

# Lithium Chloride Alleviates Neurodegeneration Partly by Inhibiting Activity of GSK3 $\beta$ in a SCA3 *Drosophila* Model

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**Abstract** Spinocerebellar ataxia type 3 (SCA3) is an autosomal dominant neurodegenerative disorder caused by the expansion of a CAG trinucleotide repeat that encodes an abnormal polyglutamine (PolyQ) tract in the disease protein, ataxin-3. The formation of neuronal intranuclear inclusions in the specific brain regions is one of the pathological hallmarks of SCA3. Acceleration of the degradation of the mutant protein aggregates is proven to produce beneficial effects in SCA3 and other PolyQ diseases. Lithium is known to be neuroprotective in various models of neurodegenerative disease and can reduce the mutant protein aggregates by inducing autophagy. In this study, we explored the therapeutic potential of lithium in a SCA3 *Drosophila* model. We showed that chronic treatment with lithium chloride at specific doses notably prevented eye depigmentation, alleviated locomotor disability, and extended the median life spans of SCA3 transgenic *Drosophila*. By means of genetic approaches, we showed that co-expressing the mutant S9E, which mimicked the phosphorylated S9 state of Shaggy as done by lithium, also partly decreased toxicity of *gmr-SCA3tr-Q78*. Taken together, our findings suggest that lithium is a promising therapeutic agent for the treatment of SCA3 and other PolyQ diseases.

**Keywords** SCA3 · Ataxin-3 · *Drosophila* model · Lithium chloride · Neuroprotective effect

## Introduction

Spinocerebellar ataxia type 3 (SCA3) is one of nine polyglutamine (PolyQ) neurodegenerative disorders caused by the expansion of a CAG trinucleotide repeat that encodes an abnormal PolyQ tract in the disease protein, ataxin-3 [1]. Patients with SCA3 suffer from progressive loss of motor coordination, dysarthria, dysphagia, oculomotor dysfunction, and peripheral neuropathy [2, 3]. Symptoms usually occur in midlife and get worsen over the next 7–29 years [4]. Until now, there is no established therapy to arrest or reverse the course of the disease.

A unifying pathological feature of SCA3 is the formation of microscopically discernible aggregation in the nucleus or cytoplasm of affected neurons. Although the significance of these aggregations of mutant proteins in the pathogenesis of SCA3 remains a matter of controversy [5], recent neuropathologic studies suggested that the accumulating proteins might interact with various targets, including several transcriptional factors or cofactors [6], proteasome components [7], and molecular chaperones [8], resulting in dysfunction of recruiting proteins in neurons.

Acceleration of the degradation of mutant proteins is proven to prevent PolyQ disease progression. These mutant proteins are degraded via both the ubiquitin proteasome system (UPS) and the macroautophagy (which we also call autophagy) machinery [9]. Along with degradation by the UPS, autophagy forms the major route for degradation of disease-causing, cytoplasmic, aggregate-prone proteins [10]. Previous studies have shown a range of PolyQ proteins, including mutant huntingtin

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(Htt) fragments, alpha-synuclein, and full-length mutant ataxin-3, which are highly dependent on autophagy for their clearance [11–13]. When autophagy is up-regulated either by inhibiting the mammalian target of rapamycin (mTOR) [14, 15] or through mTOR-independent pathways [16], the clearance of mutant PolyQ proteins is enhanced and their toxicity is attenuated. Therefore, up-regulation of autophagy may be an effective therapeutic strategy for SCA3 and other PolyQ diseases.

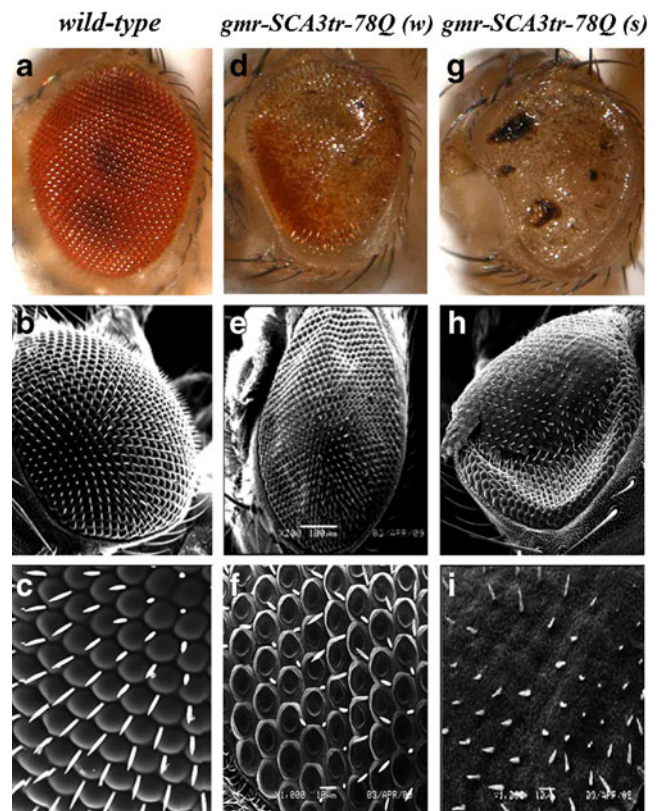
Lithium, a monovalent cation, has been used to treat bipolar disorder for more than half a century. In addition to its antidepressant effect, the ability of lithium to up-regulate autophagy, through depleting free inositol and subsequently decreasing IP3 levels (a mTOR-independent pathway of up-regulating autophagy), has recently been reported [17, 18]. Moreover, lithium has been shown to exert its neuroprotective effects by increasing levels of the anti-apoptotic factor *Bcl-2* [19] and by inhibiting the activity of caspase-3-induced glycogen synthase kinase (GSK-3) [20, 21]. It has been reported that lithium protects against polyglutamine toxicity in animal models of Huntington's disease (HD) [22] and spinocerebellar ataxia type 1 (SCA1) [23]. Because of this established function, we speculated that lithium could serve as a promising therapeutic strategy for SCA3 and other PolyQ diseases. In this study, we investigated and found the therapeutic potential of lithium chloride (LiCl) in a SCA3 *Drosophila* model.

