Fragment transgenic Huntington's disease mouse models

As mentioned in the section 'Mouse models', there is a problem with inverse correlation of genetic accuracy of the model and the phenotype or the extent of neuropathological lesions observed in the animals. A study comparing the knock-in $Hdh^{Q150/Q150}$ mice and N-terminal exon 1 fragment model, R6/2, revealed that both models had similar phenotypic and molecular characteristics, but they appeared much later in the knock-in mice (Woodman *et al.* 2007). Robust phenotype and pathology display model mice expressing the fragment of the gene containing the expanded polyQ. In case of HD, three mouse models with an N-terminal segment of mutant htt have been used in HD research, including R6/2, R6/1, and N171-82Q. The first and best characterized is the R6/2 line, which has an N-terminal end of exon 1 originally with ~150Q (Mangiarini *et al.* 1996). This model revealed that expression of only exon 1 containing expanded polyQ is sufficient to produce mice with many neuropathological features of HD. It is one of the most commonly used genetic models of HD because of a progressive and homogenous phenotype. The short survival of about 3 months and well-quantifiable phenotype makes it a great tool for experimental therapeutic interventions. Moreover, the behavioral impairment and neuropathological findings suggested that the R6/2 model corresponds to human HD to a large extent (Stack *et al.* 2005). The effects on lifespan extension, body weight, and improvements in motor performance have been used as indicators of the treatment outcome in many studies. Other tests, such as limb clasping score, grip strength, or general activity became also very useful in evaluating and comparing different therapeutic approaches. It has been found that the variability in survival and the amelioration of the phenotype with increased CAG repeat size reduces the utility in therapeutic trials and may corrupt the results (Stack *et al.* 2005). In our experience, R6/2 mice with repeat size between 125 and 145 CAG were very appropriate for testing different treatments.

The R6/1 mouse model harbors exon 1 of the human HD gene with ~116 CAG repeats (Mangiarini *et al.* 1996). This model has been not studied so extensively as R6/2. The disease starts later and has milder course than that in R6/2 with which it shares the hindlimb clasping, gait abnormalities and other motor deterioration. The later disease onset correlates with delayed appearance of htt aggregates and brain atrophy. Also, R6/1 mice usually live more than 1 year (Naver *et al.* 2003). In several treatment studies, N171-82Q mice expressing N-terminal fragment of htt gene containing exons 1 and 2 with 82 CAG was used (Schilling *et al.* 1999a). These mice have similar but less severe and more variable phenotype than R6/2. The lifespan varies between ~4 and 6 months. Interestingly, the exposure of R6/1and R6/2 mice to enriched environment delayed the disease onset and ameliorated the clasping phenotype and the loss of peristriatal cerebral volume (Hockly *et al.* 2002; van Dellen *et al.* 2000; Glass *et al.* 2004), probably because of restoration of the neurogenic process (Lazic *et al.* 2006).

More recently, HD190Q and HD150Q mice were generated expressing exon 1 htt with 190 and 150 CAG under htt promotor, respectively. The transgene also contains enhanced green fluorescent protein, which makes this model a simple and sensitive tool for *in vivo* testing of therapeutic molecules for inhibiting aggregate formation that can be visualized by a fluorescent imager. Down-regulation of mRNAs for a number of hypothalamic peptides that are known to be involved in feeding behavior and energy homeostasis was observed in these mice. The median survival of HD190Q is ~21 weeks and that of HD150Q mice 32 weeks (Kotliarova *et al.* 2005). This model however will need further phenotypic analysis.

