24. Ritson, G.P., Custer, S.K., Freibaum, B.D., Guinto, J.B., Geffel, D., Moore, J., Tang, W., Winton, M.J., Neumann, M., Trojanowski, J.Q. *et al.* (2010) TDP-43 mediates degeneration in a novel *Drosophila* model of disease caused by mutations in VCP/p97. *J. Neurosci.*, **30**, 7729–7739.
25. Watts, G.D., Wymer, J., Kovach, M.J., Mehta, S.G., Mumm, S., Darvish, D., Pestronk, A., Whyte, M.P. and Kimonis, V.E. (2004) Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. *Nat. Genet.*, **36**, 377–381.
26. Johnson, J.O., Mandrioli, J., Benatar, M., Abramzon, Y., Van, Deerlin, V.M., Trojanowski, J.Q., Gibbs, J.R., Brunetti, M., Gronka, S., Wuu, J. *et al.* (2010) Exome sequencing reveals VCP mutations as a cause of familial ALS. *Neuron*, **68**, 857–864.
27. Stolow, D.T. and Haynes, S.R. (1995) Cabeza, a *Drosophila* gene encoding a novel RNA binding protein, shares homology with EWS and TLS, two genes involved in human sarcoma formation. *Nucleic Acids Res.*, **23**, 835–843.
28. Ruden, D.M., Sollars, V., Wang, X., Mori, D., Alterman, M. and Lu, X. (2000) Membrane fusion proteins are required for oskar mRNA localization in the *Drosophila* egg chamber. *Dev. Biol.*, **218**, 314–325.
29. Shcherbata, H.R., Yatsenko, A.S., Patterson, L., Sood, V.D., Nudel, U., Yaffe, D., Baker, D. and Ruohola-Baker, H. (2007) Dissecting muscle and neuronal disorders in a *Drosophila* model of muscular dystrophy. *EMBO J.*, **26**, 481–493.
30. Chang, H.C., Dimlich, D.N., Yokokura, T., Mukherjee, A., Kankel, M.W., Sen, A., Sridhar, V., Fulga, T.A., Hart, A.C., Van Vactor, D. *et al.* (2008) Modeling spinal muscular atrophy in *Drosophila*. *PLoS One*, **3**, e3209.
31. Chee, F., Mudher, A., Newman, T.A., Cuttle, M., Lovestone, S. and Shepherd, D. (2006) Overexpression of tau results in defective synaptic transmission in *Drosophila* neuromuscular junctions. Biochem. Soc. Trans., **34**, 88–90.
32. Hirabayashi, M., Inoue, K., Tanaka, K., Nakadate, K., Ohsawa, Y., Kamei, Y., Popiel, A.H., Sinohara, A., Iwamatsu, A., Kimura, Y. *et al.* (2001) VCP/p97 in abnormal protein aggregates, cytoplasmic vacuoles, and cell death, phenotypes relevant to neurodegeneration. *Cell Death Differ.*, **8**, 977–984.
33. Fujita, K., Nakamura, Y., Oka, T., Ito, H., Tamura, T., Tagawa, K., Sasabe, T., Katsuta, A., Motoki, K., Shiwaku, H. *et al.* (2013) A functional deficiency of TERA/VCP/p97 contributes to impaired DNA repair in multiple polyglutamine diseases. *Nat. Commun.*, **4**, 1816. doi:10.1038/ncomms2828.
34. Higashiyama, H., Hirose, F., Yamaguchi, M., Inoue, Y.H., Fujikake, N., Matsukage, A. and Kakizuka, A. (2002) Identification of ter94, *Drosophila* VCP, as a modulator of polyglutamine-induced neurodegeneration. *Cell Death Differ.*, **9**, 264–273.
35. Ishigaki, S., Hishikawa, N., Niwa, J., Iemura, S., Natsume, T., Hori, S., Kakizuka, A., Tanaka, K. and Sobue, G. (2004) Physical and functional interaction between Dorfin and Valosin-containing protein that are colocalized in ubiquitylated inclusions in neurodegenerative disorders. *J. Biol. Chem.*, **279**, 51376–51385.
36. Kakizuka, A. (2008) Roles of VCP in human neurodegenerative disorders. *Biochem. Soc. Trans.*, **36**, 105–108.
37. Mizuno, Y., Hori, S., Kakizuka, A. and Okamoto, K. (2003) Vacuole-creating protein in neurodegenerative diseases in humans. *Neurosci. Lett.*, **343**, 77–80.
38. Marti, F. and King, P.D. (2005) The p95–100 kDa ligand of the T cell-specific adaptor (TSAd) protein Src-homology-2 (SH2) domain implicated in TSAd nuclear import is p97 Valosin-containing protein (VCP). *Immunol. Lett.*, **97**, 235–243.
39. Ju, J.S. and Weihl, C.C. (2010) p97/VCP at the intersection of the autophagy and the ubiquitin proteasome system. *Autophagy*, **6**, 283–285.
40. Ling, S.C., Albuquerque, C.P., Han, J.S., Lagier-Tourenne, C., Tokunaga, S., Zhou, H. and Cleveland, D.W. (2010) ALS-associated mutations in TDP-43 increase its stability and promote TDP-43 complexes with FUS/TLS. *Proc. Natl Acad. Sci. USA*, **107**, 13318–13323.
41. van Blitterswijk, M. and Landers, J.E. (2010) RNA processing pathways in amyotrophic lateral sclerosis. *Neurogenetics*, **11**, 275–290.
42. Kim, S.H., Shanware, N.P., Bowler, M.J. and Tibbetts, R.S. (2010) Amyotrophic lateral sclerosis-associated proteins TDP-43 and FUS/TLS function in a common biochemical complex to co-regulate HDAC6 mRNA. *J. Biol. Chem.*, **285**, 34097–34105.
43. Fiesel, F.C. and Kahle, P.J. (2011) TDP-43 and FUS/TLS: cellular functions and implications for neurodegeneration. *FEBS J.*, **278**, 3550–3568.
44. Ayala, Y.M., Zago, P., D'Ambrogio, A., Xu, Y.F., Petrucelli, L., Buratti, E. and Baralle, F.E. (2008) Structural determinants of the cellular localization and shuttling of TDP-43. *J. Cell Sci.*, **121**, 3778–3785.
45. Nagai, R., Hashimoto, R. and Yamaguchi, M. (2010) *Drosophila* Syntrophins are involved in locomotion and regulation of synaptic morphology. *Exp. Cell Res.*, **316**, 2313–2321.
46. Fernández-Sáiz, V. and Buchberger, A. (2010) Imbalances in p97 co-factor interactions in human proteinopathy. *EMBO Rep.*, **11**, 479–485.
47. Manno, A., Noguchi, M., Fukushi, J., Motohashi, Y. and Kakizuka, A. (2010) Enhanced ATPase activities as a primary defect of mutant valosin-containing proteins that cause inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia. *Genes Cells*, **15**, 911–922.
48. Takahashi, Y., Hirose, F., Matsukage, A. and Yamaguchi, M. (1999) Identification of three conserved regions in the DREF transcription factors from *Drosophila melanogaster* and *Drosophila virilis*. *Nucleic Acids Res.*, **27**, 510–516.
49. Stewart, B.A., Atwood, H.L., Renger, J.J., Wang, J. and Wu, C.F. (1994) Improved stability of *Drosophila* larval neuromuscular preparations in haemolymph-like physiological solutions. *J. Comp. Physiol. A*, **175**, 179–191.
50. Ly, L.L., Suyari, O., Yoshioka, Y., Tue, N.T., Yoshida, H. and Yamaguchi, M. (2013) dNF-YB plays dual roles in cell death and cell differentiation during *Drosophila* eye development. *Gene*, **520**, 106–118.

