REVIEW

Animal models of amyotrophic lateral sclerosis: a comparison of model validity

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Abstract
Animal models are necessary to investigate the pathogenic features underlying motor neuron degeneration and for therapeutic development in amyotrophic lateral sclerosis (ALS). Measures of model validity allow for a critical interpretation of results from each model and caution from over-interpretation of experimental models. Face and construct validity refer to the similarity in phenotype and the proposed causal factor to the human disease, respectively. More recently developed models are restricted by limited phenotype characterization, yet new models hold promise for novel disease insights, thus highlighting their importance. In this article, we evaluate the features of face and construct validity of our new zebrafish model of environmentally-induced motor neuron degeneration and discuss this in the context of current environmental and genetic ALS models, including *C9orf72*, mutant Cu/Zn superoxide dismutase 1 and TAR DNA-binding protein 43 mouse and zebrafish models. In this mini-review, we discuss the pros and cons to validity criteria in each model. Our zebrafish model of environmentally-induced motor neuron degeneration displays convincing features of face validity with many hallmarks of ALS-like features, and weakness in construct validity. However, the value of this model may lie in its potential to be more representative of the pathogenic features underlying sporadic ALS cases, where environmental factors may be more likely to be involved in disease etiology than single dominant gene mutations. It may be necessary to compare findings between different strains and species modeling specific genes or environmental factors to confirm findings from ALS animal models and tease out arbitrary strain- and overexpression-specific effects.

Key Words: *amyotrophic lateral sclerosis; motor neuron degeneration; face validity; construct validity; zebrafish models; mouse models; genetic models; environmental models*

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Introduction
Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the progressive degeneration of upper motor neurons (UMN) and lower motor neurons (LMN). Initially, patients display muscle weakness, fasciculations, muscle atrophy spasticity and hyperreflexia. This eventually leads to paralysis, and patients typically die from respiratory failure within 3–5 years (Al-Chalabi and Hardiman, 2013; Taylor et al., 2016). Hallmarks include astrogliosis, microgliosis, mitochondrial dysfunction, defects in axonal transport and RNA binding protein processes (Philips and Rothstein, 2015; Taylor et al., 2016), although the underlying pathogenesis driving motor neuron degeneration remains largely unknown.

There are two main forms of ALS: approximately 10% of all cases are familial ALS (fALS), the remaining 90% of cases are considered sporadic ALS (sALS) which lack evidence of a hereditary genetic component (Al-Chalabi and Hardiman, 2013; Taylor et al., 2016). sALS cases are not considered to arise from dominant genetic mutations, and it has been suggested that environmental factors may be involved in etiology (Al-Chalabi and Hardiman, 2013).

As patient tissues are only available post mortem, animal models of ALS are widely used to investigate the putative underlying pathogenic mechanisms leading to motor neuron degeneration, and the development of therapeutics (Lutz, 2018). The number of genetic animal models is increasing and the use of these models have provided critical insights into the knowledge of disease pathophysiology. However, there are inherent limitations to consider when using animal models (Babin et al., 2014; Philips and Rothstein, 2015; Lutz, 2018) and the validity of each model must be considered in order to appropriately weigh the value of their contributions to the field of ALS.

Mice are the most common species used to model ALS (Philips and Rothstein, 2015; Picher-Martel et al., 2016; Lutz, 2018). Mice have been used to understand different aspects of disease pathogenesis and genetic models have been widely used to screen therapeutics for use in clinical trials (Philips and Rothstein, 2015; Picher-Martel et al., 2016; Tosolini and Sleigh, 2017; Lutz, 2018). However, to date, therapeutics that have been developed in mouse gene models have failed to translate to effective interventions in humans, challenging the use of these models for therapeutic screening (Philips and Rothstein, 2015; Tosolini and Sleigh, 2017).

Zebrafish are emerging as an important and useful vertebrate model for studying neurodegenerative diseases generally, and ALS specifically (Babin et al., 2014). Zebrafish offer unique advantages to higher vertebrates, including: its relative ease of genetic modification; genes implicated in neurodegenerative diseases are highly conserved between humans and

zebrafish; zebrafish are efficient for toxin screening studies; and zebrafish are optically transparent as embryos. However, the use of zebrafish as a model for ALS has a number of limitations, including: the absence of UMNs in the zebrafish, the fact that it is not a mammal, and that most studies analyze zebrafish at the embryonic stage to take advantage of optical transparency (Kabashi et al., 2011; Sukardi et al., 2011; Ciura et al., 2013; Schmid et al., 2013; Babin et al., 2014; Morrice et al., 2018; Svahn et al., 2018).

