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Preclinical progression of neurodegenerative diseases

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ABSTRACT

Neurodegenerative diseases are disorders that are characterized by a progressive decline of the motor and/or cognitive function caused by a selective loss of neurons within the central nervous system. Recent advancements in the translational research have facilitated extensive insights into the molecular pathophysiology of neurodegenerative diseases. Nonetheless, a myriad of compounds that suppressed the disease progression in cellular and animal models did not exhibit efficacy in clinical trials. Perhaps, various biological, medical, and methodological factors could be attributed to unfavorable results of clinical trials of such disease-modifying therapies. Primarily, the fact that pathological changes at molecular and cellular levels precede the clinical onset by several years underscores a pressing need for the initiation of interventions before the emergence of neurological symptoms. Using exquisite biomarkers, recent studies revealed the preclinical and prodromal progression of pathophysiology, as well as compensatory brain responses in several neurodegenerative diseases. This review aims to discuss the recent advancement of biomarker studies on presymptomatic subjects and the perspective on a preventive trial of disease-modifying therapies for devastating neurological disorders.

Keywords: preclinical, prodromal, neurodegenerative disease, biomarker, preventive therapy, disease-modifying therapy

INTRODUCTION

Neurodegenerative diseases are disorders characterized by progressive impairment of the motor and/or cognitive function that are often accompanied by psychiatric disorders, including various intractable diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS) among others, for which no curable treatment has been established.[1,2)] Typically, neurological symptoms first appear after the middle age and exacerbate throughout the disease course. Histopathologically, these diseases share salient features, including the deposition of insoluble proteins, activation of glial cells, and neuronal cell loss. While most neurodegenerative diseases are sporadic, some are familial forms that exhibit Mendelian inheritance with a mutation in a gene encoding a protein that either directly or indirectly facilitates the aggregate formation. The next-generation sequencing technique revealed

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such gene mutations in patients without family history.[3] Previously, several experimental studies reported that the induction of these gene mutations caused neuronal dysfunction accompanied by mitochondrial dysfunction, axonal transport blockade, disruption of the protein quality control system, and transcriptional dysregulation in animals. These findings collectively indicate that the abnormal protein aggregation, along with consequent molecular events, underlie the progressive, selective loss of neurons and that the inhibition of protein accumulation could suppress the disease progression. Based on these hypotheses, numerous therapies targeting the molecular pathomechanism of neurodegeneration have been proposed. Although some of these agents have been shown to inhibit neuronal damage and behavioral deficits in animal models, their efficacy remains scarcely demonstrated in confirmatory clinical trials in humans.[1] The failure of the translational research on disease-modifying therapies for neurodegenerative diseases suggests several biological factors that attenuate the beneficial effects of compounds, including insidious progression of pathology, complexity in neuronal cell death pathways, intricate neuron–glial interaction, impairment of neural network, and effect of aging, as well as safety and delivery issues of compounds. Specifically, the preclinical progression of disease has garnered considerable attention as a major obstacle that hinders the development of disease-modifying therapies; this view is supported by observations that the neuron loss is apparent at the onset of neurological symptoms and that neuronal dysfunction and abnormal protein deposited in the brain are already detectable in asymptomatic carriers of a gene mutation of familial neurodegenerative disease. Hence, the prevention of such preclinical progression of neurodegeneration plays a vital role in the successful translation of interventional studies using animal and cellular models. This study offers an overview of recent advancements in research on the preclinical progression of neurodegeneration and discusses the perspectives on the translational research to cope with debilitating disorders (Table 1).

PRECLINICAL PROGRESSION OF AD

At present, over 45 million people are estimated to have dementia, and this number is witnessing continuous increase worldwide, except some European countries where a decline has been reported recently.[4] AD is the leading cause of dementia, which is characterized by the progressive memory loss and a decline in cognitive functions, as well as behavioral and psychological symptoms, including agitation, depression, anxiety, and delusions. Although most cases are el derly-onset, approximately 10% of patients manifest dementia aged <65 years. Patients with early-onset AD are often familial, with a suggestive autosomal-dominant inheritance.[5] Histopathological hallmarks of AD comprise extracellular amyloid plaques, primarily composed of Aβ peptide, and intracellular neurofibrillary tangles, both of which can be detected by positron emission computed tomography (PET) and the cerebrospinal fluid (CSF).[6] Aβ peptides are created through the proteolytic cleavage of the amyloid precursor protein (APP); this process likely instigates the intraneuronal accumulation of phosphorylated tau, which is the primary component of neurofibrillary tangles.

