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Alsin and the Molecular Pathways of Amyotrophic Lateral Sclerosis

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Abstract

Autosomal recessive mutations in the *ALS2* gene lead to a clinical spectrum of motor dysfunction including juvenile onset amyotrophic lateral sclerosis (ALS2), primary lateral sclerosis, and hereditary spastic paraplegia. The 184-kDa alsin protein, encoded by the full-length *ALS2* gene, contains three different guanine-nucleotide-exchange factor-like domains, which may play a role in the etiology of the disease. Multiple in vitro biochemical and cell biology assays suggest that alsin dysfunction affects endosome trafficking through a Rab5 small GTPase family-mediated mechanism. Four *ALS2*-deficient mouse models have been generated by different groups and used to study the behavioral and pathological impact of alsin deficiency. These mouse models largely fail to recapitulate hallmarks of motor neuron disease, but the subtle deficits that are observed in behavior and pathology have aided in our understanding of the relationship between alsin and motor dysfunction. In this review, we summarize recent clinical and molecular reports regarding alsin and attempt to place these results within the larger context of motor neuron disease.

Keywords

Amyotrophic lateral sclerosis (ALS); ALS2; Alsin; Rab5; Mouse model; Guanine-nucleotide-exchange factor; Primary lateral sclerosis; Hereditary spastic paraplegia

Introduction

First described in the nineteenth century by Jean-Martin Charcot, amyotrophic lateral sclerosis (ALS) is now recognized as the most common disorder of motor neurons [1]. ALS lies within a spectrum of heterogeneous syndromes that lead to the selective degeneration of upper and lower motor neurons. The precise etiology underlying ALS remains unknown, although the genetic and clinical evidences indicate that the causative mechanism is likely to be multifactorial [2]. ALS is largely sporadic, but in 5–10% of the cases, the disease is inherited through autosomal dominant or recessive genetic mutations [3]. Mutations in the abundant free radical scavenging enzyme superoxide dismutase 1 (SOD1) were first described 13 years ago

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and account for nearly 20% of all familial ALS [4]. Since the identification of SOD1, four other genes causative for ALS have been identified, and at least six other loci have been mapped, with the majority of the pedigrees carrying dominant modes of inheritance [2]. These genetic links are excellent tools for studying motor neuron disease, as the sporadic and familial forms appear to share a common pathogenesis based on their similar clinical and histopathological phenotypes. To date, only two loci have been mapped that carry autosomal recessive forms of ALS, and of them, only mutations in the gene *ALS2*, linked to chromosome 2q33, have been identified [5,6]. In the 5 years since mutations in *ALS2* were first identified, a multitude of in vitro studies and transgenic mouse models have been reported and have helped shed light into the mechanisms underlying ALS. In this review, we summarize these recent findings related to *ALS2* and its gene product alsin and attempt to provide a perspective of the emerging pathways that may link the different forms of motor neuron diseases.

Clinical Heterogeneity

Primary lateral sclerosis (PLS) and hereditary spastic paraplegia (HSP) are disorders that affect the motor pathways differently than ALS, and yet have also been associated with mutations in *ALS2*. Unlike ALS where both the upper and lower motor neurons are specifically compromised, only the upper motor neurons are affected in PLS [6]. The family of HSP disorders typically features progressive spasticity and weakness of lower limbs. To date, at least 12 different mutations in the 34 exons spanning the *ALS2* gene have been described in African and Asian families with juvenile ALS and PLS and in European and Asian families with hereditary spastic paralysis (see Table 1). With the exception of two recently identified homozygous missense mutations at the amino terminal [7,8], the majority of the mutations appear to result in the generation of a premature stop codon, resulting in protein instability and a loss of function [9]. The relationship between the missense mutations and alsin function is unclear.