Overall, the value of any animal model of human disease can be determined by considering different criteria of validity, such as face and construct validity (Gerlach et al., 2008; Sontag et al., 2010; Gravetter, 2012). Face validity refers to how well a model replicates the disease phenotype in humans. In other words, it assesses the similarities in pathologic features between patients and animal models. Construct validity refers to how well the mechanism used to induce the disease phenotype in animals reflects what is currently understood to cause disease in patients. For example, how similar the genetic mutation is that causes pathology in animals to the mutation in disease patients. The same applies to environmental models.

Another critical aspect of model value is predictive validity. This is a measure of how well a model can be used to predict currently unknown aspects of a disease in patients (Gerlach et al., 2008; Sontag et al., 2010; Gravetter, 2012). Predictive validity is a paramount goal in modeling a disease, but this is generally evaluated retrospectively and it is only feasible to analyze in more established models that may have predicted aspects of disease subsequently discovered in patients, such as biomarkers or therapeutic development. The mutant Cu/Zn superoxide dismutase 1 (mSOD1) mouse model may be interpreted in terms of predictive validity, and this has been reviewed previously (Greek and Hansen, 2013). Briefly, this model has been used in the development of the only U.S. Food and Drug Administration-approved ALS therapeutics, Riluzole and Edavarone which show mild therapeutic benefits (Lutz, 2018). Generally, clinical studies based on the mSOD1 mouse model have failed to translate into an intervention to reduce disease burden (Ludolph et al., 2010; Philips and Rothstein, 2015). There may be numerous reasons for the failure of therapeutic translation to patients. For example, mutant genes usually need to be highly overexpressed in order for the animal model to develop an ALS-like phenotype within its lifespan (Lutz, 2018). The overexpression of mutant genes must raise the question whether the underlying mechanisms leading to motor neuron degeneration in genetic models is similar to that in humans; more specifically, whether the mechanism in genetic models is relevant to sALS. A further point of concern is that the resulting phenotypes are highly dependent on the genetic strain of mice, where some strains show a greater tolerance to the effects from mutations (Lutz, 2018).

This aspect challenges the value of the mSOD1 model in terms of its predictive validity, although methodological pitfalls of experimental and clinical study design may also be at stake (Ludolph et al., 2010). To address these issues, there are now guidelines for preclinical animal research in ALS (Ludolph et al., 2010). However, considering that the majority of ALS models have been more recently developed, we have not included predictive validity in the current review.

Many new ALS models are emerging to represent different mutations, and these offer promise for further characterizing disease pathogenesis and more effective therapeutic development. Although each model described in, but not limited to, this review shows certain pathological features similar to ALS, they also include qualities that are not observed in patients. It may then be necessary to interpret the value of each model by considering the measures of model validity. A comparison of face and construct validity offers a method to consider the weight of each model for particular aspects of disease. For example, it may be less meaningful to investigate pathogenesis in a model that does not show evidence of cell death in motor neurons. Pathogenic mechanisms common within a number of specific gene or environmental models may be more meaningful as compared to features specific to a single model, which may be caused by species-specific or construct-related outcomes.

In this mini-review, we discuss the validity of our recent zebrafish model of environmentally-induced motor neuron degeneration. To interpret this model, we put it into context with validity criteria of other current environmental and genetic vertebrate ALS models and discuss these in order of prevalence of disease burden which each environmental and genetic model represents.