Full-length transgenic Huntington's disease mouse models

An HD mouse model with full-length human htt with 48 or 89Q manifested progressive behavioral and motor dysfunction with neuronal loss and gliosis (Reddy *et al.* 1998). A yeast artificial chromosome HD mouse model expresses the whole human htt with 128Q (YAC128) and replicates the neuropathology in HD patients (Hodgson *et al.* 1999). First symptoms, such as hindlimb clasping or gait abnormalities occur at about 3 months (Slow *et al.* 2003). Apoptosis is activated and mitochondrial dysfunction occurs in these mice as well (Fei *et al.* 2007). YAC128 appears to be a very useful model for therapeutic studies (Tang *et al.* 2009). A newer model, BAC transgenic mouse model of HD, was generated by introduction of BAC consisting of whole human htt locus of 170 kb with an expansion of 97 CAG (Gray *et al.* 2008). BAC transgenic mouse model of HD revealed that the progressive course of HD and the selective pathology of mouse brains might occur without early nuclear accumulation of aggregated htt. Synaptic pathology was observed prior to neuronal degeneration in these mice with first signs of synaptic dysfunction detectable at 3 months and a progressive synaptic pathology at 6 months (Spampanato *et al.* 2008).

Knock-in Huntington's disease mouse models

Four knock-in HD mouse models have been generated and are one of the most precise genetic replicas of conditions in humans and were expected to mimic the pathogenesis of HD better than the transgenic models. The symptoms and neuropathological findings of these mice turned out to be quite subtle with no cerebral atrophy and only rare nuclear inclusions occurring later than in transgenic mice. No shortening of the lifespan was observed in knock-in models (Shelbourne *et al.* 1999; Wheeler *et al.* 2000; Lin *et al.* 2001; Levine *et al.* 1999), therefore survival is not suitable as an endpoint in therapeutic studies. On the other hand, these mice display measurable neuropathological features and

behavioral symptoms that can be used for validation of experimental treatments.

The Hdh*Q111* line is a knock-in model with 111 CAG repeats inserted into the mouse HD gene (Wheeler *et al.* 2000). Nuclear htt immunoreactivity in ventral striatal neurons at about 6 weeks of age and later, 4.5 months, formation of N-terminal htt intranuclear inclusions in medium spiny neurons were observed in these mice. Late-onset motor deficits comprising mild gait disturbances were detected by footprint analysis at 24 months of age accompanied by reactive gliosis in affected areas of the brain. Lack of neuronal apoptosis in the striatum at this age indicates slow progression of the disease (Wheeler *et al.* 2002).

Similarly to Hdh*Q111*, in Hdh*Q94* and Hdh*Q140* knock-in mice, exon 1 of the mouse htt is replaced by mouse/human chimeric exon 1, in these cases with 94 and 140 CAG repeats, respectively. Mice expressing 94Q protein show increased repetitive rearing at night at 2 months followed by decreased locomotion activity at 4 and 6 months of age. Intranuclear microaggregates were observed at 4 months together with reduced mRNA levels in striatum. The neuronal intranuclear inclusions are widely distributed throughout striatum at 6 months of age (Menalled *et al.* 2002).

Hdh*Q140* mice develop very similar phenotype as Hdh*Q94*, however the disease starts earlier. The increased rearing occurs at 1 month, hypoactivity at 4 months and gait deficits at 12 months of age. Htt inclusions were observed from 1 month of age and there was a more widespread distribution than in Hdh*Q94* mice with intranuclear aggregates in several brain regions at age of 6 months (Menalled *et al.* 2003). Neuronal loss with reactive gliosis was found at 20–26 months of age with as much as 38% reduction in striatal volume. Alterations in dopaminergic signaling in HD mice could be at least partly attributed to the decrease in 32 kDa dopamine and cAMP-regulated phosphoprotein expression observed in the striata of Hdh*Q140* mice from 12 months of age (Heng *et al.* 2008).

