Table 1 Clinical profiles of patients with sporadic Creutzfeldt–Jakob disease

No	Age/sex	Diagnosis	Codon129/PrP^{Sc}	14-3-3/total τ	RT-QUIC	Pre-MRI duration (months)
1	69/M	Definite	MM/1	+/+	+	−2*
2	77/F	Definite	MM/1	+/+	+	19
3	75/F	Definite	ND/1+2	+/+	+	3
4	65/M	Definite	MM/2	−/−	−	12
5	69/M	Probable	MM	ND	−	0.5
6	72/F	Probable	MM	ND	ND	0.5
7	77/F	Probable	MM	−/−	+	0.5
8	72/M	Probable	MM	ND	ND	1
9	63/M	Probable	MM	+/+	+	1.5
10	88/F	Probable	MM	ND	ND	1.5
11	75/M	Probable	MV	ND	ND	1.5
12	56/M	Probable	MM	+/+	+	2
13	67/M	Probable	MM	+/+	−	2
14	70/M	Probable	MM	+/+	+	2
15	70/F	Probable	MM	+/+	+	2
16	74/F	Probable	MM	+/+	−	2
17	84/F	Probable	MM	+/−	+	2
18	85/F	Probable	MM	−/+	+	2
19	49/F	Probable	ND	+/+	+	2
20	74/F	Probable	MV	+/+	+	2.5
21	54/F	Probable	ND	ND	ND	2.5
22	61/M	Probable	MM	ND	ND	3
23	72/F	Probable	MM	+/−	−	3
24	81/F	Probable	MM	−/−	+	3
25	70/M	Probable	MM	+/+	+	6
26	83/F	Probable	MM	+/+	ND	9
27	67/F	Probable	MM	+/+	−	15
28	84/F	Probable	MM	+/+	−	26
29	57/F	Possible	MM	ND	ND	4

*MRI was obtained 2 months before the symptom onset.[2]
MM, homozygous for methionine; MV, heterozygous with methionine and valine; ND, not done; RT-QUIC, real-time quaking-induced conversion.

physicians who can refer only to hardcopies but not softcopies. Second, even for doctors who can readily refer to softcopies and thus variable DWI, the standardisation method can simplify assessment procedure without any disadvantages. Third, the standardisation can facilitate direct comparison of DWI findings from different CJD patients.

DWI and FLAIR have been reported as useful markers for the diagnosis of CJD. Of these, DWI has been assumed to be the most sensitive, although without direct evidence.[1 5 18] Hyperintensity in the cerebral cortices, the striatum or both indicates the diagnosis of CJD. The striatum hyperintensity is anterior dominant at early stages of the disease.[19] MRI lesion profiles reportedly differ among molecular subtypes of sCJD,[20 21] which was not reproduced in a recent study.[5] Zerr *et al*[4] proposed that high-signal abnormalities in caudate nucleus and putamen or at least two cortical regions (temporal, parietal or occipital lobes) either in DWI or FLAIR together with typical clinical signs can be diagnostic for probable sCJD. Based partly upon their report, 'high signal in caudate/putamen on MRI brain scan' has

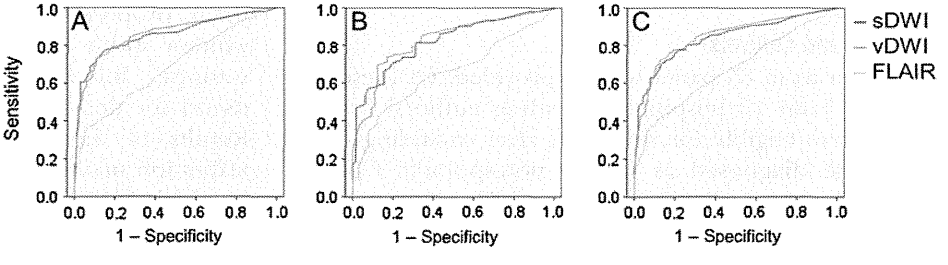

Figure 1 Receiver operating characteristic curves for each display in diagnosis of sporadic Creutzfeldt–Jakob disease. (A) Neurologists, (B) radiologists and (C) all observers. The true rate (sensitivity) is plotted as a function of the false-positive rate (1 − specificity). DWI, diffusion-weighted imaging; FLAIR, fluid-attenuated inversion recovery; sDWI, standardised DWI; vDWI, variable DWI.

Table 2 Areas under the receiver operating characteristic curves

	Neurologists	Radiologists	All observers
sDWI	0.86 (0.82 to 0.90)	0.82 (0.77 to 0.88)	0.84 (0.81 to 0.87)
vDWI	0.86 (0.82 to 0.90)	0.83 (0.77 to 0.89)	0.85 (0.82 to 0.88)
FLAIR	0.69 (0.63 to 0.75)	0.66 (0.58 to 0.73)	0.68 (0.63 to 0.72)

Means (95% CIs) are indicated.
DWI, diffusion-weighted imaging; FLAIR, fluid-attenuated inversion recovery; sDWI, standardised DWI; vDWI, variable DWI.

been used as one of the laboratory findings in the diagnostic criteria for probable sCJD in the European CJD Surveillance System (EUROCJD) since January 2010.[22] However, their criteria did not distinguish DWI and FLAIR, thereby maintaining ambiguity about the diagnostic values of MRI in situations where DWI is not available. Our data indicate that FLAIR without DWI is unreliable for the diagnosis of sCJD. On the other hand, high signals in the cerebral cortex have not been regarded as diagnostic in the EUROCJD criteria, probably because cortical abnormalities are less reliable on conventional MRI. Our results suggest that, using standardised or variable DWI but not FLAIR, cortical signals can also be used as a diagnostic marker.