Environmental Models

Bisphenol A (BPA) exposure in zebrafish

Recently, our group has developed a model of environmentally-induced motor neuron degeneration in zebrafish based on exposure of BPA (Morrice et al., 2018). BPA is a known toxin used as an industrial plasticizer to which humans are ubiquitously exposed (Jones et al., 2016). Our group found evidence that exposure to BPA induces motor neuron degeneration, which is not specific to the developmental time point of exposure. We also found preliminary evidence to suggest that activated microglia sense pathogenic stimuli at the axon terminal prior to cell death, which may suggest a retrograde mechanism of degeneration. However, a significant limitation of our model is that BPA exposure does not cause motor neuron-specific cell death, as other types of neurons also undergo cell death. This may be caused by the effect of BPA on nuclear respiratory factor 1 (NRF-1) regulated gene networks (Preciados et al., 2016). Further, because this is a new model, we have yet to further investigate different features of ALS, such as TAR DNA-binding protein 43 (TDP-43) cytoplasmic aggregation, therefore only limited features can be interpreted as measures of model validity. It is a common and valid critique that this is not a model for ALS as, historically, BPA is an unlikely candidate toxin for the disease. Rather, we are modeling the effect of a toxin on motor neurons. The rationale behind utilizing BPA as an environmental toxin is based on the premise that motor neurons may be vulnerable to toxic agents in a mechanistically-similar manner, regardless of the actual toxin. The processes that exposure to a toxic agent evokes may be similar to sporadic features that cause ALS. This preposition is not dissimilar to that behind assuming that the mechanism driving sALS and fALS pathology is equivalent, and further, as-

suming therapeutics developed from genetic fALS models will work for both fALS and sALS patients. It is unlikely that BPA is involved in ALS etiology, however this toxin may be a useful way to study features of ALS. A notable value of our model may lie in its ability to examine the microenvironment during motor neuron degeneration in a time and spatially-sensitive manner, and thus offer some insights into other various motor neuron diseases. The strengths and weaknesses of validity of our model as compared to others more specifically designed as ALS models are listed in **Additional Table 1**. In summary, BPA exposure in zebrafish shows several convincing aspects of face validity and relatively weak construct validity.

β-Sitosterol-β-d-glucoside (BSSG) exposure in mice

Cycad seed consumption is linked epidemiologically to the ALS-parkinsonism dementia complex (ALS-PDC) spectrum of neurological disorders, of which a form of ALS is a major component (Kurland, 1988). Our group has shown that BSSG, a common plant sterol found unusually in high levels in cycad, is one of the most toxic constituent in cycad flour (Wilson et al., 2002). A mouse model of sALS has been developed by our group based on dietary exposure to BSSG (Wilson et al., 2002; Tabata et al., 2008). Mice fed the flour made from raw washed cycad seeds or the synthesized form of BSSG showed progressive motor neuron degeneration which persisted and exacerbated after BSSG exposure was discontinued. The model and mimicked many aspects of the disease in humans, both phenotypically and pathologically, as summarized in **Additional Table 1**. Of note, exposure to BSSG results in a differential phenotype for rats, where rats display cognitive deficits marked by progressive degeneration in substantia nigra pars compacta (Snpc), and thus more closely resembles a Parkinsonism-like phenotype (Van Kampen et al., 2015). In summary, BSSG exposure in mice shows convincing face and construct validity, with caveats in each category.

Genetic Models

FVB-*C9orf72* BAC mouse model

Recently, a hexanucleotide repeat expansion in chromosome 9 open reading frame 72 has been identified as another gene mutation associated with fALS in which those affected carry between 23 and 5000 repeat sequences (Al-Chalabi and Hardiman, 2013; Haeusler et al., 2014). *C9orf72* mutations are the most common genetic mutation associated with ALS, although it remains unclear if disease is caused by a loss of gene function, gain of function, or dipeptide repeat protein (DPR) toxicity (Mackenzie et al., 2015; Lutz, 2018).

While different rodent models of *C9orf72* have been developed, in the following we focus on the FVB-C9orf72 BAC transgenic mouse model (Liu et al., 2016). This particular model develops many features similar to ALS, including paralysis, motor neuron loss, impaired neuromuscular junction (NMJ) integrity, RNA foci formation, DPR protein aggregation, TDP-43 cytoplasmic aggregation and reduced lifespan. However, it is important to acknowledge that this model has incomplete penetrance, where fully penetrant females develop early disease onset and death by 6 months of age. This phenotype was not observed in fully penetrant males (**Additional Table 1**) (Liu et al., 2016; Lutz, 2018). In summary, the FVB-C9orf72 BAC mouse model shows strong aspects of face validity with concerns about construct validity related to penetrance and a sex-dependent effect.

