

Alpha-Synuclein Expression in Patients with Parkinson's Disease: A Clinician's Perspective

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Although physiological function of alpha-synuclein is not yet clearly understood, accumulating evidence strongly suggests it plays a crucial role in the pathogenesis of Parkinson disease. Pathologically, alpha-synuclein is a major component of Lewy bodies, which is the pathological hallmark of Parkinson disease. Alpha-synuclein pathology is observed in the brainstem nuclei, including the dorsal motor nucleus of the vagus nerve, the locus ceruleus, and the substantia nigra in the early phase of Parkinson disease and it may spread to cerebral cortical areas in the advanced Parkinson disease and appears to have a role in the cognitive decline in Parkinson disease. Recently, it is suggested that alpha-synuclein pathology in Parkinson disease starts in the olfactory bulb or enteric nervous system and then spreads to the brainstem. In accordance with this hypothesis, alpha-synuclein pathology has been found in gastric mucosa and colonic mucosa of patients with Parkinson disease. Genetically, *SNCA* mutations including point mutation and copy number variation are known to cause familial Parkinson disease, further supporting the assumption that alpha-synuclein plays a crucial role in Parkinson disease pathogenesis. In addition, recent GWAS studies consistently show that the SNPs in *SNCA* genes are associated with risk for sporadic Parkinson disease. It is also known that variations in the promoter region or 3'UTR of *SNCA*, which increases the expression of *SNCA*, are associated with the risk for Parkinson disease. Collectively, these findings suggest that further studies on alpha-synuclein will lead to the elucidation of the mechanism of and therapy for Parkinson disease.

Key words: Parkinson disease, alpha-synuclein, neuropathology, genetics

INTRODUCTION

Parkinson's disease (PD) is a movement disorder characterized by tremor at rest, rigidity, and bradykinesia and it is the second most common degenerative disorder followed by Alzheimer's disease. Other motor symptoms manifested by patients with PD include postural instability, stooped posture, freezing of gait,

decreased facial expression, hypophonic voice, loss of dexterity, and festinating gait [1]. In addition, many patients have non-motor symptoms including: neuropsychiatric symptoms such as dementia, depression, and anxiety; sleep disorders such as insomnia, excessive daytime sleepiness, and rapid eye movement sleep disorder; autonomic nervous system symptoms such as orthostatic hypotension and micturition disorder; gastrointestinal symptoms such as constipation, difficulty in swallowing, and dyspepsia; and sensory symptoms such olfactory dysfunction, visual abnormality, and pain. The prevalence of PD increases with age and the incidence is reportedly 1-2% over the age of 60 years [2].

A large number of clinical and basic studies have been performed

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to elucidate the pathogenesis of PD. The majority of studies have investigated alpha-synuclein. Alpha-synuclein is a 140 amino acid protein that is encoded by the *SNCA* gene on human chromosome 4. It is distributed predominantly in presynaptic terminals of synapses, and is found in neural tissues at high concentrations, making up as much as 1% of total proteins in the brain. Although alpha-synuclein is recognized as a critical protein in PD, it was initially named as a non-amyloid component of plaque (NACP), which was first found in senile plaque, a pathologic characteristic of Alzheimer's disease. The physiologic function of alpha-synuclein is not yet known well. Considering its distribution in the synaptic terminal, alpha-synuclein probably takes crucial roles in synaptic plasticity, dynamics of vesicle, and in the synthesis and secretion of dopamine.

The importance of alpha-synuclein in PD began to be recognized in the late 1990s. The discovery of two crucial findings during this period triggered the study of alpha-synuclein in PD. The first was a genetic finding. In 1996, Polymeropoulos et al. [3] reported the A53T mutation of *SNCA* in a family with autosomal dominant familial PD. Through this finding, the relationship of alpha-synuclein and PD has been identified. The significance of alpha-synuclein in PD has been further supported by the findings of the other mutations of *SNCA*, including A30P and E46K in other families with inherited PD [4, 5]. The second important discovery was found in neuropathology. In 1997, Spillantini et al. [6] revealed that alpha-synuclein is the primary structural component of Lewy body, which is an intracytoplasmic inclusion characterizing PD. The finding of alpha-synuclein as the main component of Lewy bodies has underscored the critical role of alpha-synuclein in the disease mechanism of PD.

The aim of this paper is to review the clinical findings of recent studies on PD and alpha-synuclein from the perspectives of physicians. Results from animal studies or basic researches will not be covered in this review as they have been reviewed in recent literature [7].

