Wild type huntingtin toxicity in yeast: Implications for the role of amyloid cross-seeding in polyQ diseases

A. I. Alexandrov, G. V. Serpionov, V. V. Kushnirov, and M. D. Ter-Avanesyan

Bach Institute of Biochemistry, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russia

ABSTRACT. Proteins with expanded polyglutamine (polyQ) regions are prone to form amyloids, which can cause diseases in humans and toxicity in yeast. Recently, we showed that in yeast non-toxic amyloids of Q-rich proteins can induce aggregation and toxicity of wild type huntingtin (Htt) with a short non-pathogenic polyglutamine tract. Similarly to mutant Htt with an elongated N-terminal polyQ sequence, toxicity of its wild type counterpart was mediated by induced aggregation of the essential Sup35 protein, which contains a Q-rich region. Notably, polymerization of Sup35 was not caused by the initial benign amyloids and, therefore, aggregates of wild type Htt acted as intermediaries in seeding Sup35 polymerization. This exemplifies a protein polymerization cascade which can generate a network of interdependent polymers. Here we discuss cross-seeded protein polymerization as a possible mechanism underlying known interrelations between different polyQ diseases. We hypothesize that similar mechanisms may enable proteins, which possess expanded Q-rich tracts but are not associated with diseases, to promote the development of polyQ diseases.

KEYWORDS. Amyloid, Huntington disease, polyglutamine, polymerization cross-seeding, polyQ, Sup35, yeast

HUNTINGTON DISEASE AND POLYQ AMYLOIDS

At present, more than 50 diseases are known to be associated with protein misfolding, and many of them involve formation of highly ordered, β-sheet-rich fibers termed amyloids.1–3 Though amyloids can be formed by functionally- and structurally-unrelated proteins, there is a subset of disease-related amyloidogenic proteins sharing structural resemblance. The proteins of this group contain polyglutamine (polyQ) regions and expansion of these regions beyond a certain threshold causes neurodegenerative diseases accompanied by deposition of amyloid protein aggregates of these proteins. Currently, 9 dominant autosomal polyQ disorders are known, among which Huntington disease (HD) is the
most common and well studied. HD is caused by mutations that increase the number of CAG triplets in the first exon of the \textit{HTT} gene encoding the huntingtin (Htt) protein. Up to date HD has not been observed in individuals with less than 35 CAG repeats, while elongation of the polyQ tract increases the probability of appearance and severity of this disease.\textsuperscript{4-6} Mutant Htt (mHtt) with an expanded N-terminal polyQ sequence aggregates and forms insoluble granular or fibrous deposits in affected neurons, mostly in the nucleus, but also in the cytoplasm.\textsuperscript{7-9} The toxic effect of expanded polyQ proteins is related to their interference with the normal function of various proteins, which affects different cellular processes. In particular, pathological Htt impairs gene transcription and the ubiquitin-proteasome system, causes mitochondrial dysfunction, dysregulation of Ca\textsuperscript{2+} homeostasis, impairment of axonal transport and genotoxic stress.\textsuperscript{10} However, despite extensive studies, the mechanisms responsible for the above defects in HD and other polyQ diseases still remain enigmatic. Experimental models, based on mouse \textit{Mus musculus},\textsuperscript{11} fly \textit{Drosophila melanogaster},\textsuperscript{12} worm \textit{Caenorhabditis elegans}\textsuperscript{13} and yeast \textit{Saccharomyces cerevisiae}\textsuperscript{14} have been established to elaborate the reasons of mHtt toxicity on molecular and cellular levels.

