with only one symptom (Berg et al. 2012). Preliminary evidence also comes from research in high-risk populations. Non-Parkinsonian family members of patients with the *LRRK2* G2019S mutation showed more constipation and poorer color discrimination than controls (Marras et al. 2011). Therefore, preliminary evidence does suggest that multiple non-motor symptoms tend to co-occur in among individuals at higher risk for PD.

It is also important to understand when multiple pre-motor symptoms become detectable in the prodromal stage of PD. Ideally, this should be investigated in large prospective cohorts with long follow-up and repeated measurements of multiple pre-motor symptoms. In reality, we have just begun to understand the temporal relationships between individual symptoms and PD by examining existing clinical data or data from prospective cohorts (Table 1). For example, several clinical studies have consistently documented RBD onset about 10-20 years prior to PD onset (Boeve and Saper 2006; Claassen et al. 2010; Iranzo et al. 2006; Postuma et al. 2009; Schenck et al. 1996). These were studies of RBD patients diagnosed by polysomnography, and it has yet to be determined whether this clinical observation can be generalized to the general elderly population where only questionnaire-based screening for probable RBD is possible (Postuma et al. 2012a). Data on the timing of other key pre-motor symptoms are limited or inconsistent. For

example, several studies showed that constipation might precede PD clinical diagnosis by 10-20 years in men (Abbott et al. 2001; Gao X et al. 2011; Savica et al. 2009), but data are not consistent in women (Gao X et al. 2011; Savica et al. 2009). The population-based HAAS showed that hyposmia was highly predictive of PD onset within four years after symptom assessment, but not beyond (Ross et al. 2008). Two other studies among high-risk individuals (Ponsen et al. 2009; Postuma et al. 2011) showed that hyposmia predicted PD risk throughout the entire follow-up period of five years. As the assessment of temporal relationship will be bounded by the length of follow-up, future studies should have longer periods of follow-up and repeated symptom assessments. More importantly, future studies should also investigate the temporal pattern of multiple pre-motor symptoms in prodromal PD cases.

Measuring premotor symptoms for neurodegeneration research represents a substantial challenge. Studies to date have mostly used simple methods to identify pre-motor symptoms, including self-reported symptoms, self-reported diagnoses, screening tests, and structured questionnaires. For example, the sense of smell is often measured with simple screening tests such as the Brief Smell Identification Test (Ross et al. 2008) or the Sniffin' Stick Test (Hummel et al. 2001), and hyposmia is defined as a score below population norms. These simple methods have served well to establish the associations between pre-motor symptoms and PD. However, as most pre-motor symptoms are common in the elderly and are etiologically heterogeneous (Doty 2009; Leung et al. 2011), novel approaches are needed to assess various modalities of these symptoms and to identify patterns that are more specific to PD. Compared to other pre-motor symptoms, RBD is more specific; however, its diagnosis requires polysomnographic confirmation at sleep clinics. Several screening questionnaires for probable RBD (Boeve et al. 2011; Li et al. 2009; Postuma et al. 2012a; Stiasny-Kolster et al. 2007) have been developed and

validated in clinical settings, but their validities in identifying RBD patients from the general population are yet to be evaluated.

A large prospective study reported subjective complaints of motor dysfunctions such as stiffness and tremor prior to PD diagnosis (de Lau et al. 2006). In fact, subtle motor abnormalities have been quantitatively documented among individuals at high risk for PD. For example, Mirelman reported subtle gait changes among asymptomatic carriers of *LRRK2* mutation with quantified gait analyses under challenged conditions (Mirelman et al. 2011). Among RBD patients, Postuma et al. documented multiple motor abnormalities on average six to eight years prior to PD diagnosis, including voice and face akinesia, rigidity, abnormal gait, limb bradykinesia (Postuma et al. 2012b). Evaluation of subtle motor changes in addition to pre-motor symptoms may prove important in differentiating PD from other causes of non-motor symptoms. Further, the development and use of standardized assessment tools for non-motor and motor symptoms such as the NIH/NINDS common data elements for PD will greatly facilitate such research (NINDS, 2013).