***C9orf72* knockdown zebrafish model**

The zebrafish model of *C9orf72* is one of the few animal models to support a loss of function hypothesis of pathogenesis. It is for this reason that we discuss the haploinsufficiency model developed by Ciura et al. (2013), although we note that other *C9orf72* zebrafish have been developed that model a toxic gain of function (Stepto et al., 2014; Swaminathan et al., 2018; Swinnen et al., 2018). Knockdown of the zebrafish homolog to human *C9orf72* results in reduced motor function, which was rescued upon co-expression with human *C9orf72* mRNA. The rationale for modeling a loss of function mechanism is based on post-mortem tissues which show reduced *C9orf72* transcript levels. Cytoplasmic aggregation of TDP-43 was not observed in this model (**Additional Table 1**) (Ciura et al., 2013). In summary, the *C9orf72* knockdown zebrafish model shows convincing aspects of face validity, with challenges in construct validity based on an unclear disease mechanism.

Cu/Zn superoxide dismutase 1 (SOD1)-G93A mouse model

The first causative gene implicated in fALS was SOD1 (Rosen et al., 1993). The most widely used mouse model of ALS is a based on expression of the human SOD1 protein containing the G93A mutation (Philips and Rothstein, 2015; Lutz, 2018). The mSOD1 model has been instrumental in describing putative cellular dysfunction during disease pathogenesis, such as the non-cell autonomous nature of ALS (Nagai et al., 2007; Philips and Rothstein, 2015). This model displays a rapid degeneration of motor neurons which leads to paralysis and death within the first 5 months of life (further reviewed in Nardo et al. (2016)). Despite these positive aspects, there are important caveats to acknowledge. First, the mSOD1 mouse model has a propensity to spontaneously delete copy number which can directly impact the severity of disease presentation (Zwiegers et al., 2014; Lutz, 2018). This feature acts as a confounding factor in studies which have not accounted for the copy number in each animal. Also, the overexpression of human wild-type SOD1 causes axonopathy in mice, challenging the role of the mutation as the driver of pathology (Joyce et al., 2011). Although UMN deficits are absent in this model, the SOD1-G93A mouse model shows the strongest face validity as compared to the other models discussed here, however there are important caveats to acknowledge in construct validity, such as copy number and strain-dependent effects, as summarized in **Additional Table 1**.

G93R-mSOD1 zebrafish model

In **Additional Table 1**, we focus on the G93R mSOD1 zebrafish model (Ramesh et al., 2010; Joyce et al., 2011), although we note that other mSOD1 zebrafish models have been developed (Lemmens et al., 2007). This model displays evidence of adult onset, slowly progressive motor degenerative phenotype and mimics many aspects of the disease in patients, as listed in **Additional Table 1**. This model offers advantages over the mouse mSOD1 model because it is based on a slight overexpression

of mutant SOD1 and expression of wild-type SOD1 does not produce motor neuron defects in zebrafish, in contrast to mice (Lemmens et al., 2007). However, there is no evidence of muscle denervation in this model (Joyce et al., 2011). In summary, the G93R-mSOD1 zebrafish model shows convincing aspects of face validity with the notable caveat of no observed muscle denervation, and strong construct validity.

TDP43-Q331K mouse model

Another noteworthy mutation has been found in TDP-43. TDP-43 is a nuclear protein that aggregates in the cytoplasm as ubiquitinated inclusions in the majority of ALS patients and has been found mutated in 4% of fALS patients (Taylor et al., 2016). It is currently unclear if mutations in TDP-43 are caused by toxic gain or loss of function, or alternatively, if cytosolic aggregation of TDP-43 is a byproduct of an upstream pathogenic mechanism(s) (Philips and Rothstein, 2015; Taylor et al., 2016). Recent evidence show that shows that a gain and loss of TDP-43 function have a differential effect on RNA processing and suggests that both are involved in pathology (Fratta et al., 2018). Models with a mutation in TDP-43 have the potential to better represent the pathology of the majority of ALS patients. However, there have been considerable challenges in the development of TDP-43 mouse models, such as promoter-dependent effects, diet-related effects and tolerability of mutant gene expression [reviewed in Philips and Rothstein (2015) and Lutz (2018)].