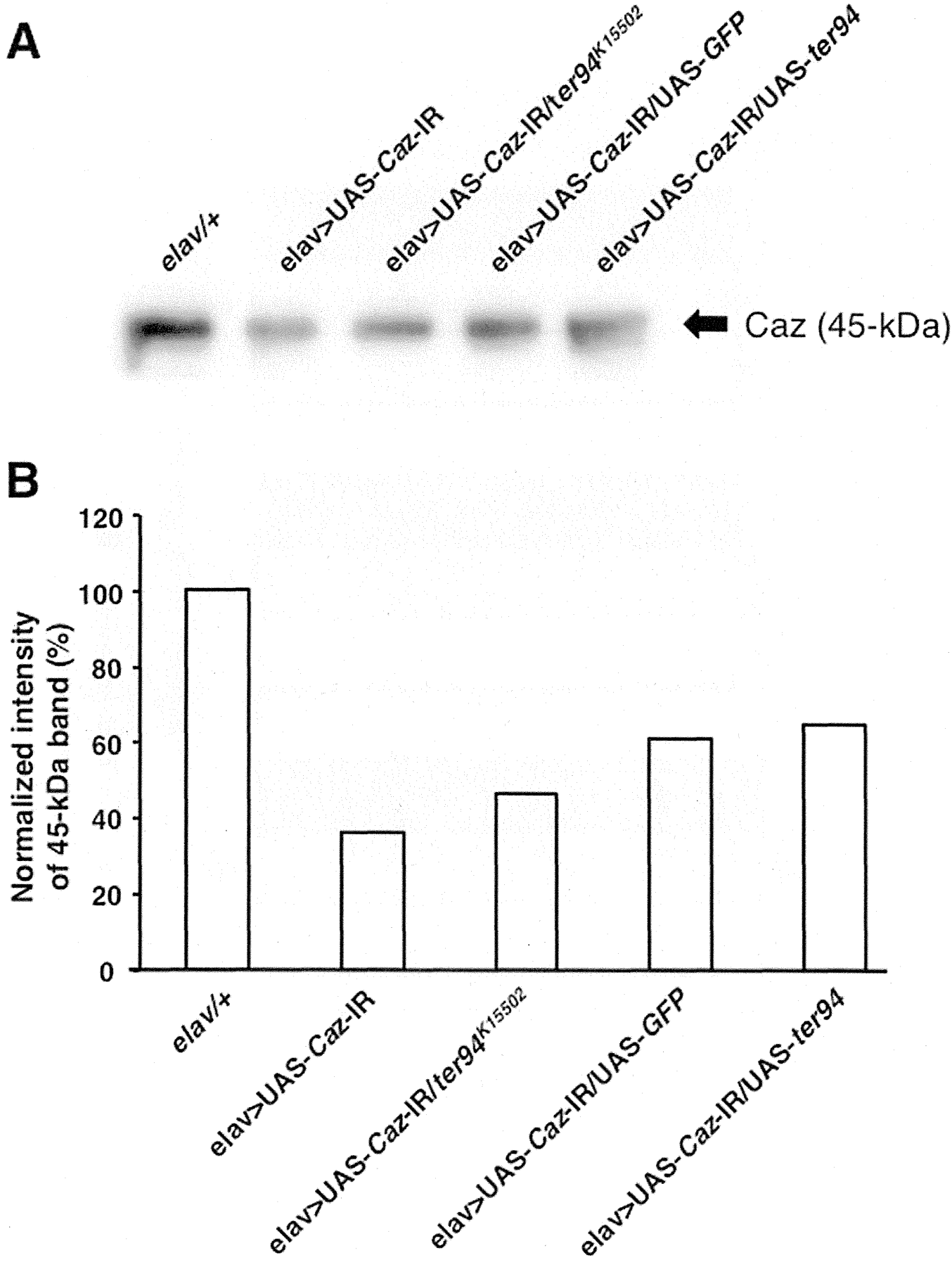
Supplementary Materials

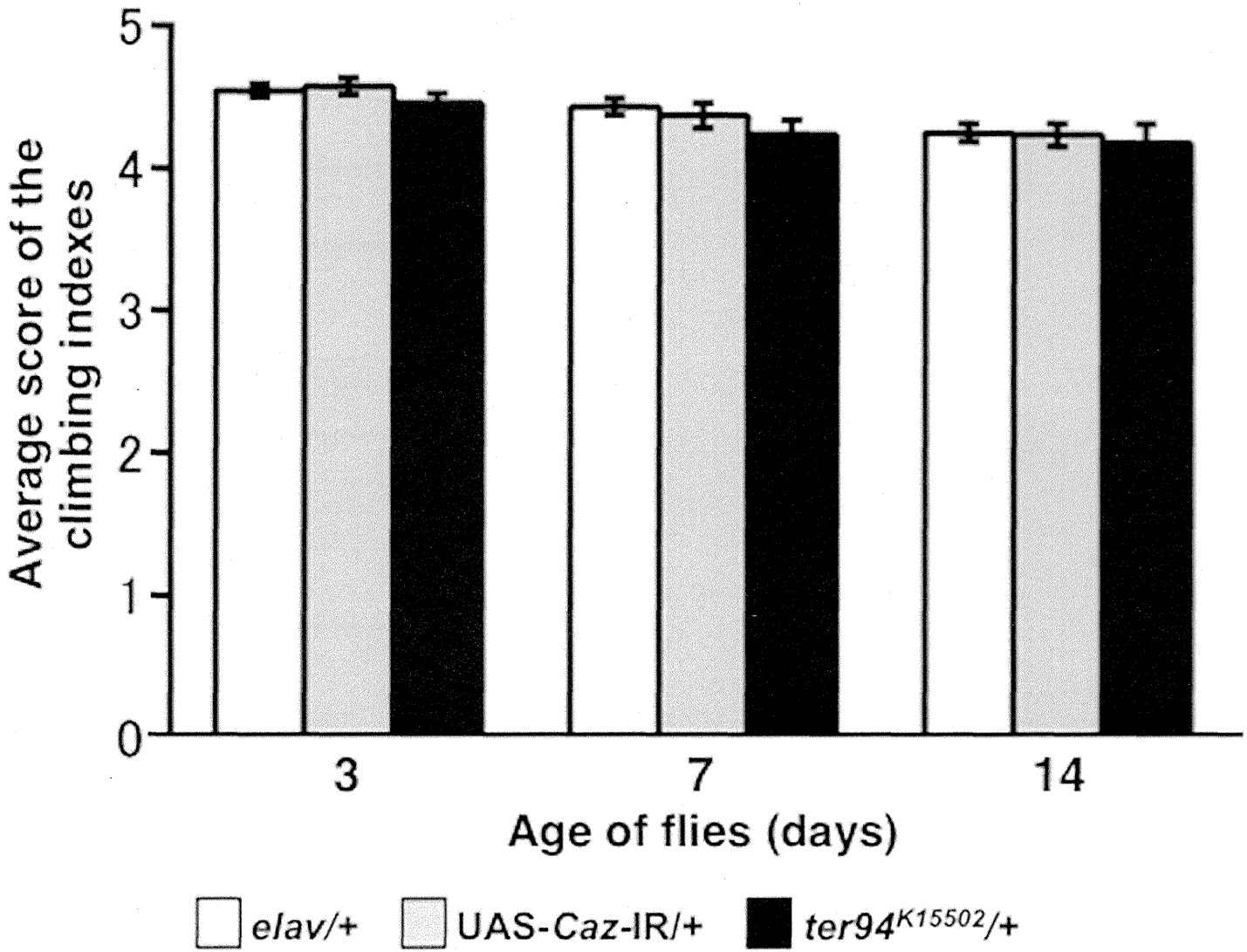
Supplementary Figure 1. Immunoblotting analysis of the CNS extracts of third instar larvae. (A) A representative result of the analysis of protein extracts from the CNS of the *elav/+*, elav>UAS-*Caz*-IR, elav>UAS-*Caz*-IR/*ter94* ^{K15502}, elav>UAS-*Caz*-IR/UAS-*GFP* and elav>UAS-*Caz*-IR/UAS-*ter94* larvae (n = 5, each). The blots are probed with the polyclonal anti-Caz antibody used in the previous study (19). A 45-kDa band (arrow) corresponds to the Caz protein. (B) Densitometric quantification of the 45-kDa bands in each fly strain used in (A). The intensity of the 45-kDa band which indicates the expression level of Caz protein is much weaker in larvae carrying elav>UAS-*Caz*-IR than in the larvae carrying *elav/+*. Besides, there is no apparent difference in the intensity of the Caz protein band between the larvae carrying elav>UAS-*Caz*-IR and elav>UAS-*Caz*-IR/*ter94* ^{K15502}. Similarly, there is no apparent difference in the intensity of the band between the larvae carrying elav>UAS-*Caz*-IR/UAS-*GFP* and elav>UAS-*Caz*-IR/UAS-*ter94*.

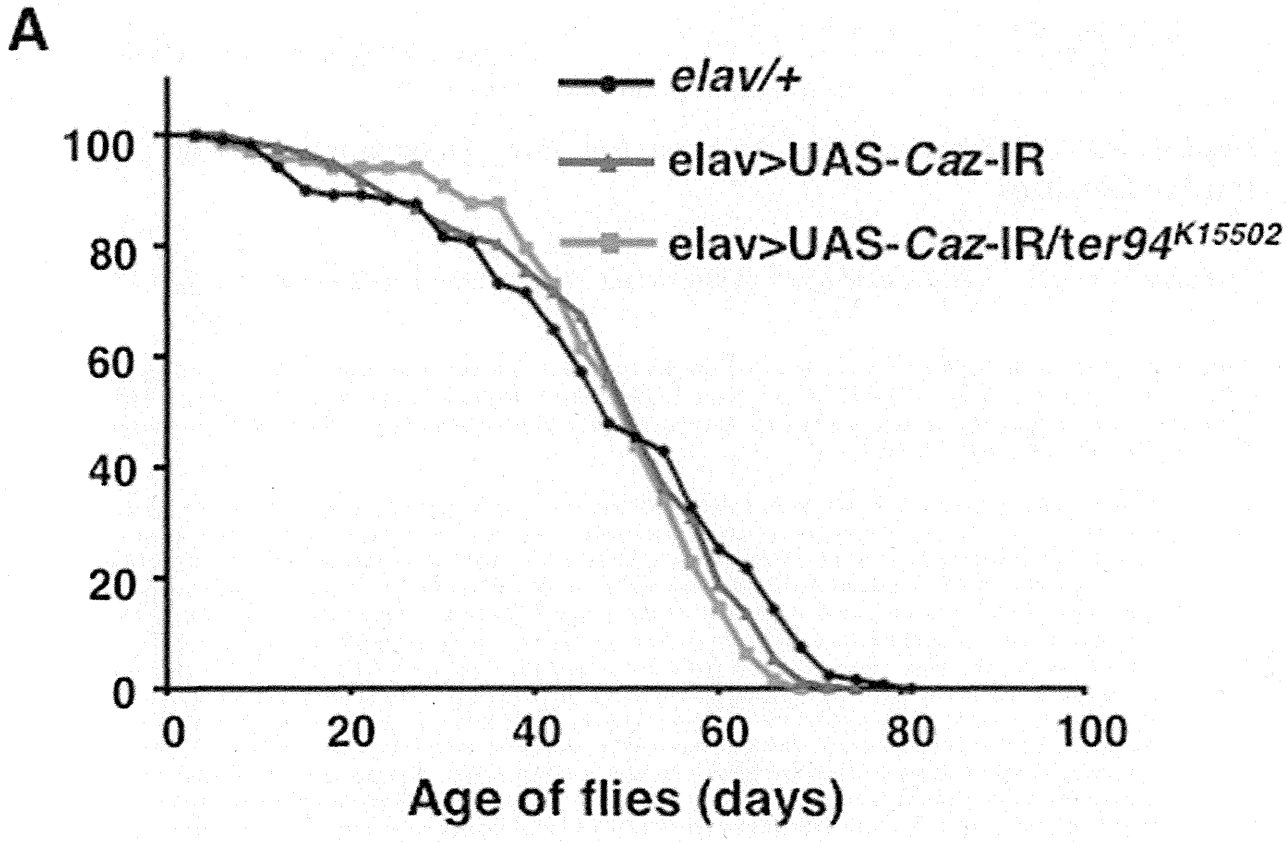
Supplementary Figure 2. The locomotive ability of each control flies, which carry *elav/+* (a driver control, n = 255), UAS-*Caz*-IR/+ (a responder control, n = 250), *ter94*^{k15502}/+ (n = 235). There are no significant differences in climbing abilities among those fly lines in each day after eclosion that was monitored until 14 days.

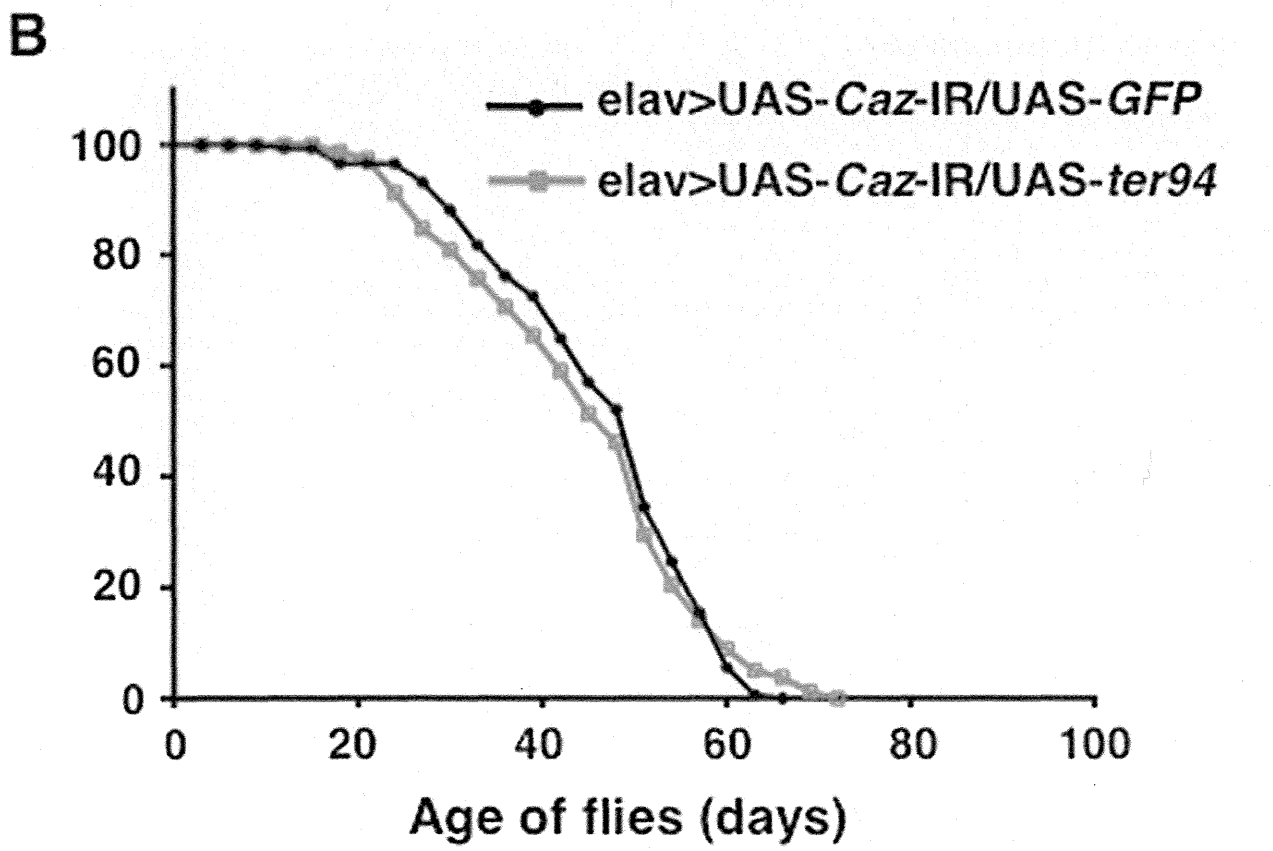
Supplementary Figure 3. Life-span analyses of flies of each genotype. Percentage survival of adult male flies of the indicated genotype is shown. (A) There are no significant differences in life spans among the control flies carrying *elav/+* (n = 151), neuron-specific *Caz*-knockdown flies carrying elav>UAS-*Caz*-IR (n = 123), and flies

carrying elav>UAS-*Caz*-IR/*ter94*^{K15502} (n = 120). (B) Similarly, there are no significant differences in life spans between flies carrying elav>UAS-*Caz*-IR/UAS-*GFP* (n = 140) and those carrying elav>UAS-*Caz*-IR/UAS-*ter94* (n = 140).


A
elav/+
elav>UAS-Caz-IR
elav>UAS-Caz-IR/ter94K15502
elav>UAS-Caz-IR/UAS-GFP
elav>UAS-Caz-IR/UAS-ter94
Caz (45-kDa)
B
Normalized intensity of 45-kDa band (%)
120
100
80
60
40
20
0
elav/+
elav>UAS-Caz-IR
elav>UAS-Caz-IR/ter94K15502
elav>UAS-Caz-IR/UAS-GFP
elav>UAS-Caz-IR/UAS-ter94


Average score of the climbing indexes
5
4
3
2
1
0
3
7
14
Age of flies (days)
elav/+
UAS-Caz-IR/+
ter94K15502/+


A
elav/+
elav>UAS-Caz-IR
elav>UAS-Caz-IR/ter94K15502
100
80
60
40
20
0
0
20
40
60
80
100
Age of flies (days)


B
elav>UAS-Caz-IR/UAS-GFP
elav>UAS-Caz-IR/UAS-ter94
100
80
60
40
20
0
0
20
40
60
80
100
Age of flies (days)

FINAL

Send Orders for Reprints to reprints@benthamscience.net

Current Medicinal Chemistry, 2014, *21*, ????-????

Peptide-Based Therapeutic Approaches for Treatment of the Polyglutamine Diseases

Toshihide Takeuchi[1,3,*], H. Akiko Popiel[1], Shiroh Futaki[3], Keiji Wada[1], and Yoshitaka Nagai[1,2,*]

[1]*Department of Degenerative Neurological Diseases, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Kodaira, Tokyo 187-8502, Japan;* [2]*Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency, Kawaguchi, Saitama 332-0012, Japan;* [3]*Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan*

Abstract: The polyglutamine (polyQ) diseases including Huntington's disease and spinocerebellar ataxias are a group of inherited neurodegenerative diseases that are caused by an abnormal expansion of the polyQ stretch in disease-causative proteins. The expanded polyQ stretches are intrinsically unstable and are prone to form insoluble aggregates and inclusion bodies. Recent studies have revealed that the expanded polyQ proteins gain cytotoxicity during the aggregation process, which may possibly cause detrimental effects on a wide range of essential cellular functions leading to eventual neuronal degeneration. Based on the pathogenic mechanism of the polyQ diseases, several therapeutic approaches have been proposed to date. Among them, here we focus on peptide-based approaches that target either aggregate formation of the polyQ proteins or abnormal cellular processes induced by the expanded polyQ proteins. Although both approaches are effective in suppressing cytotoxicity of the abnormal polyQ proteins and the disease phenotypes of animal models, the former approach is more attractive since it targets the most upstream change occurring in the polyQ diseases, and is therefore expected to be effective against various downstream functional abnormalities in a broad range of polyQ diseases. One of the major current problems that must be overcome for development of peptide-based therapies of the polyQ diseases is the issue of brain delivery, which is also discussed in this article. We hope that in the near future effective therapies are developed, and bring hope to many patients suffering from the currently untreatable polyQ diseases.

Keywords: Neurodegeneration, peptide, polyglutamine diseases, protein aggregation, therapy.