Implications for Parkinson's etiology and experimental research

An inherent implication of research on pre-motor symptoms is that it may eventually lead to a better understanding of PD etiology (Hawkes et al. 2007, 2009). The concept that pre-motor symptoms represent intermediate phenotypes prior to overt PD may offer us a vehicle to understand the role of genetics and environment in the early stages of PD development. For example, neurotoxicants or viruses may enter the body via the nasal cavity or the digestive tract (Hawkes et al. 2007, 2009), and, in susceptible individuals, may initiate Lewy pathology in the olfactory bulb or the enteric nerves (Doty 2008; Hawkes et al. 2007, 2009; Reichmann 2011);

over time, this may lead to pre-motor symptoms such as hyposmia or constipation and may eventually progress to PD. It is therefore important to identify environmental and genetic factors associated with the presence of multiple pre-motor symptoms, and more importantly factors that may prevent the progression of pre-motor symptoms to clinical PD. This concept is illustrated in Figure 2.

To the best of our knowledge, no epidemiological study has examined common etiological factors for the presence of multiple non-motor symptoms. Preliminary data are available only on risk factors for individual symptoms. The first paper on environmental risk factors for RBD was recently published by Postuma and colleagues (Postuma et al. 2012c). In this multicenter study of 347 cases and 347 controls, RBD was positively associated with pesticide exposure and head injury. However, unlike PD, RBD was more common among smokers and was not related to caffeine intake. More studies have examined risk factors associated with hyposmia. All studies found that the risk of hyposmia increases with age and is higher in men than in women (Bramerson et al. 2004; Schubert et al. 2012; Schubert et al. 2011; Siderowf et al. 2007; Siderowf et al. 2012; Vennemann et al. 2008). Data on smoking or coffee drinking and hyposmia are, however, preliminary and inconsistent (Bramerson et al. 2004; Schubert et al. 2012; Schubert et al. 2011; Siderowf et al. 2007; Siderowf et al. 2012; Vennemann et al. 2008), although current smoking is associated with higher risk of hyposmia in some studies (Schubert et al. 2012; Vennemann et al. 2008). In adults, the prevalence of constipation is higher in women and increases modestly with age (McCrea et al. 2009; Soares and Ford 2011). Other suspected risk factors for constipation include inadequate fluid or dietary fiber intake, less physical activity, concurrent use of certain medications, levels of thyroid hormone and progesterone, and a wide range of medical conditions including neurodegenerative diseases (Leung 2007; Leung et al.

2011). Therefore, risk factors for individual non-motor symptoms are diverse, and PD-related pathology is probably only a small contributor to the prevalence of each of these symptoms. Combining multiple pre-motor symptoms may rule out some diverse pathologies unrelated to PD. Further, PD is likely to be phenotypically and etiologically heterogeneous (van Rooden et al. 2011; van Rooden et al. 2009); and careful phenotyping of various PD motor and nonmotor symptoms may help us understand the interrelationship among risk factors, premotor symptoms and neurodegeneration.

While human observational studies are essential to define pre-motor symptoms and their relationship to PD development, experimental studies are needed to understand the underlying biology and to examine novel etiological hypotheses. For example, could the gastrointestinal tract or the olfactory bulb be the sites of initial exposure to a pathogenic environmental agent (Reichmann 2011)? Does pathology progress anatomically as the Braak hypothesis predicts (Pan-Montojo et al. 2012)? Does the selectivity and order of neurodegeneration in PD reflect differential sensitivity to environmental agents or some other mechanism of progression such as prion-like spreading (Dunning et al. 2012)? Experimental research that models prodromal PD may help answer these questions.

Both toxicant (e.g. MPTP, 6-OHDA, paraquat, and rotenone) and genetic (e.g. α -synuclein, LRRK2, PINK1, Parkin) based animal models have been used for PD research (Bezard et al. 2012; Blesa et al. 2012). These models were developed to mimic features of late-stage PD such as dopaminergic neuron loss and motor dysfunction, or to recapitulate particular pathogenic processes such as neuroinflammation. The extent to which current models replicate the non-motor features of PD is incompletely known at present, although recent work has revealed some

intriguing results suggesting abnormalities analogous to non-motor features of PD (Jellinger 2011; McDowell and Chesselet 2012; Smith et al. 2012).

