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Hypothesis of Ascension in Idiopathic Parkinson's Disease

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ABSTRACT

Different clinical stages are observed in idiopathic Parkinson's disease (PD). Non-motor symptoms define in particular the prodromal period of PD whereas primary motor symptoms such as bradykinesia with rigidity, resting tremor or postural instability are mandatory for the diagnosis of PD. Important non-motor symptoms are olfactory dysfunction, constipation, depression and sleep disturbances. Corresponding to the clinical course of PD, the Braak staging system postulates that the neuropathological process of PD starts in the enteric nervous system (ENS) of the gut and in the olfactory bulb. From there, Parkinson pathology spreads by transsynaptic cell-to-cell transfer via the sympathetic and parasympathetic nervous system in a rostrocranial direction. If the central nervous system is reached, typical neuropathological changes of PD with selective degeneration of dopaminergic neurons of the Substantia nigra pars compacta, the formation of Lewy bodies, reactive gliosis and progressive central neurodegeneration appear. Evidence of clinical, pathological and animal studies supporting these hypotheses are summarised in this review article. α-synuclein as PD-specific pathology was found in the olfactory bulb, the ENS, the submandibular gland, the intermediolateral nucleus of the spinal cord and the dorsal motor nucleus of the vagus nerve. In an animal model, in which mice are treated with the pesticide rotenone chronically and intragastrically, we could almost completely reproduce the typical pathological and clinical features of PD as well as their development in a chronological and regional sequence.

Introduction

Patients with idiopathic Parkinson's disease (PD) show motor and non-motor disease-related symptoms. Although this is globally accepted, the diagnosis of PD according to the UK Brain Bank criteria [1] can only be made if the combination of the motor cardinal symptoms of bradykinesia with at least one other symptom, namely muscular rigidity, rest tremor or postural instability, are clinically evident. There are first approaches to diagnosis that include non-motor complaints occurring even before the motor symptoms. The Movement Disorders Society (MDS) has established new clinical diagnostic criteria for PD. While motor symptoms continue to be regarded as constituting the core of the disease, non-motor symptoms have also been included as important criteria [2]. Meanwhile, the proposed scientific criteria of the MDS for prodromal PD are based primarily on non-motor symptoms [3]. Prodromal PD is the disease phase in which early signs of Parkinson's-specific neurodegeneration are detectable, while the diagnosis of PD cannot be made as motor symptoms are not yet fully developed. Thus, hyposmia [4, 5], sleep disturbances, in particular REM (rapid eye movement) sleep disturbance disorder [6, 7], autonomous dysfunctions, in particular constipation [8, 9], and psychiatric symptoms such as depression and anxiety disorders [10, 11] occur many years before the appearance of the first motor symptoms. This correlates well with the presumed and partially confirmed hypotheses of ascension of the underlying neurodegenerative changes. There is evidence that, in the context of the gut-brain connection via the vagal nerve of the enteric nervous system (ENS) and additionally, by means of the olfactory bulb, ascension of the Parkinson-specific pathology occurs in the form of pathological α-synuclein. On reaching the central nervous system (CNS), the main features of the Parkinsonian pathology are initiated, namely selective degeneration of the dopaminergic neurons in the substantia nigra pars compacta, formation of Lewy bodies, reactive gliosis and progressive neurodegeneration. The underlying clinical, pathological, and animal studies that support these hypotheses are presented in this review article.

Gastrointestinal Symptoms and Olfactory Disorder as Prodromal Non-motor Symptoms of Idiopathic Parkinson's Disease