\textbf{AGGREGATION AND TOXICITY OF HUMAN HUNTINGTIN IN YEAST}

Yeast \textit{S. cerevisiae} represent the simplest and genetically most tractable eukaryotic model organism which is often used to elaborate molecular bases of various human diseases including amyloid diseases and HD, in particular. As in humans, aggregation and toxicity of Htt in yeast increases with polyQ length.\textsuperscript{15} In most studies the yeast model of HD is based on cells that express the first exon of the human \textit{HTT} gene, encoding a polyQ tract with 103 glutamine residues (Htt103Q), which aggregates and strongly inhibits yeast growth, thus mimicking toxicity. The same protein with a tract of 25 glutamines, Htt25Q, is commonly used as a control, because it does not usually aggregate or cause toxicity. In yeast overproduced Htt103Q forms SDS-insoluble aggregates, which indicates their amyloid nature, since resistance to strong ionic detergents is a characteristic property of amyloids which distinguishes them from amorphous protein aggregates.\textsuperscript{16} Moreover, Htt103Q aggregates contain generic amyloid epitopes for DNA aptamer binding,\textsuperscript{17} which further supports their amyloid nature. Targeting mHtt into the nucleus of yeast cells was shown to alter transcription of a subset of genes and decrease viability.\textsuperscript{15} Cytoplasmically expressed mHtt is also toxic, and its toxicity is related to aggregation, which is stimulated by pre-existing prion amyloids of glutamine/asparagine (Q/N)-rich proteins,\textsuperscript{14,18} i.e. by cross-seeding, a process, in which polymers of one protein seed polymerization of a different protein. Notably, \textit{in vitro} studies demonstrated that the efficiency of cross-seeding inversely correlates with the structural difference between the involved proteins,\textsuperscript{19,20} and for structurally unrelated proteins it results in homopolymers of the seeded protein rather than in mixed polymers of both proteins, as was shown in one case in yeast.\textsuperscript{21} Aggregation of mHtt in yeast cells alters endocytosis, tryptophan metabolism, translation, cell cycle progression, endoplasmic reticulum-associated protein degradation and functioning of mitochondria.\textsuperscript{22-27} The molecular bases of these defects are largely unknown, however several reports have shown that mHtt cross-seeds polymerization of cellular proteins, many of which contain Q/N-rich regions.\textsuperscript{21,28,29} Among them, aggregation of the essential Sup35 protein was shown to represent a significant source of mHtt toxicity.\textsuperscript{26,30} In a similar way, in neurons mHtt aggregates can impair gene transcription by sequestration of transcription factors possessing polyQ repeats.\textsuperscript{31-33} On the other hand, yeast Q/N-rich proteins can have opposing effects on Htt, since lack or overproduction of some such proteins was shown to increase or abolish mHtt toxicity.\textsuperscript{34} Thus, it seems that mHtt aggregates induce the polymerization of a network of proteins, the perturbation of which can modulate mHtt cytotoxicity.

Wild-type Htt (wtHtt) does not aggregate on its own, although it can be sequestered in mHtt aggregates.\textsuperscript{32,34,35} However, recently we have observed that overproduced Htt25Q can form SDS-insoluble aggregates and cause toxicity, when seeded by non-toxic polyQ amyloids.
produced at moderate levels.\textsuperscript{36} Similarly to Htt\textsubscript{103Q}, Htt\textsubscript{25Q} toxicity was also related to polymerization-mediated inactivation of the Sup35 protein. Importantly, study of Sup35 co-polymerization revealed a new mode of amyloid interdependence. As we mentioned above, polymers of proteins with polyQ domains, including Htt\textsubscript{103Q}, can seed polymerization of multiple other proteins with similar domains.\textsuperscript{21,28,29,31-33,37-39} Polymers of these proteins can appear due to cross-seeding by the same initial polymer seed, or, alternatively, the process of cross-seeding can be sequential, representing a polymerization cascade, which forms a network of interdependent polymers. The latter possibility, which can be described as intermediary seeding, agrees with our results showing that polymerization of Htt\textsubscript{25Q} which was seeded by benign amyloids, can induce polymerization of Sup35 and 2 other Q/N-rich proteins, which do not aggregate in the presence of the initial seeding amyloids.\textsuperscript{36}