THE POLYGLUTAMINE DISEASES

The polyglutamine (polyQ) diseases are a group of inherited neurodegenerative disorders characterized by a common genetic mutation in the coding sequence of each disease-causative gene, in which a trinucleotide CAG repeat encoding a polyQ stretch is abnormally expanded (>35-40 repeats) [1-3]. So far, nine disorders have been recognized as such diseases, including Huntington's disease (HD), spinal and bulbar muscular atrophy (SBMA), several types of spinocerebeller ataxias (SCAs) [2]. These diseases are all characterized by the progressive degeneration and loss of neurons in various regions of the brain, resulting in progressive neurological and psychiatric symptoms such as cognitive impairment and motor disturbance. No effective treatment for the polyQ diseases has been established to date.

The molecular basis of the polyQ diseases is the abnormal expansion of a polyQ stretch in each host protein. In most cases, the threshold polyQ length for disease manifestation is around 35-40 repeats, as polyQ expansions longer than 40 repeats typically result in the polyQ diseases [3]. For example, the polyQ length in huntingtin (Htt), the causative protein of HD, ranges in size from 5 to 35 repeats in normal subjects, but is expanded to more than 40 repeats in patients with HD [4]. The polyQ length in the disease-causative protein can also affect the disease progression, as it correlates tightly with the age at onset and severity of disease [5, 6]. Animal studies have demonstrated that typical disease phenotypes such as progressive degeneration and loss of neurons in the brain can be caused by expression of the expanded polyQ stretch alone, further supporting the pathological importance of the abnormal expansion of the polyQ stretch [7-11]. These facts strongly indicate that the polyQ diseases are caused by a gain of toxic function mechanism of the expanded polyQ stretch, and are considered to be unrelated with the specific functions of each host protein.

The expanded polyQ stretches are intrinsically unstable, and are likely to form insoluble aggregates and inclusion bodies, which are a common pathological characteristic observed in the brain of polyQ disease patients as well as animal models [12, 13]. The mechanisms as to how expanded polyQ proteins form aggregates and the relationship between aggregate formation and cytotoxicity have been extensively studied [14-18]. Recent accumulating evidence strongly indicate that abnormal intermediate species such as oligomeric intermediates and even misfolded monomers of the expanded polyQ proteins which form prior to aggregates/inclusion bodies could be more toxic to neurons compared with in-

*Address correspondence to this author at the Department of Degenerative Neurological Diseases, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1 Ogawa-Higashi, Kodaira, Tokyo 187-8502, Japan; Tels: +81-42-346-1715 and +81-774-38-3211;
Faxes: +81-42-346-1745 and +81-774-32-3038;
E-mails: nagai@ncnp.go.jp and takeuchi@scl.kyoto-u.ac.jp

0929-8673/14 $58.00+.00

soluble aggregates/inclusion bodies [19-23]. Although it is still unclear which intermediate species are responsible for polyQ disease pathogenesis, these facts indicate that the expanded polyQ protein gains cytotoxicity during the aggregation process.

THERAPEUTIC APPROACHES FOR THE POLYGLUTAMINE DISEASES

Since proteins with an abnormally expanded polyQ stretch gain cytotoxicity during their aggregation process, suppression of misfolding and aggregate formation could be a potential therapeutic approach for treatment of the polyQ diseases [3]. Several studies have actually demonstrated in polyQ disease models that increasing levels of molecular chaperones [24-28] and expression of intracellular antibodies (intrabodies) [29-31] successfully reduce the eventual toxicity in neurons through suppression of polyQ protein accumulation and inclusion body formation [32, 33]. Small molecules [34-38] and peptides [39-41] that interfere with the aggregation process of the expanded polyQ protein were also shown to suppress polyQ-induced neurodegeneration in cell culture and animal models of the polyQ diseases. In addition, activation of protein degradation systems, which accelerate the clearance of the polyQ proteins, has been shown to be quite effective to suppress aggregate formation and eventual cell death [42,43]. Since suppression of polyQ aggregation is expected to broadly correct the functional abnormalities of multiple downstream cellular processes (see below), misfolding and aggregate formation of the expanded polyQ proteins are one of the most ideal therapeutic targets of the polyQ diseases [3] (Fig. **1**).

On the other hand, it is well known that polyQ disease patients as well as animal models exhibit dysfunctions in various cellular processes in the cascade of polyQ pathogenesis. This includes abnormalities in essential cellular functions including transcription [44], proteasomal degradation [45], synaptic transmission [46], axonal transport [47] and Ca^{2+} signaling pathways [48], which probably contribute to neuronal dysfunction and eventual loss of neurons in various regions of the brain [12, 49, 50]. Although the exact mechanisms as to how they eventually cause degeneration of neurons in patients of the polyQ diseases have not yet been clarified, these cellular processes that are thought to be eventually impaired in the pathogenic cascade are also potential therapeutic targets for treatment of the polyQ diseases (Fig. **1**).

In the following sections, selected examples of peptide-based therapeutic approaches focusing on these targets are introduced (Table **1**), and the current problems that must be overcome for the development of peptide-based therapies for the polyQ diseases are discussed.

PEPTIDE-BASED INHIBITORS OF POLYGLUTAMINE AGGREGATION

Therapeutic approaches targeting the polyQ stretch are particularly attractive because effective inhibitors would be expected to work generally on a broad spectrum of the polyQ diseases. Trottier *et al.* showed that the anti-polyQ monoclonal antibody 1C2 binds preferentially to longer polyQ repeats compared with short repeats [51]. Similar preferential binding to expanded polyQ proteins has been reported for the monoclonal antibodies MW1 [52] and 3B5H10 [53]. These studies led to the idea that expanded polyQ stretches may possess structurally different conformations from the shorter ones, and that potential molecules that specifically recognize and bind to such abnormal conformations could interfere with the aggregation processes of expanded polyQ proteins.

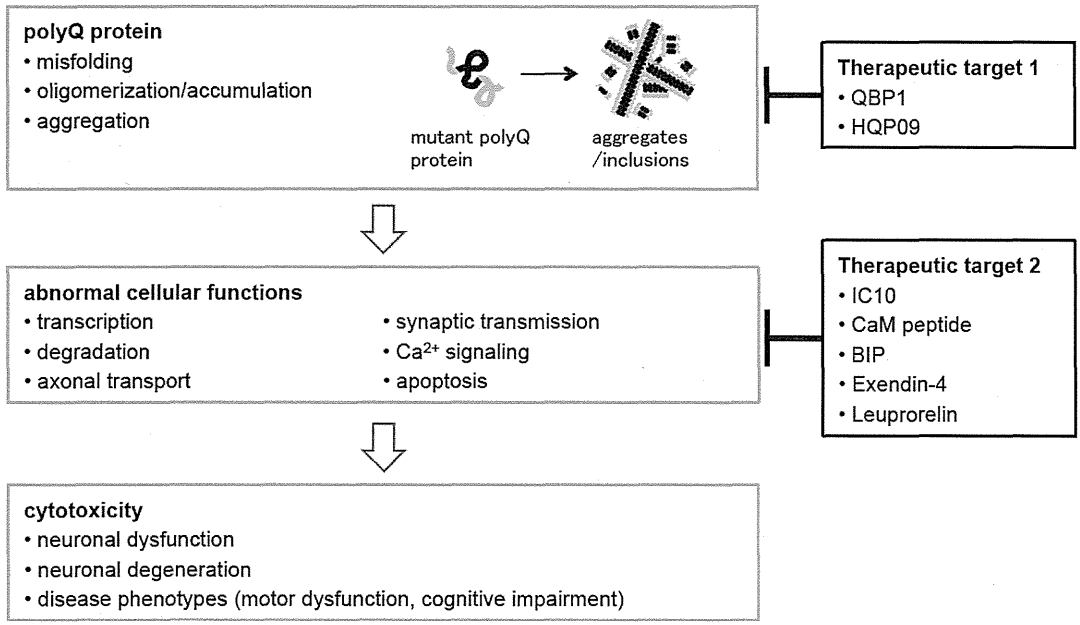


Fig. (1). Proposed mechanism of expanded polyQ protein toxicity and potential therapeutic targets for polyQ disease therapies.

Table 1. Selected examples of peptides potentially effective for the polyQ diseases.

Peptide	Sequence	Therapeutic Target	Effects *In Vitro*	Effects *In Vivo*	Ref
QBP1	SNWKWWPGIFD	polyQ aggregation	Aggregation ↓ Inclusion bodies ↓ Cytotoxicity ↓	Inclusion bodies ↓ Life span ↑ Body weight ↑	[23, 39, 54-60, 62, 63]
HQP09	Npip-Nmba-P-Nmea-Nall-Nlys-Nser[a]	polyQ aggregation	Aggregation ↓ Cytotoxicity ↓	Stabilize Ca^{2+} signaling ↓ Apoptosis ↓ Inclusion bodies	[64]
IC10	A cytosolic C-terminal tail of $InsP_3R1$ (122 amino acids)	Aberrant interaction between Htt and $InsP_3R1$	Stabilize Ca^{2+} signaling ↓	Motor function ↑ Neuronal loss ↓	[68]
CaM peptide	MKDTDSEEEIREAFRVFDKDGNGY-ISAAELRHVMTNLGEKLTDEEV (A fragment of CaM, residues 76-121)	Abberant interaction between Htt and CaM	Cytotoxicity ↓	Body weight ↑ Motor function ↑ Inclusion bodies ↓	[69-71]
BIP	VPMLK/VPTLK	Bax-induced apoptosis	Apoptosis ↓	-	[75,76]
Exendin-4	HGEGTFTSDLSKQMEEEAVRLFIEWL KNGGPSSGAPPPS	Abnormal energy metabolism	-	Motor function ↑ Life span ↑ Inclusion bodies ↓	[85]
Leuprorelin	Pyr-HWSY*L*LRP-NHEt[b,c]	Nuclear accumulation of mutant AR	-	Motor function ↑ Life span ↑ Inclusion bodies ↓	[90-92]

[a]N-substituted glycines. Npip, piperonyl; Nmba, methylbenzyl; Nmea, methoxyethyl; Nall, allyl; Nlys, aminobutyl; Nser, hydroxyethyl. [b]Pyr, pyroglutamyl. [c]*d*-amino acids in *Italics*.

QBP1

We previously took a combinatorial screening approach to search for short peptides that selectively and specifically bind to an expanded polyQ stretch, but not to a normal length polyQ stretch, using the phage display technique. Multiple rounds of screening resulted in six peptides that preferentially bind to the abnormally expanded polyQ stretch [39]. One of these peptides, QBP1 (polyQ binding peptide 1), had a particularly high affinity for the abnormal polyQ stretch with a dissociation constant (K_d) of 5.7 μM [54], and also had a suppressive effect on polyQ aggregation *in vitro* [39]. Studies focusing on its structure-activity relationship revealed that the tryptophan-rich sequence is necessary for the inhibitory activity of QBP1 [55-57]. Expression of QBP1 effectively suppressed inclusion body formation and cytotoxicity of expanded polyQ proteins in cell culture [23,39,58,59] and *Drosophila* models of the polyQ diseases [60]. Since QBP1 is poorly membrane permeable, we employed protein transduction domains (PTDs) [61] to improve the bioavailability of QBP1 by its efficient intracellular delivery. We found that the delivery efficiency of QBP1 was dramatically improved by conjugation with a PTD, leading to successful suppression of polyQ inclusions as well as polyQ-induced premature death in *Drosophila* by its oral administration [62]. The therapeutic potential of PTD-QBP1 was further investigated using a mouse model of HD. However, the therapeutic effect of PTD-QBP1 was limited to neither inhibition of body weight loss with any improvement in the other disease phenotypes nor inhibition of aggregate formation in the brains, probably due to low efficiency of PTD-QBP1 delivery to the mouse brain by intraperitoneal injections [63].

HQP09

Chen *et al.* also performed combinatorial screening to search for potential inhibitors of polyQ aggregation [64]. In contrast to our approach using peptide-based phage display libraries, they used a combinatorial library consisting of peptoids as scaffolds. Peptoids, which are oligomers of N-substituted glycines, have an advantage in developing therapeutic molecules since they are considered to be superior in stability to protease degradation, cell permeability, and structural diversity [65, 66]. They prepared a peptoid library containing 60,000 unique compounds, and screened for molecules that specifically bind to the Htt fragment with an expanded polyQ stretch. The peptoid HQP09, which was isolated from this screening process, was found to bind with high specificity to the expanded polyQ forms of Htt and ataxin-3, which is the causative protein of SCA3, and to effectively suppress polyQ aggregation *in vitro*. Interestingly, although HQP09 and QBP1 had comparable binding affinity to mutant Htt proteins, HQP09 did not show any competition with QBP1 in binding, possibly indicating that these two inhibitors recognize the abnormally expanded polyQ stretches in a different manner. The authors also tested the therapeutic activity of this peptoid, and confirmed that HQP09 reduced cytotoxicity in primary cultured neurons and decreased polyQ inclusion bodies in a mouse model of HD upon its intracerebroventricular injection. Importantly, they successfully identified the pharmacophore of HQP09 based on a structure-activity relationship study, and developed the minimal derivative peptoid HQP09-9 (4-mer, MW = 585) without significant loss of activity. Although HQP09-9 failed to exert therapeutic effects on a mouse model upon its subcu-

taneous injection probably due to poor blood-brain barrier permeability, this could be a promising lead compound for the development of drugs against a broad spectrum of the polyQ diseases.

PEPTIDE-BASED MODULATORS OF POLYGLUTAMINE TOXICITY

Another therapeutic approach is to target the various cellular dysfunctions occurring in the cascade of polyQ pathogenesis. Although the mechanisms as to how these abnormalities contribute to eventual neurodegeneration in various regions of the brain are not known, normalizing such dysfunctions has been shown to effectively reduce the toxicity of the expanded polyQ proteins and improve disease phenotypes in polyQ disease animal models.