Currently, approximately 20 different mouse models of TDP-43 have been developed, each with unique measures of face and construct validity (Lutz, 2018). In this mini-review, we discuss the TDP43-Q331K mouse model because this model is based on mild overexpression of human mutant TDP-43 (Arnold et al., 2013). Of note, the degenerative phenotype was not caused by human wild-type TDP-43. Therefore, this model offers many advantages over other mouse models. The TDP43-Q331K model is driven under the prion protein gene promoter and displays many ALS-like hallmarks such as progressive motor dysfunction, muscle atrophy, reduced NMJ integrity and motor neuron degeneration at 10 months of age. However, the motor phenotype is restricted to lower motor neurons, the progressive degeneration halts at 20 months and the ALS-like outcomes do not cause lethality in the mice. Importantly, no TDP-43 cytosolic aggregation or nuclear export is evident in this model (**Additional Table 1**) (Arnold et al., 2013; Philips and Rothstein, 2015; Lutz, 2018), although features such as ubiquitinated TDP-43 cytoplasmic inclusions have been described in other mutant TDP-43 mouse models (Xu et al., 2010; Stribl et al., 2014). In summary, the TDP43-Q331K mouse model shows convincing phenotypic features of ALS with important caveats to face validity, and this model shows problematic construct validity based on strain and promoter-dependent effects.

TDP43-A315T zebrafish model

In the following we discuss the zebrafish model of mutant TDP-43, which is based on the introduction of human mutant TDP-43 mRNA. The animals show defects in motor function, motor axons and branching, however injection with wild-type TDP-43 mRNA resulted in slight motor defects. Of note, no aggregation of TDP-43 was observed in the cytoplasm, similar to findings from the TDP43-Q331K mouse model (**Additional Table 1**) (Laird et al., 2010; Joyce et al., 2011). Together, both models suggest that the nuclear export of TDP-43 to the cytoplasm may not be the primary driver of pathogenesis but may be the result of an upstream mechanism(s). Alternatively, considering this is a hallmark in most ALS patients, the absence of this pathologic feature may reflect poorly on the face validity of these models. In the following, we have considered the latter because this a widely observed pathological finding and the mechanism is currently not well understood. In summary, the TDP43-Q331K zebrafish model shows a number of key features to show convincing aspects of face and construct validity, however gene over-expression reflects poorly in the construct validity of this model.

No animal model completely satisfies all validity criteria, thus the findings from each model should be interpreted with caution. Using older models to validate novel findings from new models may help highlight valuable data and tease out arbitrary findings resulting from strain- or overexpression-dependent effects. In terms of our zebrafish model of BPA-induced motor neuron degeneration, investigating the localization of activated microglia during pathogenesis using other environmental ALS models, such as the BSSG mouse model discussed above, may evaluate the value of our findings.

Conclusion

Many ALS models are currently available, and more are expected in the coming years (Lutz, 2018). Our recently described model of environmentally-induced motor neuron degeneration shows many aspects of face validity with pitfalls in construct validity. However, the value of our model may lie in the potential for environmentally-induced motor neuron degeneration to be more representative of the pathology underlying sALS, which environmental factors may be more likely to be involved in disease etiology than single dominant gene mutations. Further, zebrafish are valuable for investigating the dynamics of cells *in vivo*, which may be used to support findings and to address shortcomings in other animal models. Therefore, zebrafish models are a valuable addition to the current environmental and genetic ALS models.

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Additional files:
Additional Table 1: *Comparison of face and construct validity between animal models of amyotrophic lateral sclerosis.*
Additional file 1: *Open peer review report 1.*

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Additional Table 1 Comparison of face and construct validity between animal models of amyotrophic lateral sclerosis (ALS)