### IMPLICATIONS FOR AMYLOIDOSES

Despite the well-established inverse correlation between the number of CAG repeats in genes associated with polyQ diseases and the age of onset of these disorders, the length of the CAG repeats accounts for only 50–70\% of the variance in age of onset,\textsuperscript{40} suggesting that additional genetic and/or environmental factors account for the remaining variability.\textsuperscript{40,41} One type of such genetic factors is thought to be the length of polyQ in the normal version of the protein relevant for the disease.\textsuperscript{42-44} The effect of normal Htt, however, can be complex, since increasing the length of the polyQ in wtHtt mitigates HD severity if mHtt possesses a longer polyQ length and exacerbates it if mHtt has a shorter pathological polyQ.\textsuperscript{43} The mechanisms behind these effects are still unclear, though some studies show that wtHtt can co-aggregate with mHtt both in human and yeast cells.\textsuperscript{52,34,35} Co-aggregation of wtHtt with mHtt may contribute to HD pathogenesis either by a loss of wtHtt function,\textsuperscript{55} or by accelerating the aggregation of mHtt.\textsuperscript{45} Surprisingly, it was recently reported that in yeast co-production with wtHtt can ameliorate toxicity of the mutant protein.\textsuperscript{46} However, we could not reproduce this effect and, furthermore, in agreement with earlier data, our observations indicate that in yeast, aggregation of wtHtt, which occurs in the presence of mHtt aggregates,\textsuperscript{34} can contribute to cytotoxicity.\textsuperscript{36}

Accumulating data indicate that age of onset and pathogenesis of polyQ diseases can also be modulated by interaction of corresponding polyQ proteins with other disease-associated polyQ-containing proteins.\textsuperscript{47-50} For example, it was shown that the age of onset of type 2 spinocerebellar ataxia depends not only on expansion of polyQ in the SCA2 protein but also correlates with the polyQ length in the CACNA1A protein, associated with type 6 spinocerebellar ataxia.\textsuperscript{48} The toxicity modulation of one polyQ disease protein by another was also shown using a \textit{D. melanogaster} model of spinocerebellar ataxia,\textsuperscript{51} which allowed the authors to propose that functional links between corresponding genes are critical to disease severity and progression. Disease-related proteins with non-pathological polyQ domains were found in the amyloid-like inclusions of patients with polyQ diseases unrelated to these proteins,\textsuperscript{52} which also suggests that presence of these proteins in the aggregates can be a source of toxicity. Interestingly, proteins with polyQ stretches are also implicated in the pathogenesis of diseases involving aggregation of proteins with Q-rich domains, since non-pathogenic expansion of polyQ in the SCA2 protein can be a risk factor for amyotrophic lateral sclerosis associated with the TDP-43 and FUS.\textsuperscript{53} The role of interaction of the TDP-43 and FUS proteins with SCA2 in pathogenesis of amyotrophic lateral sclerosis also agrees with co-aggregation of these proteins in a \textit{D. melanogaster} model of this disease.\textsuperscript{54,55}

Published correlation studies have mostly searched for the modulating effects of polyQ length variation of disease-related polyQ proteins. However, it is likely that polyQ length polymorphism in proteins that are not associated with diseases can also modulate polyQ disease progression. This possibility is supported by results obtained in the yeast model showing that overproduction of proteins with Q/N-rich tracts can both increase\textsuperscript{34} and decrease\textsuperscript{56} toxicity of mHtt. Furthermore, our recent data showing that in yeast overproduction of various proteins with long Q-rich domains can also
cause polymerization and toxicity of wtHtt, allow speculation that in humans amyloids of such proteins may promote polyQ disease development if polyQ repeat lengths in the corresponding proteins only slightly exceed the threshold for these diseases. Possibly such amyloids can even seed polymerization of non-mutant disease-associated polyQ proteins and cause emergence of symptoms similar to that of a polyQ disease.

The role of cross-seeding in amyloid emergence can be of even more general significance, which follows from the observations that in yeast polymers of Htt103Q are also able to seed aggregation of proteins which do not possess Q/N-rich regions. This type of protein polymerization cross-seeding may provide a mechanism for the suggested predisposing role of an expansion of polyQ in disease-related proteins for neurodegenerative amyloidoses related to proteins which lack Q-rich domains. In addition, though it has not been yet modeled in yeast, it seems probable that similar cross-seeding mechanism may also provide a molecular basis for interrelations between different non-polyQ-related amyloidoses. This possibility is supported by the ability of structurally unrelated disease-associated proteins to accelerate each other’s aggregation in vivo and in vitro, though structural dissimilarity can diminish such cross-seeding activity. Importantly, interrelations between polymerization-prone proteins may be rather complex, since these proteins can seed polymerization of each other not only directly, but also through polymerization cascades. Also, even though a significant body of evidence indicates a role of protein polymerization cross-seeding in interdependence of amyloid disease emergence, such interdependence also may be facilitated by the ability of amyloids to sequester chaperones, which in turn may compromise correct protein folding, thus enabling aggregation-prone proteins to form amyloids. However, more data are needed to assess the relevance of this mechanism to amyloid diseases.