PD AND ALPHA-SYNUCLEIN: NEUROPATHOLOGICAL ASPECTS

In terms of alpha-synuclein pathology, Lewy bodies are found in several regions in the brains of patients with PD. Pathologically, the diagnostic criteria of PD are the death of dopaminergic neurons in the substantia nigra and the presence of Lewy bodies. Apart from nigra, alpha-synuclein pathologies are observed in other brainstem nuclei, including the dorsal motor nucleus of vagus (DMX) of medulla, the locus ceruleus in the pons, and the raphe nucleus. Furthermore, alpha-synuclein pathology has been found to exist in the basal forebrain, hippocampus, insular cortex, cingulated

gyrus, temporal gyrus, frontal cortex, and other parts of the brain [8]. Most patients with alpha-synuclein pathology in the substantia nigra also have alpha-synuclein pathology in the medulla or pons. On the contrary, some cases with alpha-synuclein pathology in the medulla or pons do not have alpha-synuclein pathology in the midbrain. Based on these findings, Braak et al. proposed the gradual spread of alpha-synuclein pathology [9]. According to their study, the alpha-synuclein pathology of PD begins in the DMX of the medulla and gradually spreads rostrally. Clinical symptoms of PD are manifested as alpha-synuclein pathology reaches substantia nigra and, as the disease progresses, alpha-synuclein pathology is found in the cerebral cortex. This hypothesis has attracted considerable attention and many PD investigators have aligned themselves with this hypothesis. Currently, the theory of Braak et al. has been established as the fundamental hypothesis explaining the pathogenesis and progression of PD. However, some opposing arguments also exist. The more common alpha-synuclein pathology in the medulla or pons than in the midbrain does not necessarily imply transmission of alpha-synuclein pathology from the medulla to the midbrain. Furthermore, studies on the brain of patients with PD showed that more than 15% of brains exhibited the pattern of not following the order of Braak stage progressions. Braak et al. presented dual-hit hypothesis supporting their theory in 2007 [10], suggesting that the alpha-synuclein pathology of PD is transmitted to the midbrain through two different paths. One path is from the olfactory bulb to the temporal cortex, and the other path is the transmission to DMX through the enteric nervous system. This hypothesis explains that the pathogenesis of PD is initiated by an external factor which directly generates the alpha-synuclein pathology in the olfactory bulb through the nose, or makes contact with the gastrointestinal tract by the swallowing of olfactory mucosal discharge. Autopsy findings of patients with PD have revealed the pathological findings of alpha-synuclein in the enteric nervous system of the stomach and esophagus [11, 12]. According to the outcomes of recent studies, colorectal biopsy revealed the findings of an alpha-synuclein pathology in PD patients [13].

The most convincing evidence supporting the hypothesis that alpha-synuclein pathology is transmitted through the nervous system was simultaneously reported by two independent research teams in 2008: the brain autopsy of PD patients who received fetal midbrain transplants revealed alpha-synuclein pathology within transplanted neurons [14, 15]. While fetal midbrain transplants had been attempted as a treatment method of PD in the 1990s, in recent years, it is rarely performed due to unclear efficacy, the risk of adverse effects, and ethical problems of obtaining midbrain cells from the fetus. Autopsies were performed in patients who

received fetal midbrain transplants and died after 10 years or more. Surprisingly, transplanted cells, which have aged 10 years or slightly more than 10 years, exhibited similar pathological findings of alpha-synuclein shown in host neurons. This finding implies that the alpha-synuclein pathology is transmitted from the surrounding host neurons to the transplanted neurons, as in prion diseases [16, 17]. On the other hand, other interpretations suggested that the occurrence of alpha-synuclein pathology in transplanted neurons is not due to transmission from surrounding host neurons but to the de novo intracellular alpha-synuclein pathology within the transplanted cells exposed to the alpha-synuclein pathology-promoting microenvironment which is shared by host cells. A large number of basic studies have also been carried out, supporting the transmission of alpha-synuclein pathology in PD. Additional studies will be proactively performed to further examine the mechanism of PD based on the new findings in clinical studies.

PD AND ALPHA-SYNUCLEIN: GENETIC ASPECTS

As stated in the Introduction, many genetic studies have been conducted to clarify the relationship between alpha-synuclein and PD after the discovery of the *SNCA* gene mutations in families with inherited PD. The three missense mutations of the *SNCA* gene are A53T, A30P, and E46K. Although a recent study newly found another mutation, H50Q, additional studies are needed. Although the finding of familial PD by *SNCA* missense mutation has led to clinical studies and basic studies on the pathological role of alpha-synuclein in PD, clinical features of these patients slightly differ from those of usual PD patients. PD patients with *SNCA* missense mutation are characterized by early onset age, rapid progression of the disease, and a high association of dementia. Besides the alpha-synuclein pathology, the presence of tau pathology and TDP-43 inclusion in some patients raises questions whether these patients represent 'usual' PD patients. Nevertheless, studies on the point mutation of *SNCA* have greatly contributed in understanding the mechanism of PD [18].

