Comparative analysis of genetic modifiers in Drosophila points to common and distinct mechanisms of pathogenesis among polyglutamine diseases

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Spinocerebellar Ataxia type 1 (SCA1) and Huntington’s disease (HD) are two polyglutamine disorders caused by expansion of a CAG repeat within the coding regions of the Ataxin-1 and Huntingtin proteins, respectively. While protein folding and turnover have been implicated in polyglutamine disorders in general, many clinical and pathological differences suggest that there are also disease-specific mechanisms. Taking advantage of a collection of genetic modifiers of expanded Ataxin-1-induced neurotoxicity, we performed a comparative analysis in Drosophila models of the two diseases. We show that while some modifier genes function similarly in SCA1 and HD Drosophila models, others have model-specific effects. Surprisingly, certain modifier genes modify SCA1 and HD models in opposite directions, i.e. they behave as suppressors in one case and enhancers in the other. Furthermore, we find that modulation of toxicity does not correlate with alterations in the formation of neuronal intranuclear inclusions. Our results point to potential common therapeutic targets in novel pathways, and to genes and pathways responsible for differences between Ataxin-1 and Huntingtin-induced neurodegeneration.

INTRODUCTION

Several dominantly inherited human neurodegenerative diseases result from the expansion of a translated tract of consecutive CAG trinucleotide repeats. Because CAG encodes for glutamine, these disorders are referred as polyglutamine diseases. To date, this group includes Kennedy’s disease or Spinal-Bulbar Muscular Atrophy (SBMA), Huntington’s disease (HD), Dentatorubral-Pallidoluysian Atrophy (DRPLA) and several distinct Spinocerebellar Ataxias (SCA1, SCA2, SCA3/Machado–Joseph disease, SCA6, SCA7 and SCA17). The normal function of many of the genes causing these disorders remains unclear and the corresponding proteins have no sequence similarity with each other except for the polyglutamine tract itself. Although the disease-causing proteins are widely expressed in the CNS, these disorders are characterized by loss of specific neuronal populations, resulting in characteristic patterns of neurodegeneration and clinical manifestations. In all cases, the disease only occurs once the glutamine expansion surpasses a certain threshold. The clinical symptoms usually manifest around mid-life, and become progressively worse within the next two decades of life. Furthermore, longer repeats lead to earlier age of onset with very long expansions causing juvenile cases (reviewed in 1).

It has been proposed that these disorders are caused by gain of toxic function mechanisms. In agreement with this idea, mice models expressing the expanded mutant protein show cell loss and characteristic neurodegenerative phenotypes (2,3). Therefore, even though in some cases the...
loss-of-function (LOF) of the normal gene may contribute to disease (reviewed in 4), an important component of the pathology comes from a toxic gain of function of the disease-causing protein.

There is also abundant evidence that protein context is important for polyglutamine toxicity. For example, post-transcriptional modifications outside of the polyglutamine tract are critical in determining the clinical and pathological effects of the corresponding proteins (reviewed in 2,3).

Polyglutamine diseases share a number of common features including progressive neuronal degeneration and formation of protein aggregates. These aggregates contain the mutant polyglutamine protein and other proteins such as ubiquitin, chaperones and proteasome subunits. The neuronal aggregates can be mainly nuclear [nuclear inclusions (NI)s; SCA1, SCA7 and SCA17], cytoplasmic (SCA6) or present in both cytoplasm and nucleus as in HD, DRPLA, SBMA, SCA2 and SCA3 (3,5,6). The role of NI s in pathogenesis has been a matter of much debate. At first, the predominant idea was that NI s are pathogenic as they may sequester essential proteins and interfere with their normal function. Later evidence led to the alternative view that NI s are not the main drivers of toxicity. NI s may be in fact protective by sequestering toxic oligomeric forms of the mutant protein, and preventing deleterious interactions between these oligomers and other cellular proteins (2,3,5,6).

Certain disease mechanisms may be common to all polyglutamine diseases. Protein misfolding, protein turnover, transcriptional dysregulation and apoptosis have been implicated in many of these disorders (reviewed in 2,3,8–10). However, many important specific questions remain unanswered; e.g. Are the same genes and pathways involved in transcriptional alterations in different polyglutamine diseases? Other mechanisms, such as RNA processing, axonal transport, synaptic transmission, calcium homeostasis, mitochondrial dysfunction or endoplasmic reticulum impairment have been implicated in the pathogenesis of one or more of these disorders (3,11–13). Are some of these mechanisms also common to many polyglutamine diseases? Comparative studies are required to answer these questions, and to identify common and disease-specific genetic pathways implicated in disease progression. These studies will also facilitate the development of effective therapies by identifying potential therapeutic targets.

SCA1 and HD are autosomal dominant diseases caused by expansion of a polyglutamine tract on the N-terminal region of the Ataxin-1 and Huntingtin (Htt) proteins, respectively. SCA1 is characterized by loss of coordination, impaired balance, motor and cognitive decline. These clinical features are associated with degeneration of cerebellar Purkinje cells, spinocerebellar tracts and, to a lesser extent, brain stem nuclei (14). In HD, clinical manifestations include involuntary movements (chorea), cognitive decline and psychiatric disturbances that lead to progressive dementia. These characteristic symptoms result mainly from degeneration of striatal neurons but also from degeneration of cortex neurons (4,15).

Comparative analysis of genetic modifiers of disease using animal models is a powerful approach to identify common and distinct mechanisms of pathogenesis exerted by different polyglutamine proteins. Drosophila is well suited for this type of analysis because of the surprising similarity of its genome and the human genome, the ability to manipulate up or down the activity of most of its genes and the fast pace of experimentation (16). To date, only a small number of modifier genes have been compared in fly models of neurodegenerative diseases (17). Here we take advantage of Drosophila models of HD and SCA1 to compare genetic modifiers and mechanisms of pathogenesis of Ataxin-1 and Huntingtin toxicity.