In conclusion, it should be stressed that the ability of non-pathogenic proteins with long Q-rich tracts to induce aggregation and toxicity of wild type Htt and the existence of polymerization cascades not only highlight the complex molecular nature of polyQ and possibly other amyloid diseases, but also provide a framework for a search for novel genetic factors which modify polyQ disease progression among non-disease-related proteins with polyQ tracts.

**ABBREVIATIONS**

HD  Huntington disease  
Htt  huntingtin  
mHtt  mutant Htt  
wtHtt  wild type Htt  
Htt25Q  huntingtin with 25 glutamine residues  
Htt103Q  huntingtin with 103 glutamine residues  
polyQ  polyglutamine  
Q/N-rich protein  glutamine/asparagine-rich protein

**DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST**

No potential conflicts of interest were disclosed.

**FUNDING**

This work was supported by the grant of Russian Science Foundation #14-14-00361.

**REFERENCES**

[1] Chiti F, Dobson CM. Protein misfolding, functional amyloid, and human disease. Annu Rev Biochem 2006; 75:333-66; PMID:16756495; http://dx.doi.org/10.1146/annurev.biochem.75.101304.123901

[2] Knowles TPJ, Vendruscolo M, Dobson CM. The amyloid state and its association with protein misfolding diseases. Nat Rev Mol Cell Biol 2014; 15:384-96; PMID:24854788; http://dx.doi.org/10.1038/nrm3810

[3] Nizhnikov AA, Antonets KS, Inge-Vechtomov SG. Amyloids: from pathogenesis to function. Biochem 2015; 80:1127-44

[4] MacDonald ME, Ambrose CM, Duyao MP, Myers RH, Lin C, Srinidhi L, Barnes G, Taylor SA, James M, Groot N, et al. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington’s disease chromosomes. Cell 1993; 72:971-83; PMID:8458085; http://dx.doi.org/10.1016/0092-8674(93)90585-E
AMYLOID CROSS-SEEDING AND POLYQ DISEASES 225

[5] Bates G. Huntington aggregation and toxicity in Huntington’s disease. Lancet 2003; 361:1642-4; PMID:12747895; http://dx.doi.org/10.1016/S0140-6736(03)13304-1

[6] Ross CA, Tabrizi SJ. Huntington’s disease: from molecular pathogenesis to clinical treatment. Lancet Neurol 2011; 10:83-98; PMID:21613446; http://dx.doi.org/10.1016/S1474-4422(10)70245-3

[7] Roizin L, Stellar S, Liu JC. Neuronal nuclear-cytoplasmic changes in Huntington’s chorea: electron microscope investigations. Adv Neurol 1979; 23:95-122; PMID:506869

[8] DiFiglia M. Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science 1997; 277:1990-3; PMID:9302293; http://dx.doi.org/10.1126/science.277.5334.1990

[9] Davies SW, Turmaine M, Cozens BA, DiFiglia M, Sharp AH, Ross CA, Scherzinger E, Wanker EE, Mangiarini L, Bates GP. Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. Cell 1997; 90:537-48; PMID:9267033; http://dx.doi.org/10.1016/S0092-8674(00)80513-9

[10] Takahashi T, Katada S, Onodera O. Polyglutamine diseases: where does toxicity come from? what is toxicity? where are we going? J Mol Cell Biol 2010; 2:180-91; PMID:20410236; http://dx.doi.org/10.1093/jmcb/mjq005

[11] Davies SW, Sathasivam K, Hobbs C, Doherty P, Mangiarini L, Scherzinger E, Wanker EE, Bates GP. Detection of polyglutamine aggregation in mouse models. Methods Enzym 1999; 309:687-701; http://dx.doi.org/10.1016/S0076-6879(99)09045-X

[12] Marsh JL, Pallos J, Thompson LM. Fly models of Huntington’s disease. Hum Mol Genet 2003; 12: R187-93; PMID:12925571; http://dx.doi.org/10.1093/hmg/ddg271