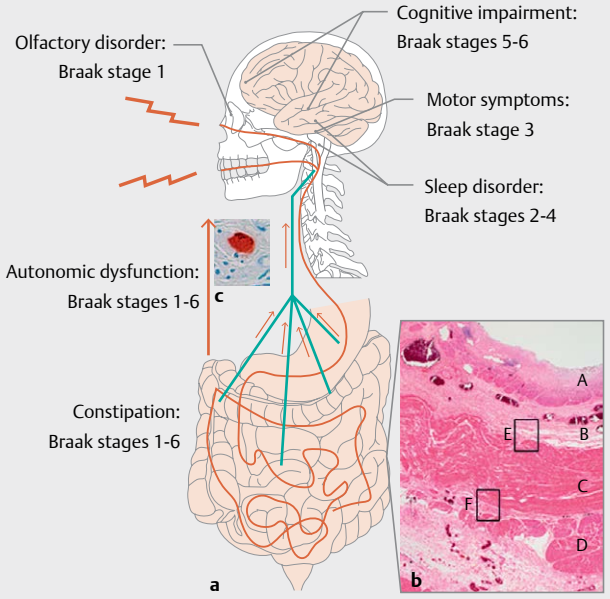
On average, one-third of all patients report having gastrointestinal symptoms; these occur in all phases of PD [12–14]. With a prevalence rate of 28–80 %, constipation is one of the most common non-motor complaints in PD [15–19], and up to 6 times more common in patients with PD compared to healthy controls of compa-

rable age and sex [8, 15, 20, 21]. Studies have also shown that constipation precedes the development of PD and is a major risk factor for this disease [8, 22–24]. In the Honolulu Heart program, 6 790 men (without PD at the time of inclusion in the study) were controlled for up to 24 years regarding the incidence of PD [22]. After an average of 12 years, 96 men developed PD. After controlling for various factors that can affect constipation, such as age, nicotine and caffeine consumption, men who did not have even one bowel movement a day had a 2.7-fold, 4.1-fold, and 4.5-fold higher risk of developing Parkinsonian syndrome than men who had one, 2 or more than 2 bowel movements a day. In another case-control study, it was shown that the incidence of constipation was higher in people who developed PD than in those who did not (incidence risk rate: 2.01) and this already 10 years before the diagnosis of PD was made [11]. A recent study using positron emission tomography showed a significantly lower concentration of acethycholinesterase in the small intestine of patients with PD compared to healthy control subjects [25]. This finding, however, did not correlate with the severity of constipation. Thus, constipation is not only an early non-motor manifestation, but at the same time also a risk factor of PD. The cause is thought to be neurodegeneration of the ENS and also CNS degeneration in the advanced stage of the disease [26]. It is also known that patients with PD have a markedly reduced olfactory function compared to controls and that this affects both the perception of threshold odours and the discrimination and identification of odours [21, 27]. This olfactory disorder is detectable in up to 90 % of patients with PD and is also a prodromal non-motor symptom of PD [28, 29].

Pathological Progression of Parkinson's Disease

The pathognomic histology of PD is Lewy bodies in neurons, Lewy neurites in dendrites and axons, loss of catecholaminergic neurons in the locus coeruleus, and loss of dopaminergic neurons in the substantia nigra. Lewy bodies and Lewy neurites are intracytoplasmatic protein aggregates, which consist mainly of α-synuclein [30]. Pathological studies suggest that there is a specific temporal and spatial pattern in the spread of neuropathological changes in PD [31, 32]. Braak and colleagues developed a system for classifying the degree of pathology (Braak stages) for Parkinson's disease, which correlated very well with the clinical symptom presentation in the different phases of disease [33–35], and over the years was adapted to the latest state of knowledge [36]. According to Braak stages currently in use, the neuropathological process of Parkinson's disease begins in the olfactory bulb, in the ENS, in the intermediolateral nucleus (IML) of the spinal cord, and in the dorsal motor nucleus of the vagus nerve (DMNV) [36–39]. The detection of α-synuclein in the olfactory bulb is associated with a neuropathologically confirmed diagnosis of advanced PD and with dementia with Lewy bodies (DLB) with a sensitivity and specificity of more than 90 %, and also correlates with the extent of synucleinopathy in other brain regions [40]. Lewy bodies and Lewy neurites were also found in the peripheral nervous system of the intestine and sympathetic and parasympathetic ganglia [41–44]. These pathological changes have been observed in Auerbach's Plexus and Meissner's plexus of the ENS and in the olfactory bulb, both in patients with diagnosed PD but also in people who did not noticeably demonstrate the classic motor symptoms of PD or the classic pathological changes in the CNS [41, 45–48]. In autopsy studies of patients with PD, α-synuclein was found in the ENS in 50–100 % of cases and in 0–52 % of cases in autopsy studies of control subjects; in in vivo studies, α-synuclein was found in the ENS in 66–100 % of patients with Parkinson's disease and in 0–8 % of control subjects [49, 50]. In contrast, phosphorylated α-synuclein was detected in 23–93 % of patients with Parkinson's, but not in control subjects [51, 52]. The analysis of the Danish National Pathology Registry showed that Lewy bodies were detectable in the gastrointestinal tract of patients with PD up to 20 years before the development of Parkinsonian syndrome [53]. Furthermore, in this study, the detection of phosphorylated α-synuclein was significantly higher in these patients compared to controls [53]. Other smaller case series also demonstrated phosphorylated α- synuclein in the gastrointestinal tract in patients in the prodromal phase of PD [54] and dementia with Lewy bodies [55, 56]. In addition, in autopsies of individuals of advanced age who had suffered from constipation but had shown no clinical symptoms of Parkinson's disease or dementia prior to their death, incidental Lewy bodies in the substantia nigra and the locus coeruleus were found, and this was associated with a reduced neuron density in the substantia nigra [57, 58]. The inclusion of both the olfactory and the gastrointestinal system in the neurodegenerative process of PD is explained by a "dual-hit" mechanism [59, 60]. Thus, a neurotrophic pathogen is inhaled and/or swallowed, which then initiates the pathological process. This is followed by an anterograde progression of the pathology of the olfactory system in the temporal lobes or retrograde progression from the gastrointestinal system via the sympathetic and parasympathetic nervous system to the brain stem (▶ **Fig. 1**) [35, 61, 62]. This neurodegenerative process appears to spread only in anatomically connected structures in the caudorostral direction and, after reaching the brain stem, spread further to cortical regions and the spinal cord [38]. The motor symptoms that clinically define PD only occur after a 60–80 % loss of dopaminergic neurons of the substantia nigra and, correspondingly, the dysfunction of the nigrostriatal dopaminergic control circuits [63]. In the further course of the disease, involvement of higher cortical structures is revealed, often by the presence of dementia [64].