We find modifier genes that are common to the SCA1 and HD models that function in protein folding/degradation, apoptosis and transcriptional regulation as well as in RNA processing and signaling. Other modifier genes have disease model specific effects. Of these, a group modulates disease progression in both SCA1 and HD Drosophila models, but in opposite ways. The observed phenotype modifications are not cell-type specific since we obtained similar results using both eye and neuro-behavioral assays.

RESULTS

Degenerative phenotypes and NI s caused by targeted expression of either SCA182Q or Htt128Q transgenes in Drosophila can be used for comparative analysis of modifier genes

The directed over expression (OE) of the transgene of interest was achieved by using the GMR-GAL4 driver, which directs expression to photoreceptor neurons and surrounding support eye cells. OE of the human Ataxin-1 protein containing a polyglutamine tract of 82 glutamines (Ataxin-182Q, Fig. 1A, top) in the eye causes an external eye phenotype consisting of ommatidial disorganization and interommatidial bristle loss that can be used to screen for genetic modifiers [Fig. 1C and B (control), (18)]. These flies expressing the SCA182Q transgene also display a retinal phenotype, characterized by a decrease in the length of the retina, tissue loss and shortened and abnormal photoreceptor neurons [Fig. 1F and E (control)].

We have also generated transgenic flies over expressing the N-terminal portion of the Huntingtin protein (amino acids 1-336) including an expanded tract of 128 glutamine repeats (Htt128Q, Fig. 1A, bottom). Unlike flies expressing Ataxin-182Q that display a severe rough external eye phenotype, the N-terminal HD model flies show mild ommatidial disorganization and bristle loss phenotypes (Fig. 1D). However, longitudinal sections show severe disorganization, tissue loss and vacuolization in the retina of these flies (Fig. 1G). This effect is even more dramatic in aged flies (Fig. 1H), demonstrating that the degenerative phenotype is progressive.

Like in SCA182Q-expressing flies, in the HD model we observe formation of neuronal NI s, one of the hallmark of both diseases [Fig. 11–K; (18) for expanded Ataxin-1 NI s]. Using the OK107-GAL4 driver we direct the expression of the Htt128Q transgene to specific neurons of the CNS. Immunofluorescence staining experiments reveal that the Htt protein is present both in large NI s and in a diffuse form that may represent soluble protein or small aggregates that cannot be resolved with the immunofluorescence method used. The percentage of cells that contain large macro-aggregates increases
During the aging process of the fly (data not shown) in agreement with the progressiveness of the model.

Using the eye phenotypes described above as a primary assay, we investigated the effects of a collection of candidate modifier genes on the SCA1 and HD models. This collection mainly includes modifier genes previously identified in a forward genetic screen using the SCA1 model (18). We found three different categories of modifiers: genes that modify SCA1 82Q and Htt 128Q-induced phenotypes similarly, genes that appear to modify only one of the models, and genes that modify the two disease models in opposite ways.

**Figure 1.** SCA1 and HD Drosophila models. (A) Schematic representation of the SCA1 82Q and Htt 128Q constructs. (Top) A human SCA1 transgene encoding the full-length Ataxin-1 protein containing 82 glutamine repeats was used to generate the SCA1 Drosophila model. (Bottom) An N-terminal fragment of the human Huntingtin cDNA encoding the first 336 amino acids of the protein (including 128 glutamine repeats) was used to generate the HD Drosophila model. In all cases the transgenes were cloned downstream of UAS binding sites to confer regulation by the GAL4 transcriptional activator. (B–H) Scanning electron microscope (SEM) photographs of the external eye (B–D) and retinal sections (E–H) of control flies (B and E), flies expressing SCA1 82Q (C and F) and flies expressing Htt 128Q (D, G and H) under the control of the GMR-GAL4 driver. Insets show a higher magnification view of the ommatidia/retina. Arrows in (E–H) indicate length of the retina. Control flies (B and E) show normal organization of the ommatidia, bristles (at the external level) as well as retina. Expression of the SCA1 82Q transgene (C and F) induces a severe disruption of the external lattice of the eye accompanied by a dramatic reduction of the length of the retina (compare with the controls in B and E, respectively). Expression of the Htt 128Q transgene (D) induces only a very mild external eye phenotype. However, at the retinal level (G and H), expression of the Htt 128Q transgene causes shortening and disorganization of the retina, as well as evident tissue loss. These phenotypes become even more evident in aged flies (H, shows 6 days old fly). Flies used for SEM analysis were raised at 27°C (B–D) and flies for paraffin vertical sections were raised at 25°C (E–H). (I–K) Confocal images of Drosophila neurons stained with anti-Huntingtin antibody. Images show high magnification views of adult ventral ganglion neurons in which Htt 128Q and UAS-CD8:GFP transgenes are expressed with the OK107-GAL4 driver. UAS-CD8:GFP labels the neuronal cytoplasm (green, I) and the Htt signal, shown in red, was detected using anti-Htt antibody (J). Confocal images reveal accumulation of Htt in the nucleus and low levels of Htt in the cytoplasm (J). Large aggregates are only found in the nucleus. (K) Merged image of (I) and (J). Genotypes: (B and E) w; GMR-GAL4/UAS-GFP. (C and F) yw, UAS-SCA1 82Q[F7]/++; GMR-GAL4/UAS-GFP. (D, G and H) w; GMR-GAL4/UAS-GFP; Htt 128Q[M64]/+. (I–K) w; FRTG13, UAS-CD8:GFP/UAS-LacZ; Htt 128Q[M64]/++; OK107-GAL4/+ . All flies shown are 1 day old except for (H), that is, 6 days old. In I–K, flies are 3 days old. Scale bar in SEM pictures is equivalent to 100 μM (10 μM in the magnification insets) and equivalent to 10 μM in the retinal paraffin sections and the immunofluorescence staining.
Table 1. Genes that modify the toxicity of Ataxin-1^{82Q} and Htt^{128Q} similarly

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<td>response</td>
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| Genes that modify SCA1^{82Q} and Htt^{128Q}-induced phenotypes similarly