[13] Faber PW, Voisine C, King DC, Bates EA, Hart AC. Glutamine/proline-rich PQE-1 proteins protect Caenorhabditis elegans neurons from huntingtin polyglutamine neurotoxicity. Proc Natl Acad Sci USA 2002; 99:17131-6; PMID:12486229; http://dx.doi.org/10.1074/jbc.M110.101527

[14] Meriin AB, Zhang X, He X, Newnam GP, Chernoff YO, Sherman MY. Huntington toxicity in yeast model depends on polyglutamine aggregation mediated by a prion-like protein Rnp1. J Cell Biol 2002; 157:997-1004; PMID:12058016; http://dx.doi.org/10.1083/jcb.200112104

[15] Hughes RE, Lo RS, Davis C, Strand AD, Neal CL, Olson JM, Fields S. Altered transcription in yeast expressing expanded polyglutamine. Proc Natl Acad Sci USA 2001; 98:13201-6; PMID:11687606; http://dx.doi.org/10.1073/pnas.191498198

[16] Kryndushkin DS, Alexandrov IM, Ter-Avanesyan MD, Kushnirov VV. Yeast [PSI+] prion aggregates are formed by small Sup35 polymers fragmented by Hsp104. J Biol Chem 2003; 278:49636-43; PMID:14507919; http://dx.doi.org/10.1074/jbc.M307996200

[17] Mitkevich OV, Kochneva-Pervukhova NV, Surina ER, Benvolensky SV, Kushnirov VV, Ter-Avanesyan MD. DNA aptamers detecting generic amyloid epitopes. Prion 2012; 6:400-6; PMID:22874671; http://dx.doi.org/10.4161/prn.20678

[18] Gokhale KC, Newnam GP, Sherman MY, Chernoff YO. Modulation of prion-dependent polyglutamine aggregation and toxicity by chaperone proteins in the yeast model. J Biol Chem 2005; 280:22809-18; PMID:15824100; http://dx.doi.org/10.1074/jbc.M500390200

[19] O’Neillain B, Williams AD, Westmark P, Wetzel R. Seeding specificity in amyloid growth induced by heterologous fibrils. J Biol Chem 2004; 279:17490-9; http://dx.doi.org/10.1074/jbc.p311300200

[20] Krebs MRH, Morozova-Roche LA, Daniel K, Robinson CV, Dobson CM. Observation of sequence specificity in the seeding of protein amyloid fibrils. Protein Sci 2004; 13:1933-8; PMID:15215533; http://dx.doi.org/10.1110/ps.04707004

[21] Urakov VN, Vishnevskaya AB, Alexandrov IM, Kushnirov VV, Smirnov VN, Ter-Avanesyan MD. Interdependence of amyloid formation in yeast: implications for polyglutamine disorders and biological functions. Prion 2010; 4:45-52; PMID:20118659; http://dx.doi.org/10.1046/j.1.11074

[22] Tauber E, Miller-Fleming L, Mason RP, Kwan W, Clapp J, Butler NJ, Outeiro TF, Muchowski PJ, Giorgini F. Functional gene expression profiling in yeast implicates translational dysfunction in mutant huntingtin toxicity. J Biol Chem 2011; 286:410-9; PMID:21044956; http://dx.doi.org/10.1074/jbc.M110.101527

[23] Meriin AB, Zhang X, Alexandrov IM, Salnikova AB, Ter-Avanesian MD, Chernoff YO, Sherman MY. Endocytosis machinery is involved in aggregation of proteins with expanded polyglutamine domains. FASEB J 2007; 21:1915-25; PMID:17341688; http://dx.doi.org/10.1096/fj.06-6878com

[24] Duenwald ML, Lindquist S. Impaired ERAD and ER stress are early and specific events in polyglutamine toxicity. Genes Dev 2008; 22:3308-19; PMID:19015277; http://dx.doi.org/10.1101/gad.1673408

[25] Bocharova NA, Sokolov SS, Knorre DA, Skulachev VP, Severin FF. Unexpected link between anaphase promoting complex and the toxicity of expanded polyglutamines expressed in yeast. Cell Cycle 2008; 7:3943-6; PMID:19066445; http://dx.doi.org/10.1083/jcb.7.24.7398