More convincing genetic evidence proving the relationship between alpha-synuclein and PD is the multiplication of *SNCA* in patients with PD. Singleton et al. [19] discovered the triplication of normal (wild-type) *SNCA* in familial PD in 2003. These patients had two times more *SNCA* gene than normal individuals with three copies of *SNCA* gene on one chromosome. This implies that even normal alpha-synuclein without mutation could generate PD when it is expressed in a greater amount than normal individuals. An increase in the amount of *SNCA* mRNA and alpha-synuclein protein was identified in serum, and brain tissue of these patients

[20, 21]. Moreover, the duplication of the *SNCA* gene (1.5 times higher amount of *SNCA* gene than normal individuals) was detected in families with inherited PD in 2004. This finding confirmed that over-expression of normal alpha-synuclein causes PD [22-25]. PD with *SNCA* triplication is characterized by early onset age and rapid progression compared to usual PD. In contrast, PD with *SNCA* duplication is similar to the onset age and progression of usual PD. This suggests the gene dosage effect where the different severity of symptoms depends on the number of copies of the *SNCA* gene. This also supports the hypothesis that alpha-synuclein is causally related to PD. However, an increase in alpha-synuclein in patients without genetic abnormality (most PD is not inherited and does not have obvious genetic abnormalities), especially in brain tissues, needs to be verified in order to establish the theory in which a greater amount of alpha-synuclein formation causes PD. Still, there is no definite evidence indicating the increased alpha-synuclein or *SNCA* mRNA in the brain of patients with non-genetic PD. Some studies even reported the decrease in alpha-synuclein or *SNCA* mRNA [26, 27]. Further studies are needed to resolve the potential methodological problems in analyzing the alpha-synuclein levels.

Given that alpha-synuclein is the key molecule in the pathogenesis of PD, the presence of the alpha-synuclein pathology in the brain tissues of patients with PD by a genetic abnormality other than *SNCA* mutation needs to be scrutinized. Mutations or polymorphisms in several other genes have been identified as causing PD or affecting PD risk (Table 1). *LRRK2* mutation is recognized as the most common genetic cause of PD and G2019S mutation is the most common form of *LRRK2* mutation. Autopsy findings revealed that alpha-synuclein pathology accounts for

Table 1. Genetic etiology of Parkinson disease

Locus	Chromosome	Gene	Inheritance
PARK1	4q	<i>SNCA</i> (point mutation)	AD
PARK2	6q	<i>PARK2</i> (parkin)	AR
PARK3	2p	?	AD
PARK4	4q	<i>SNCA</i> (multiplication)	AD
PARK5	4p	<i>UCHL1</i>	?
PARK6	1p	<i>PINK1</i>	AR
PARK7	1p	<i>DJ-1</i>	AR
PARK8	12p-q	<i>LRRK2</i>	AD
PARK9	1p	<i>ATP13A2</i>	AR
PARK10	1p	?	?
PARK11	2q	<i>GIGYF2</i>	?
PARK12	Xq	?	X-linked
PARK13	2p	<i>HTRA2</i>	?
PARK14	22q	<i>PLA2G6</i>	?
PARK15	22q	<i>FBXO7</i>	AR
PARK16	1q	?	?

about 80% of all G2019S mutant patients. However, the finding of alpha-synuclein pathology was discovered in about 43% of other LRRK2 mutant patients. A total of 10 cases of autopsy findings have been reported regarding PD resulting from mutations in the parkin gene. Among those, alpha-synuclein pathology was not found in 7 cases. A single case of the autopsy finding has been reported in the case of PINK1 and this patient showed alpha-synuclein pathology. No autopsy of DJ-1 has yet been reported [28]. In summary, alpha-synuclein appears to be involved in the pathogenesis of PD in cases of PD by other genetic abnormalities. However, more studies are needed to elucidate the relationship between alpha-synuclein and other PD-related genes, especially parkin.