The Aβ deposition is detected with PET and/or CSF even in early cases of AD and in subjects with mild cognitive impairment, with preceding the detection of tau in the CSF, indicating that the Aβ accumulation is an upstream biological change in the AD pathogenesis.[7] This hypothesis is extensively endorsed by the fact that all known causative genes in hereditary AD, *APP* and *presenilins 1 and 2* (*PSEN1* and *PSEN2*), encode proteins involved in the Aβ generation pathway. A comprehensive study on dominantly inherited AD provided crucial insights into the preclinical progression of AD by elucidating that the Aβ deposition emerges >20 years before the onset

Table 1 Preclinical markers of neurodegenerative diseases

Disease	Major symptoms	Preclinical biomarkers	Ref.
Alzheimer's disease	Cognitive impairment	PET (Aβ, tau) CSF (Aβ, tau) Cognitive composite	8–11
Parkinson's disease	Parkinsonism Cognitive impairment	DaT SPECT Transcranial sonography Polysomnography (detection of RBD)	24–26
Polyglutamine diseases			
Huntington's disease	Chorea Cognitive impairment	Symbol Digital Modality Test MRI (striatal volume)	30, 31
Spinocerebellar ataxias	Ataxia	Scale for the assessment and rating of ataxia MRI (brainstem volume)	35–37
Spinal and bulbar muscular atrophy	Muscle weakness	Serum creatinine	41, 42
Amyotrophic lateral sclerosis	Muscle weakness	CSF (dipeptide repeat proteins)* Electrophysiology (cortical hyperexcitability and motor unit number estimate)	52, 56, 57

PET, positron emission computer tomography; CSF, cerebrospinal fluid; DaT SPECT, Dopamine transporter single photon emission computed tomography (DaT SPECT; RBD, rapid eye movement sleep behavior disorder. *This marker is applicable for C9orf72-linked familial ALS.

of dementia.[8] This assumption is primarily dependent on substantial correlations between the individual age at the symptom onset and predicted values based on the parental age at the onset and mean ages at the onset by the mutation type and family.[9] Notably, similar biomarker changes and a subtle cognitive decline before the onset of dementia have also been reported in sporadic AD cases.[10,11] Overall, these studies revealed the preclinical progression of AD, which is characterized by the early deposition of extracellular Aβ followed by the intracellular accumulation of tau in both hereditary and sporadic AD. Reportedly, this view seems to underlie the lack of efficacy of disease-modifying therapies targeting Aβ and tau in clinical trials of AD.[12] Thus, preclinical biomarker changes of AD underscore the necessity for the preclinical prevention of AD pathogenesis before dementia manifests, and offer a theoretical basis for a preventive clinical trial of anti-amyloid therapies for AD.[13] Hence, it should be noted that increased regional brain activity is reported in mutation carriers of *PSEN1*, possibly suggesting a compensatory response against neural dysfunction resulting from the early Aβ pathology.[14,15]

PRECLINICAL PROGRESSION OF PD

PD is a common, age-related neurodegenerative disorder, which affects over 1% of the population aged >60 years.[16] Clinically, PD is characterized by rigidity, bradykinesia, resting tremor,

and postural instability, resulting from the degeneration of dopaminergic and non-dopaminergic neurons. At the onset of motor symptoms, >50% of dopaminergic neurons are lost, signifying that neurodegeneration precedes the symptom manifestation.[17] During the entire disease course, 30%–40% patients develop dementia, the symptoms of which comprise visual hallucination substantially overlapped by dementia with Lewy bodies (DLB).[18] A histopathological hallmark of PD is the presence of Lewy bodies, the primary component of which is phosphorylated α-synuclein, in affected dopaminergic neurons within the substantia nigra pars compacta. In addition, the deposition of α-synuclein is reported in affected cortical neurons in PD with dementia and DLB.[19] The fact that mutations or multiplications of the gene encoding α-synuclein cause the early-onset, autosomal-dominant PD underscores the implication of this protein in the central pathogenesis of the dopaminergic neuron loss. A recent experimental study suggested the interneuronal propagation of misfolded α-synuclein, providing a possible clarification for the histopathological spread of Lewy bodies as well as symptomatic progression.[20]