Meanwhile, Young *et al*[23] reported that the sensitivity and the specificity of DWI and FLAIR for the diagnosis of CJD are 91% and 95%, respectively. More recently, Vitali *et al*[5] reported that hyperintensity greater on DWI than FLAIR is diagnostic for sCJD, whereas hyperintensity greater on FLAIR than DWI is characteristic for non-prion rapidly progressive dementia. Furthermore, reduction of apparent diffusion coefficient in subcortical (striatum) hyperintensity regions on DWI is supportive for sCJD.[5 24 25] These findings can be greatly helpful for differentiating sCJD from other rapidly progressive dementia. However, assessment of FLAIR lesions tends to vary among physicians, particularly among neurologists, as shown by the present study, and standardised methods for FLAIR or apparent diffusion coefficient map have not been established until date. Therefore, clinical criteria which require DWI but not necessarily FLAIR or apparent diffusion coefficient will be more readily applicable.

As many as 13 neurologists and radiologists from different institutions participated in the observer performance study, although the sample size of patients was relatively small. Notably, the observers had various specialty backgrounds such as stroke neurologists, neurophysiologists, experts in dementia or prion disease and general and neuroradiologists. This variety simulates practical situations in which the diagnosis of suspected CJD cases may be made by physicians who do not necessarily specialise in prion disease.

This study has some limitations. First, we did not evaluate patterns of cortical involvement suggestive of sCJD[4 5] because we had to address whether DWI or FLAIR is suitable for detecting cortical lesions in the first place. Second, we did not assess the difference among sCJD subtypes[21] because majority of our cases had a typical phenotype and were homozygous for methionine; thus, they were compatible with MM1 sCJD. Until date, MM2 thalamic-type sCJD remains a diagnostic challenge in MRI-based assessment; thalamic hypoperfusion or hypometabolism on SPECT or PET can be useful.[26] Third, majority of the control patients were not those who were suspected to have CJD. However, the

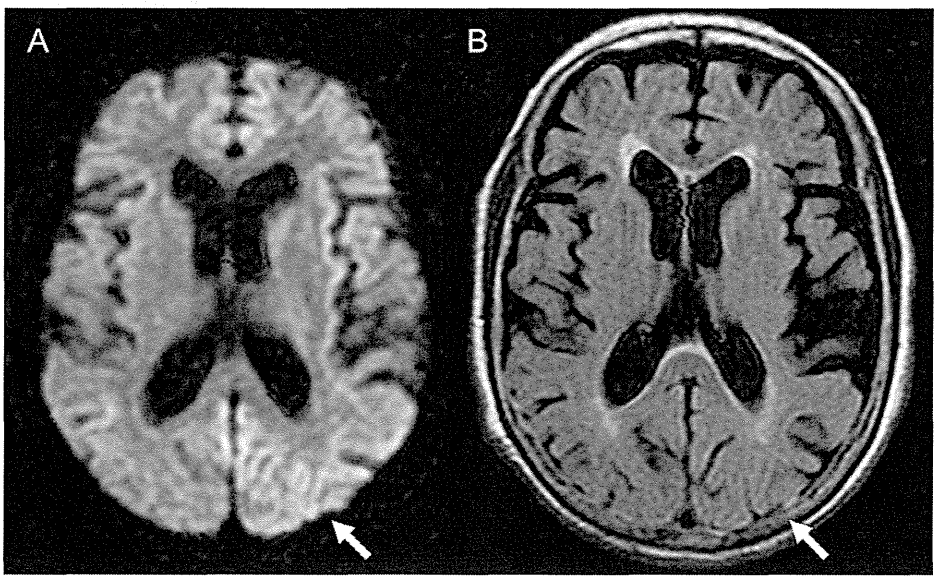

Figure 2 Representative MRI of a sporadic Creutzfeldt–Jakob disease patient (case 17). Abnormal hyperintensity in the cerebral cortex is evident on standardised diffusion-weighted imaging (A, arrow) but obscure on fluid-attenuated inversion recovery (B, arrow).

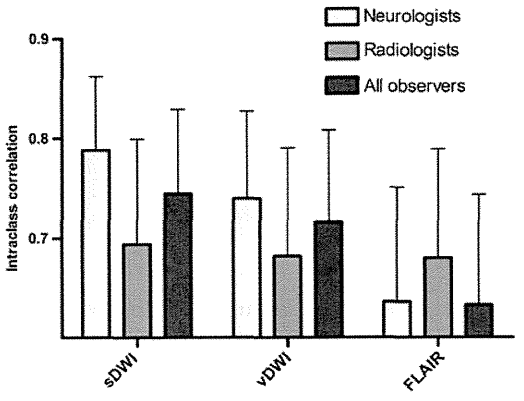

Figure 3 Intraclass correlations for each display. Error bars represent upper limits of 95% CIs. DWI, diffusion-weighted imaging; FLAIR, fluid-attenuated inversion recovery; sDWI, standardised DWI; vDWI, variable DWI.

principle aim of the present study was to establish a display method, which reliably distinguishes potentially CJD-associated signals from normal signals. Thus, our results provide a practical foundation for utilising DWI as a general diagnostic marker of sCJD when combined with previous findings.[1 4 5]

Although neuropathological confirmation of the diagnosis of sCJD was obtained in few cases, we performed RT-QUIC, a newly established CSF PrP^{Sc} amplification assay which achieved >80% sensitivity and 100% specificity for CJD.[16] Overall, 15 of 29 cases (51.7%) were pathologically proven or confirmed by RT-QUIC to have CJD. There were no significant differences in MRI findings between sCJD patients with and without positive results of CSF 14-3-3 protein, total τ protein or RT-QUIC. It will be important to further evaluate accurate diagnostic ability (sensitivity and specificity) of DWI in a prospective cohort of suspected CJD patients, that is, consecutive patients registered to the CJD surveillance who will also undergo CSF confirmation tests or neuropathological analyses.