Of course, there are also findings that cannot be clearly classified into Braak stages, raising questions about the validity of the classification system. Thus, retrospective autopsy studies have shown that in up to half of the examined patients, the pathological findings do not conform to the Braak stages [65–70]. So, for example, it was reported that in 7 % of patients with PD, no α-synuclein was detectable in DMNV despite the detection of α-synuclein in the substantia nigra and in the cortex [65]. In another study, this result was confirmed in 8.3 % of patients with PD [66]. Also, phosphorylated α-synuclein was shown in the ENS, particularly of older healthy control subjects, and not at all, or only slightly in younger control subjects [71, 72]. This suggests that the phosphorylation of α-synuclein and the accumulation of phosphorylated α-synuclein might also be a part of the process of aging [71, 73]. Furthermore, the relationship between PD-specific pathology and impaired gastrointestinal motility in patients with PD is not really understood. In studies that



▶ **Fig. 1** **a** Schematic drawing of the brain and the gastrointestinal system with a dual-hit of the olfactory system and the gastrointestinal tract via inhalation/ingestion (red thread) with rostrocranial ascension of Parkinson's pathology (red arrows) via sympathetic and parasympathetic nervous system (vagus nerve as green lines) reaching the central nervous system. Manifestation of non-motor symptoms with corresponding Braak stage (black arrows to corresponding anatomical structure). **b** Histologic intestinal section with A: mucosa, B: submucosa, **c** ring muscle, **d** longitudinal muscle and the enteric nervous system with E: Meissner's plexus and F: Auerbach's plexus. **c** α-synuclein inclusion similar to Lewy body (appearing red by immunohistochemical staining after proteinase K digestion) as Parkinson pathology.

looked for Lewy pathology in the ENS in patients with Lewy body disease, α-synuclein could be detected in the ENS of almost every patient with PD [51, 74] but neither neuronal loss of Auerbach's plexus nor a change in the neurochemical composition could be demonstrated in patients with PD compared to healthy controls [48, 75]. On the other hand, a 15 % neuronal loss per ganglion of Meissner's plexus [76] and a loss of dopaminergic neurons of Auerbach's plexus [77] were described in colonic biopsies of patients with PD from other centres. Neurochemical changes such as low concentrations of glutathione and prostaglandin [78] and high expression of glial fibrillary acidic protein and Sox 10 [79] in patients with PD suggest dysregulation and loss of enteric glial cells [79–81]. Thus, a positive correlation between gastrointestinal permeability, which is regulated by enteric glial cells, on the one hand, and the intestinal amount of α-synuclein in untreated patients, on the other, was found in the early phase of PD [82]. Enteric glial cells may thus be involved in the initiation and progression of PD in the context of gastrointestinal disorder [83]. The extent of glial markers decreases with prolonged duration of PD, suggesting a stronger role of glial cells at the onset of disease and a decrease with time [79]. Thus, the extent of enteric neuronal loss and neurochemical changes in the different autopsy studies in patients with advanced PD could be explained by the inclusion of enteric glial cells and their different behaviour during the progression of PD [75–77].