Besides motor impairment, several forms of non-motor symptoms occur in most patients with PD. An epidemiological study demonstrated that certain non-motor symptoms anteceded the onset of motor dysfunction, indicating an extended prodromal period in PD.[21] For example, autonomic impairment, such as constipation, develops 15–20 years before the onset.[22] Besides, α-synuclein deposition often presents in the colon mucosa of presymptomatic patients with PD, signifying that the peripheral pathology might expand to the central nervous system during the prodromal phase of this disease.[23] In addition, REM sleep behavior disorder (RBD) precedes the motor symptom onset by several years in patients with PD. In fact, most patients with RBD later develop PD, DLB, or multiple system atrophy (MSA), all of which are histopathologically-associated with the α-synclein pathology, suggesting that the protein accumulation underlies the pathogenesis of RBD.[24] Moreover, hyposmia, excessive daytime sleepiness, and depression could herald motor symptoms. Consistent with AD, imaging marker plays a crucial role in elucidating the preclinical progression of PD. Dopamine transporter single-photon emission computed tomography (DaT SPECT) determines presynaptic dopaminergic neuronal dysfunction in not only symptomatic patients but also presymptomatic carriers of a mutation in the *leucine-rich repeat kinase 2 (LRRK2)* gene, a leading cause of familial PD.[25,26] Likewise, pathologic substantia nigra hyperechogenicity is also detectable in *LRRK2* mutation carriers, portentous the dopaminergic degeneration at a preclinical stage of PD.[25] Alternatively, asymptomatic carriers of a mutation in *Parkin*, another causative gene of familial PD, exhibit increased activity in the ventrolateral premotor cortex during execution and perception of affective facial gestures than healthy controls, indicating a compensatory response against neural dysfunction.[27] A failure of such compensation results in the manifestation of neurological symptoms.

PRECLINICAL PROGRESSION OF POLYGLUTAMINE DISEASES

Polyglutamine diseases are a group of hereditary neurodegenerative disorders caused by an abnormal elongation of a trinucleotide CAG repeat, which encodes a polyglutamine tract, in causative genes.[28] Polyglutamine diseases include HD, spinal and bulbar muscular atrophy (SBMA), dentatorubral-pallidoluysian atrophy (DRPLA), and six forms of spinocerebellar ataxia (SCA). The diseases are inherited in an autosomal-dominant fashion except for SBMA, which presents an X-linked pattern of inheritance. Reportedly, abnormal proteins with expanded polyglutamine accumulate in neuronal and non-neuronal cells, leading to slowly progressive neurodegeneration.[28]

The fact that polyglutamine diseases are single-gene disorders facilitates the preclinical diagnosis with a genetic test. In addition, the robust correlation between the CAG repeat size

and the onset age could be used to estimate the disease onset in preclinical mutation carriers of polyglutamine diseases.[29] Hence, several studies have reported neuropsychiatric and imaging deficits during a preclinical phase of HD, the leading polyglutamine disease caused by the CAG repeat expansion in the gene-coding huntingtin (*HTT*). In addition, two large cohorts of prodromal HD, TRACK-HD and PREDICT-HD, reported subclinical motor and cognitive dysfunction, as well as a decreased volume of caudate and putamen in preclinical *HTT* gene mutation carriers.[30,31] Besides, subtle cognitive and motor deficits, especially measured with the Symbol Digit Modality Test, occur approximately 10 years before the onset when brain glucose hypometabolism and striatal atrophy emerge. Moreover, markers of neuroinflammation, including serum interleukin-6 levels, begin to alter around 15 years before the clinical diagnosis.[32] In fact, these observations are used for conducting clinical trials of early/preventive intervention HD.[33] Besides neurodegenerative changes, a compensatory reaction has been reported in early HD. For instance, reward-related fMRI signaling is enhanced in the ventral striatum, orbitofrontal cortex, and anterior insula of the *HTT* carriers whose behavioral performance is preserved, indicating that the increased activity in specific brain areas copes with the mutant huntingtin-mediated neural dysfunction.[34]