In conclusion, we suggest that hyperintensity in the cerebral cortex or striatum assessed on the standardised or variable DWI scanned with 1.5-Tesla machines can be a reliable first-line on-site diagnostic marker for sCJD.

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Competing interests None.

Patient consent Obtained.

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Contributors KF, MH, MS, TY, KSak, TH, NS, YS, KSat, SS, MY and HM: design/conceptualisation of the study. MH, KSak, TH, NS, YS, KSat, RA, KN, TM, SM and YI: acquisition of data. KF, MH, MS, RA, RK, MY and HM: analysis/interpretation of the data. MH: statistical analyses. KF, MH, MS, TY, KSak, TH, NS, YS, KSat, RA, SS, KN, TM, SM, YI, RK, MY and HM: drafting/revising the manuscript. All authors contributed to final approval of the version to be published.

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Translational Neurodegeneration

REVIEW Open Access

Neurodegenerative changes initiated by presynaptic dysfunction

Toru Yasuda[1,2], Yasuto Nakata[1], Chi-Jing Choong[1] and Hideki Mochizuki[1*]

Abstract

α-Synucleinopathies are a subgroup of neurodegenerative diseases including dementia with Lewy bodies (DLB) and Parkinson's disease (PD). Pathologically, these disorders can be characterized by the presence of intraneuronal aggregates composed mainly of α-synuclein (αSyn), which are called Lewy bodies and Lewy neurites. Recent report showed that more than 90% of αSyn aggregates are present in the form of very small deposits in presynaptic terminals of the affected neurons in DLB. However, the mechanisms responsible for presynaptic accumulation of abnormal αSyn remain unclear. In this article, we review recent findings on the involvement of presynaptic dysfunction in the initiation of neuronal dysfunctional changes. This review highlights that the presynaptic failure can be a potential trigger of the dying-back neuronal death in neurodegenerative diseases.

Introduction

Neurodegenerative diseases are age-associated and progressive disorders, which detrimentally affect patients' quality of life. Medical remedies that can fully cure the diseases are currently unavailable and invention of novel therapeutic applications is urgently required. Accordingly, it is important to identify the initial trigger(s) of the pathophysiological alterations in these diseases.

α-Synucleinopathies are a subgroup of neurodegenerative diseases including dementia with Lewy bodies (DLB), Parkinson's disease (PD), and multiple system atrophy (MSA). Pathological hallmark of these disorders is the formation of intracellular aggregates composed mainly of α-synuclein (αSyn), which are called Lewy bodies and Lewy neurites [1-3]. Pathological examination of DLB patients has identified the presence of abnormal α-synuclein (αSyn) aggregates in the presynaptic terminals [4-6]. However, the mechanisms responsible for presynaptic accumulation of abnormal αSyn remain elusive.

Role of αSyn in SNARE formation

αSyn is abundantly localized in the presynaptic nerve terminals [7,8]. The physiological functions of αSyn have yet to be defined, while several lines of evidence implicated this protein in the modulation of neurotransmitter release through the regulation of soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex formation [9-11] and size of synaptic vesicle pool [12-15]. Vesicle-associated membrane protein-2 (VAMP-2) present in the synaptic vesicles, and syntaxin and synaptosomal-associated protein of 25 KDa (SNAP-25) in the presynaptic plasma membrane form the core SNARE complex, which regulate docking and fusion of synaptic vesicles to the presynaptic membrane [16]. A recent study showed the physical interaction of αSyn with VAMP-2 promotes SNARE assembly [10]. Cysteine-string protein-α (CSPα) also participates in SNARE assembly and mutant mice lacking CSPα displayed impaired SNARE formation and premature death, but both of these phenotypes are counteracted by transgenic expression of αSyn [9,17]. On the other hand, overexpression of αSyn with no overt toxicity inhibits neurotransmitter release, due to a defective reclustering of synaptic vesicles after endocytosis [15]. Additionally, overexpressed αSyn indirectly inhibits SNARE-mediated exocytosis by sequestering arachidonic acid, which upregulates syntaxin and enhances its engagement with SNARE complex [11]. Importantly, abnormal redistribution of SNARE proteins has been observed in human PD patients and mice overexpressing a truncated form of human αSyn, which showed decreased release of dopamine (DA) in the striatum [18]. Therefore, presynaptic SNARE dysfunction is considered an initial pathogenic event in α-synucleinopathies.

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Accumulation of α-synuclein triggered by presynaptic dysfunction

In our recent study, we investigated the effects of SNARE dysfunction on endogenous αSyn using *Snap25*$^{S187A/S187A}$ mutant mice [19]. These mice have homozygous knock-in gene encoding unphosphorylatable S187A-substituted SNAP-25. *Snap25*$^{S187A/S187A}$ mutant mice present a concomitant reduction of neurotransmitter release, including serotonin and DA, from the amygdala, and develop convulsive seizures and anxiety-related behavior in general activity and light-and-dark preference tests [20]. We found that the mutant mice displayed a significant age-dependent change in the distribution of αSyn and its Ser^{129}-phosphorylated form in abnormally hypertrophied glutamatergic nerve terminals in the striatum. Electron microscopic analysis revealed the atypically condensed synaptic vesicles with concomitant mislocalization of αSyn protein to the periactive zone in the glutamatergic nerve terminals (Figure 1). However, the *Snap25*$^{S187A/S187A}$ mutant mice harbored no abnormalities in the nigrostriatal dopaminergic neurons [19]. Our results suggest that SNARE dysfunction is the initial trigger of mislocalization and accumulation of αSyn, and probably underlies the pathomechanism of α-synucleinopathies.