The genes in this group are listed in Table 1 and representative examples are shown in Figure 2. They include genes encoding signal transduction or RNA-binding proteins that were not previously known to modulate the toxicity of different polyglutamine proteins. Interestingly, this group also includes specific transcription factors that modulate the toxicity of Ataxin-1^{82Q} and Htt^{128Q} in a similar manner.

skuld (skd) is an example of a transcription factor whose partial LOF enhances the toxicity of both Ataxin-1^{82Q} and Htt^{128Q}. As shown in Figure 2B and J, one functional copy of skd (skd^{LOF}) induces a decrease in the length of the retina and promotes increased disorganization and tissue loss in the eyes of both the Ataxin-1^{82Q} and Htt^{128Q}-expressing flies, but does not have a phenotype on its own (Supplementary Fig. S1L). Controls are shown in Figures 2A and 1F for Ataxin-1^{82Q} and 2I for Htt^{128Q}, respectively.

pumilio (pum) and cpo, encode RNA-binding proteins and also modify SCA1 and HD model phenotypes similarly. OE of pum or cpo enhances both SCA1^{82Q} [Fig. 2C; (18)] and Htt^{128Q}-induced eye phenotypes (Fig. 2K, data not shown), but does not show a phenotype by itself (Supplementary Fig. S1F). Furthermore, loss of one functional copy of pum increases the retin phenotype by the SCA1 and HD Drosophila models, decreases cell loss (Fig. 2D and L, respectively), and does not display an eye phenotype alone (Supplementary Fig. S1N). Since they are RNA-binding proteins, we monitored if pum or cpo alleles would modify the Htt^{128Q} or SCA1^{82Q} mRNA levels, and found no difference with controls (Supplementary Fig. 2A and B). We also monitored the levels of Htt^{128Q} and Ataxin-1^{82Q} proteins with these modifiers in eye imaginal discs. Neither pum nor cpo had an effect on Htt^{128Q} levels. However, OE of pum did cause an increase in the Ataxin-1^{82Q} levels, whereas its LOF or OE of cpo had no effect (Supplementary Fig. S2C).

14-3-3e, encodes a protein involved in signal transduction and its OE enhances both SCA1^{82Q} and Htt^{128Q}-induced eye phenotypes (Fig. 2E and M, respectively). Note the drastic reduction in the length of the retina, accompanied with tissue loss. In contrast, 14-3-3e^{LOF} suppresses both SCA1 and HD model phenotypes as it promotes an increase in length of the retina and improves retinal integrity [Fig. 2F and N, respectively; (19)]. Modulating the expression levels of 14-3-3e does not cause a phenotype in control eyes (Supplementary Fig. S1G and O).

We have also tested the effect of an anti-apoptotic protein, Drosophila inhibitor of apoptosis (DIAP1) or thread (th), in the two models. Confirming previous results (17), we find that OE of DIAP1 strongly suppresses the SCA1^{82Q}-induced eye phenotype (Fig. 2G). We also found that DIAP1^{OE} suppresses the Htt^{128Q} eye phenotypes (Fig. 2O) and does not have a phenotype on its own (Supplementary Fig. S1H). In contrast, SCA1^{82Q} or Htt^{128Q}-expressing flies that also carry a LOF allele of DIAP1 (th^{(5)}) show a clear enhancement of the eye phenotypes when compared with SCA1^{82Q} or Htt^{128Q} controls (Fig. 2H and P, respectively; note the decrease in the length of the retina as well as increase in tissue loss). DIAP1^{LOF} (th^{(5)}) does not display an eye phenotype on its own (Supplementary Fig. S1P).

Genes that modify only Ataxin-1^{82Q} toxicity

This group includes modifiers of SCA1^{82Q}-induced toxicity previously reported (18), as well as new Ataxin-1^{82Q} modifiers reported here. They include both enhancers; i.e. heat-shock RNA o (Hsro), Sc2, Rpd3, C-terminal-binding protein (dClBP) and GstS1 as well as suppressors; i.e. pasilla (ps), and Nucleoporin-44A (Nup44A) of Ataxin^{82Q} toxicity. In the context of the HD model, however, we were unable to detect modification of the eye phenotype by these genes (Table 2, data not shown).