## Results

### SCA3tr-Q78 Transgenic Fly Exhibits Neurodegenerative Phenotypes

To apply an incisive tool to explore the therapeutic potential of lithium, we have previously established a *Drosophila* model of SCA3 expressing forms of human gene encoding ataxin-3 protein in the developing eyes and in neurons using the GAL4/UAS system [24]. Compared to wild-type *Drosophila* phenotype (Fig. 1a–c), expression of a truncated form of the ataxin-3 protein with 78 polyglutamines produced deleterious neurodegenerative phenotypes. Weaker expression of SCA3tr-Q78 in eyes resulted in a mild disruption of the regular, external lattice of the eye in adult flies (Fig. 1d–f); stronger expression of SCA3tr-Q78 showed a greater loss of cell integrity, and the pigmentation of the adult flies was completely faded with black point-like necrosis (Fig. 1g–i). The fly eyes underwent late-onset, progressive degeneration during adult life.

To study the toxicity of SCA3tr-Q78 in neurons, flies homozygous for the neuronal driver *nrv2-Gal4* were crossed to flies of genotype *UAS-SCA3tr-Q78/TM3* and the percentage of flies with the *nrv2-SCA3tr-Q78* transgene was evaluated. Expression of SCA3tr-Q78 in neurons caused decreased

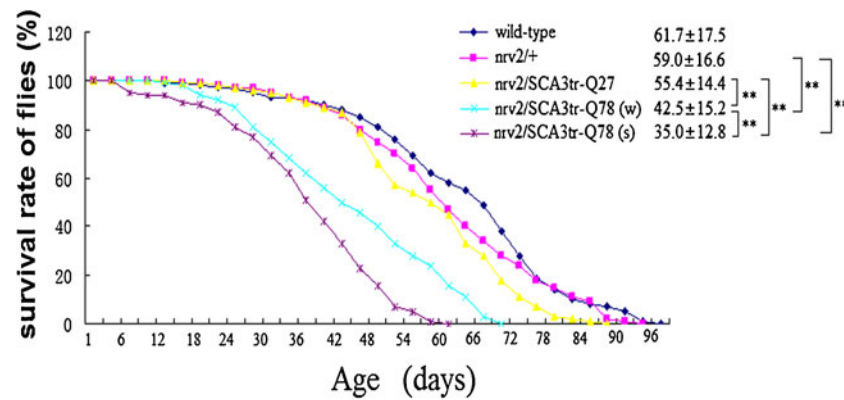


**Fig. 1** Expanded polyglutamine protein leads to eye degeneration of adult flies. **a, d, g** Dissecting microscopic images of the adult eyes of 15-day-old flies. **b, e, h** Dissecting microscopic images of the adult eyes of 15-day-old flies ( $\times 80$ ). **c, f, i** Dissecting microscopic images of the adult eyes of 15-day-old flies ( $\times 200$ ). Compared to wild-type *Drosophila* phenotype (**a, b, c**), the eyes of flies expressing weak (**d, e, f**), and strong (**g, h, i**) SCA3tr-Q78 proteins were mildly to severely disrupted, respectively. Weak (**d, e, f**) lines showed a slight disruption of the regular, external lattice of the eye in adult flies. Strong lines (**g, h, i**) showed severely disrupted eye morphology, with loss of pigmentation, collapsed, and even appeared point-like necrosis during adult life

locomotor ability and reduced life spans in adult flies (Figs. 2 and 3), and the effects were more dramatic in flies of genotype *nrv2-SCA3tr-Q78 (s)* (Figs. 2 and 3). All these results showed that expression of SCA3tr-Q78 protein in flies resulted in late-onset, progressive, neurodegenerative phenotypes, which were quite similar to features of SCA3/MJD patients, thus allowed us to study a range of effects of LiCl treatment on SCA3 pathogenesis in vivo.

### Lithium Chloride Treatment Prevents Against PolyQ-Induced Eye Depigmentation in SCA3tr-Q78 Transgenic *Drosophila*

Since previous study had shown that lithium could be reliably given to *Drosophila* through fly food and without lethality [25], we chose to administer fly with drug-containing medium. Daily doses of LiCl at 1, 5, 10, 15, 20, and 50 mM were administered to SCA3 *Drosophila* model before crosses. We



**Fig. 2** Expanded polyglutamine protein causes decreased life span in SCA3 transgenic *Drosophila* model. Differences in life span of female SCA3 transgenic *Drosophila* model were shown. As time went by, the life span of each transgenic flies decreased in a repeat length-dependent

manner, and the most serious phenotypes can be seen when SCA3tr-Q78 protein is strongly expressed. The mean life span and SD are shown,  $**p < 0.01$  ( $n = 100$ )

carefully observed the external morphology of *gmr-SCA3tr-Q78 Drosophila* eyes in detail. Consistent with a previous study [22], prolonged treatment with LiCl at certain range (1–20 mM) resulted in a dramatic improvement of eye depigmentation at 5 days after eclosion, with obvious increase of pigmentation, decreased black point-like necrosis, and nearly normal ommatidial pattern of the photoreceptor rhabdomeres (Fig. 4). The most notable improvement was observed at the range of 15–20 mM (Fig. 4d, e, i, j, n, o). Possibly due to a very narrow therapeutic window of lithium [26] and a lower tolerance in fly compared with humans [25], when treated with LiCl at the dose of 50 mM, no eggs of parental flies survived to adulthood; the parental flies also died after treatment with LiCl for 10 days.