Another knock-in mouse model is $Hdh^{(CAG)150}$ with insertion of 150 CAG expansion into exon 1 of the mouse htt homolog. $Hdh^{(CAG)150}$ mice have late-onset phenotype with neuronal intranuclear inclusions present predominantly in the striatum (Lin *et al.* 2001; Tallaksen-Greene *et al.* 2005; Heng *et al.* 2007). Significant behavioral phenotype does not appear within first year and only after 70–100 weeks of age in homozygous mice, weight loss, decreased activity, diminished rotarod performance, and clasping was observed. At age of 100 weeks, both homozygous and heterozygous $Hdh^{(CAG)150}$ mice exhibited resting tremor, unsteadiness, and staggering gait. Gliosis was significantly increased by 14 months. By 27 weeks, nuclear htt immunoreactivity and ubiquitin-positive inclusions were initially present within the matrix compartment and later, at 70–100 weeks of age, intranuclear inclusions were observed in most striatal neurons. Striatal dopamine D_1 and D_2 receptor binding sites were reduced at 100 weeks of age in both homozygous and heterozygous mice with a 50% neuronal loss and a 40% reduction in striatal volume. The D_1 and D_2 receptors were, however, already diminished at age of 70 weeks with no loss of striatal neurons suggesting that neuronal dysfunction precedes neurodegeneration (Heng *et al.* 2007).

Mouse models of other polyglutamine diseases

Transgenic mouse models bearing either truncated or full-length cDNA of respective genes for SCA1–3 and 7, DRPLA, and SBMA, YAC mouse models for SCA3 and SBMA and knock-in mouse lines for SCA1 and SCA7 have been generated (Yamada *et al.* 2008; Marsh *et al.* 2009). Models of all polyQ diseases showed accumulation of mutant proteins in neuronal nuclei except SCA2, where the expanded ataxin-2 was localized in the cytoplasm (Huynh *et al.* 2000).

Polyglutamine mouse models and preclinical therapeutic testing

Recently, concerns about applicability of different mouse models for preclinical therapeutic testing have been raised. Especially, the suitability of the models with truncated forms of mutant polyQ proteins have been discussed because they might represent very artificial systems not reflecting the real conditions observed in human diseases. Usually, these truncated transgenes are expressed from more than one copy of the cDNA per cell resulting in an over-expression and intensified toxicity. However, this should not be a prerequisite for avoiding the fragment models for initial experimental treatments. It has been shown that in the full-length HD mouse models, the cleaved form of mutant htt is the mediator of cytotoxicity (Graham *et al.* 2006). If a therapy is able to ameliorate the neuropathological and behavioral phenotype of an 'exaggerated' state, the more subtle damages observed in full-length or knock-in mouse models would be expected to be manageable with the same therapy and in an optimal situation with more pronounced beneficial outcome or with lower doses of the drug reducing the risk of unwanted side effects. Moreover, some of the fragment models, such as R6/2, are so well characterized and have been employed in many studies that they are the best choice for at least initial *in vivo* treatment testing and comparing the outcomes with preceding trials. Although this is not true in some therapeutic strategies, such as those targeting the proteolytic cleavage of the intact proteins. For other treatments, such as those targeting polyQ protein aggregation or clearance, the fragments models are very useful because of the robust expression of the transgenes and constant appearance of neuronal inclusions. For an eventual translation of the particular treatment into the clinic, however, the results from a fragment model should be validated in a second mouse model with a full-length transgene or in a knock-in model.

Therapeutic approaches for polyglutamine diseases

Experimental treatments of polyQ diseases could be divided into those targeted at the pathological cascades of the disease, such as preventing the cellular damage and to those intercepting downstream deterioration. To the former category belong the therapies decreasing the levels and inhibiting the aggregation of the mutant protein, while the approaches targeting the toxic effects of the polyQ protein such as mitochondrial dysfunction and oxidative stress, transcriptional abnormalities, UPS impairment, excitotoxicity, or apoptotic pathways belong to the latter. Some treatments can target both aspects, for example amiloride or its homolog benzamil decreases the intracellular levels of mutant htt by activating the impaired UPS (Wong *et al.* 2008), enabling the degradation of other ubiquitinated cellular proteins.

Despite many experimental therapeutic studies (Beal and Ferrante 2004; Li *et al.* 2005; Giampa *et al.* 2009), unfortunately only a few have been translated into clinical trials. This is however not always a result of a failure or toxicity of the therapeutic agent itself in preclinical confirmatory studies. The clinical studies in polyQ diseases patients are difficult to perform because of slow progression of these diseases, low incidence, and inter- and intrafamilial variability in the disease course.