[26] Kochneva-Pervukhova NV, Alexandrov AI, Ter-Avanesyan MD. Amyloid-mediated sequestration of
essential proteins contributes to mutant huntingtin toxicity in yeast. PLoS One 2012; 7:e29832; PMID:22537994; http://dx.doi.org/10.1371/journal.pone.0029832

[27] Papsdorf K, Kaiser CJO, Drazic A, Grötzinger SW, Haelnner C, Eisenreich W, Richter K. Polyglutamine toxicity in yeast induces metabolic alterations and mitochondrial defects. BMC Genomics 2015; 16:662; PMID:26335097; http://dx.doi.org/10.1186/s12864-015-1831-7

[28] Kryndushkin D, Pripuzova N, Burnett BG, Shewmaker F. Non-targeted identification of prions and amyloid-forming proteins from yeast and mammalian cells. J Biol Chem 2013; 288:27100-11; PMID:23926098; http://dx.doi.org/10.1074/jbc.M113.485359

[29] Nizhnikov AA, Alexandrov AI, Ryzhova TA, Mitkevich OV, Dergalev AA, Ter-Avanesyan MD, Gal-kin AP. Proteomic screening for amyloid proteins. PLoS One 2014; 9:e116003; PMID:25549323; http://dx.doi.org/10.1371/journal.pone.0116003

[30] Zhao X, Park Y-N, Todor H, Moomau C, Masison D, Eisenberg E, Greene LE. Sequestration of Sup35 by aggregates of huntingtin fragments causes toxicity of [PS1+] yeast. J Biol Chem 2012; 287:23346-55; PMID:22573320; http://dx.doi.org/10.1074/jbc.M111.287748

[31] Perez MK, Paulson HL, Pendse SJ, Sainoz SJ, Bonini NM, Pittman RN. Recruitment and the role of nuclear localization in polyglutamine-mediated aggregation. J Cell Biol 1998; 143:1457-70; PMID:9852144; http://dx.doi.org/10.1083/jcb.143.6.1457

[32] Kazantsev A, Preisinger E, Deinovskiy A, Goldgaber D, Housman D. Insoluble detergent-resistant aggregates form between pathological and nonpathological lengths of polyglutamine in mammalian cells. Proc Natl Acad Sci U S A 1999; 96:11404-9; PMID:105600189; http://dx.doi.org/10.1073/pnas.96.20.11404

[33] Nucifora FC, Sasaki M, Peters MF, Huang H, Cooper JK, Yamada M, Takahashi H, Tsuji S, Troncoso J, Dawson VL, et al. Interference by huntingtin and atrophin-1 with CBP-mediated transcription leading to cellular toxicity. Science 2001; 291:2423-8; PMID:11264541; http://dx.doi.org/10.1126/science.1056784

[34] Duennwald ML, Jagadish S, Giorgini F, Muchowski PJ, Lindquist S. A network of protein interactions determines polyglutamine toxicity. Proc Natl Acad Sci U S A 2006; 103:11051-6; PMID:16832049; http://dx.doi.org/10.1073/pnas.0604548103

[35] Busch A, Engemann S, Lurz R, Okazawa H, Lehr-rach H, Waneker EE. Mutant huntingtin promotes the fibrillogenesis of wild-type huntingtin: a potential mechanism for loss of huntingtin function in Huntington’s disease. J Biol Chem 2003; 278:41452-61; PMID:12888569; http://dx.doi.org/10.1074/jbc.M303354200

[36] Serpionov GV, Alexandrov AI, Antonenko YN, Ter-Avanesyan MD. A protein polymerization cascade mediates toxicity of non-pathological human huntingtin in yeast. Sci Rep 2015; 5:18407; PMID:26673834; http://dx.doi.org/10.1038/srep18407

[37] Yamanaka T, Miyazaki H, Oyama F, Kurosawa M, Washizu C, Doi H, Nukina N. Mutant Huntingtin reduces HSP70 expression through the sequestration of NF-Y transcription factor. EMBO J 2008; 27:827-39; PMID:18288205; http://dx.doi.org/10.1038/emboj.2008.23