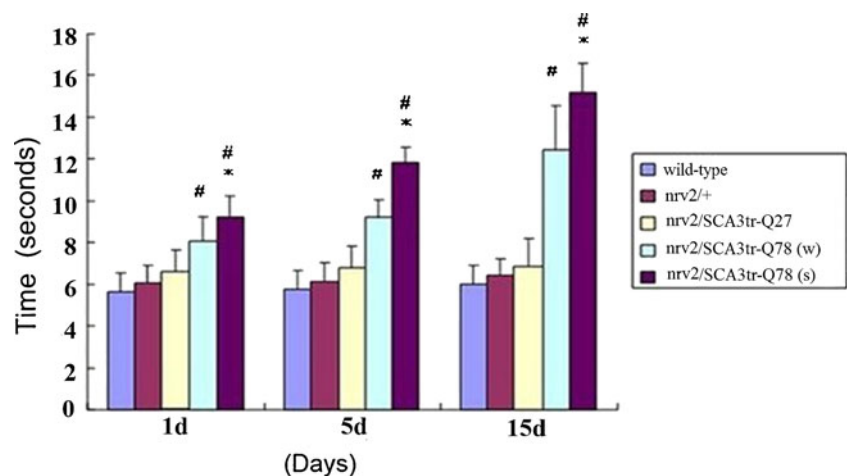
#### Lithium Chloride Ameliorates Locomotion Disability of SCA3tr-Q78 Transgenic *Drosophila*

To test whether LiCl could also protect against toxicity selectively involved in neurons, we analyzed the locomotor ability of *nrv2-SCA3tr-Q78* flies using the positive phototactic

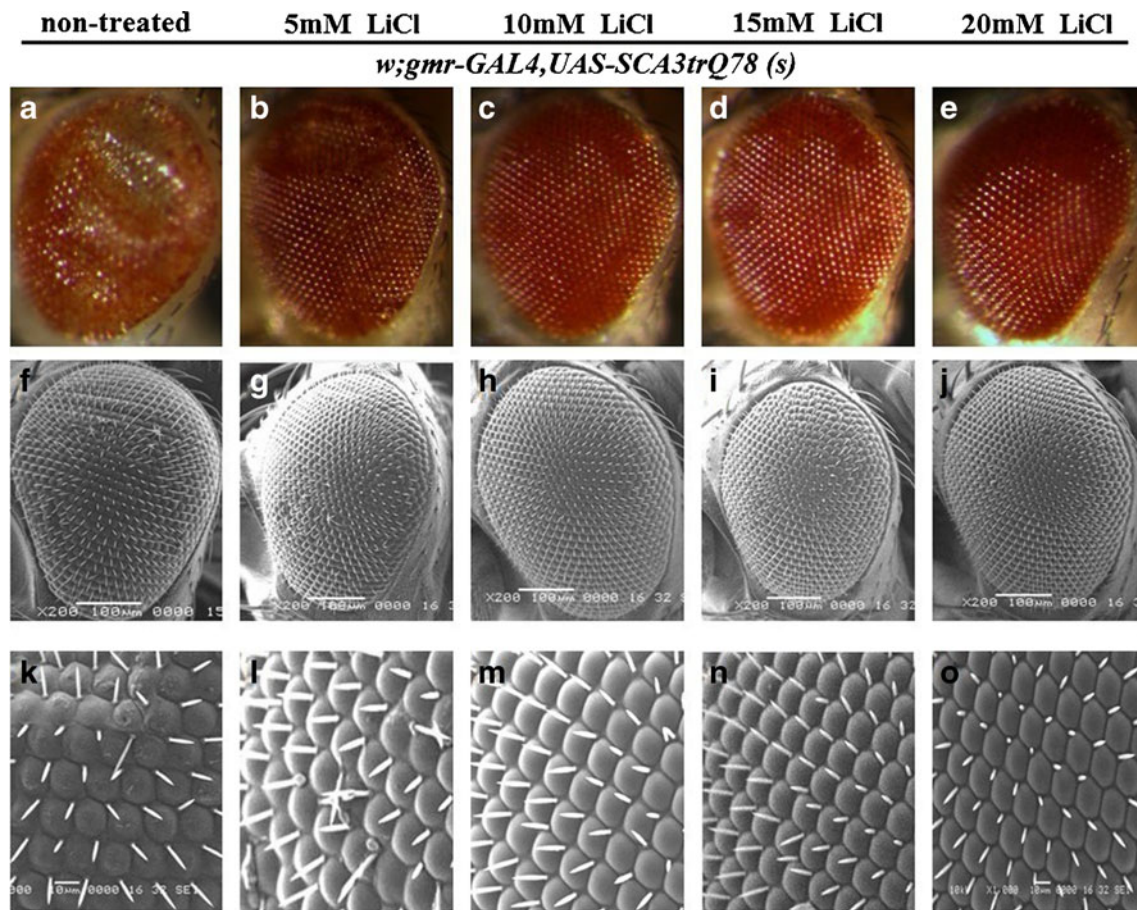
climbing assay as described previously [27]. We randomly selected age-matched female flies into a 25-ml pipette and dropped them to the bottom by knocking the pipette. A fiberoptic lamp illuminated the pipette from the top and we measured the time for the first five flies to reach a line 17.5 cm from the bottom. By this method, we evaluated the climbing ability of flies at 1, 5, and 15 days after eclosion.

As can be obviously seen in Fig. 5a, when LiCl was administered at doses from 0 to 10 mM, all of the 1-day-old SCA3tr-Q78 transgenic flies (eclose from the pupal case for 1 day) showed a dose-dependent increase in locomotor ability compared to non-treated controls ( $P < 0.05$ ), but as the doses gradually increased to 15 mM, the locomotor ability of 1-day-old *nrv2-SCA3tr-Q78 (s)* flies showed a mild decrease compared to flies treated at 10 mM. After treated at a dose of 20 mM, all 1-day-old SCA3tr-Q78-expressing flies required more time to cross the line, suggesting an optimum therapeutic dose after which the locomotor ability may be further impaired. In contrast, when treated with LiCl at the same doses over time, the *nrv2-SCA3tr-Q27* flies showed no