Therapies aimed at the polyglutamine proteins

Gene silencing

Decreasing the levels of the mutant protein and thus preventing the downstream deteriorating effects appears to be one of the best strategies. The therapeutic potential of down-regulating abnormal gene expression has been demonstrated in a tetracycline-regulated mouse model of HD (Yamamoto *et al.* 2000) and a doxycycline-regulated SCA1 mouse model (Zu *et al.* 2004). The nuclear inclusions, which formed after induction of the mutant htt expression, disappeared when the expression was shut down. Also the behavioral phenotype was ameliorated, suggesting that therapeutic approaches aimed either at inhibition of mutant htt expression or its degradation might be effective. Several techniques targeting polyQ protein expression have been explored including siRNA or short-hairpin RNA (Bonini and La Spada 2005). This strategy however appeared to be not feasible in humans because of the non-specific ablation of the normal gene copy. On the other hand, this problem could be overcome as single nucleotide polymorphisms have been identified with some of them being suitable to siRNA targeting (Lombardi *et al.* 2009; Pfister *et al.* 2009). Recently, microRNAs suppressing the expression of ataxin-1 mRNA by specific binding to its 3′-untranslated region and decreasing the levels of the protein were identified and successfully tested in cells (Lee *et al.* 2008). For diseases with pathology at limited localizations such as retina in SCA7 this approach is suitable. On the other hand, targeting wide CNS pathology in other polyQ diseases would be more challenging.

Enhancement of protein degradation

Enhancing the degradation of mutant proteins is another therapeutic approach and the attempts to increase the autophagic clearance of mutant htt resulted in reduced htt toxicity in N171-82Q transgenic mice and in the *Drosophila* and the zebrafish HD models (Sarkar *et al.* 2007b; Williams *et al.* 2008). The activations of both mammalian target of rapamycin-dependent (e.g. by rapamycin analog CCI-779) or -independent (e.g. by lithium, calpain inhibitors, etc.) pathways modulation were beneficial and the combination treatment resulted in additive protection against polyQ-related neurodegeneration (Sarkar *et al.* 2008). Lithium also improved the neurological and pathological findings in SCA1(154/2Q) knock-in mice (Watase *et al.* 2007). There is not much knowledge about regulation of the enzymatic UPS activity. While a relatively large-scale of UPS inhibitors exists, no chemical compound actually activating the UPS activity has been available. Recently, amiloride, a well-known potassium-sparing diuretic drug widely used in clinics, and its derivative, benzamil, have been reported to reduce the polyQ aggregation and toxicity in HD models. Benzamil ameliorated brain pathology, motor deficits, and increased the lifespan of R6/2 mice (Wong *et al.* 2008). Another compound shown to increase enzymatic UPS activity is Y-27632, a rho-associated kinases inhibitor, which already has been used in clinical trials because of its anti-ischemic, antivasospastic, and antihypertensive effects (Lai and Frishman 2005). Interestingly, this compound also enhanced the macroautophagy activity, and this unique effect of modulating both main cellular degradation pathways led to reduced levels and reduced aggregation of mutant htt, ataxin-3, AR, and atrophin-1 in cell systems (Bauer *et al.* 2009). Although Y-27632 was previously shown to reduce the polyQ toxicity in *Drosophila* model of HD (Pollitt *et al.* 2003), this treatment needs to be tested further in mouse models.

Inhibition of aggregation

One of the first therapeutic approaches in polyQ diseases has been aimed at the prevention of aggregation. The therapeutic potential of small molecules able to prevent directly the formation of polyQ aggregates has been shown in several studies. For example treatment of R6/2 mice with Congo Red or trehalose significantly increased the mice survival by 16.4% and 11.3%, respectively (Sanchez *et al.* 2003; Tanaka *et al.* 2004). In a recent study, however, chronic administration of Congo Red did not improve the phenotype and lifespan significantly, but this could have been a consequence of limited ability of this compound to cross the blood–brain barrier (Wood *et al.* 2007). Trehalose has been

reported to activate macroautophagy in an mammalian target of rapamycin-independent manner (Sarkar *et al.* 2007a) so the observed beneficial effect in mice might result from the stabilization of the expanded polyQ protein and its enhanced degradation. Conserving mutant htt in the native non-toxic conformation has been thought to prevent the cytotoxic effect of the mutant protein. PolyQ-binding peptide 1 was shown to prevent conversion of the expanded polyQ into aggregation-prone β-sheet-rich conformation and prevent neurodegeneration in a *Drosophila* model of HD (Nagai *et al.* 2003, 2007).