Effect of SNAP-25 dysfunction

Previous studies using neural preparations showed that the neurotransmitter release is regulated by protein kinase C, which phosphorylates Ser^{187} residue in SNAP-25, augmenting exocytosis of synaptic vesicles [21,22]. Patch-clamp analysis showed chromaffin cells that overexpressed the S187A mutant form of SNAP-25 had impaired rate of presynaptic vesicle pool refilling [23]. Recently, we reported that *Snap25*$^{S187A/S187A}$ mice showed reduced DA and serotonin release in amygdala [20]. In human DLB brains, more than 90% of αSyn aggregates are located in the presynaptic terminals in the form of small deposits [4-6]. This is consistent with our findings of abnormal accumulation of αSyn in presynapses [19], suggesting that this process is the initial pathological event in DLB, eventually leading to the death and degeneration of neuronal cells [24]. Another finding that lends support to the role of αSyn aggregates in the presynaptic terminals in DLB is the lack of histopathological changes in the dopaminergic terminals in our study [19].

Role of αSyn in nerve terminals

In experiments on glutamate release conducted in hippocampal slices prepared from αSyn knockout mice [25], paired-pulse facilitation was significantly weaker, and high-frequency-induced long-term potentiation and frequency facilitation were not observed. These findings suggest that αSyn contributes to mobilization of glutamate-containing vesicles from the reserve pool [25]. αSyn may act as a positive regulator of neurotransmitter release at presynaptic terminals. Therefore, presynaptic accumulation of αSyn observed in our *Snap25*$^{S187A/S187A}$ mice might reflect a compensatory response to a possible SNARE dysfunction-related chronic shortage of neurotransmitter release in the VGLUT1-positive nerve terminals [19].

Relation between glutamatergic and dopaminergic nerve terminals in the striatum

In the striatum, the medium spiny neurons, which constitute more than 90% of all striatal neurons, receive

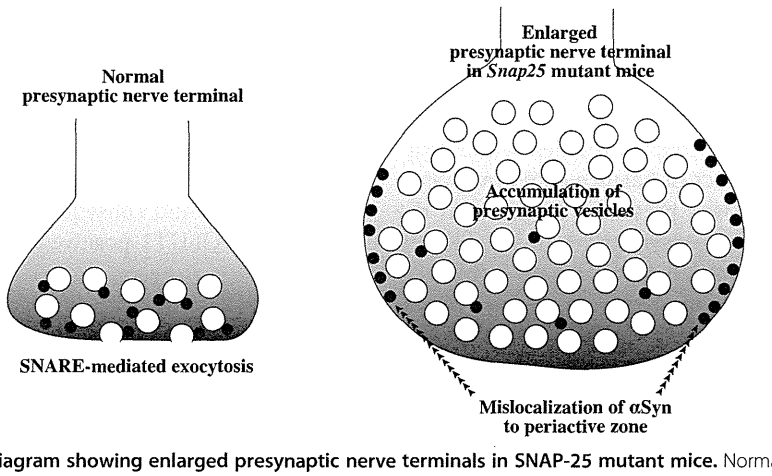

Figure 1 A hypothetical diagram showing enlarged presynaptic nerve terminals in SNAP-25 mutant mice. Normal presynaptic nerve terminals with uniform distribution of vesicles were observed in wild type mice while abnormally enlarged presynaptic nerve terminals with condensed synaptic vesicles and predominant localization of αSyn proteins in the periactive zones were found in SNAP-25 mutant mice, suggesting that SNARE dysfunction leads to presynaptic accumulation of endogenous αSyn and perturbations to the finely-tuned balance between exocytosis and endocytosis.

input from glutamatergic axons that contact the spine head and dopaminergic axons that synapse with the dendritic spine neck. DA released from dopaminergic axons regulates the release of glutamate via D_2-like receptors on the corticostriatal nerve terminals [26,27]. We found no significant changes in the striatal tissue levels of DA and its metabolites in *Snap25*$^{S187A/S187A}$ mice. These findings confirmed the results reported in our previous study using the same mouse model, in which the microdialysis analysis revealed marked reduction of DA release from the amygdala [20]. In another *in vitro* study using PC12 cells, phosphorylation of SNAP-25 at S187 potentiated calcium-dependent DA release and recruitment of synaptic vesicles containing DA [28-30]. These observations suggest decreased striatal DA release in *Snap25*$^{S187A/S187A}$ mice, resulting in increased demand for neurotransmitter release at glutamatergic nerve terminals. Thus, presynaptic accumulation of αSyn might reflect a possible compensatory response to low DA inhibitory control over cortical glutamatergic drive.

Correlation of VAMP-2 with αSyn

Increased expression of VAMP-2 protein accompanied increased αSyn expression in the striatum of *Snap25*$^{S187A/S187A}$ mice [19]. Binding of the carboxy terminus of αSyn to the amino terminus of VAMP-2 primes subsequent SNARE complex assembly [31]. Therefore, the increased VAMP-2 level might also reflect a compensatory response to the impaired synaptic vesicle release by enhancing SNARE complex formation in concert with increased αSyn.