The clinical heterogeneity and presence of two existing transcripts that encode long (1,659 amino acids) and short (396 amino acids) forms of alsin have led to studies searching for correlations between genotypes and the observed phenotypes. Alsin, predicted to have a molecular mass of 184 kD, has three putative guanine nucleotide exchange (GEF) domains: regulator of chromosome condensation 1 (RCC1) like domains (termed RLD), a diffuse B cell lymphoma (Dbl) homology/pleckstrin homology (DH/PH) domain, and a vacuolar protein sorting 9 (VPS9) domain (Fig. 1a). Additionally, eight consecutive membrane occupation and recognition nexus (MORN) motifs have been identified in the region between the DH/PH and VPS9 domains [5,6]. Of the reported mutations to date, only four (261delA, 553delA, G660A, and 1130delAT) are found in the RCC1 domain region expected to affect both forms of the protein, and of these, the two nearest to the amino-terminus (261delA and 553delA) result in frame shift mutations that are responsible for the development of juvenile ALS (ALS2) [5,6, 10]. The other two mutations that keep most of the short form of alsin intact primarily affect upper motor neurons [8,11]. This observation has led to speculation that an intact short form of alsin may protect lower motor neurons and be responsible for the varying severity of clinical phenotypes [6]. Under that hypothesis, an intact short form would lead to solely JPLS or HSP, with the differences between them depending on the integrity of individual protein domains of the long form. No neuropathological data currently exist from any of the affected families, so the hypothesis is based on diagnostic tests used for determining region specific motor dysfunction. The physiological significance of the short form is supported by the fact that mutations disrupting both alsin splicing variants have later disease onsets compared to those solely affecting the long form, with the symptoms being detected before 24 months of age.

However, a number of observations after the initial identification of the disease-causing mutations suggest that a simple genotype–phenotype correlation between the presence and

absence of short form of alsin may not exist for these ALS2 cases. Nearly half of the patients in the Tunisian kindred that developed juvenile ALS through ALS2 mutations did not show EMG denervation, a diagnostic criteria for motor neuron degeneration [12]. Additionally, the 1130delAT mutation affects both forms of the alsin protein and yet leads to the development of infant-onset HSP without affecting the lower motor neurons [13]. An in vitro study on the stability of each of the truncated proteins generated by the disease causing mutations found that all of the mutants were unstable and surprisingly decayed at a half-life comparable to the equally unstable alsin short form, further suggesting that the splice variants may be an unsuitable marker for clinical phenotype [9].

Since the identification of the first mutations in *ALS2*, a myriad of papers have been published regarding the protein expression, potential GEF domain functions, and mechanism of alsin in different expression systems. In the following section, we will discuss some of the emerging pathways that have been linked to the various mutations found in *ALS2* by integrating reported in vitro data with four independent studies on *ALS2* knockout mice.

ALS2 and *ALS2CL* Expression

Multiple groups detected ubiquitous expression of *ALS2* mRNA in the central nervous system and non-neuronal tissues, with the cerebellum and kidney showing the highest enrichment and spinal cord and heart showing the lowest [5,6]. With the exception of the liver, which appears to show selective augmentation of the short form, the distribution of both splice variants appears to be similar at the mRNA level. Protein expression largely mirrors mRNA expression, although full-length alsin appears to be far less abundant than SOD-1, and the short form is undetectable, possibly due to a transient half-life [9,14].

Interestingly, a novel protein was identified through a BLAST search of the GenBank/DDBJ/EMBL database with a high degree of homology to the C-terminal region of alsin and termed ALS2CL. This protein contains similar repeated MORN motifs found between flanking PH and VPS9 domains. Northern blot analysis of human tissues has revealed that the expression of ALS2CL mRNA is high in the heart and kidney and low in the brain and skeletal muscle [15].

Guanine-Nucleotide-Exchange Factor Activities

The identification of three putative GEF domains has led to a number of in vitro dissections of *ALS2*-linked GEF activity. It is difficult to detect endogenous alsin through immunocytochemistry techniques in mouse primary neurons because of a lack of alsin-specific antibodies. Evaluation of endogenous expression in rat embryonic hippocampal neurons, however, has been possible and demonstrates that alsin localizes primarily with small punctate structures in the dendrites, axon, cell body, membrane ruffles, and growth cones [16,17]. The presence of alsin in growth cones, which are structures abundant in actin that guide axons to their targets, suggests a link between alsin and members of the Rho subfamily. Rac GTPases, Rho, and Cdc42, which are classified as Rho subfamily proteins, mediate actin dynamics related to neuritic outgrowth and are known to be activated by GEFs containing DH/PH domains [18].