Each classification system will have weak points and deviating results should always be expected. Thus, the distribution of Lewy bodies in PD might be more diverse than hitherto suspected. However, the different study results could also be due to the different cohorts and techniques used as well as the detection of different types of α-synuclein or the inclusion of patients with incidental Lewy-body disease (ILBD) and not with PD [84–87]. An ILBD is defined by the presence of cerebral Lewy bodies in autopsies of individuals without evidence of a neurodegenerative disease before their death [88, 89]. It is assumed that 5–24 % of the elderly population have ILDB [69, 84, 90] and could correspond to the preclinical stage of synucleinopathy [91]. Nevertheless, detection of Lewy bodies is essential for the pathological diagnosis of PD, whereas it does not appear to be specific for prodromal PD [84].

Evidence for Ascension Hypothesis using Animal Models for Parkinson's Disease

Currently, there is no animal model that fully maps the temporal and spatial spread of Parkinson pathology corresponding to the Braak stages. Some animal models may present clinical symptoms such as hypokinesia and symptomatic effects of levodopa and dopamine agonists, while other pathological changes such as the loss of tyrosine hydroxylase-positive and dopaminergic neurons in the substantia nigra, the loss of tyrosine hydroxylase-positive neurons in the ENS or α- synuclein inclusions in the substantia nigra and in the ENS. In the different animal models generated by toxins, not only the nature of the toxin (for example, MPTP, the pesticide rotenone or herbicide paraquat) [92–94] but also the route of administration (systemic, local) [95–97] plays an essential role. Pathogenic environmental factors must be able to overcome physiological defence mechanisms, such as the mucous membranes of the nasopharyngeal and gastrointestinal system, before they can be absorbed. Thus, the administration route of the toxin is crucial for triggering the pathology of PD in a realistic and natural form. The chronic intranasal administration of MPTP induces motor symptoms in mice, a reduction in the striatal dopamine content and the loss of tyrosine hydroxylase in the substantia nigra and in the striatum [96]. While chronic inhalation of rotenone did not produce any clinical or pathological changes typical of PD in mice and rats [96], systemic administration induced Parkinson's-typical pathological changes with nigrostriatal neurodegeneration and formation of α-synuclein inclusions similar to Lewy bodies [95, 98]. Furthermore, a degeneration of CNS structures could be detected, which is not usually associated with the PD and this process also showed no progression [98]. Pan-Montojo et al. [97] applied low doses of rotenone in wild-type mice in a chronic intragastric fashion (by means of a gastric tube) with the aim of inducing neuropathological changes typical of Parkinson's. Rotenone was administered at such low doses that it had only a local effect on the ENS, but not a systemic effect. In mice treated for 1.5 months, typical Parkinsonian neuropathological changes occurred, such as α-synuclein aggregation in the ENS, IML and DMNV, while changes in the substantia nigra or motor symptoms were not observed. Both α-synuclein aggregation and a loss of dopaminergic neurons in the substantia nigra pars compacta as well as motor symptoms could

be demonstrated in mice treated for 3 months. In this mouse model, a temporal and spatial progression of the Parkinsonian pathology corresponding to the Braak stages and the clinical symptom presentation in patients with PD could be simulated. Inden et al. [99] have also shown that chronic oral administration of rotenone in mice resulted in selective nigrostriatal dopaminergic neurodegeneration, motor symptoms, and cytoplasmic accumulation of α-synuclein in dopaminergic neurons. Interestingly, the loss of dopaminergic neurons in the substantia nigra pars compacta occurred asymmetrically, corresponding to the presentation in humans with PD. Tasselli et al. [100] were able to show the neurodegeneration of the substantia nigra in mice after 4 weeks of chronic oral rotenone treatment, but no change in gastrointestinal motility or neuropathological changes of the ENS could be detected. What is certain is that the administration of rotenone leads to an accumulation of α-synuclein [95, 101–104]