**Fig. 3** Differences in locomotor ability of SCA3 transgenic *Drosophila* model. X-axis represents observing time, and the locomotor ability of SCA3 transgenic *Drosophila* model at the 1st day, the 5th day, and the 15th day after eclosion was shown. Number sign represents comparison to wild-type flies,  $p < 0.05$ . Asterisk represents comparison to *nrv2/SCA3tr-Q78(w)*,  $p < 0.05$ . Error bars represent SEM ( $n = 200$ )







**Fig. 4** Lithium chloride prevents against PolyQ-induced eye depigmentation in SCA3 *Drosophila* model. **a–e** Dissecting microscopic images of the eyes of 5-day-old adult flies ( $\times 80$ ). **f–j** Scanning electron microscopic images of the eyes of 5-day-old adult flies ( $\times 200$ ). **k–o** Scanning electron microscopic images of the eyes of 5-day-old adult flies ( $\times 1,000$ ). **a, f, k** Non-treated flies expressing strong SCA3tr-Q78 in the eyes showed severely degeneration, as seen by loss of depigmentation, disorganization

of the ommatidial array, and abnormal bristles. Genotype: *w; gmr-GAL4, UAS-SCA3tr-Q78(s)*. **b, g, l** The eye degeneration was less pronounced when treated with LiCl at a dose of 5 mM, and with the dosage increasing, the degenerative eye phenotypes of flies were gradually restored towards almost normal (**d, e, i, j, n, o**), as seen by an obvious increase of pigmentation, an ordered array of ommatidia, and interspersed bristles

remarkable difference in the locomotor ability compared to non-treated controls (Fig. 5a). Similar results were observed at later time points (Fig. 5b, c). Thus, our data suggested that prolonged treatment with LiCl at a certain range positively benefited the SCA3 phenotypes, for which the proper doses might be from 10 to 15 mM.

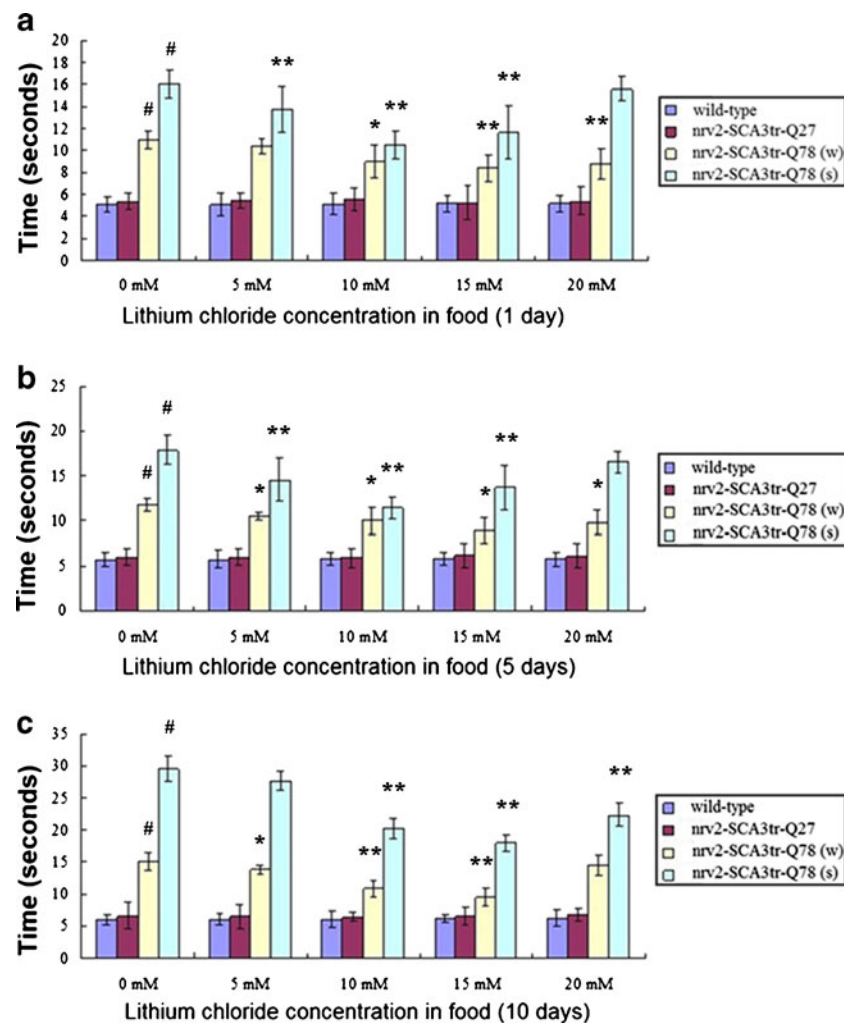
#### Lithium Chloride Extends Survival Time of SCA3tr-Q78 Transgenic *Drosophila*

To further confirm the positive effects of LiCl on the SCA3 phenotypes, we then tested whether LiCl affected the survival time of *nrv2-SCA3tr-Q78* flies. As shown in Fig. 6, all SCA3tr-Q78 transgenic flies treated with LiCl at doses from 5 to 15 mM had a striking extension of median life spans than the corresponding controls. The median life spans of the *nrv2-SCA3tr-Q78 (w)* and *nrv2-SCA3tr-Q78 (s)* increased

from the original means of 47 and 41 days to 62 and 52 days, respectively. But when the doses were increased to 20 mM (Fig. 6), the median life spans of the two fly strains (39 and 36 days,  $n=200$ ) did not significantly differ from that of non-treated controls (43 and 35 days,  $n=200$ ), further confirming the hypothesis that the beneficial effects of LiCl could be masked if used at higher doses. Thus, our results showed that LiCl could rescue PolyQ-induced toxicity in SCA3tr-Q78 transgenic *Drosophila* when used at an appropriate dose.