IC10 peptide

Since aberrant interactions between mutant Htt and various proteins often cause abnormalities in downstream cellular functions [50], disruption of such interactions could be a promising therapeutic approach. Bezprozvanny and coworkers found that the polyQ expanded Htt protein specifically binds to type 1 inositol 1,4,5-triphosphate receptor ($InsP_3R1$) and facilitates its activity, indicating that abnormal neuronal Ca^{2+} signaling may play an important role in HD pathogenesis [48, 67]. Since mutant Htt specifically binds to the C-terminal cytosolic region of $InsP_3R1$ (IC10 fragment), they hypothesized that introduction of the IC10 peptide into neurons would normalize Ca^{2+} signaling and eventual neurodegeneration by interfering with the abnormal interaction between mutant Htt and $InsP_3R1$[48]. They indeed found that viral vector-mediated expression of the IC10 peptide effectively stabilized neuronal Ca^{2+} signaling, improved motor dysfunctions and reduced neuronal loss in a mouse model of HD [68].

CaM-peptide

Mutant Htt also associates with calmodulin (CaM) with a higher affinity than wild-type Htt, and this interaction facilitates a wide range of downstream cellular functions. Muma and coworkers prepared several deletion mutants of CaM and found that a fragment corresponding to 76-121 amino acids of CaM (CaM-peptide) is responsible for binding with mutant Htt [69]. They demonstrated that expression of CaM-peptide reduced cytotoxicity by disrupting the abnormal interaction between endogenous CaM and mutant Htt in cellular models [69, 70] and improved disease phenotypes including body weight loss and motor dysfunctions in a mouse model of HD [71]. The studies on both IC10 and CaM peptides strongly indicate that abnormal interactions of the expanded polyQ proteins is critical for polyQ disease pathogenesis, and that molecules targeting these abnormal interactions may be promising lead compounds for polyQ disease treatment.

BIP

It has been reported that expanded polyQ proteins directly induce apoptosis. Mutant Htt with expanded polyQ stretch was shown to activate p53 and increase the expression level of Bax, a proapoptotic member of the Bcl-2 family of proteins that play a key role in programmed cell death in neurons [72]. Similarly, activation of Bax and subsequent cell death has also been shown in cells expressing polyQ-expanded ataxin-3 and ataxin-7, causative proteins of SCA3 and SCA7, respectively [73, 74]. Matsuyama, Yokota and coworkers found that the proapoptotic activity of Bax is normally suppressed by Ku70, a cytoprotective protein that interacts with Bax and prevents its mitochondrial translocation, while in SCA3, mutant ataxin-3 abnormally stimulates the acetylation of Ku70, which results in dissociation of Bax from Ku70 and promotes the subsequent activation of apoptosis [75]. Importantly, expression of Ku70 effectively blocked mutant ataxin-3-induced cell death, which strongly indicates that approaches targeting the activation process of Bax could be effective for suppression of the eventual apoptosis induced by expanded polyQ proteins [75]. To develop peptide-based suppressors of Bax-induced apoptosis, they identified the Bax-binding domain of Ku70 and designed a penta-peptide, Bax-inhibiting peptide (BIP) derived from this domain [76]. BIP is particularly promising since this is cell-permeable and effectively suppresses the mitochondrial translocation of Bax and subsequent apoptotic cell death [75].

Exendin-4

Although the polyQ diseases are considered primarily as neurological disorders, patients also exhibit peripheral symptoms. In HD, it is known that patients suffer from various metabolic abnormalities including progressive weight loss, appetite dysfunction and poor glycemic control [77-80]. Similarly, mouse models of HD also exhibit these symptoms, together with impaired glucose metabolism in both brain and periphery and elevated blood glucose levels [81, 82]. This is probably due to the significant toxicity caused by high levels of mutant Htt in peripheral tissues including the pancreatic islet cells, leading to decrease in β-cell mass and impaired insulin release capacity [81, 82]. Since molecules that improve abnormal energy metabolism such as creatine have been shown to work as a neuroprotective agent and to delay the onset of motor dysfunction in a mouse model of HD [83], therapeutic approaches targeting this diabetic-like condition may be promising. Exendin-4 (Ex-4) is an agonist for glucagon-like peptide-1 receptor, and is used as a peptide drug for diabetes to improve glucose regulation [84]. Martin *et al.* tested the effects of Ex-4 on a mouse model of HD, and found that daily administration of Ex-4 by subcutaneous injection improved motor dysfunction and extended the life span of HD mice [85]. They also found that Ex-4 injection significantly promoted pancreatic β-cell growth and reduced Htt aggregates in the pancreas as well as in the brain cortex [85]. This study strongly indicates that therapeutic approaches targeting not only the central pathophysiologies but also the peripheral symptoms could be an effective strategy for treatment of the polyQ diseases.

Leuprorelin

SBMA is an adult-onset motor neuron disease, which is caused by the expansion of a polyQ stretch in the androgen receptor (AR) [86]. Although the specific pathogenic mechanisms of SBMA still remain unclear, the nuclear accumulation of abnormal AR proteins is thought to be respon-

sible for neuronal toxicity. Since testosterone binds to AR as a ligand and induces its nuclear translocation, reduction of the testosterone level would lead to a decrease in the eventual nuclear accumulation of mutant AR and to lower cytotoxicity [87, 88]. This idea is actually supported by the experimental fact that surgical castration significantly improved motor dysfunctions of a mouse model of SBMA [87, 89]. Katsuno *et al.* tested the effects of leuprorelin, a lutenizing hormone-releasing hormone (LHRH) peptide agonist that reduces testosterone release from the testis, and found that subcutaneous injection of leuprorelin reduced the nuclear accumulation of mutant AR in muscle and spinal cord, and improved the motor dysfunctions and extended the life span of a SBMA mouse model [90]. Furthermore, they conducted a series of clinical trials of leuprorelin including a randomized, placebo-controlled trial in a large cohort of 204 SBMA patients from 14 hospitals in Japan [91, 92]. Although clinical outcomes of leuprorelin administration for 48 weeks were limited to suppression of nuclear AR accumulation and decreased serum levels of testosterone with no significant improvement of motor functions, there is a possibility that leuprorelin could be effective in long-term trials in early-phase SBMA patients.

FUTURE DIRECTIONS

In this review, we introduced selected studies focusing on the development of peptide-based therapies for treatment of the polyQ diseases. Among them, the therapeutic approach focusing on aggregate formation of the expanded polyQ stretch, which targets the most upstream change occurring in the polyQ diseases, is considered to be most attractive because potential inhibitors are expected to suppress a large number of downstream functional abnormalities in a broad range of the polyQ diseases. However, efficient delivery into brains is always problematic in developing peptide-based drugs [93], as aggregation inhibitors developed by us [63] and by Chen *et al.* [64] both failed to demonstrate therapeutic effects on mouse models via their subcutaneous or intraperitoneal administration. Since both QBP1 and HQP09 have been shown to possess high potential to specifically and selectively suppress mutant polyQ-induced cytotoxicity, it is highly likely that they would be promising leads for development of polyQ disease drugs if given the ability to efficiently translocate across the blood-brain barrier (BBB). Therefore, it is quite clear that one of the future directions that we should progress toward is to re-design these potential peptide inhibitors into BBB-permeable molecules. Elucidating the structural basis as to how QBP1 and HQP09 inhibit aggregate formation of the expanded polyQ stretch would be helpful towards designing their small chemical analogues with high BBB permeability without loss of its inhibitory activity. Another direction is to develop effective delivery systems using carrier molecules which would efficiently deliver cargoes to the brain. Potential carriers include cell-penetrating peptides (CPPs, protein transduction domains/PTDs) [94-96], viral vectors [97, 98] and liposomes [99, 100], which may enable these peptide inhibitors to translocate through the BBB and to perform their therapeutic activities in specific regions of the brain. We hope that in the near future therapeutic approaches that are widely effective against the polyQ diseases are developed, and bring hope to many patients suffering from the currently untreatable polyQ diseases.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

We thank James R. Burke, Warren J. Strittmatter, Shinya Oishi and Nobutaka Fujii for their helpful discussions. The authors' work on the polyglutamine diseases is supported in part by Grants-in-Aid for Scientific Research on Priority Areas (Advanced Brain Science Project, and Research on Pathomechanisms of Brain Disorders to Y.N.) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan; by Grants-in-Aid for Scientific Research (B) (to Y.N.) and Challenging Exploratory Research (to Y.N.) from the Japan Society for the Promotion of Science (JSPS), Japan; by a Grant-in-Aid for the Research Committee for Ataxic Diseases (to Y.N.) from the Ministry of Health, Labor and Welfare, Japan; and by a grant from Core Research for Evolutional Science and Technology (CREST) of the Japan Science and Technology Agency (to Y.N.). T.T. is grateful for the JSPS research fellowship.

ABBREVIATIONS

AR	=	Androgen receptor
Bax	=	Bcl-2 associated X protein
BBB	=	Blood-brain barrier
Bcl2	=	B-cell lymphoma 2
BIP	=	Bax-inhibiting peptide
CaM	=	Calmodulin
Ex-4	=	Exendin-4
HD	=	Huntington's disease
Htt	=	Huntingtin
$InsP_3R1$	=	Type 1 inositol 1,4,5-triphosphate receptor
K_d	=	Dissociation constant
LHRH	=	Lutenizing hormone-releasing hormone
MW	=	Molecular weight
PolyQ	=	Polyglutamine
PTD	=	Protein transduction domain
QBP1	=	PolyQ binding peptide 1
SBMA	=	Spinal and bulbar muscular atrophy
SCA	=	Spinocerebeller ataxia

REFERENCES

[1] Gusella, J.F.; MacDonald, M.E. Molecular genetics: unmasking polyglutamine triggers in neurodegenerative disease. *Nat. Rev. Neurosci.,* **2000**, *1*, 109-115.

[2] Orr, H.T.; Zoghbi, H.Y. Trinucleotide repeat disorders. *Annu. Rev. Neurosci.,* **2007**, *30*, 575-621.

[3] Nagai, Y.; Popiel, H.A. Conformational changes and aggregation of expanded polyglutamine proteins as therapeutic targets of the

polyglutamine diseases: exposed beta-sheet hypothesis. *Curr. Pharm. Des.,* **2008**, *14*, 3267-3279.

[4] The Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell,* **1993**, *72*, 971-983.

[5] Doyu, M.; Sobue, G.; Mukai, E.; Kachi, T.; Yasuda, T.; Mitsuma, T.; Takahashi, A. Severity of X-linked recessive bulbospinal neuronopathy correlates with size of the tandem CAG repeat in androgen receptor gene. *Ann. Neurol.,* **1992**, *32*, 707-710.

[6] Igarashi, S.; Tanno, Y.; Onodera, O.; Yamazaki, M.; Sato, S.; Ishikawa, A.; Miyatani, N.; Nagashima, M.; Ishikawa, Y.; Sahashi, K.; *et al.* Strong correlation between the number of CAG repeats in androgen receptor genes and the clinical onset of features of spinal and bulbar muscular atrophy. *Neurology,* **1992**, *42*, 2300-2302.

[7] Mangiarini, L.; Sathasivam, K.; Seller, M.; Cozens, B.; Harper, A.; Hetherington, C.; Lawton, M.; Trottier, Y.; Lehrach, H.; Davies, S. W.; Bates, G. P. Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell,* **1996**, *87*, 493-506.

[8] Ikeda, H.; Yamaguchi, M.; Sugai, S.; Aze, Y.; Narumiya, S.; Kakizuka, A. Expanded polyglutamine in the Machado-Joseph disease protein induces cell death *in vitro* and *in vivo*. *Nat. Genet.,* **1996**, *13*, 196-202.

[9] Burright, E.N.; Clark, H.B.; Servadio, A.; Matilla, T.; Feddersen, R.M.; Yunis, W.S.; Duvick, L.A.; Zoghbi, H.Y.; Orr, H.T. SCA1 transgenic mice: a model for neurodegeneration caused by an expanded CAG trinucleotide repeat. *Cell,* **1995**, *82*, 937-948.

[10] Warrick, J.M.; Paulson, H.L.; Gray-Board, G.L.; Bui, Q.T.; Fischbeck, K.H.; Pittman, R.N.; Bonini, N.M. Expanded polyglutamine protein forms nuclear inclusions and causes neural degeneration in Drosophila. *Cell,* **1998**, *93*, 939-949.

[11] Faber, P.W.; Alter, J.R.; MacDonald, M.E.; Hart, A.C. Polyglutamine-mediated dysfunction and apoptotic death of a Caenorhabditis elegans sensory neuron. *Proc. Natl. Acad. Sci. USA,* **1999**, *96*, 179-184.

[12] Ross, C.A. Polyglutamine pathogenesis: emergence of unifying mechanisms for Huntington's disease and related disorders. *Neuron,* **2002**, *35*, 819-822.

[13] Shao, J.; Diamond, M.I. Polyglutamine diseases: emerging concepts in pathogenesis and therapy. *Hum. Mol. Genet.,* **2007**, *16 Spec No. 2*, R115-R123.

[14] Scherzinger, E.; Lurz, R.; Turmaine, M.; Mangiarini, L.; Hollenbach, B.; Hasenbank, R.; Bates, G. P.; Davies, S.W.; Lehrach, H.; Wanker, E.E. Huntingtin-encoded polyglutamine expansions form amyloid-like protein aggregates *in vitro* and *in vivo*. *Cell,* **1997**, *90*, 549-558.

[15] Scherzinger, E.; Sittler, A.; Schweiger, K.; Heiser, V.; Lurz, R.; Hasenbank, R.; Bates, G.P.; Lehrach, H.; Wanker, E.E. Self-assembly of polyglutamine-containing huntingtin fragments into amyloid-like fibrils: implications for Huntington's disease pathology. *Proc. Natl. Acad. Sci. USA,* **1999**, *96*, 4604-4609.

[16] Chen, S.; Berthelier, V.; Yang, W.; Wetzel, R. Polyglutamine aggregation behavior *in vitro* supports a recruitment mechanism of cytotoxicity. *J. Mol. Biol.,* **2001**, *311*, 173-182.