Binding assays with Rho subfamily members reveal a specific interaction of solely Rac1 with an alsin fragment containing the DH/PH domains [16]. To test whether the DH/PH domain of alsin has selective GEF activity for any of the members of the Rho subfamily, pull-down assays in CHO cells were performed with co-transfected RhoA, Rac1, Cdc42, and alsin. Only Rac1 activity was significantly augmented with alsin overexpression, and this stimulation was dependent on an intact DH/PH domain and not replicated when the domain was excised in an altered construct [16,17]. Interestingly, when the experiment was repeated with a truncated

alsin fragment consisting of solely the DH/PH domain, Rac1 was not stimulated, indicating that the other alsin domains are necessary for Rho GEF mediation. This link between Rac1 and alsin is further strengthened by an observation that cellular toxicity and reduced axonal growth associated with knockdown of alsin protein using siRNA can be rescued with Rac1 overexpression [19]. A similar correlation is found through overexpression of alsin in primary rat neurons, which increases the length of the longest neurite. Furthermore, PAK1, an effector of Rac1 shows an enhanced activity in cells containing transfected full-length alsin, but not alsin lacking the DH/PH domain [16,17]. Thus, the DH/PH domains of alsin appear to have selective GEF activity for Rac1, and a role for alsin in axonal guidance is suggested.

While studies of endogenous expression of *ALS2* have provided the framework for the link between the DH/PH domain and Rac1, subcellular fractionation of brain tissue and overexpression of alsin has yielded clues regarding the function of the other domains. Although a cytoplasmic pool of alsin exists, the majority of alsin subsist in membrane fractions containing secretory trafficking machinery, with enrichment in endosomal membranes [9,16]. Distribution of transfected full-length alsin protein is primarily cytosolic, with only a small minority localizing to vesicular-like structures [9,14,16]. These alsin-positive structures appear to be early endosomal as several groups have reported co-localization of alsin with the early endosome antigen 1 (EEA1) and Rab5 [8,11]. Rab GTPases typically regulate intracellular protein and membrane trafficking events, and three isoforms of Rab5 exist in mammalian systems: Rab5a, Rab5b, and Rab5c [20]. Rab5 primarily associates with early endosomes and clathrin-coated vesicles and is necessary for early endosomal fusion and for regulation of several G-protein coupled receptors (GPCRs) [20]. The ability of Rab5 to mediate endocytic transport is dependent on its activation through binding with GTP. The VPS9 domain is an obvious candidate out of the alsin domains to likely influence Rab5 dynamics, as VPS9 domain containing proteins Rabex-5 and Rin1 both exhibit specific Rab5GEF activity [21]. The carboxy-terminal region of alsin containing the VPS9 domain interacts directly with all three isoforms of Rab5 using yeast-two hybrid systems and in vitro binding assays [14,16].

To determine whether alsin activates Rab5 and promotes endosomal fusion, multiple groups have transfected full-length alsin or truncated forms containing either the VPS9 domain alone or the VPS9 domain with other domains. While all forms can stimulate the release of bound GDP from Rab5 subfamily GTPases in a cell-free GDP dissociation assay, overexpression of full-length alsin is unable to stimulate Rab5-mediated endosome fusion as efficiently as truncated forms lacking the RLD domains [14,16]. Analyses of a range of alsin deletion constructs suggest that multiple domains influence Rab5GEF activity. While an N-terminal fragment lacking the VPS9 domain not surprisingly lacks Rab5 GEF activity, C-terminal peptides containing solely the VPS9 are also unable to promote Rab5GEF activity [14]. ALS2CL, which contains MORN motifs and flanking PH and VPS9 domains, but lacks RCC1 and DH domains, has a higher binding affinity to Rab5 than alsin, but it does not exhibit Rab5-GEF activity [15]. This interplay between alsin domains in regulating Rab5 activity results in interesting observations on the endocytic pathway.

Overexpression of wild-type Rab5a (Rab5) in cell lines promotes endosomal fusion resulting in the formation of large endosomal structures [14,16]. The degree of fusion is enhanced if a constitutively active form of Rab5 (Rab5Q) is expressed instead of Rab5 [14,22]. Co-expression of Rab5 and a truncated alsin construct with solely MORN motifs and the VPS9 domain fail to replicate the enhanced endosomal fusion observed with the Rab5Q construct alone. This difference, however, is mitigated if the DH/PH domain is included with the MORN motifs and VPS9 domain, suggesting that the DH/PH domain has a role in enhancing Rab5 activity too [14]. This element of the DH/PH domain perhaps explains the clear functional differences between alsin and ALS2CL. In contrast to the observed enlargement of early endosomal antigen 1 (EEA1) positive endosomes when expressing alsin with Rab5, co-

expression of ALS2CL and Rab5 in HeLa cells induces a reorganization of EEA1 negative endosomes by forming some tubular structures [15].