Accumulated α-Synuclein as a Pathological Correlate of Parkinson's Disease

Mitochondrial dysfunction caused by a complex I defect [105–107], oxidative stress [108], inflammation and protein deficiency [109, 110] are crucial mechanisms in the pathogenesis of PD. Environmental toxins such as rotenone cause exocytosis and release of α-synuclein from enteric and sympathetic neurons into the extracellular space [111–113]. The released α-synuclein can be absorbed by neurons and retrograded to the soma [114, 115]. In addition to transmission from neuron to neuron, transmission to non-neuronal cells is possible. Transmitted α-synuclein can trigger aggregation of endogenous α-synuclein in neurons [116]. This accumulated α-synuclein affects the mitochondrial complex I activity, reduces mitochondrial function, and causes oxidative stress in the neurons [108, 117, 118]. Different species of neurons react with different sensitivity to the loss of mitochondrial complex I activity. Thus, the combination of rotenone and endogenous dopamine results in selective toxicity of dopaminergic neurons in cell cultures from the mesencephalon of mice and rats and in the substantia nigra pars compacta of mice [95, 119]. Central dopaminergic neurons also appear to have a higher sensitivity to the accumulation of intracellular α-synuclein than, for example, neurons of the DMNV and the IML [97]. These results show that the dopaminergic neurons of the substantia nigra have intrinsic sensitivity to complex I defects. Furthermore, a proinflammatory effect of α-synuclein might exacerbate the degeneration of the dopaminergic neurons. Thus extracellular α-synuclein leads to the release of inflammatory factors such as cytokines and activates microglia, which in turn leads to an inflammatory reaction [120–122]. Furthermore, a study showed that the combination of low-dose rotenone with an inflammogen works synergistically and leads to a selective degeneration of dopaminergic neurons, which shows that inflammatory changes can increase neurodegeneration [110]. The accumulation of Lewy bodies is, however, not restricted to the dopaminergic neurons of the substantia nigra, but also occurs in other dopaminergic, glutamatergic, noradrenergic, serotonergic, histaminergic, and cholinergic neurons. This is quite in agreeement with the clinically variable phenotypic presentation of the PD, especially with regard to non-motor subtypes [51, 123–125]. Finally, cells exposed to neuron-mediated α-synuclein show signs of apoptosis, such as the decay of the cell nucleus and caspase 3 activation [117, 126].

Ascension of Pathology from ENS to CNS

It is postulated that the Parkinsonian pathology propagates in the form of accumulated α-synuclein by means of sympathetic and parasympathetic nerves from the ENS to the CNS. α-synuclein is released from neurons into the extracellular space where it is either free or associated with exosomes [127] and can be taken up via endocytosis from neighbouring neurons and neuronal precursor cells [116, 126]. In a mouse model with Parkinson-like pathology, it was shown that when α-synuclein was transferred to transplanted neuronal precursor cells where it accumulated and formed inclusion bodies similar to Lewy bodies [126, 128]. Also, in autopsy studies of patients with PD who had received fetal mesencephalic tissue transplants, accumulated α-synuclein could be detected in the transplanted neurons, suggesting that there must have been a transfer of α-synuclein from the "host" to the transplant neurons [129, 130]. In a mouse model, the progress of Parkinsonian-like pathology could be stopped by interrupting the connection between the enteric neurons and the sympathetic or parasympathetic neurons [113]. Thus, hemivagotomy or partial sympathectomy carried out before the occurrence of motor symptoms delayed the occurrence of these symptoms, but not the occurrence of gastrointestinal symptoms, in rotenone-treated and operated mice compared to rotenone-treated but non-operated mice [31]. Furthermore, the amount of accumulated α-synuclein in choline acetyltransferase (ChAT)-positive neurons was significantly greater in the DMNV contralateral to the hemivagotomy compared to the DMNV ipsilateral to the hemivagotomy [113] in the rotonone-treated and hemivagotomized mice. This asymmetry could not be explained by neuronal cell death in the ipsilateral DMNV, which can be a consequence of hemivagotomy [131]. Thus, hemivagotomy seems to stop the progression of Parkinsonian-like pathology in the sense of transmission of accumulated α-synuclein from the ENS via the vagal nerve to the DMNV [113]. These results are supported by another study, which also showed an active axonal transport of α-synuclein from the intestine to the DMNV via the vagus nerve in a time-dependent fashion after injection of various forms of α-synuclein into the intestinal wall in rats [132]. In contrast to other studies, the transport of α-synuclein did not lead to neuronal cell death, and exogenous α-synuclein could not be demonstrated in the DMNV or the substantia nigra [132]. Whether this is a dose-dependent effect of the amount of α-synuclein injected, whether a certain incubation time is required after injection of α-synuclein into the intestinal wall, or whether exogenous α-synuclein failed to initiate aggregation of endogenous α-synuclein in neurons, is currently not clear [116, 133].