[17] Yang, W.; Dunlap, J.R.; Andrews, R.B.; Wetzel, R. Aggregated polyglutamine peptides delivered to nuclei are toxic to mammalian cells. *Hum. Mol. Genet.,* **2002**, *11*, 2905-2917.

[18] Wetzel, R. Physical chemistry of polyglutamine: intriguing tales of a monotonous sequence. *J. Mol. Biol.,* **2012**, *421*, 466-490.

[19] Kuemmerle, S.; Gutekunst, C.A.; Klein, A.M.; Li, X.J.; Li, S.H.; Beal, M.F.; Hersch, S.M.; Ferrante, R.J. Huntington aggregates may not predict neuronal death in Huntington's disease. *Ann. Neurol.,* **1999**, *46*, 842-849.

[20] Klement, I.A.; Skinner, P.J.; Kaytor, M.D.; Yi, H.; Hersch, S.M.; Clark, H.B.; Zoghbi, H.Y.; Orr, H.T. Ataxin-1 nuclear localization and aggregation: role in polyglutamine-induced disease in SCA1 transgenic mice. *Cell,* **1998**, *95*, 41-53.

[21] Saudou, F.; Finkbeiner, S.; Devys, D.; Greenberg, M.E. Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. *Cell,* **1998**, *95*, 55-66.

[22] Arrasate, M.; Mitra, S.; Schweitzer, E.S.; Segal, M.R.; Finkbeiner, S. Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature,* **2004**, *431*, 805-810.

[23] Nagai, Y.; Inui, T.; Popiel, H.A.; Fujikake, N.; Hasegawa, K.; Urade, Y.; Goto, Y.; Naiki, H.; Toda, T. A toxic monomeric conformer of the polyglutamine protein. *Nat. Struct. Mol. Biol.,* **2007**, *14*, 332-340.

[24] Cummings, C.J.; Mancini, M.A.; Antalffy, B.; DeFranco, D.B.; Orr, H.T.; Zoghbi, H.Y. Chaperone suppression of aggregation and altered subcellular proteasome localization imply protein misfolding in SCA1. *Nat. Genet.,* **1998**, *19*, 148-154.

[25] Warrick, J.M.; Chan, H.Y.; Gray-Board, G.L.; Chai, Y.; Paulson, H.L.; Bonini, N.M. Suppression of polyglutamine-mediated neurodegeneration in Drosophila by the molecular chaperone HSP70. *Nat. Genet.,* **1999**, *23*, 425-428.

[26] Sittler, A.; Lurz, R.; Lueder, G.; Priller, J.; Lehrach, H.; Hayer-Hartl, M.K.; Hartl, F.U.; Wanker, E.E. Geldanamycin activates a heat shock response and inhibits huntingtin aggregation in a cell culture model of Huntington's disease. *Hum. Mol. Genet.,* **2001**, *10*, 1307-1315.

[27] Katsuno, M.; Sang, C.; Adachi, H.; Minamiyama, M.; Waza, M.; Tanaka, F.; Doyu, M.; Sobue, G. Pharmacological induction of heat-shock proteins alleviates polyglutamine-mediated motor neuron disease. *Proc. Natl. Acad. Sci. USA,* **2005**, *102*, 16801-16806.

[28] Fujikake, N.; Nagai, Y.; Popiel, H.A.; Okamoto, Y.; Yamaguchi, M.; Toda, T. Heat shock transcription factor 1-activating compounds suppress polyglutamine-induced neurodegeneration through induction of multiple molecular chaperones. *J. Biol. Chem.,* **2008**, *283*, 26188-26197.

[29] Lecerf, J.M.; Shirley, T.L.; Zhu, Q.; Kazantsev, A.; Amersdorfer, P.; Housman, D.E.; Messer, A.; Huston, J.S. Human single-chain Fv intrabodies counteract *in situ* huntingtin aggregation in cellular models of Huntington's disease. *Proc. Natl. Acad. Sci. USA,* **2001**, *98*, 4764-4769.

[30] Colby, D.W.; Chu, Y.; Cassady, J.P.; Duennwald, M.; Zazulak, H.; Webster, J.M.; Messer, A.; Lindquist, S.; Ingram, V.M.; Wittrup, K.D. Potent inhibition of huntingtin aggregation and cytotoxicity by a disulfide bond-free single-domain intracellular antibody. *Proc. Natl. Acad. Sci. USA,* **2004**, *101*, 17616-17621.

[31] Wolfgang, W.J.; Miller, T.W.; Webster, J.M.; Huston, J.S.; Thompson, L.M.; Marsh, J.L.; Messer, A. *Proc. Natl. Acad. Sci. USA,* **2005**, *102*, 11563-11568.

[32] Nagai, Y.; Fujikake, N.; Popiel, H.A.; Wada, K. Induction of molecular chaperones as a therapeutic strategy for the polyglutamine diseases. *Curr. Pharm. Biotechnol.,* **2010**, *11*, 188-197.

[33] Butler, D.C.; McLear, J.A.; Messer, A. Engineered antibody therapies to counteract mutant huntingtin and related toxic intracellular proteins. *Prog. Neurobiol.,* **2012**, *97*, 190-204.

[34] Heiser, V.; Scherzinger, E.; Boeddrich, A.; Nordhoff, E.; Lurz, R.; Schugardt, N.; Lehrach, H.; Wanker, E.E. Inhibition of huntingtin fibrillogenesis by specific antibodies and small molecules: implications for Huntington's disease therapy. *Proc. Natl. Acad. Sci. USA,* **2000**, *97*, 6739-6744.

[35] Heiser, V.; Engemann, S.; Brocker, W.; Dunkel, I.; Boeddrich, A.; Waelter, S.; Nordhoff, E.; Lurz, R.; Schugardt, N.; Rautenberg, S.; Herhaus, C.; Barnickel, G.; Bottcher, H.; Lehrach, H.; Wanker, E.E. Identification of benzothiazoles as potential polyglutamine aggregation inhibitors of Huntington's disease by using an automated filter retardation assay. *Proc. Natl. Acad. Sci. USA,* **2002**, *99 Suppl 4*, 16400-16406.

[36] Tanaka, M.; Machida, Y.; Niu, S.; Ikeda, T.; Jana, N.R.; Doi, H.; Kurosawa, M.; Nekooki, M.; Nukina, N. Trehalose alleviates polyglutamine-mediated pathology in a mouse model of Huntington disease. *Nat. Med.,* **2004**, *10*, 148-154.

[37] Wang, J.; Gines, S.; MacDonald, M.E.; Gusella, J.F. Reversal of a full-length mutant huntingtin neuronal cell phenotype by chemical inhibitors of polyglutamine-mediated aggregation. *BMC. Neurosci.,* **2005**, *6*, 1.

[38] Ehrnhoefer, D.E.; Duennwald, M.; Markovic, P.; Wacker, J.L.; Engemann, S.; Roark, M.; Legleiter, J.; Marsh, J.L.; Thompson, L.M.; Lindquist, S.; Muchowski, P.J.; Wanker, E.E. Green tea (-)-epigallocatechin-gallate modulates early events in huntingtin misfolding and reduces toxicity in Huntington's disease models. *Hum. Mol. Genet.,* **2006**, *15*, 2743-2751.

[39] Nagai, Y.; Tucker, T.; Ren, H.; Kenan, D.J.; Henderson, B.S.; Keene, J.D.; Strittmatter, W.J.; Burke, J.R. Inhibition of polyglutamine protein aggregation and cell death by novel peptides identified by phage display screening. *J. Biol. Chem.,* **2000**, *275*, 10437-10442.

[40] Kazantsev, A.; Walker, H.A.; Slepko, N.; Bear, J.E.; Preisinger, E.; Steffan, J.S.; Zhu, Y.Z.; Gertler, F.B.; Housman, D.E.; Marsh, J.L.; Thompson, L.M. A bivalent Huntingtin binding peptide suppresses polyglutamine aggregation and pathogenesis in Drosophila. *Nat. Genet.,* **2002**, *30*, 367-376.

[41] Popiel, H.A.; Nagai, Y.; Onodera, O.; Inui, T.; Fujikake, N.; Urade, Y.; Strittmatter, W.J.; Burke, J.R.; Ichikawa, A.; Toda, T. Disruption of the toxic conformation of the expanded polyglutamine stretch leads to suppression of aggregate formation and cytotoxicity. *Biochem. Biophys. Res. Commun.,* **2004**, *317*, 1200-1206.

[42] Bauer, P.O.; Goswami, A.; Wong, H.K.; Okuno, M.; Kurosawa, M.; Yamada, M.; Miyazaki, H.; Matsumoto, G.; Kino, Y.; Nagai, Y.; Nukina, N. Harnessing chaperone-mediated autophagy for the selective degradation of mutant huntingtin protein. *Nat. Biotechnol.,* **2010**, *28*, 256-263.

[43] Shoji-Kawata, S.; Sumpter, R.; Leveno, M.; Campbell, G.R.; Zou, Z.; Kinch, L.; Wilkins, A.D.; Sun, Q.; Pallauf, K.; MacDuff, D.; Huerta, C.; Virgin, H.W.; Helms, J.B.; Eerland, R.; Tooze, S.A.; Xavier, R.; Lenschow, D.J.; Yamamoto, A.; King, D.; Lichtarge, O.; Grishin, N.V.; Spector, S.A.; Kaloyanova, D.V.; Levine, B. Identification of a candidate therapeutic autophagy-inducing peptide. *Nature,* **2013**, *494*, 201-206.

[44] Steffan, J.S.; Kazantsev, A.; Spasic-Boskovic, O.; Greenwald, M.; Zhu, Y.Z.; Gohler, H.; Wanker, E.E.; Bates, G.P.; Housman, D.E.; Thompson, L.M. The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription. *Proc. Natl. Acad. Sci. USA,* **2000**, *97*, 6763-6768.

[45] Bence, N.F.; Sampat, R.M.; Kopito, R.R. Impairment of the ubiquitin-proteasome system by protein aggregation. *Science,* **2001**, *292*, 1552-1555.

[46] Romero, E.; Cha, G.H.; Verstreken, P.; Ly, C.V.; Hughes, R.E.; Bellen, H.J.; Botas, J. Suppression of neurodegeneration and increased neurotransmission caused by expanded full-length huntingtin accumulating in the cytoplasm. *Neuron,* **2008**, *57*, 27-40.

[47] Gunawardena, S.; Her, L.S.; Brusch, R.G.; Laymon, R.A.; Niesman, I.R.; Gordesky-Gold, B.; Sintasath, L.; Bonini, N.M.; Goldstein, L.S. Disruption of axonal transport by loss of huntingtin or expression of pathogenic polyQ proteins in Drosophila. *Neuron,* **2003**, *40*, 25-40.

[48] Tang, T.S.; Tu, H.; Chan, E.Y.; Maximov, A.; Wang, Z.; Wellington, C.L.; Hayden, M.R.; Bezprozvanny, I. Huntingtin and huntingtin-associated protein 1 influence neuronal calcium signaling mediated by inositol-(1,4,5) triphosphate receptor type 1. *Neuron,* **2003**, *39*, 227-239.

[49] Tobin, A.J.; Signer, E.R. Huntington's disease: the challenge for cell biologists. *Trends Cell Biol.,* **2000**, *10*, 531-536.

[50] Harjes, P.; Wanker, E.E. The hunt for huntingtin function: interaction partners tell many different stories. *Trends Biochem. Sci.,* **2003**, *28*, 425-433.

[51] Trottier, Y.; Lutz, Y.; Stevanin, G.; Imbert, G.; Devys, D.; Cancel, G.; Saudou, F.; Weber, C.; David, G.; Tora, L.; Agid, Y.; Brice, A.; Mandel, J. Polyglutamine expansion as a pathological epitope in Huntington's disease and four dominant cerebellar ataxias. *Nature,* **1995**, *378*, 403-406.

[52] Bennett, M.J.; Huey-Tubman, K.E.; Herr, A.B.; West, A.P., Jr.; Ross, S.A.; Bjorkman, P.J. A linear lattice model for polyglutamine in CAG-expansion diseases. *Proc. Natl. Acad. Sci. USA,* **2002**, *99*, 11634-11639.

[53] Miller, J.; Arrasate, M.; Brooks, E.; Libeu, C.P.; Legleiter, J.; Hatters, D.; Curtis, J.; Cheung, K.; Krishnan, P.; Mitra, S.; Widjaja, K.; Shaby, B.A.; Lotz, G.P.; Newhouse, Y.; Mitchell, E.J.; Osmand, A.; Gray, M.; Thulasiramin, V.; Saudou, F.; Segal, M.; Yang, X.W.; Masliah, E.; Thompson, L.M.; Muchowski, P.J.; Weisgraber, K.H.; Finkbeiner, S. Identifying polyglutamine protein species in situ that best predict neurodegeneration. *Nat. Chem. Biol.,* **2011**, *7*, 925-934.

[54] Okamoto, Y.; Nagai, Y.; Fujikake, N.; Popiel, H.A.; Yoshioka, T.; Toda, T.; Inui, T. Surface plasmon resonance characterization of specific binding of polyglutamine aggregation inhibitors to the expanded polyglutamine stretch. *Biochem. Biophys. Res. Commun.,* **2009**, *378*, 634-639.

[55] Ren, H.; Nagai, Y.; Tucker, T.; Strittmatter, W.J.; Burke, J.R. Amino acid sequence requirements of peptides that inhibit polyglutamine-protein aggregation and cell death. *Biochem. Biophys. Res. Commun.,* **2001**, *288*, 703-710.

[56] Tomita, K.; Popiel, H.A.; Nagai, Y.; Toda, T.; Yoshimitsu, Y.; Ohno, H.; Oishi, S.; Fujii, N. Structure-activity relationship study on polyglutamine binding peptide QBP1. *Bioorg. Med. Chem.,* **2009**, *17*, 1259-1263.