#### Inhibition of GSK3 $\beta$ /Shaggy Protects Against PolyQ-Mediated Eye Depigmentation of SCA3tr-Q78 Transgenic *Drosophila*

The fact that lithium serves as an inhibitor of GSK-3 $\beta$  to perturb Wnt pathway has been widely accepted [20–22]. A recent study, using pharmacological or genetic approaches,



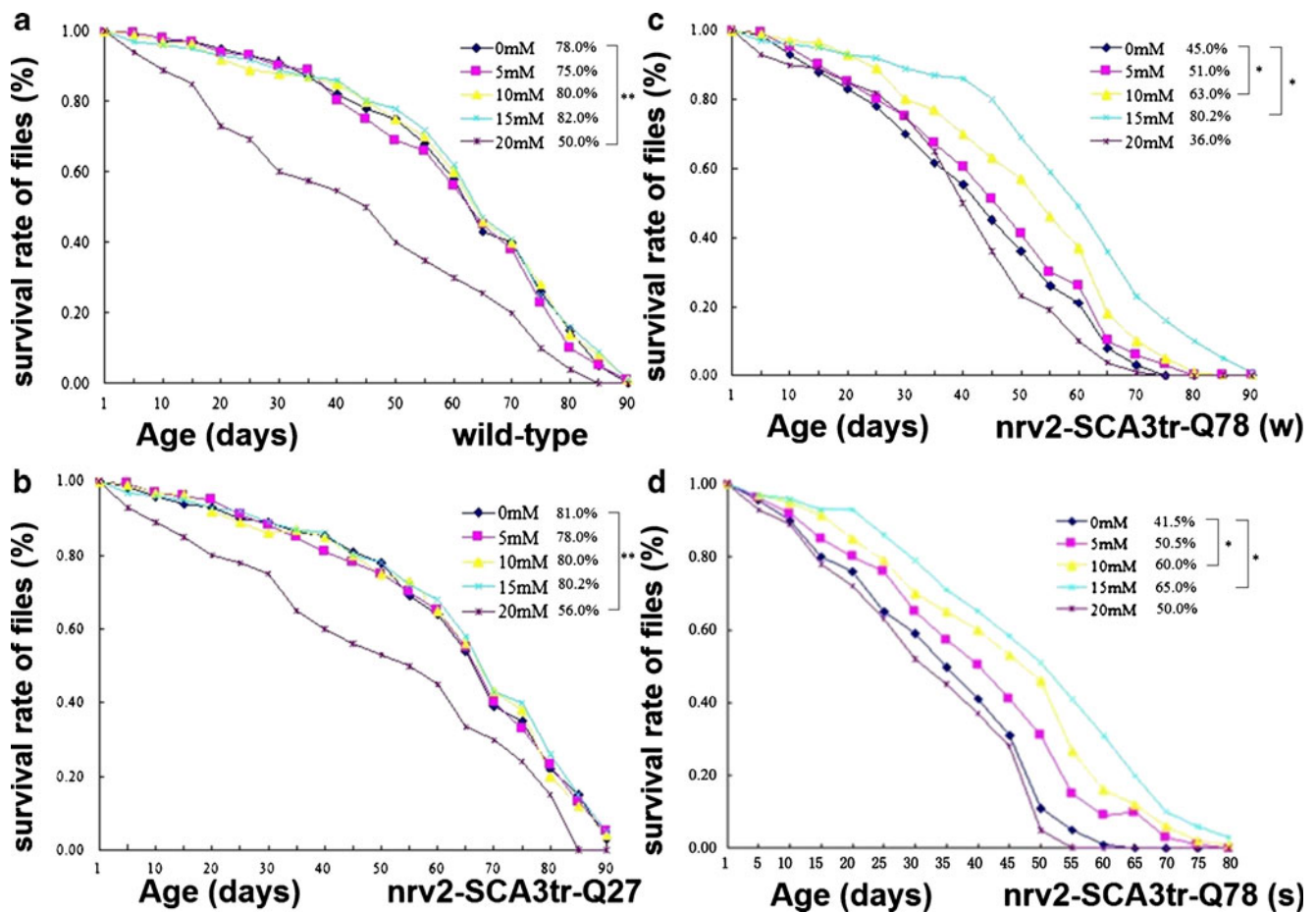
**Fig. 5** Lithium chloride ameliorates climbing ability of SCA3 *Drosophila* model. Climbing ability of *wild-type* and SCA3tr-Q78-expressing flies on LiCl-containing medium was tested at 1 day (**a**), 5 days (**b**), and 15 days (**c**) after eclosion. *Number sign* represents comparison to wild-type flies without treating with LiCl,  $p < 0.05$ . *Asterisk* represents comparison to the same genotype flies without treating with LiCl,  $p < 0.05$ . *Double asterisks* represent comparison to the same genotype flies without treating with LiCl,  $p < 0.01$ . *Error bars* represent SEM ( $n = 200$ ). When treated with LiCl at doses from 5 to 10 mM, although wild-type and *nrv2-*

*SCA3tr-Q27* flies showed no differences in behavior compared to non-treated controls, weak and strong expression of *nrv2-SCA3tr-Q78* flies showed a significant dose-dependent increase in performance. When treated with LiCl at a dose of 15 mM, even the climbing ability of *nrv2-SCA3tr-Q78 (s)* flies was gradually reduced, which was better than the non-treated controls. When treated with LiCl at a dose of 20 mM, the climbing ability of some *nrv2-SCA3tr-Q78* flies showed no differences compared with that of non-treated controls

has found that inhibition of GSK-3 $\beta$  could rescue toxicity of aggregate-prone proteins in *Drosophila* models of HD [22] and Alzheimer's disease (AD) [28]. To test whether inhibition of GSK-3 $\beta$  by LiCl also rescues the PolyQ-induced toxicity in SCA3tr-Q78 transgenic *Drosophila*, we co-expressed in the eyes a dominant negative form of the kinase Shaggy (Sgg) (i.e., the GSK-3 fly ortholog), the S9E mutant, which mimics an inactive state of the GSK-3 $\beta$ . Although a heterozygous S9E mutation (i.e., absence of one functional copy of S9E) had no effect on the toxicity of *gmr-SCA3tr-Q78* (Fig. 7b, e, h), a homozygous S9E mutation could partly suppress PolyQ-induced eye depigmentation, with decreased dark black spots, more regular compound eye structure, and

increased pigment cells (Fig. 7c, f, i). Thus, our results indicated that the effect of LiCl in protecting against PolyQ-mediated eye depigmentation can partly be explained by the inhibition of GSK3 $\beta$ /shaggy.