Central to this work is the availability of validated methods to evaluate olfaction, gastrointestinal function, sleep disturbances, or anxiety/depression in experimental animals. The technical difficulties of reliably determining the presence or absence of these non-motor features in experimental animals are not trivial. Furthermore, the mechanistic relationship between abnormalities observed in the commonly used assays in experimental animals and the analogous symptoms in human patients is uncertain, especially for complex behavioral traits such as depression and anxiety. Nonetheless, ways to evaluate these non-motor symptoms have been reported in mice, rats, primates and zebrafish. Each of these animals shows phylogenetic conservation of neuroanatomical structures involved in early Braak stages of PD pathology, suggesting that they might be employed as models to study pre-motor PD.

So far, a number of animal models of PD have shown either non-motor functional abnormalities or pathology outside the substantia nigra. Olfactory function has been shown to be abnormal in MPTP treated rodents (Schintu et al. 2009), transgenic mice expressing α -synuclein under the *thy1* promoter (Thy1- α Syn) (Fleming et al. 2008), and mice expressing reduced levels of the vesicular monoamine transporter (VMAT) (Taylor et al. 2009). Sleep and circadian rhythm are known to be disrupted in rodents treated with MPTP (Laloux et al. 2008), rats treated with rotenone (Garcia-Garcia et al. 2005), and in Thy1- α Syn and VMAT2 deficient mice (Taylor et al. 2009). Gastrointestinal function has been shown to be abnormal in MPTP treated mice (Anderson et al. 2007), rotenone treated rats (Drolet et al. 2009), Thy1- α Syn mice (Wang et al. 2008; Wang et al. 2012), mice expressing mutant human α -synuclein from a P1 artificial chromosome containing its endogenous regulatory elements (Kuo et al. 2010), and VMAT2

deficient mice (Taylor et al. 2009). These findings are of interest because they demonstrate that toxicant exposures and genetic manipulations used to induce motor signs of PD can also induce non-motor features. This suggests that at least some of the neuronal populations underlying non-motor symptoms share susceptibility with dopamine neurons to agents implicated in motor PD pathogenesis. This is consistent with a model in which common etiological mechanisms could underlie both motor and non-motor components of the disease.

Interestingly, a few of the models have shown ordered progression from non-motor to motor symptoms. Thy1- α Syn transgenic mice showed α -synuclein inclusions in the olfactory bulb and deficits in olfactory function on multiple tests by 3 months of age (Fleming et al. 2008). By this time point, animals also showed sleep abnormalities that progressively worsened (Kudo et al. 2011) and progressive reduction in stool frequency (Wang et al. 2012). These changes preceded loss of striatal dopamine, which did not occur until 14 months of age (Lam et al. 2011). Similarly, VMAT2 deficient mice demonstrated progressive non-motor symptoms prior to the onset of motor deficits (Taylor et al. 2009). Gastrointestinal dysfunction was seen at 2 months, olfactory defects by 5 months, and anxiety-like behavior at 6 months. L-DOPA responsive hypokinesia and loss of striatal tyrosine hydroxylase terminals were present by 18 months, and loss of nigral dopamine neurons worsened between 18 and 24 months (Caudle et al. 2007). Data from both models imply that a systemic abnormality affecting all cells can result in specific abnormalities of neuronal populations implicated in non-motor and motor PD with replication of some of the temporal course.

These data do not yet allow us to distinguish between a model for pathogenic progression in which the temporal course of the disease is dictated by the differential vulnerability of various neuronal groups to a systemic abnormality and an alternative model in which pathology spreads

anatomically from one site of the nervous system to another to produce progressive symptoms. Much recent attention has been given to the idea that α -synuclein has prion-like properties and that α -synucleinopathy can spread from a site of initial pathology to other regions of the central nervous system (CNS) by axonal transport and cell-to-cell spread (Luk et al. 2012). In this regard, it is noteworthy that the pathology in both Thy1- α Syn (Fleming et al. 2004) and VMAT2 deficient mice (Taylor et al. 2009) is dependent on the presence of α -synuclein. However, several alternative explanations for the progression of disease are equally consistent with the available data and further studies will be necessary to determine whether progression can be arrested by interventions that prevent the transport or transmission of pathological α -synuclein species, or whether additional cellular factors dictate the differential vulnerability of neuronal groups involved in non-motor symptoms.