Pathological changes in glutamatergic nerve terminals

Presynaptic neurotransmitter release is mediated by the synaptic vesicle cycle, consisting of exocytosis followed by endocytosis and recycling. Exocytosis incorporates synaptic vesicles into the presynaptic terminal membranes and increases the surface area, while endocytosis retrieves excess plasma membrane components followed by recycling to form other synaptic vesicles. Under normal conditions, the dynamics of balance between exocytosis and endocytosis are well preserved to maintain the correct surface area of the presynaptic terminal [32,33]. However, a mutant leucine-rich repeat kinase 2 (LRRK2) bacterial artificial chromosome (BAC) transgenic mouse model showed enlarged axonal endings in the striatal dopaminergic neurons, suggesting imbalance between exocytotic membrane addition and endocytic retrieval [34]. Excessive accumulation of presynaptic vesicles and enlargement of the VGLUT1-positive nerve terminals was also observed in *Snap25*$^{S187A/S187A}$ mice [19]. Taking into consideration the synaptic vesicle cycle, our findings suggest that the balance of the cycle is likely biased toward decreased endocytosis.

The enlarged VGLUT1-positive nerve terminals of *Snap25*$^{S187A/S187A}$ mice showed concomitant accumulation of αSyn and p-αSyn [19]. Kramer and Schulz-Schaeffer [5] have previously reported that 90% or even more of αSyn aggregates in DLB cases were located at the presynapses in the form of very small deposits. In parallel, dendritic spines were retracted, whereas the presynapses were relatively preserved, suggesting that neurotransmitter deprivation may explain the cognitive impairment in DLB [5,6]. While the presynaptic aggregates did not contain much p-αSyn in their examination [5,6], widespread varicosities and dot-like structures containing p-αSyn are commonly observed in αSyn-transgenic mouse model and human DLB brains [35,36]. This may represent axonal transport defects and presynaptic dysfunctions [35,36]. Recent study showed that mutant αSyn (A53T) diminished levels of various motor proteins in neurons [37], supporting this scenario. Alternatively, excessive amount of misfolded αSyn and p-αSyn may aggregate at synapses, physically preventing the targeting of other presynaptic proteins [5]. In experiments using *Caenorhabditis elegans* overexpressing human αSyn, four genes related to the endocytosis process were identified as genetic modifiers for αSyn toxicity [38]. They included two subunits of the adaptor protein (AP) complex 2, which interacts with clathrin and promotes presynaptic clathrin-mediated vesicle recycling [39]. Furthermore, proteomics analysis revealed that p-αSyn also preferentially interacted with the proteins involved in endocytosis, including clathrin heavy chain and subunit of AP-2 and AP-1 complexes, over the non-phosphorylated αSyn [40]. Clathrin-mediated recycling of exocytosed synaptic vesicles occurs in the periactive zone, a region adjacent to the active zone where synaptic vesicle is endocytosed [33]. Similarly, in *Snap25*$^{S187A/S187A}$ mice, immunoelectron microscopy showed preferential localization of αSyn at the periactive zone of excitatory presynaptic nerve terminals. This might reflect the interaction of αSyn and p-αSyn with the proteins involved in clathrin-mediated endocytosis. Taking these findings together, presynaptic accumulation of αSyn and p-αSyn could disturb the endocytosis process and consequently contribute to the development of VGLUT1-positive terminal enlargement [19].

Presynaptic accumulation of αSyn

Presynaptic accumulation of αSyn is considered an early event in the pathogenesis of α-synucleinopathies [4-6]. Mice overexpressing human αSyn showed presynaptic accumulation of αSyn and low DA release in the striatum. Stoica et al. [41] reported a "dying back" type of neuronal alteration, progressing from the dendrites to the axon and then to the perikaryon and nucleus in a spontaneously inherited autosomal recessive rat model for PD that overexpressed αSyn in mesencephalic area.

Transmission electron microscopy (TEM) examination revealed that the retrograde pathological process in substantia nigra and striatum starts at the synaptic level by marked presynaptic accumulation of αSyn followed by post-synaptic degeneration of axonal terminals, dendrites and spine alterative changes and perikaryal aggregation of mitochondria with relative preservation of neuronal nuclei. These findings were associated with abnormal distribution of SNARE proteins, which colocalized with αSyn aggregates. Similarly, accumulation of SNARE proteins and αSyn were reported in the striatum of PD patients [18]. These observations suggest that SNARE dysfunction likely occurs at an early stage of pathogenesis in nigrostriatal dysfunction observed in PD. Considering the findings observed in the VGLUT1-positive nerve terminals, we expected that SNARE dysfunction might have induced presynaptic accumulation of αSyn, which consequently result in the development of neurodegenerative changes in the nigrostriatal system. However, contrary to our expectation, *Snap25*$^{S187A/S187A}$ mice showed no significant neurodegenerative changes in nigrostriatal dopaminergic neurons, suggesting that SNARE dysfunction alone was insufficient to cause nigrostriatal degeneration as observed in PD, and appeared to be a downstream event associated with abnormal accumulation of αSyn.

Conclusion

In conclusion, SNARE dysfunction leads to accumulation of endogenous αSyn in the corticostriatal nerve terminals. Presynaptic accumulation of αSyn is considered to be an early key event in the pathogenesis of α-synucleinopathies. Although the "prion-like" propagation hypothesis of αSyn, including tau and TAR DNA-binding protein 43 kDa, is currently receiving considerable attention worldwide, our findings provide an insight to understanding of the possible mechanisms that lead to presynaptic accumulation of endogenous αSyn. Moreover, given that SNAP-25 is reduced in the striatum of MSA brains [42], we speculate that a discontinuous pattern of αSyn pathologies usually found in MSA, *i.e.* glial cytoplasmic inclusions (GCIs) in the putaminal oligodendrocytes, and neuronal cytoplasmic inclusions (NCIs) and neuronal nuclear inclusions (NNIs) in the cortex [43,44], might be potentially linked through the presynaptic accumulation of αSyn in the corticostriatal neurons. Further investigations on the *Snap25* mutant mice with genetic ablation of αSyn would contribute to understanding the essential role of redistributed αSyn.