In recent years, many studies have attempted to clarify the relationship between PD and a certain single nucleotide polymorphism (SNP) through a genome-wide association study (GWAS). Numerous GWASs have shown that several SNPs in *SNCA* increases the risk of PD [29], and this has been supported by results of functional studies. Of these, one of the most well studied examples is the length of dinucleotide repetition in the *SNCA* promoter of REP1. A large number of clinical studies have repeatedly verified that the risk of PD increases as the repetitions of REP1 increase [30] and the functional studies have shown that the number of REP1 repetitions actually increased the expression of the *SNCA* gene. Furthermore, the amount of alpha-synuclein in the serum of patients is known to increase as the number of REP1 increases. The outcome also implies a direct relationship between alpha-synuclein and PD. The association of PD with other SNPs of *SNCA* has been identified through GWAS, and some SNPs of the 3' untranslated region (3'UTR) have increased the risk of PD. Patients with a SNP in 3'UTR of *SNCA*, rs356219, reportedly exhibited a high concentration of alpha-synuclein in serum than in patients without SNP [31].

Recent studies have presented the relationship between DNA methylation of the *SNCA* gene and PD. According to Jowaed et al. [32] and Matsumoto et al. [33], a decrease in DNA methylation status was observed in the *SNCA* promoter region of intron1 in patients with PD. Consequently, *SNCA* mRNA transcription and alpha-synuclein protein expression increased. However, no difference in the methylation status of the *SNCA* promoter was found in a study performed using DNA from white blood cells (WBC) of PD patients [34]. Therefore, additional studies are needed. A recent study suggested the association of PD and microRNA, another mechanism regulating the expression of genes. Experimental studies have demonstrated that *SNCA* expression can be regulated by manipulating a microRNA binding site in 3'UTR of the *SNCA* gene [35, 36], and interestingly a recent

study reported a variation in mir-153 binding site in 3'UTR of the *SNCA* gene in a patient with Parkinson disease [37].

PD AND ALPHA-SYNUCLEIN: BIOLOGICAL SAMPLES

As studies have verified the pathological role of alpha-synuclein in patients with PD, other studies have attempted to apply this pathogenesis in the diagnosis and prognosis of PD.

Until now, no specific diagnostic marker of PD has been identified yet. The diagnosis of PD is still determined solely by the clinical findings. Radiological tests and laboratory findings are used only as supporting tools in diagnosing PD. In these circumstances, the development of a biological marker is crucial for a more precise diagnosis and the prediction of disease before the onset of symptoms. Many studies are currently conducted to find the biomarker in this regard.

Peripheral blood is one of the most accessible biological samples. Several studies have verified an increase in alpha-synuclein in the blood of PD patients compared with normal individuals [38]. However, alpha-synuclein is mostly present in RBCs that contain more than 95% of all alpha-synuclein in the blood [39]. Thus, the alpha-synuclein level measured in the blood sample is inevitably influenced by the minimal difference in the degree of hemolysis and accordingly it is inappropriate to use the blood level of alpha-synuclein for diagnostic purposes. Increases in alpha-synuclein protein and *SNCA* mRNA in WBCs from PD patients have been reported [40]; however, others reported no difference [41]. Along with the measurement of alpha-synuclein level in the peripheral blood, studies to measure alpha-synuclein level in cerebrospinal fluid (CSF) are also being carried out. The advantage of measuring the alpha-synuclein level in CSF is that it contains no or only a small amount of RBCs; however, again the contamination by hemolysis cannot be controlled completely. Despite these flaws, recent studies have identified a decrease of alpha-synuclein in the CSF of PD patients compared with that of normal individuals [42, 43]. This outcome aligns with the finding of reduced amyloid protein levels in the CSF of patients with Alzheimer's disease and has drawn attention to the similarity of two proteins regarding the disease mechanism. In a model of neuron injury by alpha-synuclein, oligomer is considered more toxic than monomer. As a result, attempts have been made to measure the alpha-synuclein oligomer level in body fluids. Studies have shown that although the total alpha-synuclein level is decreased in the CSF of PD patients, the alpha-synuclein oligomer level was higher than that of normal individuals [44].

Some studies have attempted to diagnose PD through alpha-synuclein in other biological samples or tissue specimens. Devic

et al. [45] presented an increase of alpha-synuclein in the saliva of PD patients. In addition, an increase of alpha-synuclein has been found in minor salivary gland biopsies [46, 47], tissues from skin biopsy [48, 49], stomach biopsy [50], and colonic biopsy [51], and in fibroblasts obtained from the skin [52]. However, more studies are needed. These findings are anticipated to aid the development of a premortem biomarker crucial in the diagnosis, clarification of the mechanism, and new treatment methods of PD.

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