The hypothesis that an environmental agent could provoke pathology at an anatomical site of entry that then progresses to involve other structures, culminating in degeneration of the substantia nigra, has received some preliminary experimental support. For example, Jang et al (Jang et al. 2009) reported that, in mice, intranasal injected H5N1 influenza virus travelled from the enteric nervous system (ENS) into the CNS and eventually caused degeneration of dopaminergic neurons. Further, this sequence was accompanied by chronic neuroinflammation with microglial activation and elevated expression of cytokines and other pro-inflammatory biomarkers (Jang et al. 2012). These findings imply that initiating pathogenic events can provoke distinct secondary mechanisms underlying disease progression, with the important implication that environmental agents that trigger early events in PD pathogenesis may no longer be present at the end stage of the disease, when tissue samples are generally available for analysis.

The gastrointestinal tract is potentially an important site for exposure to environmental agents, and the suggestion that α -synuclein pathology in the ENS may be one of the first abnormalities in PD patients has promoted interest in the possibility of modeling pathology in the ENS and its subsequent progression to the CNS. Transgenic mice expressing human α -synuclein under its own regulatory elements showed prominent ENS pathology, but no progression to other features of PD (Kuo et al. 2010), suggesting that a second event was necessary to promote disease progression. Recent reports showed that intragastric rotenone caused α -synuclein aggregation in mice, following a staged pattern that was consistent with the Braak hypothesis (Pan-Montojo et al. 2010; Pan-Montojo and Funk 2010), and resection of the autonomic nerves prevented this progression (Pan-Montojo et al. 2012). These interesting observations are yet to be replicated by other laboratories and their interpretation consequently remains speculative. However, the local microenvironment of the gastrointestinal tract remains a potentially significant factor in dictating initiating pathogenic events, and is worthy of further investigation. This might also encompass evaluation of the role of the gut microbiome, which could be experimentally manipulated in animal models to determine whether alterations can initiate PD pathology or modulate the time course of onset of pathology and progression. Although few empirical data exist regarding the role of the microbiome in PD, microbiome influences the immune system, gastrointestinal mobility, and the metabolism of nutrients and other exogenous chemicals (Grenham et al. 2011), all of which may potentially contribute to the development of PD. Similarly, experimental animals could be exposed to toxicants through the gastrointestinal tract to evaluate whether etiologically-implicated exogenous agents can provoke the earliest pathological changes of PD or modulate their appearance in experimental models. These studies could provide valuable mechanistic insights and generate further hypotheses to be addressed in human studies.

Finally, although this review focuses on PD, it is important to point out that research on pre-motor symptoms may have broader implications as many of these symptoms have been linked to other neurological diseases. For example, hyposmia is associated with higher risk of cognitive decline and Alzheimer's disease (Wilson et al. 2009; Wilson et al. 2007), and RBD precedes Lewy body dementia and multiple system atrophy (Schenck et al. 2013). Further, olfactory dysfunction has been documented in schizophrenic patients and individuals at high risk for schizophrenia (Moberg et al. 2013). Therefore research on pre-motor symptoms may eventually provide novel insights into the natural history and etiology of neurodegeneration and related conditions in addition to PD, and into the complex interrelationships among these conditions.

Conclusion

Pre-motor symptoms of PD may offer us an excellent opportunity to identify populations at higher risk for PD and to understand early disease etiology. Further research is needed to understand whether the presence of multiple pre-motor symptoms is predictive of PD. Animal experiments may help to understand the biology of these non-motor symptoms and test novel etiological hypothesis. At the current time, our understanding of these pre-motor symptoms is still in its infancy and the research calls for close multidisciplinary collaborations among clinicians, epidemiologists, basic scientists, and geneticists.

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Table 1: Prospective evidence on selected pre-motor symptoms in large population-based studies