The Function of the RLD Domain

While much of the current literature suggests that DH/PH and VPS9 domains with the MORN motifs are necessary to promote Rab5 activity in the endocytic pathway, an opposing role for the RLD domain has been defined. Recently, two motor neuron disease-related missense mutations (C156Y and G540E) have been identified within the RLD domain, indicating that this domain also plays a very important role in the normal function of alsin [7,8]. The RLD domain structure consists of a seven-bladed propeller formed from internal amino acid repeats [23]. When only the RLD domain of alsin is expressed in cell lines, a diffuse staining similar to full-length alsin over-expression is observed, indicating a cytosolic distribution [14,16]. Expression of alsin lacking the RLD domain in the cell lines, however, results in extensive vesicular localization associated with early endosome markers [9,14]. Therefore, the RLD domain of alsin may prevent the association of activated Rab5 with its downstream effectors located in early endosomes, suggesting that this domain acts as a negative regulator of Rab5-mediated endosomal fusion. As seen from our 3D profile of the RCC1 mutations (see Fig. 1b), it appears that the C156Y mutation, which is close to the Ran-binding site, may lead to protein instability due to the larger size of the tyrosine side chain compared to the cysteine side chain. The G540E mutation, however, is not on the predicted binding sites, and the apparent difference is that the amino acid change alters the polarity and acidity of the side chain. This may compromise the interactions with other proximal residues and the surrounding water network. It will be interesting to study whether these two missense mutations affect the subcellular localization and Rab5 GEF activity of alsin.

Additionally, like in other RLD-containing proteins, the RLD domain of alsin may serve as a potential protein–protein interaction domain. To decipher the molecular network in which alsin is involved, we screened proteins interacting with its RLD domain. We found that the RLD domain of alsin interacted with the PSD-95/Dlg/ZO-1 (PDZ) domains of glutamate receptor interacting protein (GRIP1). Glutamate-mediated excitotoxicity, primarily mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) type glutamate receptors (AMPAR) in spinal motor neurons, is one of the major mechanisms believed to contribute to the onset of sporadic ALS [24]. AMPARs are tetramers composed of various combinations of the GluR1-4 subunits in which GluR2 is particularly important, as GluR2-containing AMPARs are calcium-impermeable and neurons lacking GluR2-containing AMPARs are more vulnerable to excitotoxicity [24]. The presentation of GluR2 at the synaptic surface is dynamically regulated through its binding proteins, including GRIP1. GRIP1 transports GluR2 to dendrites and anchors GluR2 in both postsynaptic membrane and intracellular compartments [25,26]. Loss of alsin altered the subcellular localization of GRIP1, which correlated with a reduction of GluR2 at the cell/synaptic surface of *ALS2*$^{-/-}$ neurons, resulting in an increased vulnerability of these neurons to excitotoxicity [27].

ALS2 Knockout Mice

One confound that has arisen in these studies in vitro is the variability between cell lines, as investigators have observed that overexpression of *ALS2* yields different alsin distributions in COS-7, SW-13, and HEK293 cells [14,28]. This inherent variability is not necessarily addressed with the development of *ALS2* knockout mice, as strain backgrounds and differences in breeding regimens result in only partial agreement between models. These mouse models, however, are excellent tools for studying the loss of function inheritance believed to affect the different kindred associated with *ALS2*-related motor dysfunction.