Genes that modify SCA1^{82Q}- and Htt^{128Q}-induced phenotypes in opposite ways

Of all the genes tested, this group is perhaps the most intriguing. In Table 3, we list the genes that induce a clear modification of both SCA1^{82Q} and Htt^{128Q}-induced phenotypes, but in opposite directions. Therefore, these modifiers are good candidates to uncover specific mechanisms of Ataxin-1^{82Q} and Htt^{128Q} toxicity. Representative examples of this interesting class of modifiers are described below in more detail.
Figure 2. Genes that modify SCA182Q and Htt128Q-induced phenotypes similarly. Paraffin retinal sections of flies expressing either SCA182Q (A–H) or Htt128Q (I–P) transgenes are shown. Arrows indicate retina’s length. SCA1 and HD model controls are shown in (A) and (I), respectively. SCA182Q flies carrying one functional copy of skd show an enhanced phenotype characterized by disorganization and decrease in the length of the retina as well as tissue loss (B). In Htt128Q flies, partial LOF of skd also induces an enhancement of the Htt128Q-induced eye phenotype, most evident by a reduction of the length of the retina (J). (C) SCA182Q transgenic flies over expressing pum show a more severe eye phenotype than controls. These flies have a more disorganized retina with some tissue loss (compare with the control in A). In Htt128Q flies, pum OE clearly increases the amount of tissue loss (K). Decreased levels of pum, on the other hand, are responsible for an increase in the length and organization of the retina both in SCA1 and HD models (D and L, respectively). In the HD model (L) note also that pum LOF induces a decrease in tissue loss compared with control in (I). OE of 14-3-3 is a strong enhancer of both SCA182Q and Htt128Q eye phenotypes (E and M). Note the dramatic decrease in the length of the retina that 14-3-3 OE causes in both models, as well as an increase in tissue loss in the SCA1 model (E). (F and N) Decreased levels of 14-3-3, on the other side, suppress both SCA182Q and Htt128Q eye phenotypes. Note improved organization of the retina as well as decrease in tissue loss. (G) SCA182Q flies that also over express DIAP1 display a retinal phenotype very similar to wild-type flies (compare with 1E). Note the improvement of the retinal organization as well as length. The improvement is also evident in Htt128Q flies over expressing DIAP1 (O). Decreased levels of DIAP1 also induce a modification of the SCA182Q and Htt128Q eye phenotypes (H and P, respectively). Note the decrease in the length of the retina, along with increased tissue loss. skdLOF, pum (OE and LOF) and DIAP1 (OE and LOF) do not display an eye phenotype by themselves (Supplementary Fig. S1). Genotypes: (A) yw, UAS-SCA182Q[F7]/þ; GMR-GAL4/þ; UAS-myc-DIAP1; GMR-GAL4/þ; Htt128Q[M64]/+; (B) yw, UAS-SCA182Q[F7]/þ; GMR-GAL4/þ; pum[tt]103/+; (C) yw, UAS-SCA182Q[F7]/þ; GMR-GAL4/þ; pum[tt]103/+; (D) yw, UAS-SCA182Q[F7]/þ; GMR-GAL4/þ; skd[F7]/þ; (E) yw, UAS-SCA182Q[F7]/þ; GMR-GAL4/þ; skd[F7]/þ; 14-3-3e2810B10/þ; (F) yw, UAS-SCA182Q[F7]/þ; GMR-GAL4/þ; 14-3-3e2810B10/þ; (G) yw, UAS-SCA182Q[F7]/UAS-myc-DIAP1; GMR-GAL4/þ; 14-3-3e2810B10/þ; (H) yw, UAS-SCA182Q[F7]/þ; GMR-GAL4/þ; Htt128Q[M64]/þ; (I) w, GMR-GAL4/UAS-GFP; Htt128Q[M64]/þ; (J) w, GMR-GAL4/UAS-GFP; Htt128Q[M64]/þ; (K) w, GMR-GAL4/UAS-GFP; Htt128Q[M64]/þ; (L) w, GMR-GAL4/UAS-GFP; Htt128Q[M64]/þ; (M) w, GMR-GAL4/UAS-GFP; Htt128Q[M64]/þ; (N) w, GMR-GAL4/UAS-GFP; Htt128Q[M64]/þ; (O) w, UAS-myc-DIAP1/þ; GMR-GAL4/þ; Htt128Q[M64]/þ; (P) w, GMR-GAL4/UAS-GFP; Htt128Q[M64]/þ; All SCA182Q transgenic flies shown are 1 day old and all Htt128Q flies are 6 days old. All flies were raised at 25°C. Scale bar is equivalent to 10 μM.
the sensitivity of the retina and improves tissue integrity when compared (Fig. 3E and G). However, lowering [Fig. 3C and D (control)] and increased length of the retina levels would modulate the stability of Htt 128Q. Interestingly, Conversely, in the HD model, OE of Akt1 degeneration caused by OE of Htt 128Q, and does not cause an Ataxin-1 82Q flies (Fig. 3A and B). Internally, organization and degeneration of the ommatidia of eye phenotype by itself (Fig. 3; Supplementary Fig. S1B). the disorganization of photoreceptors in the retina of on its own (Supplementary Fig. S1J). We have previously loss of [Fig. 3J and H (control)]. Loss of one functional copy of Akt1 does not cause an eye phenotype on its own (Supplementary Fig. S1J). We have previously reported that Akt1 OE induces the stabilization and accumulation of Ataxin-1 82Q (19) therefore, we investigated if Akt1 levels would modulate the stability of Htt 128Q Interestingly, we find that decreased levels of Akt1 correlate with an increase in Htt 128Q levels in the eye disc of Htt 128Q animals, while they do not have an effect on the Htt 128Q mRNA levels (Supplementary Fig. S2A and C). This suggests that either the Htt 128Q is stabilized, or that it is not being degraded.

**Table 2. Genes that modify Ataxin-1 82Q toxicity only**

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**Table 3. Genes that modify the toxicity of Ataxin-1 82Q and Htt 128Q differently**

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Akt1. Ataxin-1 and Huntingtin have been reported as substrates of Akt1, a serine/threonine kinase involved in numerous cell survival pathways (19,20). OE of Akt1 enhances SCA1 82Q-induced eye and retinal degeneration but suppresses degeneration caused by OE of Htt 128Q, and does not cause an eye phenotype by itself (Fig. 3; Supplementary Fig. S1B). Externally, OE of Akt1 is responsible for an increased disorganization and degeneration of the ommatidia of Ataxin-1 82Q flies (Fig. 3A and B). Internally, Akt1 OE increases the disorganization of photoreceptors in the retina of Ataxin-1 82Q animals and also causes tissue loss (Fig. 3E and F). Conversely, in the HD model, OE of Akt1 increases the thickness of the retina and improves tissue integrity when compared with flies expressing Htt 128Q alone (Fig. 3H and I). Furthermore, reducing the endogenous levels of Akt1 with one copy of a LOF allele ameliorates the SCA1 82Q-induced eye phenotype. These flies have improved organization of the ommatidia [Fig. 3C and D (control)] and increased length of the retina (Fig. 3E and G). However, lowering Akt1 levels enhances the Htt 128Q-induced phenotype, as shown by a reduction in the length of the retina [Fig. 3J and H (control)]. Loss of one functional copy of Akt1 does not cause an eye phenotype on its own (Supplementary Fig. S1J). We have previously reported that Akt1 OE induces the stabilization and accumulation of Ataxin-1 82Q (19) therefore, we investigated if Akt1 levels would modulate the stability of Htt 128Q Interestingly, we find that decreased levels of Akt1 correlate with an increase in Htt 128Q levels in the eye disc of Htt 128Q animals, while they do not have an effect on the Htt 128Q mRNA levels (Supplementary Fig. S2A and C). This suggests that either the Htt 128Q is stabilized, or that it is not being degraded.