Cystamine may reduce expanded polyQ aggregation by inhibition of TG that is thought to crosslink expanded polyQ proteins and facilitate their aggregation. In HD brains, increased TG activity was observed (Karpuj *et al.* 1999). In one study, where the treatment of R6/2 mice started at age of 7 weeks, cystamine did not affect aggregate formation, while improving the mice phenotype (Karpuj *et al.* 2002). This could have been a result of caspase inhibition and antioxidant activity of cystamine (Lesort *et al.* 2003). In another study though, where the treatment started at 3 weeks, the htt aggregation decreased by 68% in striatum and by 47% in neocortex (Dedeoglu *et al.* 2002). The survival improvement was high in both studies with 12% and 19.5% increase, respectively.

Another strategy to reduce the misfolding, oligomerization, and aggregation of polyQ proteins is to increase the cellular levels of molecular chaperones such as Hsp70. The up-regulation not only interferes with the aggregation process but may also enhance the degradation of the mutant protein through UPS. Beside over-expression of HSPs, several promising compounds have been introduced which induce the expression of HSPs. Geranylgeranylacetone is an acyclic isoprenoid compound, used as an oral anti-ulcer drug, strongly induced HSPs expression in different tissues (Hirakawa *et al.* 1996). Oral administration of geranylgeranylacetone enhanced the expression of Hsp70, Hsp90, and Hsp105 through induction of heat-shock factor 1 in CNS, where it inhibited nuclear accumulation of mutant AR leading to improved motor behavior and increased survival of transgenic SBMA mice (Katsuno *et al.* 2005). An Hsp90 inhibitor, 17-(allylamino)-17-demethoxygeldanamycin (17-AAG) has also been demonstrated to ameliorate neurodegeneration in SBMA mice (Waza *et al.* 2005). Treatment with 17-AAG dissociated p23 (Hsp90 cochaperone) from the Hsp90–AR complex and facilitated the proteasomal degradation of the mutant AR. Recently, it has been reported that 17-AAG suppressed polyQ-induced neurodegeneration in *Drosophila* models of SCA3 and HD via activation of heat-shock factor 1 which in turn up-regulated molecular chaperones Hsp40, Hsp70, and Hsp90. 17-AAG reduced the lethality of SCA3 and HD *Drosophila* models by 74.1% and 46.3%, respectively (Friedman *et al.* 2008). An analog of 17-AAG, 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin has recently been reported to induce expression of Hsp40 and Hsp70, degrade the pathogenic AR protein, and to ameliorate the phenotype in SBMA transgenic mice (Tokui *et al.* 2009).

Therapies aimed at downstream effect of the expanded polyglutamine protein

Besides attempting to cut off the polyQ-related pathology at the level of the mRNA or polyQ protein itself, a number of treatments targeting the downstream pathogenic events have been tested.