To rule out the possibility that improvement of PolyQ-mediated eye depigmentation resulted from GAL4 levels but not because of the expression of the S9E mutant, we have also investigated the effects of an active Sgg in flies expressing SCA3tr-78Q protein. The expression of the kinase Sgg enhanced the toxicity of *gmr-SCA3tr-Q78*. Flies co-expressing SCA3tr-78Q and a heterozygous Sgg in eyes showed more serious eye depigmentation, including a greater loss of pigment cells, rough eye collapse, and a highly



**Fig. 6** Lithium chloride extends mean life span of SCA3 *Drosophila* model. Longevity curves of wild-type (a), *nrv2-SCA3tr-Q27* (b), *nrv2-SCA3tr-Q78 (w)* (c), and *nrv2-SCA3tr-Q78 (s)* (d) flies are shown in this figure. When treated with LiCl at doses ranging from 5 to 15 mM, the mean life spans of the wild-type and *nrv2-SCA3tr-Q27* flies showed no difference compared to that of the same genotype flies without treating with LiCl. When treated with LiCl at a dose of 20 mM, the mean life spans of the two fly strains decreased to a greater extent compared to that of the corresponding control ( $p < 0.01$ ). When treated with LiCl at doses ranging from 5 to 15 mM, the mean life spans of the

*nrv2-SCA3tr-Q78 (w)* and *nrv2-SCA3tr-Q78 (s)* flies extended compared to that of flies without treating with LiCl ( $p < 0.05$ ), although the extension of life span did not reach to the level of wild-type flies. When treated with LiCl at a dose of 20 mM, the mean life spans of the two fly strains were shown not different from that of the corresponding control. The median survival rates of all genotype flies were shown in this figure. Asterisk represents comparison to the same genotype flies without treating with LiCl,  $p < 0.05$ . Double asterisk represents comparison to the same genotype flies without treating with LiCl,  $p < 0.01$  ( $n = 200$ )

disordered ommatidial array (Fig. 8b, e, h). More deleterious effects were observed with a homozygous Sgg co-expression (Fig. 8c, f, i). Thus, our results further confirmed that inhibition of the Sgg activity could partly protect against PolyQ-mediated eye depigmentation.

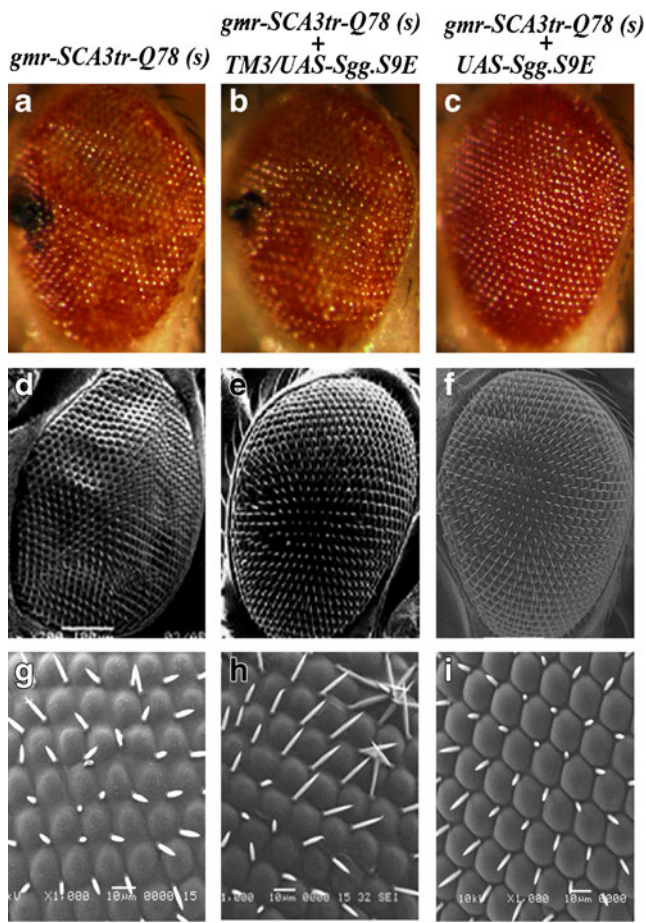
## Discussion

SCA3/MJD is an autosomal dominant neurodegenerative disorder characterized by a complex and pleiotropic phenotype, involving predominantly the cerebellar, oculomotor, pyramidal, extrapyramidal, and peripheral systems [29]. The main pathological lesions can be observed in the spinocerebellar system, as well as in the cerebellar dentate nucleus

[29]. Previous studies [30, 31], in combination with our study results, have shown that overexpression of human ataxin-3 with an expanded polyglutamine repeat in flies leads to the progressive, late-onset neurodegenerative phenotypes. *Drosophila*, as an ideal and classical model to imitate and study inherited human neurological diseases, has a complex nervous system, organized into neural center much like the human brain [31]. Genes especially those involving the molecular mechanisms of PolyQ-mediated neurodegeneration between humans and flies are highly conserved [31]. It has been proved feasible to apply flies to elucidate mechanisms of the disease onset and progression related to the cerebellar lesions in PolyQ diseases [15, 24, 32].