Regarding SCA, a multicenter longitudinal study reported mild ataxia and gray matter atrophy in the brainstem and cerebellum in mutation carriers of SCA1 and SCA2.[35] In addition, delayed central motor conduction time and reduced sensory action potentials have been confirmed in the prodromal phase of SCA2.[36,37] As demonstrated in other neurodegenerative diseases, asymptomatic mutation carriers of *ataxin-3*, the causative gene of Machado–Joseph disease (MJD or SCA3), exhibit enhanced glucose metabolism in the parietal and temporal cortices, besides decreased glucose utilization in the cerebellum and brainstem, suggesting defense mechanism against neurodegeneration in preclinical MJD.[38]

SBMA is a late-onset neuromuscular disorder attributed to the expansion of a CAG repeat in the gene-coding androgen receptor (AR) and exclusively affects males.[39] Patients with SBMA are characterized by declined serum levels of creatine, which results from both neurogenic muscle atrophy and impaired muscular uptake of creatine.[40] In addition, serum creatinine levels correlate with scores of the motor function and start declining >10 years before the onset of symptoms, including muscle weakness, signifying the preclinical progression of SBMA.[41,42] The electrophysiological evaluation portrays signs of chronic denervation and decreased sensory action potentials in female carriers of the CAG expansion in AR, although they do not exhibit the overt motor phenotype.[43] Overall, these observations imply that the pathogenic AR-induced motor neuron degeneration is likely compensated in preclinical male SBMA subjects and asymptomatic female carriers of the AR mutation.

PRECLINICAL PROGRESSION OF ALS

ALS, or Lou Gehrig's disease, is a fatal neurodegenerative disease affecting both upper and lower motor neurons.[44] Although ALS is characterized by the progressive muscle weakness and wasting throughout the body, bladder and bowel sphincters, and the external ocular muscles are typically spared even at an advanced stage. The ALS prognosis is extremely poor—death due to respiratory failure and/or bulbar palsy typically occurs 3–5 years from the onset of weakness.[45] Although most ALS cases are sporadic, 5%–10% of patients have a positive family history. The pivotal histopathological finding of sporadic ALS is the loss of motor neurons in the primary motor cortex and anterior horn of the spinal cord, as well as aggregates of the 43-kDa TAR DNA-binding protein (TDP-43) in residual neurons.[46,47] In addition, point mutations in the

glycine-rich domain of the gene encoding TDP-43 have been determined as disease-causing mutations of familial and sporadic ALS, signifying a pathogenic role of TDP-43 in ALS.[48] Over 30% of sporadic patients with ALS develop frontotemporal dementia during the disease progression, whereas subjects with frontotemporal lobar degeneration (FTLD), accompanied by TDP-43 pathology, often develop motor neuron disease, further suggesting a strong molecular and pathological correlation between ALS and FTLD.[49]

An extensive GGGGCC hexanucleotide repeat expansion in the first intron of the *C9orf72* gene is a leading genetic cause of ALS in Caucasians, accounting for 30%–40% of familial cases and 5% patients with sporadic ALS in Europe, although this mutation is far less common in Asia.[50] The *C9orf72* gene mutation causes FTLD and pathologically results in the intraneuronal aggregation of TDP-43, as well as sporadic ALS cases. A pathological hallmark of *C9orf72*-related ALS/FTLD is the aggregation of five dipeptide repeat proteins (DPRs) in affected neurons, resulting from repeat associated non-ATG (RAN) translation, and DPRs have been reported to accumulate in the brain before TDP-43 pathology.[51] The poly-GP, which is one of the DPRs, is detectable in the CSF of asymptomatic *C9orf72* mutation carriers, signifying a preclinical molecular change in this disease.[52] In addition, alteration of miRNA expression profiles in the CSF has also been reported in mutation carriers of *C9orf72*.[53] An extensive study on preclinical subjects with FTLD associated with *C9orf72*, *progranulin (GRN)*, or *microtubule-associated protein tau (MAPT)*, reported a decreased volume of insula 10 years before the estimated onset of dementia, along with the emergence of mild cognitive impairment around 5 years before the clinical onset.[54]