Modification of transcription

Several compounds modulating the deregulated gene transcription have been tested in different models of polyQ diseases. Especially, targeting histone methylation and acetylation is thought to achieve normalization of transcription disrupted by expanded polyQ proteins. Histone deacetylase inhibitors such as suberoylanilide hydroxamic acid (SAHA) and sodium butyrate (SB) have been demonstrated to ameliorate the phenotype in polyQ disease models. SAHA has been tested in R6/2 mice in which it improved the motor phenotype however no amelioration in body weight was observed and higher doses of SAHA were toxic (Hockly *et al.* 2003). The effect of SB was investigated in R6/2 mice and besides the improvement in motor phenotype it also delayed the body weight loss and extended the lifespan by more than 20%. Moreover, SB increased not only the acetylation of histones H3 and H4 but also of SP1 resulting in improved transcriptional regulation in R6/2 brains (Ferrante *et al.* 2003). In DRPLA transgenic mice expressing full-length atrophin-1 with 118Q (Atro-118Q14), SB ameliorated the motor impairments and extended the lifespan (Ying *et al.* 2006). Treating AR-97Q SBMA mice expressing full-length AR with SB mitigated the neuromuscular phenotype and increased survival (Minamiyama *et al.* 2004). Another histone deacetylase inhibitor, phenylbutyrate, improved survival, and attenuated gross brain atrophy and ventricular enlargement in N171-82Q HD transgenic mice (Gardian *et al.* 2005).

The inhibition of histone H3 methylation with mithramycin resulted in a great extension of lifespan in R6/2 mice accompanied by improvement in motor performance and striatal pathology (Ferrante *et al.* 2004). The improvement of H3 and H4 modification profile after chromomycin and mithramycin treatment was observed also in N171-82Q mouse model (Stack *et al.* 2007). Another drug shown to mitigate the transcriptional dysbalance is the phosphodiesterase type IV inhibitor rolipram, which increases the phosphorylation and activity of CREB. Rolipram ameliorated the neuropathological findings, slowed the progression of neurological phenotype and increased the survival in R6/2 mice. Moreover, BDNF, whose expression is impaired in HD (Zuccato *et al.* 2003), was induced in treated mice through

restored function of CREB (DeMarch *et al.* 2008). In another study, it was shown that rolipram also prevented the sequestration of CBP into nuclear aggregates (Giampa *et al.* 2009).

In the AR-97Q SBMA mouse model, a drug specifically disrupting the interaction of mutant AR with CBP, ASC-J9 (dimethylcurcumin), reduced the SBMA symptoms and reversed muscular atrophy in treated mice. This effect was accompanied by restoration of vascular endothelial growth factor 164 expression (Yang *et al.* 2007).

In a *C. elegans* HD transgenic model expressing N-terminal htt with 128Q, treatment with resveratrol resulted in amelioration of mutant polyQ toxicity and it also reduced cell death in neuronal cells derived from HdhQ111 knock-in mice. The underlying mechanism was shown to be an enhanced activation of silent mating type information regulation-2 protein through activation of *daf-16*, a member of the forkhead box family of Forkhead transcription factors (Parker *et al.* 2005). Resveratrol treatment appears to be very promising candidate for support therapy of polyQ diseases.

Mitochondria, energy metabolism, and antioxidants

Different compounds improving energy metabolism defects or reducing oxidative stress in polyQ diseases have been successfully tested in mouse models. Reduced concentrations of creatine and phosphocreatine were observed in basal ganglia of HD patients (Sanchez-Pernaute *et al.* 1999). Creatine administration stabilized the MPT, prevented ATP depletion, and increased the protein synthesis. In R6/2 mice, the treatment with 2% creatine ameliorated the brain pathology, improved the phenotype and increased the lifespan by 17.4% (Ferrante *et al.* 2000). Similarly, in N171-82Q HD mice, the survival extended by 19.3% with 2% dietary creatine treatment (Andreassen *et al.* 2001a). Other drugs preventing the depletion of ATP, or increasing the activities of mitochondrial complexes II–III seen in HD, such as dichloroacetate and triacetyluridine were successfully tested in R6/2 mice with moderate but significant effects (Andreassen *et al.* 2001b; Saydoff *et al.* 2006).

Antioxidants such as α-lipoic acid, coenzyme Q_{10}, clioquinol, tauroursodeoxycholic acid (also antiapoptotic effect), or BN82451 have been proven effective in R6/2 mouse lines (Giampa *et al.* 2009). Especially coenzyme Q_{10}, an antioxidant, a cofactor of the mitochondrial electron transport chain and inhibitor of MPT, extended the survival of R6/2 mice by up to 26.3% while greatly improving the motor perfomance and reducing weight loss and nuclear inclusions (Smith *et al.* 2006). Clioquinol administration reduced the striatal atrophy, decreased nuclear inclusion formation, ameliorated the HD phenotype development, and enhanced the lifespan in R6/2 mice by 20% (Nguyen *et al.* 2005).