Mushroom-body expressed. OE of mub, a RNA-binding protein, suppresses (Fig. 4A, B, E and F), and a heterozygous LOF allele of mub enhances the SCA1 82Q-induced eye phenotype [Fig. 4C–E and G; (18)]. Note that neither mub OE nor mub LOF have an eye phenotype on their own (Supplementary Fig. S1C and K, respectively). While mub OE induces an increase in the length of the retina of flies expressing SCA1 82Q, the heterozygous mub LOF allele causes a reduction of the retinal thickness and promotes tissue loss in the retinas of SCA1 82Q/mub LOF flies. Interestingly, OE of mub strongly enhances the Htt 128Q-induced eye phenotype, dramatically reducing the length of the retina when compared with Htt 128Q-expressing control flies (Fig. 4H and I). On the other hand, the mub LOF allele is a weak suppressor of the Htt 128Q-induced eye phenotype, promoting a mild increase in the length of the retina [Fig. 4J and H (control)]. Since mub is an RNA-binding protein, we monitored the levels of SCA1 82Q and Htt 128Q mRNA, as well as the Ataxin-1 82Q and Htt 128Q protein levels (in eye imaginal discs), and found no significant effect of mub in larvae (Supplementary Fig. S2).

**Vibrator (vib).** vib encodes a Drosophila orthologue of mammalian proteins implicated in lipid transport. vib is involved in phosphatidylinositol transfer (21), it plays a role in actin-based processes and signal transduction (22), and a recent report relates its function to cytokinesis (23).

In the SCA1 Drosophila model, increased levels of vib suppress the Ataxin-1 82Q eye phenotype (Fig. 5). At the external level, vib OE improves the organization of the ommatidia and eye bristles of flies expressing SCA1 82Q [Fig. 5B and A (control)]. Internally, it is responsible for an increase in the length of the retina in SCA1 82Q/vib OE flies in comparison with flies expressing SCA1 82Q alone (Fig. 5C and D). In the HD Drosophila model, vib OE is an enhancer of the eye phenotype, severely reducing to almost 50% the length of the retina of Htt 128Q flies, but does not display an eye phenotype on its own [Fig. 5F and E (control); Supplementary Fig. S1D].

Because no LOF alleles of vib were available at the time of this study, only OE alleles were tested. However, even though only one allele is shown, two distinct vib OE alleles were analyzed, and the results obtained for both alleles in the two fly models are identical (data not shown).

**CG14438.** CG14438 encodes a protein with 21 zinc finger domains whose function is unknown. OE of CG14438 is one of the strongest suppressors of the Ataxin-1 82Q eye phenotype we have recovered. At the external level, it improves the organization of the ommatidia [Fig. 6B and A (control)]. Internally, CG14438 OE strongly suppresses the phenotype of the retina whose length and organization are improved in SCA1 82Q/CG14438 OE flies compared with SCA1 82Q controls. [Fig. 6F and E (control)]. On the other hand, LOF of CG14438 enhances the SCA1 82Q-induced eye phenotype, but does not display an eye phenotype on its own (Figs. 6C–G and Supplementary Fig. S1M). Externally the ommatidia and...
interoommatidial bristles are more disorganized [Fig. 6C and D (control)], and internally the retina is thinner and also less organized [Fig. 6G and E (control)]. In contrast, in the HD model, OE of CG14438 enhances the Htt128Q eye phenotype, but does not have an eye phenotype when expressed alone (Fig. 6H and I; Supplementary Fig. S1E). The length of the retina of Htt128Q/CG14438 OE flies is dramatically reduced and there is also an increase in its disorganization in comparison to flies expressing Htt128Q alone (Fig. 6I; compare with Fig. 6H). Conversely, the LOF allele causes an increase in length of the retina, as well as a decrease in retinal tissue loss when compared with control flies (Fig. 6J and H).

Validation of the Ataxin-182Q and Htt128Q eye modifier genes in a motor performance assay

To investigate whether the genetic interactions described above in the eye are also valid in the CNS, we carried out a motor performance test that measures the climbing ability of flies as function of age. We used the nervana-GAL4 driver, which directs expression of the transgenes of interest to the CNS, and measured the number of flies that are able to climb above a 5 cm line marked in the vials in 18 s. Using this assay we tested specific genes from Table 3 that do not display a phenotype by themselves when the nervana-GAL4 driver is used.
Wild type or other control strains perform well in the climbing assay until late in life (Fig. 7A and B). In contrast, SCA1 82Q and Htt 128Q-expressing flies perform well only as young adults, becoming progressively more impaired as they age (Fig. 7A and B, respectively). In SCA1 82Q-expressing flies, climbing performance is impaired by day 20–21 (when only 50% of the flies are still able to climb) in comparison to control flies that only start to lose their ability to climb by day 32 (Fig. 7A). Similarly, in the HD model, by day 24, the climbing performance is impaired (only 50% of the flies are still able to climb), whereas in the control, at this age, 70% of the flies are still able to climb (Fig. 7B).

As shown in Figure 7A, OE of the Ataxin-1 82Q suppressors mub and vib improves the climbing performance of the SCA1 82Q-expressing flies. Flies co-expressing mutant Ataxin-1 and either mub or vib in addition to SCA1 82Q it takes between 28 and 25 days, respectively, to observe a similar motor impairment.

The results obtained with the climbing assay for the HD model also mimic the results obtained in the eye: OE of mub and vib worsens the Htt 128Q-induced motor impairments (Fig. 7B). The climbing performance of Htt 128Q/mubOE or...
the percentage of flies able to climb. Note that mut\textsuperscript{OE} and vib\textsuperscript{OE} do not have a climbing phenotype on their own (Fig. 7A and B).

Therefore, the observation that certain modifier genes have opposite effects on Htt\textsuperscript{128Q} and SCA\textsubscript{1}\textsuperscript{82Q}-induced toxicity is not only valid in the eye, but it is also true when using a motor behavior test based on expression of these proteins in the CNS.