Competing interest

The authors have no conflict of interest.

Authors' contribution

TY, YN, and CJC wrote the paper. HM supervised the work. All authors read and approved the final manuscript.

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The promises of stem cells: stem cell therapy for movement disorders

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SUMMARY

Despite the multitude of intensive research, the exact pathophysiological mechanisms underlying movement disorders including Parkinson's disease, multiple system atrophy and Huntington's disease remain more or less elusive. Treatments to halt these disease progressions are currently unavailable. With the recent induced pluripotent stem cells breakthrough and accomplishment, stem cell research, as the vast majority of scientists agree, holds great promise for relieving and treating debilitating movement disorders. As stem cells are the precursors of all cells in the human body, an understanding of the molecular mechanisms that govern how they develop and work would provide us many fundamental insights into human biology of health and disease. Moreover, stem-cell-derived neurons may be a renewable source of replacement cells for damaged neurons in movement disorders. While stem cells show potential for regenerative medicine, their use as tools for research and drug testing is thought to have more immediate impact. The use of stem-cell-based drug screening technology could be a big boost in drug discovery for these movement disorders. Particular attention should also be given to the involvement of neural stem cells in adult neurogenesis so as to encourage its development as a therapeutic option.

1. Introduction

Scientists have known of the existence of stem cells, the unspecialized cells found in all multicellular organisms that can self-renew through self-division and differentiate into diverse specialized cell types, for over a century. Yet it has been only since the late 1990s, when human embryonic stem cells were first cultured in the laboratory, that the field of stem cell research has become the focus of intense scientific interest.

There are essentially three kinds of stem cells: embryonic stem (ES) cells, which are isolated from the inner cell mass of blastocysts; adult stem cells, which are found in various developed tissues such as bone marrow cells; and induced pluripotent stem (iPS) cells, which are artificially derived from a non-pluripotent cell, typically an adult somatic cell, by inducing a "forced" expression of specific genes.

One of the most astounding applications of stem cells is in the treatment and cure of a wide variety of movement disorders including Parkinson's disease (PD), multiple system atrophy (MSA) and Huntington's disease (HD). Several ways of how stem cells are being explored and used in both basic and clinical applications of current movement disorders research include disease modeling, drug toxicity screening/drug discovery, gene therapy and cell replacement therapy.

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In most cases, it is difficult to obtain the damaged cells in a disease and to study them in detail. Stem cells, either carrying the disease gene or engineered to carry disease genes, offer an alternative for laboratory studies. Researchers are able to model disease processes *in vitro* and perform more relevant and informative biological assays, thereby better understanding the mechanisms underlying the disease. Stem cells have also been used in the laboratory to screen for new drugs. It has been revealed that very few drugs have been tested on human-diseased cells before human testing. Liver and heart toxicity problems account for about 30% of drugs that fail in early-stage clinical trials, indicating a need for more efficient means of drug toxicity testing before clinical trials. The use of stem cells with specific diseases may correct this situation. Furthermore, given their unique regenerative abilities, stem cells offer the possibility of a renewable source of cell replacement therapies for neurological diseases.

However, stem cell research has been controversial and has raised ethical dilemmas primarily concerning the creation, treatment, and destruction of human embryos inherent to research involving ES cells. The recent discovery of iPS cells, hailed as a potential alternative to ES cells, provides researchers with a unique tool to derive neurons from patient-specific iPS cells for the study of neurological diseases. More importantly, iPS cell research obviates many ethical and resource-related concerns posed by ES cells while prospectively matching their potential for scientific use.

In recent years, the discovery of constitutive ongoing neurogenesis in the adult human brain has challenged the traditional view of a fixed circuitry in functionally normal brains, and has raised high hopes that the adult brain may have the capacity for self-renewal

after injury, thereby sidestepping the need for transplantation. Primary neural precursor cells reside in specialized zones called "neurogenic niches". A population of neural stem cells (NSCs) preserves enough germinal character to maintain neurogenesis throughout life and, once differentiated, their daughter cells integrate into already existing neuronal networks. Whether adult neurogenesis can be induced under certain circumstances in regions that lack constitutive adult neurogenesis remains controversial, but several studies have reported the isolation of NSCs from different regions of the adult brain, including the substantia nigra pars compacta (SNc). Therefore, there has been considerable interest within the scientific community to gain understanding of the possible correlation between neurogenesis and pathogenesis of movement disorders, which could help the future development of novel therapeutic intervention.

2. Stem cell therapy for PD and MSA

There has been a long history of fetal tissue transplantation for the treatment of patients with advanced PD. Despite the wake of a long series of encouraging open-label studies, initial enthusiasm for cell replacement therapy by grafting fetal neuronal precursor cells into the striatum has vanished after two double-blind placebo-controlled clinical trials showing only moderate symptomatic improvement and the occurrence of severe disabling dyskinesia. These problems should be solved before fetal tissue transplantation can be considered a therapeutic option for PD [1].

Studies have shown ES cell transplanted into the brains of PD rat model differentiated into dopaminergic neurons, restoring partial neural function [2]. PD rodent models subjected to engraftment of dopaminergic neurons derived from human ES cells demonstrated complete behavioral restoration and motor function improvement. Similarly, parkinsonian monkeys receiving transplantation showed excellent DA neuron survival, function and lack of neural overgrowth, indicating potential for the development of cell-based therapies in PD [3].