Excitotoxicity

The overactivation of NMDA glutamate receptors and subsequent excitotoxicity in striatal neurons leading to their death has been thought to have a role in pathogenesis of HD and electrophysiological studies in brain slices from R6/2 mice indeed suggested complex changes in glutamatergic transmission (Cepeda *et al.* 2003). Therefore, compounds intercepting excessive glutamate release have been tested for therapeutic purposes. Treatment of R6/2 mice with a glutamate antagonist, riluzole, decreased the body weight loss, affected the initial locomotor activity, significantly increased the mean survival (10.2%), and appeared to modulate the aggregation process (Schiefer *et al.* 2002). Although the beneficial effects of riluzole were not confirmed in a recent study (Hockly *et al.* 2006), clinical trials were carried out in HD patients. Unfortunately, the latest 3-year trial showed no beneficial effect (Landwehrmeyer *et al.* 2007). Two drugs affecting the levels of glutamate in synaptic cleft through stimulation of the presynaptic metabotropic glutamate receptor 2 (mGluR2) or inhibition of the post-synaptic mGluR5, LY379268, and 2-methyl-6-(phenylethynyl)-pyridine, respectively were also tested for their ability to combat HD in R6/2 mice (Schiefer *et al.* 2004). Similar to riluzole, both compounds significantly increased the lifespan of R6/2 mice. In one study, the administration of remacemide (NMDA antagonist) or coenzyme Q_{10} prolonged the survival of R6/2 mice by 15.5% and 14.5%, respectively, and reduced motor deficits, weight loss, and aggregate formation. When the drugs were combined, the survival extended by 31.8% (Ferrante *et al.* 2002). This combination was not so effective in N171-82Q mice but the lifespan still improved by more than 20% (Schilling *et al.* 2001). The clinical trial using either of these drugs or in combination, however displayed no significant effects in HD patients (Huntington Study Group 2001). Another NMDA antagonist, memantine, has been reported to retard the disease progression in HD patients (Beister *et al.* 2004).

Apoptosis

From antiapoptotic drugs, minocycline appeared to be a good candidate for therapeutic trials. Minocycline was shown to inhibit the mitochondrial release of apoptosis inducing factor, proapoptotic protein Smac/Diablo, and cytochrome *c*, while decreasing the cleavage of proapoptotic factor Bid and activating caspases 1, 3, 8, and 9 (Wang *et al.* 2003). Intraperitoneal administration of minocycline to R6/2 mice extended the lifespan by 13.5% (Chen *et al.* 2000). When administered perorally, minocycline failed to improve the symptoms in R6/2 mice (Smith *et al.* 2003). In another study, minocycline and coenzyme Q_{10} improved survival of R6/2 mice 11.2% and 14.6%, respectively, and when administered together, the neuropathology and phenotype outcome improved when compared with separate treatments and the

lifespan extension was 18.2% (Stack *et al.* 2006). The results of the clinical studies using minocycline produced promising data (Bonelli *et al.* 2004) but a long-term clinical trial should be conducted to definitely evaluate the benefits of minocycline in HD patients.

Inhibition of caspases could comprise double beneficial effect in polyQ pathogenesis. First, decreased generation of caspase-cleaved fragments of mutant proteins may result in reduced toxicity and second, execution of apoptosis may be blocked. A broad caspase inhibitor, z-Val-Ala-Asp-fluoromethylketone, improved the rotarod performance of R6/2 mice and extended the lifespan by 25% (Ona *et al.* 1999). The combined administration of caspases 1 and 3 inhibitors, Tyr-Val-Ala-Asp-chloromethylketone and Asp-Glu-Val-Asp-aldehyde-fluoromethylketone, respectively, increased the survival by 17.3% (Chen *et al.* 2000).