[38] Mitsui K, Doi H, Nukina N. Proteomics of polyglutamine aggregates. Methods Enzymol 2006; 412:63-76; PMID:17046652; http://dx.doi.org/10.1016/S0076-6879(06)12005-4

[39] Wear MP, Kryndushkin D, O’Meally R, Sonnenberg JL, Cole RN, Shewmaker FP. Proteins with intrinsically disordered domains are preferentially recruited to polyglutamine aggregates. PLoS One 2015; 10: e0136362; PMID:26317359; http://dx.doi.org/10.1371/journal.pone.0136362

[40] Paradisi I, Hernández A, Arias S. Huntington disease mutation in Venezuela: age of onset, haplotype analyses and geographic aggregation. J Hum Genet 2008; 53:127-35; PMID:18157708; http://dx.doi.org/10.1007/s10038-007-0227-1

[41] The U.-Venezuela Collaborative Research Project, Wexler NS, Lorimer J, Porter J, Gomez F, Moskowitz C, Shackell E, Marder K, Penchaszadeh G, Roberts SA, et al. Venezuelan kindreds reveal that genetic and environmental factors modulate Huntington’s disease age of onset. Proc Natl Acad Sci USA 2004; 101:3498-503; PMID:14993615; http://dx.doi.org/10.1073/pnas.0308679101

[42] Djoussé L, Knowlton B, Hayden M, Almqvist EW, Brinkman R, Ross C, Margolis R, Rosenblatt A, Durr A, Dode C, et al. Interaction of normal and expanded CAG repeat sizes influences age at onset of Huntington disease. Am J Med Genet A 2003; 119A:279-82; http://dx.doi.org/10.1002/ajmg.a.20190

[43] Aziz NA, Jurgens CK, Landwehrmeyer GB, van Room-Mom WMC, van Ommen GJB, Stijnen T, Roos RAC. Normal and mutant HTT interact to affect clinical severity and progression in Huntington disease. Neurology 2009; 73:1280-5; PMID:19776381; http://dx.doi.org/10.1212/WNL.0b013e3181bd1121

[44] França MC, Emmel VE, D’Abreu A, Maurer-Morelli CV, Secolin R, Bonadia LC, da Silva MS, Nucci A, Jardim LB, Saraiva-Pereira ML, et al. Normal ATXN3 allele but not CHIP polymorphisms modulates age at onset in Machado–Joseph disease. Front Neurol 2012; 3:164.

[45] Slepkó N, Bhattacharyya AM, Jackson GR, Steffan JS, Marsh JL, Thompson LM, Wetzel R. Normal-repeat-length polyglutamine peptides accelerate aggregation nucleation and cytotoxicity of expanded
polyglutamine proteins. Proc Natl Acad Sci USA 2006; 103:14367-72; PMID:16980414; http://dx.doi.org/10.1073/pnas.0602348103

[46] Saleh AA, Bhadra AK, Roy I. Cytotoxicity of mutant huntingtin fragment in yeast can be modulated by the expression level of wild type huntingtin fragment. ACS Chem Neurosci 2014; 5:205-15; PMID:24377263; http://dx.doi.org/10.1021/cn400171d

[47] Jardim L, Silveira I, Pereira ML, do Ceu Moreira M, Mendonca P, Sequeiros J, Giugliani R. Searching for modulating effects of SCA2, SCA6 and DRPLA CAG tracts on the Machado-Joseph disease (SCA3) phenotype. Acta Neurol Scand 2003; 107:211-4; PMID:12614315; http://dx.doi.org/10.1034/j.1600-0404.2003.00046.x

[48] Pulst S-M, Santos N, Wang D, Yang H, Huynh D, Velazquez L, Figueroa KP. Spino cerebellar ataxia type 2: polyQ repeat variation in the CACNA1A calcium channel modifies age of onset. Brain 2005; 128:2297-303; PMID:16000334; http://dx.doi.org/10.1093/brain/awh586

[49] de Castilhos RM, Furtado GV, Gheno TC, Schaeffer P, Russo A, Barsottini O, Pedroso JL, Salarini DZ, Vargas FR, de Lima MADFD, et al. Spino cerebellar ataxias in Brazil–frequencies and modulating effects of related genes. Cerebellum 2014; 13:17-28; PMID:23943520; http://dx.doi.org/10.1007/s12311-013-0510-y