It was recently shown that reprogramming mouse embryonic fibroblasts with four transcription factors Oct4, Sox2, Klf4, and c-Myc induces pluripotency [4], enabling generation of iPS cells from patients with a variety of diseases [5]. iPSC-derived midbrain dopaminergic neurons from a patient with a triplication in the α-synuclein gene (SNCA) showed accumulation of α-synuclein, inherent overexpression of markers of oxidative stress, and sensitivity to peroxide-induced oxidative stress, precisely recapitulating the cause of disease in the patients [6,7]. Comparably, PARK2 iPSC-derived neurons exhibited mitochondrial dysfunction associated with increased oxidative stress and α-synuclein accumulation, resembling pathogenic changes in patient brains [8]. Neurons derived from mutant PINK1 iPS cells displayed impaired recruitment of lentivirally expressed Parkin to mitochondria, increased mitochondrial copy number and upregulation of PGC-1α, an important regulator of mitochondrial biogenesis, upon mitochondrial depolarization [9]. LRRK2 mutant iPSC-derived DA neurons demonstrated increased susceptibility to oxidative stress, consistent with existing understanding of early PD phenotypes [10]. Such disease-specific iPS cells offer an unprecedented opportunity to recapitulate both normal and pathologic human tissue formation *in vitro*, thereby facilitating disease investigation and drug development.

Furthermore, generation of iPS cells provides a new avenue for transplantation therapy as it can avoid immunorejection, a major complication in current transplantation medicine. Wernig et al. [11] reported upon transplantation into the fetal mouse brain, iPSC-derived neural precursor cells extensively differentiate into glia and neurons. Functional recovery was observed after transplantation of iPSC-derived midbrain dopamine neurons into the adult brain of Parkinsonian rats. Risk of tumor formation from grafted cells was minimized by the separation of contaminating pluripotent cells and committed neural cells using fluorescence-activated cell sorting. Encouraging data from rodent studies then prompted subsequent assessment in a primate model. Kikuchi et al. [12] observed that human iPSC-derived neural progenitor cells grafted in the brain of a primate PD model survived as dopaminergic neurons for as long as six months, implying the therapeutic potential of human iPS cells. Direct reprogramming of mouse and human fibroblasts into induced neural stem cells (iNSCs) has been proven feasible with a single factor, Sox2. iNSCs express NSC markers and resemble wild-type NSCs in their morphology, self-renewal, ability to form neurospheres and differentiate into several types of mature neurons as well as astrocytes and oligodendrocytes, indicating multipotency. Importantly, implanted iNSCs can survive and integrate in mouse brains without tumorigenic potential. As an additional merit, this method allows shortening of the duration for neuronal cell creation from fibroblasts [13].

Adult stem cells comprise mesenchymal stem cells, hematopoietic stem cells, ectodermal stem cells and so on. Scientific interest in adult stem cells is spotlighted on their ability to divide or self-renew indefinitely, and generate all the cell types of the organ from which they originate, potentially regenerating the entire organ from a few cells. Numerous studies using expanded and/or induced bone marrow-derived mesenchymal stem cells have been reported for animal models and yet only three clinical studies with intracerebral or intravasal application of these cells have been reported for PD and MSA patients. In two open-label studies, subventricular application of both allogenic and autologous bone marrow-derived mesenchymal stem cells showed improvement of motor behavior as reflected by reduction of UPDRS ON and OFF scores in most but not all PD patients [14,15]. In a randomized placebo-controlled trial involving a small number of cognitively intact MSA-C patients, mesenchymal stem cell therapy was safe and was able to delay the progression of neurological deficits with functional improvement in the follow-up period in some of the patients [16].

3. Adult neurogenesis in Parkinson's disease

Increasing evidence points to the presence of adult neural stem cells in many areas of the mammalian brain, mainly in the hippocampus and subventricular zone (SVZ) near the lateral ventricle. It is well known that changes occurring in the SVZ depend upon the pathological condition. Dopamine is an important molecule in neurogenesis. Therefore many investigators now focus on neurogenesis in PD. Höglinger et al. [17] reported reduction in the numbers of proliferating cells in the SVZ of postmortem brains of PD patients, implying that generation of neural precursor cells is impaired in PD as a consequence of dopaminergic denervation. However, controversy regarding neurogenesis in the SVZ in PD models persists. Some groups reported decreased neural precursor proliferation while some reported increased neural precursor proliferation in the SVZ of PD models.

Likewise, whether dopaminergic neurogenesis occurs in the adult substantia nigra (SN) in PD brains or in PD animal models remains a matter of debate. So we evaluated nigral neurogenesis in animal models and PD autopsy brains. We first performed retroviral labeling in a PD rodent model and observed efficient labeling of proliferating cells in SN with retroviral transduction of green fluorescent protein. But many of these labeled cells became microglia and none had differentiated into tyrosine-hydroxylase (TH)-positive neurons. Second, staining for intrinsic markers of neurogenesis showed that there were no proliferating cells in the SN of PD patients but a large number of polysialylated neural cell

adhesion molecule (PSA-NCAM)-positive cells were detected in SN pars reticulata (SNr) of some PD patients. In rat and primate models, dopamine-depleted hemispheres showed more PSA-NCAM staining than the intact side. A small number of TH and PSA-NCAM double positive cells, indicative of newly differentiated dopaminergic neurons, were detected [18]. However, no TH and PSA-NCAM double positive cells in PD patients were detected. Despite not being conclusive enough, these results suggest enhanced neural reconstruction in PD, which may be important in the design of new therapies against the progression of PD.