A large epidemiological study supports the findings of these animal model studies [134]. Here it was shown that patients who underwent truncal vagotomy, but not superselective, had a low risk for the development of PD than the general population. The incidence of PD in vagotomized persons with a follow-up of more than 20 years was 0.65 per 1 000 person-years, compared to 1.28 per 1 000 person-years in adapted controls who had no vagotomy (adapted for the year of birth, sex, so-called index date, which

means that the control person had no vagotomy after the vagotomy date of the corresponding Parkinsonian patient). These results demonstrate the long prodromal phase of PD and the vagus nerve as a rostrocranial pathway of the Parkinsonian pathology to the CNS. In the animal model, vagotomized mice showed a lower dopaminergic neuronal cell death in the substantia nigra pars compacta ipsilateral to the vagotomy side than mice that were not vagotomised after 4 months of chronic gastric rotenone treatment [113]. Soon after Parkinson's pathology reaches the DMNV, it appears to spread further to the CNS. In summary, the spread of Parkinsonian pathology appears to be due to an intact synaptic linkage. Thus, only neurons with synaptic connection to the ENS show pathological changes after gastric rotenone treatment [97].

Summary

Non-motor symptoms, in particular olfactory disturbances and constipation, appear many years ahead of Parkinson's disease with motor symptoms; these becomes clinically manifest only when there is a degeneration of 60–80 % of the dopaminergic neurons in the substantia nigra. In addition to these non-motor symptoms, the pathological process runs its course decades before the clinical manifestation of the PD according to the UK Brain Bank criteria. The olfactory and gastrointestinal systems can serve as contact points to the environment as an entry site for pathogenic substances via inhalation and swallowing. The Braak stages postulate that the pathological process of PD begins in the olfactory bulb and in the ENS, and in the form of α-synuclein, spreads rostrocranially by transsyptic cell-to-cell transport mechanism via the sympathetic and parasympathetic nervous system over the DMNV and IML to the CNS. Thus, PD follows clinically and also pathologically a specific temporal and spatial sequence. Epidemiological, clinical, in particular autopsy studies and animal model studies have confirmed decisive aspects of the Braak stages. However, there are also conflicting study results such as the loss of nerve cells in the ENS as part of the pathological process, the role of enteric glial cells and the gastrointestinal microbiome, as well as different results concerning neurochemical changes in the gastrointestinal tract and the relationship between constipation and neuropathological changes. Furthermore, the Lewy body pathology is binding for the diagnosis of PD but not specific for idiopathic PD in the prodromal phase, since even incidental Lewy pathology could be demonstrated especially in the elderly, and of course α-synuclein in other synucleinopathies. This should be kept in mind in discussions about detection of α-synuclein in biospies, in particular from the gastrointestinal tract, as a promising biomarker for PD. Currently, lacking adequate specificity and sensitivity, it has no diagnostic use in clinical routine in suspected PD. In the future, however, testing for phosphorylated α-synuclein in tissue samples could be carried out in the course of routine gastrointestinal screening in combination with controlling for the presence of non-motor symptoms of PD as a clinical test battery for staging risk of possible PD. This would enable, on the one hand, investigation of neuroprotective therapies and, on the other, defining additional predisposing factors for PD that influence the progress rate or the course of the disease and the phenotypic variability of the PD.

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Conflict of Interest

The authors declare they have no conflict of interest.

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