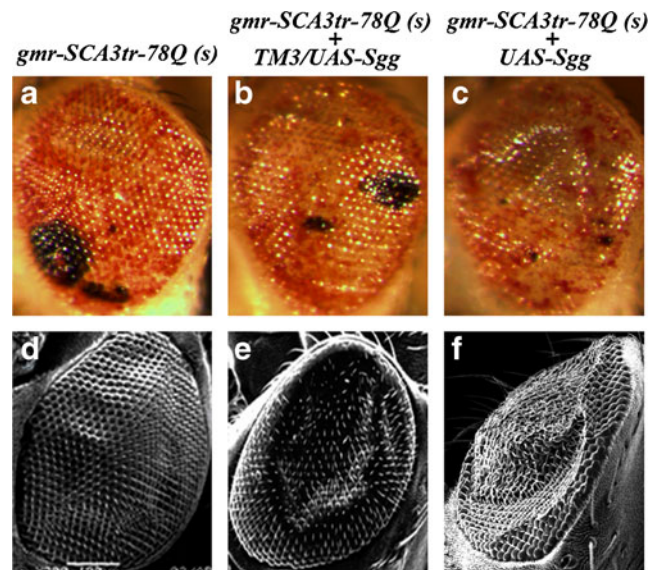
Here, by using a faithful *Drosophila* model of SCA3/MJD, we showed that lithium administered at an appropriate dose





**Fig. 7** *Sgg.S9E* alleviates PolyQ-mediated eye depigmentation in SCA3 *Drosophila* model. **a–c** Dissecting microscopic images of the eyes of 5-day-old adult flies ( $\times 80$ ). **d–f** Scanning electron microscopic images of the eyes of 5-day-old adult flies ( $\times 200$ ). **g–i** Scanning electron microscopic images of the eyes of 5-day-old adult flies ( $\times 1,000$ ). **a, d, g** Strong expression of SCA3tr-Q78 resulted in severe degeneration of the fly eyes. Genotype: *w; gmr-GAL4, UAS-SCA3tr78Q(s)*. **b, e, h** Co-expression of a heterozygous S9E mutation with SCA3tr-Q78 had no effect on the phenotypes of degenerative eyes. Genotype: *Gmr-GAL4/UAS-SCA3tr-Q78; TM3/UAS-Sgg.S9E*. **c, f, i** Co-expression of a homozygous S9E mutation with SCA3tr-Q78 partially alleviated eye degeneration. When the dark black spots disappeared, the restored structures of ommatidia and bristles formation were observed. Genotype: *Gmr-GAL4/UAS-SCA3tr-Q78; UAS-Sgg.S9E*

reduced neurodegenerative phenotypes mediated by the mutant ataxin-3. Eye depigmentation was alleviated in LiCl-treated SCA3 *Drosophila*, and the effect was enhanced by increasing the dose within a certain range. Treatment with LiCl increased the locomotor ability of all *nrv2-SCA3tr-Q78* fly strains, and with increasing dose and administration time, the effects first peaked and then declined. An extension of the *Drosophila* life span was apparent in *nrv2-SCA3tr-Q78* fly strains treated at 10–15 mM, and when LiCl reached doses of 20 mM, the average life span of treated flies was gradually reduced. In addition, the previous study on the effect of lithium in SCA1 mice model also revealed that lithium attenuated toxicity of the full-length mutant ataxin-1 with 154 glutamine



**Fig. 8** *Sgg* enhances PolyQ-mediated eye depigmentation in SCA3 *Drosophila* model. **a–c** Dissecting microscopic images of the eyes of 5-day-old adult flies ( $\times 80$ ). **d–f** Scanning electron microscopic images of the eyes of 5-day-old adult flies ( $\times 200$ ). **g–i** Scanning electron microscopic images of the eyes of 5-day-old adult flies ( $\times 1,000$ ). **a, d, g** Strong expression of SCA3tr-Q78 resulted in severe degeneration of the fly eyes. Genotype: *w; gmr-GAL4, UAS-SCA3tr78Q(s)*. **b, e, h** Co-expression of a heterozygous *Sgg* with SCA3tr-Q78 enhanced the toxic effect on the phenotypes of degenerative eyes. Genotype: *Gmr-GAL4/UAS-SCA3tr-Q78; TM3/UAS-Sgg*. **c, f, i** Co-expression of a homozygous *Sgg* with SCA3tr-Q78 produced severely disrupted internal morphology of eyes, with a great loss of cell integrity, a highly disordered ommatidial array, and abnormal bristles. Genotype: *Gmr-GAL4/UAS-SCA3tr-Q78; UAS-Sgg*

repeats [23]. Although the therapeutic window and toxic potential of lithium in human may be different from that in *Drosophila* or mouse models, similar beneficial effects of chronic lithium treatment on PolyQ-mediated neurodegeneration may provide with strong evidence for the significance of lithium as a potential treatment strategy for SCA3 and other PolyQ diseases.

The mechanism underlying neuroprotective effects by lithium has been extensively studied in various cellular and animal models. Up to now, at least two approaches, induction of autophagy and inhibition of GSK3 $\beta$ , have been put forward to be involved in lithium's neuroprotective effects against PolyQ-induced toxicity [17, 21]. In this study, we explored whether at least some of the neuroprotective effects of lithium in our SCA3 *Drosophila* model could be attributed to inhibition of GSK3 $\beta$ /shaggy.

GSK-3 is a serine/threonine kinase with  $\alpha$  and  $\beta$  isoforms, which is expressed ubiquitously in mammalian tissues [33]. Lithium can act as a competitive inhibitor of magnesium, thereby inhibiting the kinase activity of GSK-3 [34–36]; lithium also inhibits GSK-3 by enhancing phosphorylation of GSK-3 $\alpha$  at Ser21 or GSK-3 $\beta$  at Ser9 [36]. Chronic treatment with lithium has been shown to cause an

increase in the phosphorylation of Ser-9 of GSK3 $\beta$  in mouse models of SCA1 [23] and AD [28], resulting in reduced toxicity of aggregate-prone proteins. Accordingly, by using genetic approach, we showed that co-expressing the mutant S9E [37], which mimicked the phosphorylated S9 state of sgg as done by lithium, also partly decreased the toxicity of *gmr-SCA3tr-Q78*. Possibly due to lithium's amelioration of the phenotypes of SCA3 *Drosophila* through more than one mechanism, the effects of the mutant S9E were not such remarkable as that of lithium. Thus, our results suggested that the lithium's neuroprotective effects might be at least partly explained by its roles in GSK3 $\beta$ /shaggy inhibition.