[50] Du Montcel ST, Durr A, Bauer P, Figueroa KP, Ichikawa Y, Brussino A, Forlani S, Rakowicz M, Schöls L, Mariotti C, et al. Modulation of the age at onset in spino cerebellar ataxia by CAG tracts in various genes. Brain 2014; 137:2444-55; PMID:24972706; http://dx.doi.org/10.1093/brain/awu174

[51] Lessing D, Bonini NM. Polyglutamine genes interact to modulate the severity and progression of neurodegeneration in drosophila. PLoS Biol 2008; 6: e29; PMID:18271626; http://dx.doi.org/10.1371/journal.pbio.0060029

[52] Uchihara T, Fujigasaki H, Koyano S, Nakamura A, Yagishita S, Iwabuchi K. Non-expanded polyglutamine proteins in intranuclear inclusions of hereditary ataxias–triple-labeling immunofluorescence study. Acta Neuropathol 2001; 102:149-52; PMID:11563629

[53] Elden AC, Kim H-J, Hart MP, Chen-Plotkin AS, Johnson BS, Fang X, Armakola M, Geser F, Greene R, Lu MM, et al. Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. Nature 2010; 466:1069-75; PMID:20740007; http://dx.doi.org/10.1038/nature09320

[54] Bonini NM, Gitter AD. Model organisms reveal insight into human neurodegenerative disease: ataxin-2 intermediate-length polyglutamine expansions are a risk factor for ALS. J Mol Neurosci 2011; 45:676-83; PMID:21660502; http://dx.doi.org/10.1007/s12031-011-9548-9

[55] Farg MA, Soo KY, Warrach ST, Sundaramoorthy V, Blair IP, Atkin JD. Ataxin-2 interacts with FUS and intermediate-length polyglutamine expansions enhance FUS-related pathology in amyotrophic lateral sclerosis. Hum Mol Genet 2013; 22:717-28; PMID:23172909; http://dx.doi.org/10.1093/hmg/ddz479

[56] Kayatekin C, Matlock KES, Hesse WR, Guan Y, Chakrabortee S, Russ J, Wanker EE, Shah J V, Lindquist S. Prion-like proteins sequester and suppress the toxicity of huntingtin exon 1. Proc Natl Acad Sci U S A 2014; 111:12085-90; PMID:25092318; http://dx.doi.org/10.1073/pnas.1412504111

[57] Kim J-M, Hong S, Kim GP, Choi YJ, Kim YK, Park SS, Kim JE, Seon BS. Importance of low-Range CAG expansion and CAA interruption in SCA Parkinsonism. Arch Neurol 2007; 64:1510; PMID:17923635; http://dx.doi.org/10.1001/archneur.64.10.1510

[58] Yamashita C, Tomiyama H, Funayama M, Inamizu S, Ando M, Li Y, Yoshino H, Araki T, Ichikawa T, Ehara Y, et al. The evaluation of polyglutamine repeats in autosomal dominant Parkinson’s disease. Neurobiol Aging 2014; 35:1779.e17-1779.e21; PMID:24534762; http://dx.doi.org/10.1016/j.neurobiolaging.2014.01.022

[59] Gratuze M, Cibani G, Cicchetti F, Planel E. Is Huntington’s disease a tauopathy? Brain 2016; 139:1014-25; PMID:26969684; http://dx.doi.org/10.1093/brain/aww021

[60] Jellinger KA. Interaction between pathogenic proteins in neurodegenerative disorders. J Cell Mol Med 2012; 16:1166-83; PMID:22176890; http://dx.doi.org/10.1111/j.1582-4934.2011.01507.x

[61] Gotz J. Formation of neurofibrillary tangles in p301l Tau transgenic mice induced by Abeta 42 fibrils. Science 2001; 293:1491-5; PMID:11520988; http://dx.doi.org/10.1126/science.1062097

[62] Arslan F, Hong JY, Kanneganti V, Park S-K, Liebman SW. Heterologous aggregates promote novo prion appearance via more than one mechanism. PLoS Genet 2015; 11:e1004814; PMID:25568955; http://dx.doi.org/10.1371/journal.pgen.1004814