Symptoms	Study	Age ^a , years	Years of follow-up	No. cases	Assessment	Primary results, RR/HR/OR(95%CI)	Timeline, years prior to PD ^b
Hyposmia	HAAS, men only (Ross et al. 2008)	79.7 ± 4.1 (71-95)	Up to 8 years	35	BSIT score <6	Lowest vs. top two quartiles: 5.2 (1.5-25.6) for the 1 st 4 years, 0.3 (0.0–2.7) for the 2 nd 4 years of follow-up	Within 4 years
Constipation ^c	HAAS, men only (Abbott et al. 2007)	60 (51-75)	Up to 24 years	96	Self-reported bowel movement frequency	<1/day vs >2/day: 4.5 (1.2-16.9)	Could be 12 or more years
	REP(Savica et al. 2009)	--	--	196	Medical record review: constipation diagnosis or laxative use	2.5 (1.5-4.1)	Could be 20 or more years
	HPFS, men only (Gao X et al. 2011)	~54-89	Up to 6 years only	156	Self-reported bowel movement frequency	≤2/week vs. daily: 5.0 (2.6-9.6)	Six years and probably more
Daytime sleepiness ^d	NHS, women only (Gao X et al. 2011)	~36-61	Up to 24 years	402	Self-reported bowel movement frequency	Within 6 years of follow up: 2.2 (0.8-6.1), no association beyond 6 years	May limit to 6 years of follow-up
	HAAS men only (Abbott et al. 2005)	77 (71-93)	Up to 8 years	43	Self-report: single question	2.8 (1.1-6.4)	0.5-4.9 years
RBD ^e	NIH-AARP DH (Gao J et al. 2011)	52-71	4-10 years	770	Self-reported hours ≥1 vs 0 hours of daytime napping	1.5 (1.2-1.9)	4-10 years
	Mayo Clinic (Claassen et al. 2010)	21-60	Only if ≥15 years	9 PD out of 27 RBD	Clinical diagnosis	---	15-50 years
	Barcelona, Spain (Iranzo et al. 2006)	74.1(61–86)	>2 years	7 PD out of 44 RBD	Clinical diagnosis	---	Could be 6-18 years
	Minnesota, men only (Schenck et al. 1996)	54.5	-	11 PD out of 29 RBD	Clinical diagnosis	---	Could be by 10-29 years

Symptoms	Study	Age ^a , years	Years of follow-up	No. cases	Assessment	Primary results, RR/HR/OR(95%CI)	Timeline, years prior to PD ^b
Depression	Montreal (Postuma et al. 2009)	65.4 ± 9.3	-	19 PD out of 93 RBD	Clinical diagnosis	---	On average preceded by 11 years
	EPIC-Norfolk (Ishihara-Paul et al. 2008)	41-80	Median 8 years	175	Structured questionnaire	Lifetime major depression 2.1 (1.4-2.9)	Similar results for first episode of depression before or after age 40
Anxiety	REP (Bower et al. 2010)	48.3 (20-69)	Mean 29, up to 45	156	MMPI	Quartile 4 vs. 1-3: 1.16 (0.81-1.66)	
	REP (Shiba et al. 2000)		51 years (8–87 yrs)	196	Medial record review	1.9 (1.1-3.2)	Within 5 years
	EPIC-Norfolk (Ishihara-Paul et al. 2008)	41-80	Median 8 years	175	Structured questionnaire	2.7 (1.5-4.7)	
	HPFS, men only (Weisskopf et al. 2003)	56.0 (42-77)	Up to 12 years	189	Crown–Crisp anxiety index	Score ≥4 vs 0-1: 1.5 (1.0-2.1)	Could be >2 years
	REP (Bower et al. 2010)	48.3 (20-69)	Mean 29, up to 45	156	MMPI	Quartile 4 vs.1-3: 1.63 (1.16-2.27) / men 2.03 (1.28-3.24)/ women 1.29 (0.79-2.10)	
	REP (Shiba et al. 2000)		51 years (8–87)	196	Medial record review	2.2 (1.4-3.4), Slightly attenuated even restricted to more than 20 years prior to index date	Could be >20 years

^a Means ± standard deviations and/or range are presented. ^b The time from measurement of non-motor symptoms to PD diagnosis are our best estimates. These estimates could however be misleading as they were bounded by the length of follow-up and inclusion criteria. ^c The HAAS and HPFS/NHS used the frequency of bowel movement as an indicator for constipation. ^d The NIH-AARP DH used daytime napping duration as a surrogate for daytime sleepiness. ^e Based on follow-ups of RBD patients

Abbreviations: BSIT: brief smell identification test; CI: confidence interval; EPIC: European Prospective Investigation into Cancer; HAAS: Honolulu Asia Aging Study; HPFS: Health Professionals Follow-up Study; HR: Hazard Ratio; MMPI: Minnesota Multiphasic Personality Inventory; NHS: Nurse’s Health Study; OR: odds ratio; RBD: rapid eye movement sleep behavior disorder; REP: Rochester Epidemiology Project; RR: relative risk

Figure Legends

Figure 1. A hypothesis on the development of pre-motor symptoms among individuals who will or will not develop PD in lifetime. The green lines represent the joint prevalence of multiple pre-motor symptoms by age: solid for future PD cases, and dashed for non-cases. The red line represents motor signs among future PD patients. The blue line represents the loss of dopaminergic neurons in the substantia nigra pars compacta of PD patients which underlies cardinal motor signs. PD diagnosis is made based on cardinal motor signs (red) when approximately 50% of the dopaminergic neurons in the substantia nigra have been lost (threshold shown as the black dotted line). Individuals at high risk for PD (solid green line) will develop multiple pre-motor symptoms years before onset of PD motor signs; for individuals who will not develop PD (dashed green line), the joint prevalence of these symptoms remain at low level even at older age.