Alteration of Ataxin-1\textsuperscript{82Q} and Htt\textsuperscript{128Q} protein aggregation in NIs does not correlate with suppression or enhancement of neurotoxicity

We investigated whether genes modifying the toxicity of Ataxin-1\textsuperscript{82Q} and Htt\textsuperscript{128Q} also modify their aggregation into NIs. With this goal in mind, we generated flies that over express the SCA\textsubscript{1}\textsuperscript{82Q} and Htt\textsuperscript{128Q} transgenes under the control of OK107-GAL4, a driver directing expression to well-defined subset of CNS neurons (Fig. 8A and B).

Figure 8C and D shows the quantification of neurons that contain Ataxin-1 or Huntingtin NIs respectively. Note that, with this driver and in the specific experimental conditions used, we observe that ~43% of the SCA\textsubscript{1}\textsuperscript{82Q}-expressing neurons have NIs, whereas ~13% of the neurons that express Htt\textsuperscript{128Q} have NIs.

Figure 8C shows that OE of the Ataxin-1\textsuperscript{82Q} suppressor CG14438 leads to an increase in the percentage of neurons containing Ataxin-1\textsuperscript{82Q} NIs, when compared with appropriate SCA\textsubscript{1}\textsuperscript{82Q} controls that over express LacZ. Conversely, flies that express SCA\textsubscript{1}\textsuperscript{82Q} and carry the CG14438\textsubscript{LOF} allele have fewer neurons with Ataxin-1\textsuperscript{82Q} NIs than the corresponding controls. In addition, OE of CG14438 also correlates with increased Htt\textsuperscript{128Q} levels detected in eye imaginal discs (Supplementary Fig. S2C). On the other hand, OE of vib or the E3 ligase CHIP, also suppressors of Ataxin-1\textsuperscript{82Q} toxicity [this report and (24)], decrease the percentage of neurons that contain NIs. This observation is valid for both vib alleles tested (Fig. 8C, data not shown). The levels of vib did not affect the total amount of Ataxin-1\textsuperscript{82Q} protein detected in eye imaginal discs (Supplementary Fig. S2C).

Figure 8D shows that in the HD model, OE of CG14438 (an enhancer of Htt\textsuperscript{128Q} toxicity) also increases the percentage of neurons with NIs. We could not detect alterations in NI formation with the CG14438\textsubscript{LOF} allele. OE of vib (enhancer of Htt\textsuperscript{128Q}) increases the percentage of neurons where Htt\textsuperscript{128Q} NIs can be detected, and this observation was valid for both alleles. However, in the HD model OE of CHIP (a suppressor of Htt\textsuperscript{128Q} toxicity, Table 1) dramatically increases in the percentage of neurons containing NIs. In summary, we identified genes modifying toxicity that also alter NI formation, but we found no correlation between modification (suppression or enhancement) of the toxicity and modification (increase or decrease) in the formation of NIs.

DISCUSSION

We have used Drosophila models of SCA1 and HD for a comparative analysis of genetic factors involved in Ataxin-1 and Huntington-induced neurotoxicity. We found genetic modifiers...
involved in a variety of cellular functions that affect similarly the SCA1 and HD *Drosophila* models. For other genes we did not detect modification of Huntingtin toxicity. Surprisingly, we also identified modifier genes that have antagonistic effects on Ataxin-1 and Huntingtin toxicity. We found no consistent correlation between modifier gene activity and NI formation.

**Genes that modify the phenotypes of SCA1 and HD *Drosophila* models similarly**

Among this group of modifiers there are genes involved in protein folding, protein turnover, apoptosis and transcriptional regulation (Table 1). These are cellular processes that have been implicated in polyglutamine toxicity in general, and also in SCA1<sup>82Q</sup> and Htt<sup>128Q</sup>-induced neurodegeneration (9,25–27). The observation that they modulate similarly the Ataxin-1<sup>82Q</sup> and Htt<sup>128Q</sup> phenotypes in *Drosophila* provides further validation for these fly models, and suggests that novel modifiers identified in *Drosophila* may also be relevant to SCA1 and HD pathogenesis. Interestingly, we found specific transcription factors among the common modifiers suggesting that at least some of the genetic pathways responsible for altered transcription are common in HD and SCA1.

Other modifiers in this group are genes for which there was previously little evidence of their involvement in both
In a number of neurodegenerative diseases and muscular dystrophies (3,11). These include disorders caused by expansion of non-translated repeats that lead (FXTAS, DM1 and DM2), or are suspected to lead (SCA8, SCA10, SCA12), to gain of RNA toxicity (28,29). Other neurodegenerative diseases may be caused by abnormal alternative splicing [FTDP-17 (30) and ALS (31)], or by mutations in factors involved in splicing or mRNA localization [SMA (32)]. We reported that RNA-binding proteins form a functional class among the Ataxin-1 genetic modifiers (18). In addition, Ataxin-1 itself was later shown to have RNA-binding activity in vitro (33), and whether other polyglutamine diseases besides SCA1 implicate RNA-mediated mechanisms remains unclear. In the case of pum, we find that more pum correlates with more Ataxin-1 in eye imaginal discs, which suggests that pum may promote the translation of SCA1 mRNA or cause the stabilization of Ataxin-1\textsuperscript{82Q}. Our data provides strong support for the hypothesis that RNA-binding proteins also have a role in Htt\textsuperscript{128Q}-induced neurodegeneration, and suggests that abnormal RNA-processing may be a more general pathogenic mechanism in neurodegenerative disorders than previously anticipated.

Two other genes not previously known to modify Ataxin-1\textsuperscript{82Q} and Htt\textsuperscript{128Q} toxicity in a similar manner are 14-3-3e and RhoGAPp190, both of which participate in a variety of signal transduction pathways. RhoGAP proteins mediate growth factor dependent regulation of GTPases (34), and have been implicated in cytoskeletal reorganization and axonal growth (35). 14-3-3 proteins are involved in many cellular processes, and they are binding partners of a large number of phosphoproteins (36). We found that loss-of-function of 14-3-3e suppresses, and OE enhances, both Ataxin-1\textsuperscript{82Q} and Htt\textsuperscript{128Q} toxicity. In the case of Ataxin-1\textsuperscript{82Q} we understand the mechanism behind these modifications. Upon phosphorylation of Ataxin-1\textsuperscript{82Q} by Akt, 14-3-3e binds and stabilizes the phosphorylated protein, which leads to its accumulation and neurotoxic effects (19).