[57] Hamuro, L.; Zhang, G.; Tucker, T.J.; Self, C.; Strittmatter, W.J.; Burke, J.R. Optimization of a polyglutamine aggregation inhibitor peptide (QBP1) using a thioflavin T fluorescence assay. *Assay Drug Dev. Technol.,* **2007**, *5*, 629-636.

[58] Takahashi, Y.; Okamoto, Y.; Popiel, H.A.; Fujikake, N.; Toda, T.; Kinjo, M.; Nagai, Y. Detection of polyglutamine protein oligomers in cells by fluorescence correlation spectroscopy. *J. Biol. Chem.,* **2007**, *282*, 24039-24048.

[59] Takahashi, T.; Kikuchi, S.; Katada, S.; Nagai, Y.; Nishizawa, M.; Onodera, O. Soluble polyglutamine oligomers formed prior to inclusion body formation are cytotoxic. *Hum. Mol. Genet.,* **2008**, *17*, 345-356.

[60] Nagai, Y.; Fujikake, N.; Ohno, K.; Higashiyama, H.; Popiel, H.A.; Rahadian, J.; Yamaguchi, M.; Strittmatter, W.J.; Burke, J.R.; Toda, T. Prevention of polyglutamine oligomerization and neurodegeneration by the peptide inhibitor QBP1 in Drosophila. *Hum. Mol. Genet.,* **2003**, *12*, 1253-1259.

[61] Futaki, S.; Hirose, H.; Nakase, I. Arginine-rich peptides: methods of translocation through biological membranes. *Curr. Pharm. Des.,* **2012**, *19*, 2863-2868.

[62] Popiel, H.A.; Nagai, Y.; Fujikake, N.; Toda, T. Protein transduction domain-mediated delivery of QBP1 suppresses polyglutamine-induced neurodegeneration in vivo. *Mol. Ther.,* **2007**, *15*, 303-309.

[63] Popiel, H.A.; Nagai, Y.; Fujikake, N.; Toda, T. Delivery of the aggregate inhibitor peptide QBP1 into the mouse brain using PTDs and its therapeutic effect on polyglutamine disease mice. *Neurosci. Lett.,* **2009**, *449*, 87-92.

[64] Chen, X.; Wu, J.; Luo, Y.; Liang, X.; Supnet, C.; Kim, M.W.; Lotz, G.P.; Yang, G.; Muchowski, P.J.; Kodadek, T.; Bezprozvanny, I. Expanded polyglutamine-binding peptoid as a novel therapeutic agent for treatment of Huntington's disease. *Chem. Biol.,* **2011**, *18*, 1113-1125.

[65] Simon, R.J.; Kania, R.S.; Zuckermann, R.N.; Huebner, V.D.; Jewell, D.A.; Banville, S.; Ng, S.; Wang, L.; Rosenberg, S.; Marlowe, C.K.; Spellmeyer, D.C.; Tan, R.; Frankel, A.D.; Santi, D.V.; Cohen, F.E.; Bartlett, P.A. Peptoids: a modular approach to drug discovery. *Proc. Natl. Acad. Sci. USA,* **1992**, *89*, 9367-9371.

[66] Zuckermann, R.N.; Kodadek, T. Peptoids as potential therapeutics. *Curr. Opin. Mol. Ther.,* **2009**, *11*, 299-307.

[67] Tang, T.S.; Tu, H.; Orban, P.C.; Chan, E.Y.; Hayden, M.R.; Bezprozvanny, I. HAP1 facilitates effects of mutant huntingtin on inositol 1,4,5-trisphosphate-induced Ca release in primary culture of striatal medium spiny neurons. *Eur. J. Neurosci.,* **2004**, *20*, 1779-1787.

[68] Tang, T.S.; Guo, C.; Wang, H.; Chen, X.; Bezprozvanny, I. Neuroprotective effects of inositol 1,4,5-trisphosphate receptor C-terminal fragment in a Huntington's disease mouse model. *J. Neurosci.,* **2009**, *29*, 1257-1266.

[69] Dudek, N.L.; Dai, Y.; Muma, N.A. Protective effects of interrupting the binding of calmodulin to mutant huntingtin. *J. Neuropathol. Exp. Neurol.,* **2008**, *67*, 355-365.

[70] Dudek, N.L.; Dai, Y.; Muma, N.A. Neuroprotective effects of calmodulin peptide 76-121aa: disruption of calmodulin binding to mutant huntingtin. *Brain Pathol.,* **2010**, *20*, 176-189.

[71] Dai, Y.; Dudek, N.L.; Li, Q.; Fowler, S.C.; Muma, N.A. Striatal expression of a calmodulin fragment improved motor function, weight loss, and neuropathology in the R6/2 mouse model of Huntington's disease. *J. Neurosci.,* **2009**, *29*, 11550-11559.

[72] Bae, B.I.; Xu, H.; Igarashi, S.; Fujimuro, M.; Agrawal, N.; Taya, Y.; Hayward, S.D.; Moran, T.H.; Montell, C.; Ross, C.A.; Snyder, S.H.; Sawa, A. p53 mediates cellular dysfunction and behavioral abnormalities in Huntington's disease. *Neuron,* **2005**, *47*, 29-41.

[73] Chou, A.H.; Yeh, T.H.; Kuo, Y.L.; Kao, Y.C.; Jou, M.J.; Hsu, C.Y.; Tsai, S.R.; Kakizuka, A.; Wang, H.L. Polyglutamine-expanded ataxin-3 activates mitochondrial apoptotic pathway by upregulating Bax and downregulating Bcl-xL. *Neurobiol. Dis.,* **2006**, *21*, 333-345.

[74] Wang, H.L.; Yeh, T.H.; Chou, A.H.; Kuo, Y.L.; Luo, L.J.; He, C.Y.; Huang, P.C.; Li, A.H. Polyglutamine-expanded ataxin-7 activates mitochondrial apoptotic pathway of cerebellar neurons by

upregulating Bax and downregulating Bcl-x(L). *Cell. Signal.,* **2006**, *18*, 541-552.

[75] Li, Y.; Yokota, T.; Gama, V.; Yoshida, T.; Gomez, J.A.; Ishikawa, K.; Sasaguri, H.; Cohen, H.Y.; Sinclair, D.A.; Mizusawa, H.; Matsuyama, S. Bax-inhibiting peptide protects cells from polyglutamine toxicity caused by Ku70 acetylation. *Cell Death Differ.,* **2007**, *14*, 2058-2067.

[76] Yoshida, T.; Tomioka, I.; Nagahara, T.; Holyst, T.; Sawada, M.; Hayes, P.; Gama, V.; Okuno, M.; Chen, Y.; Abe, Y.; Kanouchi, T.; Sasada, H.; Wang, D.; Yokota, T.; Sato, E.; Matsuyama, S. Bax-inhibiting peptide derived from mouse and rat Ku70. *Biochem. Biophys. Res. Commun.,* **2004**, *321*, 961-966.

[77] Aziz, N.A.; Swaab, D.F.; Pijl, H.; Roos, R.A. Hypothalamic dysfunction and neuroendocrine and metabolic alterations in Huntington's disease: clinical consequences and therapeutic implications. *Rev. Neurosci.,* **2007**, *18*, 223-251.

[78] Powers, W.J.; Videen, T.O.; Markham, J.; McGee-Minnich, L.; Antenor-Dorsey, J.V.; Hershey, T.; Perlmutter, J.S. Selective defect of in vivo glycolysis in early Huntington's disease striatum. *Proc. Natl. Acad. Sci. USA,* **2007**, *104*, 2945-2949.

[79] Gaba, A.M.; Zhang, K.; Marder, K.; Moskowitz, C.B.; Werner, P.; Boozer, C.N. Energy balance in early-stage Huntington disease. *Am. J. Clin. Nutr.,* **2005**, *81*, 1335-1341.

[80] Lodi, R.; Schapira, A.H.; Manners, D.; Styles, P.; Wood, N.W.; Taylor, D.J.; Warner, T.T. Abnormal in vivo skeletal muscle energy metabolism in Huntington's disease and dentatorubropallidoluysian atrophy. *Ann. Neurol.,* **2000**, *48*, 72-76.

[81] Hurlbert, M.S.; Zhou, W.; Wasmeier, C.; Kaddis, F.G.; Hutton, J.C.; Freed, C.R. Mice transgenic for an expanded CAG repeat in the Huntington's disease gene develop diabetes. *Diabetes,* **1999**, *48*, 649-651.

[82] Bjorkqvist, M.; Fex, M.; Renstrom, E.; Wierup, N.; Petersen, A.; Gil, J.; Bacos, K.; Popovic, N.; Li, J.Y.; Sundler, F.; Brundin, P.; Mulder, H. The R6/2 transgenic mouse model of Huntington's disease develops diabetes due to deficient beta-cell mass and exocytosis. *Hum. Mol. Genet.,* **2005**, *14*, 565-574.

[83] Ferrante, R.J.; Andreassen, O.A.; Jenkins, B.G.; Dedeoglu, A.; Kuemmerle, S.; Kubilus, J.K.; Kaddurah-Daouk, R.; Hersch, S.M.; Beal, M.F. Neuroprotective effects of creatine in a transgenic mouse model of Huntington's disease. *J. Neurosci.,* **2000**, *20*, 4389-4397.

[84] Greig, N.H.; Holloway, H.W.; De Ore, K.A.; Jani, D.; Wang, Y.; Zhou, J.; Garant, M. J.; Egan, J.M. Once daily injection of exendin-4 to diabetic mice achieves long-term beneficial effects on blood glucose concentrations. *Diabetologia,* **1999**, *42*, 45-50.

[85] Martin, B.; Golden, E.; Carlson, O.D.; Pistell, P.; Zhou, J.; Kim, W.; Frank, B.P.; Thomas, S.; Chadwick, W.A.; Greig, N.H.; Bates, G.P.; Sathasivam, K.; Bernier, M.; Maudsley, S.; Mattson, M.P.; Egan, J.M. Exendin-4 improves glycemic control, ameliorates brain and pancreatic pathologies, and extends survival in a mouse model of Huntington's disease. *Diabetes,* **2009**, *58*, 318-328.

[86] Katsuno, M.; Tanaka, F.; Adachi, H.; Banno, H.; Suzuki, K.; Watanabe, H.; Sobue, G. Pathogenesis and therapy of spinal and bulbar muscular atrophy (SBMA). *Prog. Neurobiol.,* **2012**, *99*, 246-256.

[87] Katsuno, M.; Adachi, H.; Kume, A.; Li, M.; Nakagomi, Y.; Niwa, H.; Sang, C.; Kobayashi, Y.; Doyu, M.; Sobue, G. Testosterone reduction prevents phenotypic expression in a transgenic mouse model of spinal and bulbar muscular atrophy. *Neuron,* **2002**, *35*, 843-854.

[88] Takeyama, K.; Ito, S.; Yamamoto, A.; Tanimoto, H.; Furutani, T.; Kanuka, H.; Miura, M.; Tabata, T.; Kato, S. Androgen-dependent neurodegeneration by polyglutamine-expanded human androgen receptor in Drosophila. *Neuron,* **2002**, *35*, 855-864.

[89] Chevalier-Larsen, E.S.; O'Brien, C.J.; Wang, H.; Jenkins, S.C.; Holder, L.; Lieberman, A.P.; Merry, D.E. Castration restores function and neurofilament alterations of aged symptomatic males in a transgenic mouse model of spinal and bulbar muscular atrophy. *J. Neurosci.,* **2004**, *24*, 4778-4786.

[90] Katsuno, M.; Adachi, H.; Doyu, M.; Minamiyama, M.; Sang, C.; Kobayashi, Y.; Inukai, A.; Sobue, G. Leuprorelin rescues polyglutamine-dependent phenotypes in a transgenic mouse model of spinal and bulbar muscular atrophy. *Nat. Med.,* **2003**, *9*, 768-773.

[91] Banno, H.; Katsuno, M.; Suzuki, K.; Takeuchi, Y.; Kawashima, M.; Suga, N.; Takamori, M.; Ito, M.; Nakamura, T.; Matsuo, K.; Yamada, S.; Oki, Y.; Adachi, H.; Minamiyama, M.; Waza, M.; Atsuta, N.; Watanabe, H.; Fujimoto, Y.; Nakashima, T.; Tanaka, F.; Doyu, M.; Sobue, G. Phase 2 trial of leuprorelin in patients with spinal and bulbar muscular atrophy. *Ann. Neurol.,* **2009**, *65*, 140-150.

[92] Katsuno, M.; Banno, H.; Suzuki, K.; Takeuchi, Y.; Kawashima, M.; Yabe, I.; Sasaki, H.; Aoki, M.; Morita, M.; Nakano, I.; Kanai, K.; Ito, S.; Ishikawa, K.; Mizusawa, H.; Yamamoto, T.; Tsuji, S.; Hasegawa, K.; Shimohata, T.; Nishizawa, M.; Miyajima, H.; Kanda, F.; Watanabe, Y.; Nakashima, K.; Tsujino, A.; Yamashita, T.; Uchino, M.; Fujimoto, Y.; Tanaka, F.; Sobue, G. Efficacy and safety of leuprorelin in patients with spinal and bulbar muscular atrophy (JASMITT study): a multicentre, randomised, double-blind, placebo-controlled trial. *Lancet Neurol.,* **2010**, *9*, 875-884.

[93] Craik, D.J.; Fairlie, D.P.; Liras, S.; Price, D. The Future of Peptide-based Drugs. *Chem. Biol. Drug Des.,* **2013**, *81*, 136-147.

[94] Asoh, S.; Ohta, S. PTD-mediated delivery of anti-cell death proteins/peptides and therapeutic enzymes. *Adv. Drug Deliv. Rev.,* **2008**, *60*, 499-516.