Animal model	Pros	Cons	Reference
BPA exposure: zebrafish			Morrice et al. (2018)
Face validity	Reduced motor behaviour; lower motor neuron degeneration, loss of neuromuscular junction (NMJ) integrity; microglia activation.	The model is based in a developing embryo; non-motor neuron specific degeneration; zebrafish cannot be used to model upper motor neurons (UMN) defects.	
Construct validity	Toxin-induced motor neuron degeneration; non-developmentally dependent effect.	Exposure to bisphenol A (BPA) has not previously been associated with ALS; exposure dose not relevant to human exposure.	
BSSG exposure: mice			Wilson et al. (2002); Tabata et al. (2008)
Face validity	Progressive motor dysfunction persists after BSSG exposure has been discontinued; loss of motor neurons; reduced NMJ integrity; astrocytosis; evidence of microgliosis; neuronal cell death in pathologically-relevant regions.	Differential cellular pathology in rats showing degeneration in substantia nigra pars compacta (Snpc).	
Construct validity	Toxin-induced motor neuron degeneration; previously association with ALS on Guam; equivalent dietary route of exposure.	Exposed to isolated toxin or washed form of the cycad seed flour, not total flour constituents.	
FVB-C9orf72 BAC: mouse			Liu et al. (2016)
Face validity	Paralysis, motor neuron loss; reduced NMJ integrity; accumulation of antisense RNA foci, DPR protein and TDP-43 cytosolic aggregation, reduced lifespan.	Strain-dependent effects.	
Construct validity	Transgene models a disease-causing mutation in humans.	Unclear mechanism of pathogenesis, transgenesis-dependent effect, different *C9orf72* models show differences in phenotypes, incomplete penetrance, gender dependent effects on phenotype of fully penetrant mice.	
C9orf72 knockdown: zebrafish			Ciura et al. (2013)
Face validity	Motor deficits and reduced motor axon length with was rescued with human *C9orf72* mRNA.	Developmental onset; no cytoplasmic TAR DNA-binding protein 43 (TDP-43) aggregation; zebrafish cannot be used to model UMN defects.	
Construct validity	Models one possible mechanism of a disease-causing mutation in humans; phenotype can be rescued upon co-expression with human *C9orf72* mRNA.	Unclear mechanism of pathogenesis; based on zebrafish homologue of the human gene.	
SOD1-G93A: mouse			Nardo et al. (2016)
Face validity	Progressive motor dysfunction; loss of motor neurons; axonal deinnervation; impaired NMJ integrity; proteinopathy; mitochondrial dysfunction; glutamate mediated excitotoxicity; oxidative stress; axonal transport defects; microgliosis; astrocytosis; impaired glial function; T cell invasion into the central nervous system (CNS); premature death.	No evident UMN degeneration; early onset; strain-dependent effects.	
Construct validity	Transgene models a disease-causing mutation in humans; different mutations in Cu/Zn superoxide dismutase 1 (SOD1) produce similar motor defects.	Based on overexpression of mutant SOD1; transgene copy number variation impacts disease phenotype; expression of human wild-type SOD1 produces axonopathy.	
G93R-mSOD1: zebrafish			Ramesh et al. (2010)
Face validity	Adult onset lower motor neuron loss; progressive reduction in motor function; abnormal NMJ structure, muscle atrophy; decreased survival.	No evidence of muscle denervation; zebrafish cannot be used to model UMN defects.	
Construct validity	Transgene models a mutation in a pathologically relevant gene; no motor defects with expression of wild-type SOD1.	Threefold expression of mutant SOD1 (mSOD1) required.	
TDP43-Q331K: mouse			Arnold et al. (2013)
Face validity	Progressive motor dysfunction; motor neuron and axon degeneration; reduced integrity of NMJ; muscle atrophy, astrocytosis and microglia infiltration; defects in RNA processing.	Limited period of progressive degeneration; no TDP-43 cytosolic aggregation; no UMN loss or defects; ALS-like features do not result in death organism.	
Construct validity	Transgene models a disease-causing mutation in humans.	Unclear mechanism of TDP-43 in ALS pathology; unclear if prions are involved in pathology; promoter-dependent effects; gene copy number effects, strain-dependent effects.	
TDP43-A315T: zebrafish			Kabashi et al. (2010); Laird et al. (2010)
Face validity	Reduced motor function; reduced motor axon length and aberrant branching.	No TDP-43 cytosolic aggregation; zebrafish cannot be used to model UMN defects	
Construct validity	Injection of human TDP-43 mRNA representing disease-causing mutations in humans; dose dependent effects; reproducible effects with injection of different TDP-43 mutations.	Unclear mechanism of TDP-43 in ALS pathology; overexpression of mutant gene; wild-type mRNA produced only minor motor defects.	