In the case of Htt\textsuperscript{128Q} the mechanism of modification is less clear. We expect, however, that it is different from the Ataxin-1\textsuperscript{82Q} mechanism because Akt has a protective role in Htt-induced neurodegeneration [(20) and this report]. 14-3-3 proteins co-localize with Huntingtin perinuclear inclusions in cell culture (37), and interact with HAP-1, a protein involved in intracellular trafficking (38). Thus, 14-3-3e may modify Htt\textsuperscript{128Q} toxicity by modulating the trafficking impairments caused by this expanded protein.

**Genes that modify the phenotypes of SCA1 but not HD Drosophila models**

These are Ataxin-1\textsuperscript{82Q} modifier genes that do not alter Htt\textsuperscript{128Q}-induced toxicity in our assays and experimental conditions. They include genes in some of the functional categories discussed above (Table 2). Although negative results are difficult to interpret, some of these genes may account for differences between SCA1\textsuperscript{82Q} and Htt\textsuperscript{128Q}-induced toxicity.

![Figure 7. Modification of SCA1\textsuperscript{82Q} and Htt\textsuperscript{128Q} neuronal phenotypes in a motor performance assay. Quantification of the climbing performance of SCA1\textsuperscript{82Q} (A) and Htt\textsuperscript{128Q} (B) flies as a function of age. The nervana-GAL4 driver was used to direct expression of the mutant proteins to the CNS.](https://academic.oup.com/hmg/article-abstract/17/3/376/599582/713036164982828/26Dec2018)
Modifier genes that have antagonistic effects on the phenotypes of SCA1 and HD models

Surprisingly, we found modifier genes that are common to both disease models but have antagonistic effects on SCA1\(^{82Q}\) and Htt\(^{128Q}\)-induced toxicity (Table 3).

Of all the genes in this group, the best understood is Akt. As described above, Akt phosphorylates Ataxin-1 promoting binding of 14-3-3 proteins. This leads to increased Ataxin-1 stability and thus causes neuronal degeneration (19). Huntingtin is also a substrate of Akt, and phosphorylation of Htt at Ser421 is crucial for neuroprotection mediated by the insulin-like growth factor-1 (IGF-1) signaling pathway (20,39). Akt also protects from Htt toxicity by phosphorylating Arfaptin 2 and promoting proteasome function (40). Interestingly, we...
observe increased levels of Htt\textsuperscript{128Q} protein in animals with reduced Akt activity; this is consistent with the hypothesis that reduced Akt phosphorylation of Arfaptin 2 leads to increased proteasome impairment. Because the N-terminal Htt fragment in Htt\textsuperscript{128Q} flies lacks Ser421, Akt-mediated protection against Htt\textsuperscript{128Q} toxicity is likely indirect and mediated by CG17184, the Drosophila orthologue of Arfaptin 2.

\textit{mub} encodes the Drosophila orthologue of human poly(rC)-binding protein 3, and both are members of a conserved family of proteins that contain three RNA-binding KH domains, and have been implicated in mRNA stabilization, translational activation and translational silencing (41). OE of \textit{mub} ameliorates Ataxin-1\textsuperscript{82Q} but enhances Htt\textsuperscript{128Q} phenotypes in the eye; conversely, \textit{mub}\textsuperscript{LOF} aggravates Ataxin-1\textsuperscript{82Q} and suppresses Htt\textsuperscript{128Q} phenotypes (Fig. 4). These results were reproduced independently in a motor performance assay indicating that these interactions are not specific to the eye, and confirming their relevance to neurons (Fig. 7). These observations further suggest that RNA-mediated mechanisms are relevant to Huntingtin neurotoxicity.

OE of \textit{vib} suppresses SCA1\textsuperscript{82Q} and enhances Htt\textsuperscript{128Q}-induced toxicity, both in the eye and motor performance assays (Fig. 5). \textit{vib} encodes a conserved phospholipid transporter, which are proteins involved in lipid metabolism and membrane trafficking, and implicated in numerous cell-signaling pathways (42). Accumulating evidence suggests that the metabolism of phospholipids in neural membranes is abnormal in neurodegenerative diseases, such as Friedreich ataxia (43) and Alzheimer’s Disease (44). Less evidence is available for the involvement of phospholipid metabolism and signaling in HD, and particularly in SCA1. However, Huntingtin has been implicated in membrane trafficking (45,46). Also, it has been suggested that electrostatic interactions with acidic phospholipids mediate binding of Huntingtin to membranes (47). The genetic interactions between \textit{vib} and Ataxin-1\textsuperscript{82Q} and Htt\textsuperscript{128Q} suggest that abnormal phospholipid metabolism may be a mechanism of pathogenesis also in polyglutamine diseases.

OE of CG14438 suppresses Ataxin-1\textsuperscript{82Q} and enhances Htt\textsuperscript{128Q} eye phenotypes; conversely its LOF enhances Htt\textsuperscript{128Q} phenotypes (Fig. 6). \textit{CG14438} is an uncharacterized gene encoding a long protein (3321 amino acids). It contains 21 C2H2-type zinc-fingers, which are domains that can mediate interactions to DNA, RNA or other proteins. The unknown function of this Drosophila gene provides little insight into possible mechanisms by which it may modulate SCA1\textsuperscript{82Q} and Htt\textsuperscript{128Q} –induced toxicity. However, we find that suppression by CG14438\textsuperscript{OE} correlates with increased Ataxin-1\textsuperscript{82Q} protein levels (Supplementary Fig. S2) and more NIs in SCA1\textsuperscript{82Q} flies (Fig. 8). This suggests that the CG14438\textsuperscript{OE} may suppress Ataxin-1\textsuperscript{82Q}-induced toxicity by promoting its aggregation and reducing the levels of soluble toxic oligomers.