[95] Blumling Iii, J.P.; Silva, G.A. Targeting the brain: advances in drug delivery. *Curr. Pharm. Biotechnol.,* **2012**, *13*, 2417-2426.

[96] Malakoutikhah, M.; Teixidó, M.; Giralt, E. Shuttle-mediated drug delivery to the brain. *Angew. Chem. Int. Ed.,* **2011**, *50*, 7998-8014.

[97] Salegio, E.A.; Samaranch, L.; Kells, A.P.; Forsayeth, J.; Bankiewicz, K. Guided delivery of adeno-associated viral vectors into the primate brain. *Adv. Drug Deliv. Rev.,* **2012**, *64*, 598-604.

[98] Hester, M.E.; Foust, K.D.; Kaspar, R.W.; Kaspar, B.K. AAV as a gene transfer vector for the treatment of neurological disorders: novel treatment thoughts for ALS. *Curr. Gene Ther.,* **2009**, *9*, 428-433.

[99] Garbayo, E.; Ansorena, E.; Blanco-Prieto, M.J. Brain drug delivery systems for neurodegenerative disorders. *Curr. Pharm. Biotechnol.,* **2012**, *13*, 2388-2402.

[100] Micheli, M.R.; Bova, R.; Magini, A.; Polidoro, M.; Emiliani, C. Lipid-based nanocarriers for CNS-targeted drug delivery. *Recent Pat. CNS Drug Discov.,* **2012**, *7*, 71-86.

Received: ???????????????? **Revised:** ???????????????? **Accepted:** ????????????????

その他のFTLD—異常蛋白の視点から

上山盛夫　藤掛伸宏　永井義隆

はじめに

前頭側頭葉変性症(FTLD：frontotemporal lobar degeneration)は前頭葉と側頭葉の神経が進行性に変性・脱落する神経変性疾患である．病理型としては，多くの神経細胞あるいはグリア細胞内に封入体が認められるFTLDと，封入体が認められないFTLD-ni(no inclusions)に大別され，封入体が認められるものは，蓄積する蛋白質によりFTLD-tau，FTLD-TDP，FTLD-FUS，およびFTLD-UPS(ubiquitin proteasome system)の4型に分類されている[1]．本稿ではFTLD-tau以外の封入体が認められるFTLD-Uについて異常蛋白質の視点から紹介する．また，近年FTLDの原因として同定された*C9orf72*遺伝子変異による異常蛋白質の蓄積についても最新の知見を紹介する．

うえやま もりお　(独)国立精神・神経医療研究センター神経研究所/疾病研究第四部
ふじかけ のぶひろ　同
ながい よしたか　同 室長

FTLD-TDP

タウ陰性でユビキチン陽性の封入体を伴ったFTLD-Uにおいて，封入体を構成する主要蛋白質は不明であったが，2006年にRNA結合蛋白質であるTAR DNA-binding protein of 43(TDP-43)が蓄積していることが明らかにされた[2,3]．FTLD-Uのうち TDP-43陽性封入体の病理所見を示すものはFTLD-TDPと分類され，FTLDの45%を占める[4]．さらに，筋萎縮性側索硬化症(ALS：amyotrophic lateral sclerosis)においても TDP-43が蓄積していることが明らかにされたことから，FTLDとALS両疾患の病態には共通の分子基盤があると考えられるようになった．TDP-43はFTLD-TDP患者脳内において疾患特異的なリン酸化や断片化を受けており，特にC末断片の凝集・蓄積が観察される[5,6]．TDP-43のC末端には凝集性に富むプリオン様ドメインが存在し(図1)，断片化により凝集性が増すことから，このC末断片が患者脳における凝集・蓄積

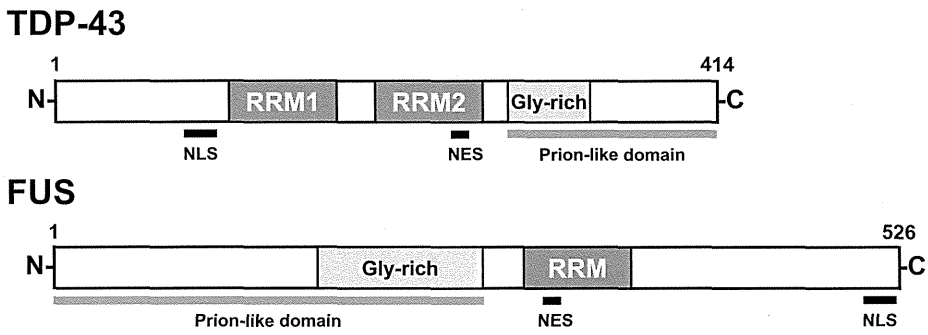


図1　TDP-43とFUSの蛋白質構造

TDP-43は414残基，FUSは526残基のアミノ酸からなり，その分子内にはRNA結合に関わるRNA認識モチーフ(RRM：RNA recognition motif)，蛋白質-蛋白質相互作用に関わるグリシンリッチ領域(Gly-rich)を含むプリオン様ドメイン(Prion-like domain)，核移行シグナル(NLS：nuclear localization signal)，および核外移行シグナル(NES：nuclear export signal)が存在する．

0289-0585/15/¥500/論文/JCOPY

に関与すると予測される．異常なTDP-43の凝集・蓄積メカニズムおよび細胞間伝播は未解明であったが，2013年にNonakaらは，患者脳由来の不溶性TDP-43を培養細胞内に導入すると，本来は可溶性であった細胞内のTDP-43が凝集体を形成することを示し，異常TDP-43の凝集性が正常TDP-43に伝播すること，さらにこのTDP-43の凝集性は細胞間でも伝播することを明らかにした．これらの結果から，患者脳の不溶性TDP-43はプリオン様の特性を保持していると結論付けている[7]．培養細胞を用いた*in vitro*の実験ではあるが，TDP-43の凝集・蓄積モデルを樹立し，細胞間伝播までを示した上記の研究は興味深いものであり，今後，動物モデルや患者病理の解析からTDP-43のプリオン様の性質獲得と病態の進行との関連性の解明が期待される．

FTLD-FUS

2008年に家族性ALS(ALS6)の原因としてFUS変異が同定され，ALS6患者の病理学的解析からFUSが神経細胞質内の封入体の構成成分であることが明らかにされた．続いて，TDP-43陰性封入体を伴ったaFTLD-U(atypical FTLD-U)，NIFID(neuronal intermediate filament inclusion disease)，とBIBD(basophilic inclusion body disease)において，神経細胞およびグリア細胞の細胞質にFUS陽性の封入体が認められることが明らかになり[8~10]，これらはまとめてFTLD-FUSと分類された．RNA結合蛋白質であるFUSはTDP-43と同様に，分子内に凝集性に富むプリオン様ドメインを持っていることから(図1)，FTLD-TDPで認められている異常蛋白質の伝播がFTLD-FUSでも生じる可能性が示唆される．しかし，FUSが凝集・蓄積するメカニズムは未解明であり，今後の研究が必要である．

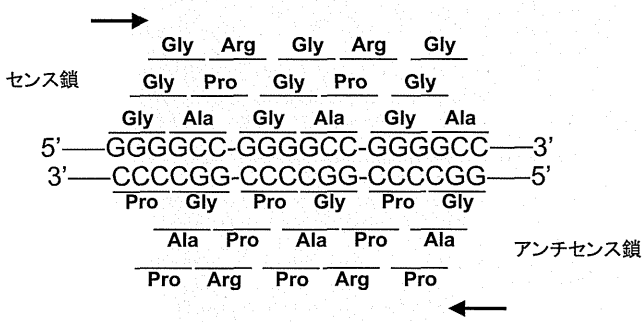


図2　*C9orf72*遺伝子GGGGCC異常伸長リピート由来のジペプチドリピート蛋白質

C9-FTLD/ALS患者において*C9orf72*遺伝子非翻訳領域の6塩基(GGGGCC)異常伸長リピート配列はセンス鎖およびアンチセンス鎖の両方向に転写される．さらに，ATGに依存しない翻訳(RANT：repeat-associated non-ATG translation)により5種類のジペプチドリピート(DPR：dipeptide repeat)蛋白質が産生される．Gly：グリシン，Arg：アルギニン，Pro：プロリン，Ala：アラニン

FTLD-UPS

FTLD-Uのうち TDP-43陰性およびFUS陰性で，未だその主要構成成分の不明な封入体を伴うものはFTLD-UPSと分類されている．そのうちの一部において*CHMP2B*(charged multivesicular body protein 2B)の遺伝子変異が明らかにされた[11]．CHMP2Bはエンドソーム輸送選別複合体を構成する分子で，リソソームにおけるユビキチン化蛋白質の分解に関与することが知られており，CHMP2Bの機能不全によりユビキチン化蛋白質が細胞質内に残留し，封入体を形成していると考えられる．

最近の話題 — C9-FTLD/ALS

2006年にALS-FTD家系の連鎖解析から第9番染色体に原因遺伝子座があると報告された．その遺伝子変異は長らく不明であったが，2011年にこの家族性ALS-FTDの原因遺伝子変異として染色体9p21領域に存在する*C9orf72*遺伝子の非翻訳領域に6塩基(GGGGCC)リピート配列の異常伸長が発見された[12,13]．この*C9orf72*遺伝子変異はヨーロッパおよび北米の家族性と孤発性FTLD/ALSの最も高頻度な発症原因であり，本邦でも紀伊半島のFTLD/ALS家系を中心に報告されている．*C9orf72*遺伝子変異によるFTLD/ALS(C9-FTLD/ALS)患者ではTDP-43陽性を示す封入体が脳の広範囲でみられ，FTLD-TDPに分類されているが，小脳，海馬，および前頭側頭新皮質ではTDP-43陰性の封入体が認められる[14]．C9-FTLD/ALSと同様な遺伝子非翻訳領域にあるリピート配列の異常伸長に起因するSCA8，DM1，およびFXTASなどの一群の疾患では，転写された異常伸長リピートRNAを鋳型として開始コドンATGに依存しない翻訳(RANT：repeat-associated non-ATG translation)により，

異常伸長したポリアミノ酸リピート蛋白質が産生されることが知られている．C9-FTLD/ALSにおいても，異常伸長GGGGCCリピートRNAからRANTにより翻訳されたポリ(グリシン-アルギニン)，ポリ(グリシン-プロリン)，ポリ(グリシン-アラニン)，ポリ(プロリン-アラニン)，およびポリ(プロリン-アルギニン)の5種類のジペプチドリピート(DPR：dipeptide repeat)蛋白質(図2)の存在が示され，これらがTDP-43陰性封入体に蓄積していることが明らかになった[15,16]．続いて，DPR蛋白質のうちポリ(グリシン-アラニン)，ポリ(グリシン-アルギニン)，およびポリ(プロリン-アルギニン)は，培養細胞に発現させると細胞死を引きおこすことが示され[17~19]，さらにショウジョウバエモデルにおいても，ポリ(グリシン-アルギニン)とポリ(プロリン-アルギニン)が神経変性を引きおこすことが示され[20]，DPR蛋白質の一部は神経細胞毒性を持つことが明らかにされた．以上のことから，DPR蛋白質の蓄積がC9-FTLD/ALS発症の原因として考えられるようになり，病理型として新たにFTLD/ALS-DPRとして分類することが提唱されている[15]．しかしながら，各DPRの細胞毒性はモデルによって一致しておらず，今後の検証が必要である．また，すべての封入体がDPR陽性ではなく，TDP-43陽性を示す封入体もみられる理由は不明であり，今後の研究が必要である．

むすび

近年の遺伝学的・生化学的研究の発展により，FTLDにおいて封入体を構成する主要蓄積蛋白質が次々と同定された．しかし，未だにFTLD-UPSにおいて蓄積する異常蛋白質は不明であり，蓄積蛋白質が解明されたFTLD-TDPやFTLD-FUSにおいても凝集・蓄積および伝播のメカニズムは様々な仮説が提唱されているが，多くは未解明である．今後，FTLD-TDPについては，細胞モデルを用いた研究から得られた異常TDP-43の凝集・蓄積および伝播に関する知見を，動物モデルで検証することが期待される．また，他のFTLDについてもFTLD-TDPの研究が起因となり，異常蛋白質の凝集・蓄積，伝播メカニズムの解明が進むことが期待されている．