4. Stem cell therapy for Huntington's disease

Huntington's disease (HD) is characterized by a loss of brain striatal neurons that occurs as a consequence of an expansion of a cytosine adenine guanine (CAG) trinucleotide repeat encoding polyglutamine (polyQ) in the first exon of the Huntingtin gene. Therapeutic strategies are largely based on the amelioration of mutant huntingtin-related metabolic impairment and cellular toxicity. Yet cell replacement may be a potential therapy when cell death has become prominent in later stages of the disease. Numerous preclinical studies reported the efficiency of human fetal striatal tissue in providing functional recovery in various rodent and non-human primate models of striatal neuronal loss. On this basis, several clinical trials have assessed fetal cell transplantation for treatment in HD patients. Delivery of fetal striatal primordium into the caudate putamen of patient's brain was done via surgical stereotactic method. Yet due to heterogeneity in experimental design and small sample size, these clinical trials provided divergent data and reported modest improvements even in the best of cases. Some patients showed symptomatic improvement following the transplant but disease progression ensued with no greater survival. To address this issue, three ongoing randomized controlled clinical trials are reassessing fetal graft efficacy.

The ethical and immunological concerns associated with fetal allografts, along with the practical need to obtain tissue that is precisely staged, accurately dissected and freshly collected, imply that availability of fetal tissues for cell transplantation in the brain is likely to be extremely limited. Thus there is an urgent and active search for alternative sources. Human ES serve as a readily renewable source of potential medium spiny neurons for cell replacement therapy in HD patients. *In vivo* differentiation of striatal progenitor derived from human ES cells into striatal neurons following xenotransplantation into adult rats has first been described by Aubry et al. [19], opening an avenue of human ES cell therapy for HD. However, long-term proliferation of human neural progenitors leads to xenograft overgrowth in the rat brain, hindering its clinical use. HD-specific iPS cells have also been generated and reproduced CAG-repeat-expansion-associated gene expression phenotypes upon differentiation into neural cells, representing a well-characterized resource to elucidate the disease mechanism in HD and providing a human stem cell platform for screening new candidate therapeutics [20]. Also, An et al. [21] reported that iPS cells derived from the HD patient could be corrected by the replacement of the expanded CAG repeat with a normal repeat using homologous recombination, and the correction persists upon differentiation into striatal neurons *in vitro* and *in vivo*. Notably, correction of the HD-iPSCs normalized pathogenic HD signaling pathways and reversed disease phenotypes in neural stem cells. The ability to make patient-specific, genetically corrected iPS cells from HD patients is crucial for the eventual use of these cells in cell replacement therapy.

As mentioned earlier, neurogenesis has recently been observed in the adult human brain, suggesting the possibility of endogenous neural repair. Curtis et al. [22] first reported augmentation of neurogenesis as reflected by increased progenitor cell proliferation in the subependymal layer adjacent to the caudate nucleus, in response to neuronal cell loss in the caudate nucleus in HD. Degree of cell proliferation increased with pathological severity and increasing CAG repeats in the HD gene. Most importantly, proliferating cells were shown to express neuronal markers, indicating the generation of neurons and glial cells in diseased human brain. These results provide evidence for the regenerative potential of the human brain. Further, on the basis that ependymal overexpression of brain-derived neurotrophic factor (BDNF) stimulates neuronal addition to the adult striatum from subependymal progenitor cells while Noggin potentiates this process by suppressing subependymal gliogenesis and increasing progenitor availability, Cho et al. [23] found that BDNF and Noggin induced striatal neuronal regeneration, delayed motor impairment, and extended survival in R6/2 huntingtin transgenic mice, suggesting a new therapeutic strategy for HD.

5. Challenges of stem cell research

Before stem cells can be used to treat a myriad of disorders, many technical obstacles that hinder the clinical use must be overcome. The first major concern is that ES- and iPS-derived grafts have been reported to induce formation of teratomas. The tumor formation depends on the extent to which the cells are selectively enriched and differentiated prior to transplantation. Contamination with undifferentiated multipotent cells permits teratogenesis in the host. There are a number of successful engraftments of human ES cells-derived cells within the brain as treatment for PD and HD without tumor formation. But these studies were conducted in rodents and did not include long assessment periods. This problem may be solved with the establishment of safe stem cells incorporated with an anti-tumorigenic system by virus-mediated suicidal gene introduction [24]. These suicidal genes can serve as cell death switches that halt potentially deadly reactions.

Second, human ES cells express low levels of human leukocyte antigen class I molecules in both undifferentiated and differentiated states and might elicit immune responses. To address this issue, researchers found that short-term immune-dampening treatment enables human embryonic stem cells to avoid rejection after transplantation. Breakthrough of iPS cells also potentially allows generation of patient-specific donor cells that would likely, although not certainly, evade rejection as autograft. However, some researchers opined that iPSC-derived neurons will not be suitable for transplantation until the oncogenes and retroviruses used are replaced with more controlled methods of reprogramming. The problems that remain would likely be overcome through years of intensive research.

Recently a critical issue regarding clinical use of unapproved stem cell treatment in many clinics in some countries has been revealed. Those clinics claimed success in treating patients, including PD patients, but none has published data from controlled clinical trials. PD experts expressed concerns that these treatments might provide anecdotal, poorly controlled and transient improvement in patients and were dubious if the infused cells would survive for more than a few days in patients because so far there are neither scientific nor clinical data to support long-term benefits of hematopoietic or neural stem cell therapies for PD patients. Leading researchers now emphasize the need to strictly regulate stem cell therapy by requiring the organizations using stem cells to register their research and clinical activities, source of stem cells and ethical procedures.

6. Conclusion

In summary, stem cell research has made tremendous progress to date, offering new and promising potentials for the use of

these cells as therapeutic agents. However, it has also been the subject of much debate, and a great deal of research is required to overcome the existing technical hurdles including tumorigenesis and immune response so as to enable development of novel approaches that could be translated into effective and well-tolerated clinical application. Though in its infancy, generation of iPS cells is a breakthrough in stem cell research that, in the long term, may lessen the need to use human ES cells that is always at the crux of ethical concerns.

Conflict of interests

The authors have no conflicts of interest to declare.

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