Alterations of Ataxin-1\textsuperscript{82Q} and Htt\textsuperscript{128Q} protein aggregation in NIs do not correlate with suppression or enhancement of neurotoxicity

For some modifier genes we investigated whether modulation of the eye and motor performance phenotypes correlates with changes in the formation of NIs. We found no direct correlation between NI formation and toxicity: for two Ataxin-1\textsuperscript{82Q} suppressors (\textit{vib}\textsuperscript{OE} and CHIP\textsuperscript{OE}) the percentage of neurons containing NIs was reduced, whereas for a third suppressor the percentage of neurons containing NIs was increased (CG14438\textsuperscript{OE}). In the case of Htt\textsuperscript{128Q}, we found increased percentage of neurons containing NIs for both enhancers (CG14438\textsuperscript{OE} and \textit{vib}\textsuperscript{OE}) and a suppressor (CHIP\textsuperscript{OE}) of the Htt\textsuperscript{128Q}-induced toxicity. These results are consistent with the idea that some modifiers may function by reducing the levels of mutant protein, while others function downstream of protein synthesis/clearance. Thus, our results suggest that NI formation is not a good read-out of polyglutamine-induced neuronal toxicity, and argue for caution when interpreting results from chemical screens in which the screening assay is based on aggregation of the mutant protein.

In summary, this comparative study of SCA1 and HD models in Drosophila has lead to identifying novel modifier genes that fall in two important categories. Those modifying SCA1\textsuperscript{82Q} and Htt\textsuperscript{128Q}-induced neurotoxicity in a similar manner point to new potential therapeutic targets that may be common to both diseases. A second class of modifiers genes have antagonistic effects on SCA1\textsuperscript{82Q} and Htt\textsuperscript{128Q}-induced toxicity, and point to disease-specific mechanisms of pathogenesis.

MATERIALS AND METHODS

Fly strains and constructs

SCA1\textsuperscript{82Q} and SCA1\textsuperscript{30Q} lines were previously described (18). Htt\textsuperscript{128Q} flies were generated by sub-cloning a portion of N-terminal of the human Huntingtin protein (amino acids 1–336, including 128 glutamine repeats) into the Drosophila transformation vector pUAST (48). Transgenic flies were obtained following standard procedures for embryonic germ line injection and transformation. Similar transgenes were also generated with 16 and 44 glutamines.

Flies were maintained on standard cornmeal medium. Crosses were performed at 25°C or 27°C in order to induce medium or high expression levels of the transgenes. In this study, the \textit{GMR-GAL4}, \textit{nervana}-GAL4 (both obtained from Bloomington Stock Center (Indiana University, IN, USA) and OK107-GAL4 drivers (\textit{w}; \textit{FRTG13}, \textit{UAS-CD8:GFP};OK107-GAL4—a gift from Dr Hugo Bellen) were used.

All stocks used were obtained from the Bloomington Stock Center with the exception of the following stocks: obtained from Exelixis (EP(2)2417, EP(3)3623, EP(3)561, EP(3)513, EP(3)5114, EP(3)674, EP(3)1303, EP(2)686, EP(3)3375, EP(2)2300, EP(3)3463, EP(3)3378, EP(3)3461 and EP(3)1357) or from private collections: UAS-\textit{Akt1}, UAS-\textit{DPi3K}\textsuperscript{OE} wt, UAS-\textit{14-3-3-\epsilon}gse and 14-3-3-\epsilonuq (19); \textit{ps} and \textit{ps}1449 were a gift from Dr Deborah Andrew (49). UAS-\textit{DIAP1} by was kindly provided by Dr Bruce Hay.

Scanning electron microscopy

Flies collected on the day of eclosion were serially dehydrated in ethanol, critical point dried, metal coated and analyzed in a Jeol JSM 6100 microscope.
Retinal paraffin sections

Adult fly heads were dissected, fixed in 4% formaldehyde overnight, dehydrated in serial dilutions of ethanol and embedded in Paraplast Plus paraffin. Ten micrometer sections were obtained using an Olympus Cut4055 microtome and afterwards re-hydrated. Sections were stained with hematoxilin (Sigma), dehydrated and mounted using Permount mounting medium (Fischer Chemicals). Samples were visualized in a Nikon Microphot-FXA microscope (Nikon) and the tissue morphology was analyzed. Images were obtained using the AxionCam MRc camera from Zeiss.

Climbing assay

SCA1\textsuperscript{820} and Htt\textsuperscript{128Q} flies were crossed with nervana-GAL4 driver and climbing ability was analyzed throughout their lifespan. The climbing performance is determined by counting the number of flies that climb above a 5 cm line in 18 s, after being tapped to the bottom of an empty culture vial. Groups of 30 virgin female flies were kept in individual vials and their climbing performance was recorded, at least, every 2 days. A minimum of two replicas per genotype was tested.

Immunofluorescence

Adult ventral ganglia of aged flies were dissected in phosphate-buffered saline (PBS), fixed in 4% formaldehyde for 30 min overnight, dehydrated in serial dilutions of ethanol and embedded in Paraplast Plus paraffin. Ten micrometer sections were used. For the PCR step, the primers used were: Htt\textsuperscript{128Q} and 5'-CACCGACCAAAGAAAGAAACTTTCA-3' and 5'-TTTAA TTTCTTATAGAGCTGACCTGA-3'. SCA1, 5'-CTG CAGTTTGGCCGCTCTCT-3' and 5'-GAGCTAAAGAAGG TGGAAGACTTTCA-3'. RP49, 5'-GAGCTAAAGAAGG TGGAAGACTTTCA-3'.

Immunofluorescence quantification

Carried out as previously described (19). Eye imaginal discs from age-matched larvae were stained with anti-Htt (5374 Chemicon) or anti-Ataxin-1 (11NQ). Tissue was mounted and flattened. Stacks of confocal slices were obtained through the thickness of each eye disc. Each stack was flattened and signal of the slices summed. The average intensity for each eye disc was calculated in the area with positive immunofluorescence staining (Supplemental Fig. 2) using Image J and the average value from four different eye discs per genotype was plotted in the chart.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG Online.

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Conflict of Interest statement: None declared.

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