文　献

1) Mackenzie IRA, Neumann M, Bigio EH, et al. Nomenclature and nosology for neuropathologic subtypes of frontotemporal lobar degeneration：an update. Acta Neuropathol. 2010；119：1-4.
2) Arai T, Hasegawa M, Akiyama H, et al. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Biochem Biophys Res Commun. 2006；351：602-11.
3) Neumann M, Sampathu DM, Kwong LK, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science. 2006；314：130-3.
4) Ling S-C, Polymenidou M, Cleaveland DW. Converging mechanisms in ALS and FTD：disrupted RNA and protein homeostasis. Neuron. 2013；79：416-38.
5) Hasegawa M, Arai T, Nonaka T, et al. Phosphorylated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Ann Neurol. 2008；64：60-70.
6) Igaz LM, Kwong LK, Xu Y, et al. Enrichment of C-terminal fragments in TAR DNA-binding protein-43 cytoplasmic inclusions in brain but not in spinal cord of frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Am J Pathol. 2008；173：182-94.
7) Nonaka T, Masuda-Suzukake M, Arai T, et al. Prion-like properties of pathological TDP-43 aggregates from diseased brains. Cell Rep. 2013；4：124-34.
8) Neumann M, Rademakers R, Roeber S, et al. A new subtype of frontotemporal lobar degeneration with FUS pathology. Brain. 2009；132：2922-31.
9) Neumann M, Roeber S, Kretzschmar HA, et al. Abundant FUS-immunoreactive pathology in neuronal intermediate filament inclusion disease. Acta Neuropathol. 2009；118：605-16.
10) Munoz DG, Neumann M, Kusaka H, et al. FUS pathology in basophilic inclusion body disease. Acta Neuropathol. 2009；118：617-27.
11) Holm IE, Isaacs AM, Mackenzie IRA. Absence of FUS-immunoreactive pathology in frontotemporal dementia linked to chromosome 3 (FTD-3) caused by mutation in the *CHMP2B* gene. Acta Neuropathol. 2009；118：719-20.
12) DeJesus-Hemandez M, Mackenzie IR, Boeve BF, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of *C9ORF72* causes chromosome 9p-linked FTD and ALS. Neuron. 2011；72：245-56.
13) Renton AE, Majounie E, Waite A, et al. A hexanucleotide repeat expansion in *C9ORF72* is the cause of chromosome 9p21-linked ALS-FTD. Neuron. 2011；72：257-68.
14) AI-Sarraj S, King A, Troakes C, et al. p62 positive, TDP-43 negative, neuronal cytoplasmic and intranuclear inclusions in the cerebellum and hippocampus define the pathology of *C9orf72*-liked FTLD and MND/ALS. Acta Neuropathol. 2011；122：691-702.
15) Mori K, Weng S-M, Arzberger T, et al. The *C9orf72* GGGGCC repeat is translated into aggregating dipeptide-repeat proteins in FTLD/ALS. Science. 2013；339：1335-8.
16) Gendron TF, Bieniek KF, Zhang Y-J, et al. Antisense transcripts of the expanded *C9ORF72* hexanucleotide repeat form nuclear RNA foci and undergo repeat-associated non-ATG translation in c9FTD/ALS. Acta Neuropathol. 2013；126：829-44.
17) May S, Hornburg D Schludi MH, et al. *C9orf72* FTLD/ALS-associated Gly-Ala dipeptide repeat proteins cause neuronal toxicity and Unc119 sequenstration. Acta Neuropathol. 2014；128：485-503.
18) Zhang Y-J, Jansen-West K, Xu Y-F, et al. Aggregation-prone c9FTD/ALS poly (GA) RAN-translated proteins cause neurotoxicity by inducing ER stress. Acta Neuropathol. 2014；128：505-24.
19) Kwon I, Xiang S, Kato M, et al. Poly-dipeptides encoded by the C9ORF72 repeats bind nucleoli, impede RNA biogenesis, and kill cells. Science. 2014；345：1139-45.
20) Mizielinska S, Grönke S, Niccoli T, et al. *C9orf72* repeat expansions cause neurodegeneration in *Drosophila* through arginine-rich proteins. Science. 2014；345：1192-4.

ポリグルタミン病における神経変性

4

永井義隆
NAGAI Yoshitaka/国立精神・神経医療研究センター神経研究所疾病研究第四部

ポリグルタミン病は，CAGリピート配列の異常伸長という特徴的な遺伝子変異に起因する遺伝性疾患であるため，分子生物学を基盤とした病態研究が他疾患に先んじて進展してきた．遺伝子改変マウスの解析から，神経症状は神経細胞死に至る前の可逆性神経機能障害に起因することが明らかになった．その神経機能障害は，個々の神経細胞の自律的(cell-autonomous)な障害だけでなく，グリアなどを含む非細胞自律的(non-cell-autonomous)なネットワーク障害に起因すると考えられるようになった．その中でも，シナプスを介する神経伝達の障害は発症前から存在することが示唆され，発症後からでも神経症状を改善できる治療標的となる可能性がある．

Key words
- ポリグルタミン病
- オートファジー性細胞死
- 神経機能障害
- 非細胞自律的
- シナプス神経伝達

はじめに

アルツハイマー病，パーキンソン病，筋萎縮性側索硬化症(ALS)，ハンチントン病，脊髄小脳失調症などに代表される神経変性疾患は，それぞれ特定の領域の神経細胞群が進行性に変性・脱落した結果，さまざまな神経・精神症状を呈する原因不明の疾患群と元来定義されていた．しかしながら，遺伝性を示す神経変性疾患においては，分子遺伝学的解析から大多数の原因遺伝子変異が同定され，アミロイド前駆蛋白質，タウ，α-シヌクレイン，SOD1，TDP-43，huntingtin，ataxinなど多くの遺伝子変異により構造異常(ミスフォールディング)・凝集を起こしやすい変異蛋白質が産生されることが明らかにされた．一方，孤発性の神経変性疾患においても，これらの蛋白質が凝集して神経細胞内外に封入体として蓄積することが従来から知られていた．以上のことから，異常蛋白質のミスフォールディング・凝集により，共通に神経変性が引き起こされるという普遍的な発症分子メカニズムが想定され，これらの疾患はコンフォメーション病もしくはミスフォールディング病と総称されている[1]．本稿では，コンフォメーション病のモデル疾患としてポリグルタミン病に着目し，その神経変性メカニズムの解明を目指した研究から明らかになった最新の知見を紹介する．

1 ポリグルタミン病

ポリグルタミン(PolyQ)病とは，ハンチントン病，脊髄小脳失調症(SCA)1，2，3，6，7，17型，歯状核赤核淡蒼球ルイ体萎縮症，球脊髄性筋萎縮症など9疾患の総称であり，これらの疾患は，それぞれ別の原因遺伝子内にあるグルタミンをコードするCAGリピート配列の異常伸長という

共通の遺伝子変異により発症する．このCAGリピート配列は健常人にも存在する配列(正常約4～35リピート)であるが，PolyQ病患者では約35～40リピートから100リピート以上に異常伸長しており，その閾値は各疾患でおおむね共通している．また，CAGリピート数と疾患の発症年齢・重症度とが強く相関することが知られている．さらに，これらの原因蛋白質はPolyQ鎖以外には相同性を認めず，多くが優性遺伝性で1つの対立遺伝子の変異のみで発症することから，PolyQ病は異常伸長PolyQ鎖自身が原因蛋白質の生理的機能とは無関係に神経毒性を獲得(gain of toxic function)することにより発症すると考えられている．その発症メカニズムとしては，異常伸長したPolyQ鎖を持つ変異蛋白質がミスフォールディング・凝集を生じて封入体として神経細胞内に蓄積し，その結果，細胞レベル・個体レベルでさまざまな機能異常を引き起こし，最終的に神経変性を引き起こすと考えられている[2][3]．

PolyQ病は上述のように遺伝子異常により定義されており，この点で，歴史的に臨床症状から疾患概念が確立されてきたパーキンソン病や病理学的に定義されてきたアルツハイマー病とは疾患概念の階層性が異なり，より厳密な疾患概念であると言える．PolyQ病のひとつであるハンチントン病は，ヒト遺伝性疾患の中でも人類史上初めてポジショナルクローニングにより原因遺伝子座の決定に成功した疾患であり，その後の爆発的に進展した分子遺伝学的研究の幕開けとして，このことは特筆に値する．その後の神経変性疾患の研究においても，環境要因の寄与が少なく，ほぼ遺伝的要因のみに発症が規定されているというPolyQ病の特徴は，遺伝子異常を基盤とした分子生物学的な病態研究の進展において他疾患をリードしている．さらに，PolyQ鎖長が神経毒性と強く相関することから，晩発性神経変性疾患のモデル化において，より早期に明瞭な表現型を発症する遺伝学的実験モデルの作製に適しており，これらの特徴から異常蛋白質ミスフォールディング・凝集による共通の神経変性メカニズムの解明に大きく貢献している．実際に，ハンチントン病の変異遺伝子を導入した重度の表現型を呈するトランスジェニックマウスがいち早く樹立され，これまで患者脳では見つかっていなかった封入体が発見され[4]，その後，患者脳の病理学的解析でも確認された[5]．このように分子遺伝学の進展により，従来の症候学→病理病態学→病因解明という疾患研究の流れが，遺伝学→分子病態学→病理病態学という新しい流れへと大きな変遷を遂げた．

ポリグルタミン病における神経細胞死

神経変性とは，神経細胞(群)の細胞死，脱落を指す．それでは，PolyQ病における神経細胞死はどういう細胞死であろうか？ 細胞死は，形態学的特徴から，能動的なプログラム細胞死であるアポトーシスと，外的要因による受動的な細胞死であるネクローシスに従来分類されてきた．神経変性疾患における細胞死は，明らかに外的要因によるネクローシスとは異なるため，これまでアポトーシスの関与が疑われてきた．実際に培養細胞モデルを用いた研究から，数時間～数日間で生じる細胞死においては，確かにアポトーシス実行因子であるカスパーゼの活性化などが報告されてきた．しかしながら，マウスなどの*in vivo*モデルや患者脳のように，長期間かかって緩徐進行性に生じる神経細胞死においては，典型的なアポトーシス像は認められないことが明らかになった．このように，神経変性疾患における神経細胞死のメカニズムは，まだ十分には解明されていない．

一方，プログラム細胞死にはアポトーシスではなく，むしろネクローシス様の形態を呈するものが知られているが，その詳細は明らかではない．Clarkeが提唱した2型細胞死は，細胞質中に多数のオートファゴソーム様空胞，リソソームの出現を特徴としており[6]，これは現在ではいわゆるオートファジー性細胞死(autophagic cell death)に相当すると考えられる．ハンチントン病などPolyQ病患者やモデルマウスにおける神経細胞死は，形態的にはアポトーシスの特徴を欠き，エンドソーム，リソソームの蓄積を認めることから，むしろオートファジー性細胞死の関与が示唆されている[7][8]．アルツハイマー病患者脳でも，多数のリソソーム・オートファゴソーム様空胞を伴う顆粒空胞変性が認められ，パ

ーキンソン病においても病理学的にはアポトーシスに加えてオートファジー性変性が認められる．以上のことから，PolyQ病を含む神経変性疾患においては，カスパーゼ依存的なアポトーシスの関与は少なく，むしろオートファジー性細胞死により緩徐進行性の神経変性・細胞死が引き起こされると考えられている[9]．

ポリグルタミン病における神経機能障害

神経変性疾患における神経症状は，これまでは神経細胞群の変性・脱落の結果，その欠落症状として出現すると考えられていた．しかしながら，PolyQ病患者由来の変異遺伝子を導入したさまざまな遺伝子改変モデルマウスが樹立され，発症前からの経時的な病態解析が可能となった結果，ハンチントン病モデルマウスにおいて神経症状が発症する時点では，著明な神経細胞死は認められないことが明らかになった[4]．さらに驚くべきことに，遺伝子発現誘導システムによるハンチントン病モデルコンディショナルマウスを用いて，発症後からでも異常伸長PolyQ蛋白質の発現を遮断すると神経症状が改善することが示され，PolyQ病の神経症状は神経細胞死よりもむしろ可逆性の神経機能障害に起因すると考えられるようになった[10]（図1）．このことから，従来は発症時には神経細胞死が進行・完成しており難治性と考えられていた神経変性疾患に対し，この神経機能障害を標的とした治療により発症後からでも病態進行を阻止し，症状を改善できる可能性が示唆され，これらの難病の克服へ向けて大きな希望がもたらされた．また，これまで患者死後脳を用いた病理学的解析から「神経変性疾患は神経細胞の脱落・変性に起因する」と定義されていた疾患概念が，部分的にでも覆る可能性が示唆された．このこともまた，分子遺伝学を基盤にした新たな疾患研究の潮流がもたらした賜物であると言えよう．

それでは，PolyQ病における神経機能障害の実体とはどういうものであろうか？ これまでにPolyQ病における神経機能障害として，転写調節障害，ユビキチン・プロテアソーム系やオートファジー・リソソーム系など蛋白質分解システムの障害，細胞内輸送・軸索輸送障害，ミトコンドリア障害，小胞体ストレスなど，さまざまな機能障害が明らかにされている[3]．しかし，これらはすべて細胞内レベルでの機能障害であり，これらが個体レベルでの神経症状にどのようにつながるのかは未解明である．

遺伝子改変技術の発達により，脳部位特異的に異常伸長PolyQ蛋白質を発現する，あるいは脳部位特異的に発現を遮断したハンチントン病モデルコンディショナルマウスが作製された．これらの解析の結果，神経症状は線条体や大脳皮質など個々の神経細胞内での機能障害にのみ起因するのではなく，両者のネットワーク障害の寄与が重要であることが明らかにされた[11][12]．また，グリア特異的に異常伸長PolyQ蛋白質を発現するSCA7モデルマウスを用いて，神経細胞間だけでなく神経－グリア間のネットワーク障害も神経機能障害に関わることが明らかになった[13]．さらに，球脊髄性筋萎縮症や

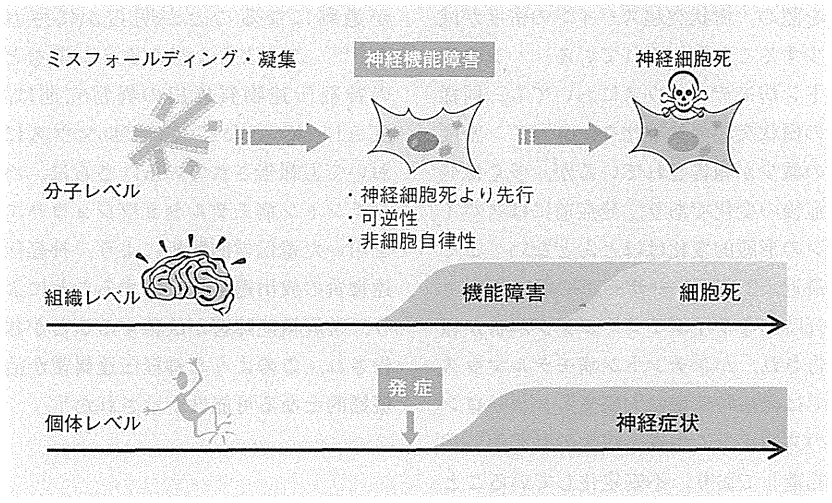


図1　ポリグルタミン病の神経症状は可逆性神経機能障害に起因する