Another major causative gene of familial ALS is *copper/zinc superoxide dismutase (SOD1)* on chromosome 21, which accounts for 10%–20% of autosomal-dominant patients, both in Asia and Europe.[55] An electrophysiological study reported that the short-interval intracortical inhibition (SICI) is decreased in asymptomatic *SOD1* mutation carriers, indicating that cortical hyperexcitability precedes the onset of motor symptoms in SOD1-linked familial ALS.[56] Furthermore, the decreased motor unit number estimate, another electrophysiological marker of the motor neuron degeneration, is detectable in asymptomatic carriers of *SOD1* mutation.[57] Although little information is available about preclinical biomarker changes in sporadic ALS, an epidemiological study suggested altered profiles of lipid metabolism in the asymptomatic phase of sporadic ALS.[58]

SUMMARY

Lately, the preclinical phase has been garnering considerable attention as a therapeutic window of neurodegenerative diseases. Early changes in the neurological function, such as cognition and movement, imaging, and electrophysiology exist in preclinical carriers of a causative gene mutation, as well as in asymptomatic subjects who are most likely to develop a sporadic neurodegenerative disease later. Thus, the onset of biological events is considerably earlier than that of primary clinical symptoms. In particular, the brain activity in specific regions is upregulated in preclinical subjects of various neurodegenerative diseases, indicating that compensatory neuronal reactions maintain the neural function at early stages of the disease, and that symptoms manifest when such compensation collapses (Fig. 1). Hence, these views are of utmost importance for developing preventive therapies, which is essential for the successful inhibition of the disease progression in every neurodegenerative disorder.

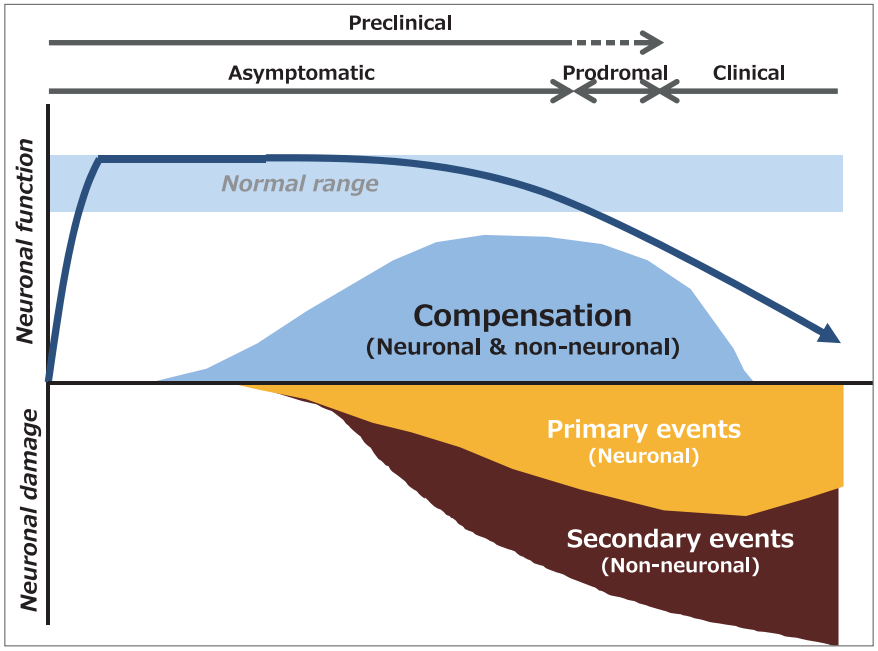

Fig. 1 Time course of neurodegeneration
Accumulation of abnormal proteins instigates primary of molecular changes in neurons followed by secondary events such as glial alteration and neuroinflammation. Compensatory responses cope with neural dysfunction and maintain neurological function at an early stage, but breakdown of such defense machinery leads to manifestation of clinical symptoms. The definition of "preclinical" has not been fully established. It includes prodromal phase, when patients develop mild, auxiliary symptoms, in some studies, while other researches define preclinical as being free from any symptoms. In this review, we adopted the former definition.

COMPETING INTERESTS

The authors have no conflicts of interest.

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