In conclusion, we have demonstrated that lithium used at an appropriate dose alleviates the phenotypes in SCA3 *Drosophila* model and that this neuroprotection is at least partly associated with lithium's action on GSK3 $\beta$ /shaggy inhibition. Although the dose and duration of lithium administration need to be further explored in clinical aspect, its therapeutic effects in our SCA3 *Drosophila* model bring hope and clues for therapy of SCA3 patients in the future.

## Materials and Methods

### *Drosophila* Stocks and Crosses

All fly stocks were raised on nutritional media containing a mixture of cornmeal, glucose, sucrose, agar, 10 % methylparaben, and yeast powder. The growth incubators were maintained at 25 °C on a 12:12-h light/dark cycle at 60–70 % relative humidity. The *w*<sup>1118</sup> strain was used as the background strain. *GMR-GAL4* and *NRV2-GAL4* were P {long*GMR-GAL4*}2 [38] and P {nrv2-*GAL4.S*}3 [39], respectively. They were obtained from the Bloomington *Drosophila* Stock Center. The *UAS-SCA3tr-Q27*, *UAS-SCA3tr-Q78* (*w*), and *UAS-SCA3tr-Q78* (*s*) [31] fly strains were also received from the Bloomington *Drosophila* Stock Center. Male flies expressing UAS constructs were crossed to female flies expressing the neuron-specific promoter *nrv2* or the eye-specific promoter *gmr*, and flies P (*w*; *gmr-GAL4*/+; *UAS-SCA3tr-Q27*/+), P (*w*; *gmr-GAL4*/+; *UAS-SCA3tr-Q78*(*w*)/+), P (*w*; *gmr-GAL4*/*UAS-SCA3tr-Q78*(*s*)/+), P(*w*; *nrv2-GAL4*/+; +/+; *UAS-SCA3tr-Q27*/+), P(*w*; *nrv2-GAL4*/+; +/+; *UAS-SCA3tr-Q78*(*w*)/+), and P (*w*; *nrv2-GAL4*/+; *UAS-SCA3tr-Q78*(*s*)/+; +/+) were generated.

To generate the mutant S9E in the *gmr-SCA3tr-Q78*(*s*) flies, the *UAS-Sgg.S9E* strain, which harbors a mutation that mimics the S9 phosphorylation [37], was purchased from the Bloomington *Drosophila* Stock Center. The virgin female flies, *w*; +/+; *UAS-Sgg.S9E/TM3* were crossed with male flies, *w*; *Cyo/Sco*; *TM3/TM6*. The resultant F1 virgin females, *w*; *Cyo*/+; *UAS-Sgg.S9E/TM3* were crossed with *w*; *Sco*/+; *UAS-Sgg.S9E/TM6* males. The F2 male flies, *w*; *Cyo/Sco*; *UAS-*

*sgg.S9E* were crossed with *w*; *UAS-SCA3tr-Q78*(*s*); *TM3/TM6* females. The resultant F3 flies, *w*; *UAS-SCA3tr-Q78*(*s*)/*Cyo*; *TM3/UAS-Sgg.S9E*, can be distinguished by its wing and chaeta phenotype. Each virgin females were crossed with *w*; *gmr-GAL4*/*Cyo*; *TM3/TM6* males. The final flies were *w*; *UAS-SCA3tr-Q78*(*s*)/*gmr-GAL4*; *TM3/UAS-Sgg.S9E* and *w*; *UAS-SCA3tr-Q78*(*s*)/*gmr-GAL4*; *TM6/UAS-Sgg.S9E*.

### Lithium Treatment

All flies were exposed to different doses of LiCl mixed with instant fly food at the first day before crosses. They were reared on drug-containing media during the following stages: flies before crosses, egg, larva, pupa, and adults. To ensure its stability and efficacy, LiCl was first prepared at 100 mM concentration in ion-free water and then added at appropriate concentrations in standard medium after it was cooled to below 50 °C. Food colorants were used as an indicator to ensure the uniformity of the drug-containing media.

### *Drosophila* Eye Morphology

Proboscises were removed from heads of 5-day-old adult flies and fixed in 2.5 % glutaraldehyde for 2 h, followed by 1 % osmic acid for 1 h. They were then rinsed in PBS, dehydrated in serial dilutions of acetone, and replaced by serial dilutions of isoamyl acetate. Tissues were embedded in OCT (Tissue Tech) and frozen by immersion in liquid nitrogen. Adult flies eyes were viewed on a 1000 cx electron microscope (JSM-6490LV, Japan, Hitachi).

### Positive Phototaxis Assay

The locomotor ability of flies was analyzed as described [27]. Ten flies were randomly selected from each sample and placed into a 25-ml pipette that was sealed at the top with wax film to prevent escape animals (*n*=200 animals per group for each genotype). A fiberoptic lamp illuminated the pipette from the top. After adapting for 8 s, we knocked the bottom of the pipette to drop the flies and recorded time for the first five flies to cross a line 17.5 cm from the bottom. Five trials were completed for each sample. ANOVA and rank-sum tests were used to test the differences in climbing ability among groups. A *p* value less than 0.05 was considered significant. Data were presented as the mean  $\pm$  SEM.

### Lifespan Analyses

All 1-day-old flies were transferred to glass vials containing 2 ml of the LiCl-containing food. In a vial, 20 flies were reared carefully to keep a good condition (*n*=200 female flies per group for each genotype). Fresh solutions of LiCl were prepared daily at concentrations of 1, 5, 10, 15, and



20 mM, in standard cornmeal. In each vial, 2 ml of the standard media or of the LiCl-containing food was added. The dead flies were counted every day at 10:00 a.m. and survivors were transferred to freshly prepared food. Three trials were completed for each group.  $\chi^2$  statistics was used to test the difference of life spans among groups. A *p* value less than 0.05 was considered significant.

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