Figure 2. A hypothesis on risk factors, pre-motor symptoms, and PD. Environmental or genetic factors may initiate neurodegeneration through mechanisms such as neuroinflammation; in susceptible individuals, this may first lead to pre-motor symptoms years before PD clinical onset; if this neurodegeneration continues without effective intervention, pre-motor symptoms may eventually progress into overt PD; however with interventions such as drinking coffee, this pre-motor progression may be halted before it becomes irreversible.

Figure 1.

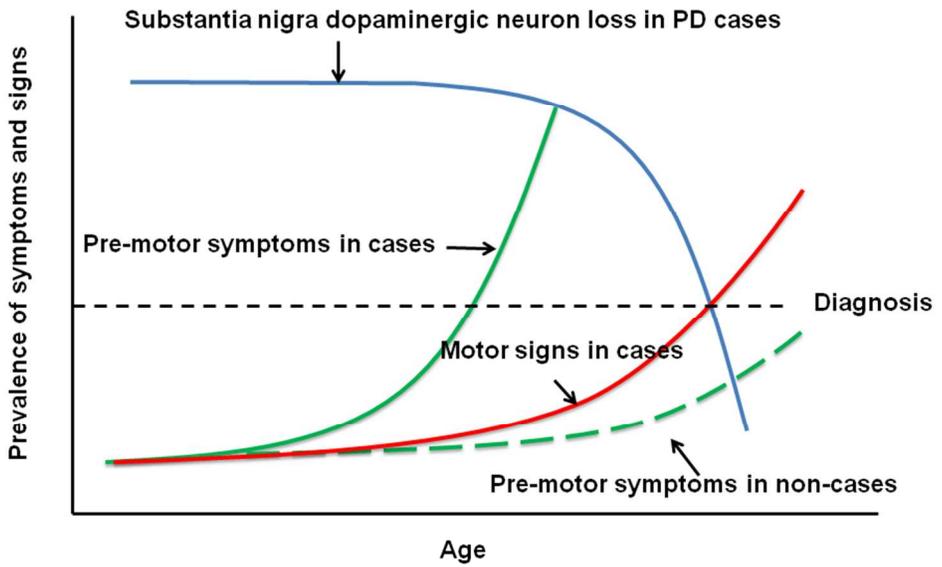


Figure 1

254x190mm (96 x 96 DPI)

Figure 2.

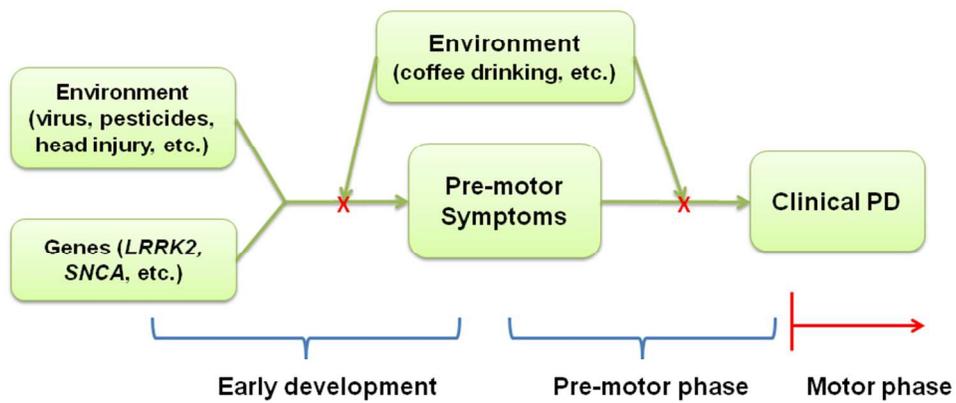


Figure 2

254x190mm (96 x 96 DPI)