Other treatment possibilities

Selective serotonin reuptake inhibitors are a group of drugs widely used for the treatment of patients with depression and severe anxiety disorders (Blier and de Montigny 1994). In addition to restoration of serotonin levels in striatum, the treatment of N171-82Q mice with paroxetine reduced brain atrophy, delayed the onset of motor dysfunction, attenuated weight loss, and increased the survival (Duan *et al.* 2004). Another selective serotonin reuptake inhibitor, sertraline, had similar effects in N171-82Q mice. Upon this treatment, increased BDNF levels, preserved levels of Hsp70 and Bcl-2 in the brains, and enhanced neurogenesis were observed (Duan *et al.* 2008). The enhanced neurogenesis and increased BDNF levels were also reported in R6/2 mice treated with sertraline (Peng *et al.* 2008). Importantly, the effective levels of sertraline in mice were comparable to those achievable in humans (Duan *et al.* 2008).

Control of Ca^{2+} homeostasis seems to be a promising therapeutic strategy for polyQ diseases and particularly the modulation of the IP3R1 activity in ER membrane could provide a good target (Bezprozvanny 2007; Chen *et al.* 2008b). Inhibiting the excessive Ca^{2+} release from intracellular storages would potentially result in reduced deteriorating events such as calpain activation or mitochondrial dysbalance leading to caspase and apoptosis activation. Although there has been a focus on identifying downstream effectors to address the particular pathogenic event without disturbing other physiological or protective cellular processes, in this case, the relatively proximal component of the intracellular Ca^{2+}-dependent network, down-regulation of IP3R1 activity, could be evaluated more extensively.

Another strategy to advance toward an effective therapy of polyQ diseases is the combination of drugs targeting different aspects of these disorders. As mentioned in the section 'Excitotoxicity', the synchronous utilization of some compounds resulted in increased therapeutic effects in some cases (coenzyme Q_{10} with remacemide or minocycline). Combinations of a treatment targeting the polyQ protein abnormal processing with another treatment aimed at a downstream pathogenic process are of particular interest. For example, the concomitant treatment of R6/2 mice with cystamine (prevents polyQ aggregation by TG inhibition) and mithramycin (restores the polyQ-mediated transcriptional dysregulation) extended the mean survival by 40% and markedly improved the neuropathological findings more than a single treatment by any of these two drugs (Ryu *et al.* 2006).

As described in the section 'Role of nuclear localization of mutant polyglutamine proteins', testosterone binds to AR and promotes its uptake by nucleus, the primary site of SBMA pathogenesis (Stenoien *et al.* 1999; Katsuno *et al.* 2002). Treatment aimed at testosterone blockage by castration has been shown to be beneficial in SBMA transgenic mice (Katsuno *et al.* 2002). Chronic adminitration of a luteinizing hormone-releasing hormone analog, leuprorelin, caused decreased serum levels of testosterone in male transgenic AR-97Q mice resulting in amelioration of neuromuscular phenotype and increased lifespan (Katsuno *et al.* 2003). Importantly, phase 2 clinical trial showed beneficial effects of leuprorelin in SBMA patients and is a good premise for large-scale clinical trials of androgen deprivation (Banno *et al.* 2009).

In last decade, a great progress has been achieved in understanding the pathomechanism of polyQ diseases, but, unfortunately, the therapy development does not keep up. The challenge for the next decade will be the translation of promising therapeutic strategies from preclinical trials to clinical use.

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Ⅲ．研究成果の刊行に関する一覧表

1．雑誌

欧文

著者名	論文題目	雑誌名	巻：頁,西暦年号
Doumanis, J., Wada, K., Kino, Y., Moore, A.W. & Nukina, N.	RNAi screening in Drosophila cells identifies new modifiers of mutant huntingtin aggregation.	*PLoS One*	**4**;e7275 (2009).
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Takeda A, Saito N, Baba T, Kikuchi A, Sugeno N, Kobayashi M, Hasegawa T, Itoyama Y.	Functional imaging studies of hyposmia in Parkinson's disease	J Neurol Sci	(in press)
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2．単行本

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