Unlike the striking phenotypes generated from transgenic mice overexpressing mutations in SOD1 [2], mice deficient in *ALS2* have largely failed to recapitulate symptoms of motor neuron disease [29-32]. That is somewhat surprising given the clear loss of function mutations that afflict the different reported kindred, but as all the knockouts generated resulted in an embryonic deletion of *ALS2*, there may be compensatory gene effects in the mouse. The four different *ALS2* mouse knockouts were generated independently with variations in targeting vector, embryonic stem (ES) cell line, and probably housing conditions. As a result, while several observations are relatively conserved across the different reported mouse knockouts, some discrepancies are notable and help illustrate the difficulties in assigning phenotypes to mouse models. *ALS2* knockouts appear to be grossly normal, viable, fertile, and capable of a life span similar to wild-type littermates [29-32]. Characteristic pathological markers and behavioral paradigms in mouse models for ALS have evolved largely from the impressive collection of data on the *SOD1* transgenic mice [33]. As a result, behavioral tests measuring motor coordination, locomotor activity, and muscle strength were performed with standard apparatuses such as an accelerating rotarod, a motion sensitive activity box, and a grip strength meter. Despite the absence of overt signs of hind limb weakness that are hallmarks of the *SOD1* transgenic mice, *ALS2*$^{-/-}$ mice perform poorly on the accelerating rotarod compared to their wild-type littermates [29,32]. This inability to remain on the rotarod for longer latencies was independent of an observed weight augmentation evident in aged *ALS2* knockouts [29]. The enrichment of alsin in the cerebellum led us to test whether motor learning, a cerebellar-dependent task measured through repeated trials on the rotarod, was affected in *ALS2*-deficient mice. We found a reduced motor learning capability in aged *ALS2*$^{-/-}$ mice, and demonstrated that the deficit was restricted to the motor system, as the Y-maze test for learning and memory failed to detect a difference between mouse cohorts [29]. Other observed deficits in the *ALS2* knockouts were decreased running speed, heightened anxiety, and hypoactivity [29,31, 32]. These deficits were not noted in all the reported mice, but similar discrepancies in behavioral phenotyping have been noted in mice derived from different 129 strains [34,35]. Additionally, we reported that *ALS2*$^{-/-}$ mice develop peripheral lymphopenia and had higher proportions of hematopoietic stem and progenitor cells in which the stem cell factor-induced cell proliferation was up-regulated [36]. A direct link between ALS and the lymphohematopoietic system comes from clinical observations that ALS patients had marked lymphopenia with reductions in $CD2^+$ and $CD8^+$ T cells in comparison to age-matched controls [37]. An HIV-associated ALS disorder seen in many patients further suggests that reduced immunity from viral infections may play a key role in ALS pathogenesis [38,39]. Recently, a significant decrease of white blood cells was observed in the $SOD1^{G93A}$ transgenic mouse model of ALS, further supporting a link between immunodeficiency and ALS disease onset [40].

Pathologies From ALS2 Mice

The aforementioned strain and targeting vector differences may explain the heterogeneity in observed pathology in the mouse models. While all the *ALS2* knockout models failed to demonstrate any loss of upper or lower motor neurons, there appears to be a decrease in the number of motor neuron axons and a decrease in cerebellar purkinje cells [30]. There is also a reported progressive enhancement of astrocytic, microglial, and macrophagic staining in the brain and spinal cord of *ALS2*$^{-/-}$ mice, suggesting an activated inflammatory response [30]. An examination of spinal cord tissue identified regions of swollen and degenerating axons present in the lateral column that were absent in the dorsal column [32]. Due to the prominent examples of altered endocytic trafficking with overexpression of *ALS2* in culture, several groups examined whether similar results were obtained in cells derived from the knockout mice. Interestingly, the expression levels of proteins implicated in endosomal fusion including Rab5 and Rac1 were relatively unchanged in Western blot analysis of brain extracts from wild-type and knockout mice [31]. Despite these similar levels of protein, the dynamics of

endosomal transport and fusion appear to be compromised in cells derived from *ALS2* knockouts. *ALS2*$^{-/-}$ fibroblasts treated with epithelium growth factor (EGF) show a delay in EGF-receptor-mediated endocytosis, which is supported by a similar study on neurons with brain-derived growth factor [30,31]. In addition, a recent report reveals a significant accumulation of large insulin-like growth factor 1 (IGF1) receptor puncta in *ALS2*$^{-/-}$ neurons after IGF1 treatment, which induces a Rab5-dependent internalization of IGF1 receptors [31]. Furthermore, brain cytosol derived from *ALS2*$^{-/-}$ mice, which are added to labeled endosomes, show far less fusion activity than cytosol from wild-type mice [31]. Thus, so far, the experiments on cultures derived from the *ALS2* knockout mice support previous in vitro data regarding an endocytic role for alsin. In contrast, *ALS2*-deficient neurons fail to display alterations in neuritic outgrowth despite the observations of endogenous alsin in growth cones, which argues against a role for alsin in neurogenesis.

Conclusions

Although mutations in *ALS2* lead to clinical variations of motor dysfunction in humans, mice lacking in alsin surprisingly show only subtle deficits. Perhaps, conditional mouse models that allow for temporal control of alsin loss will allow us to avoid the pitfalls of potential compensatory mechanisms in the mouse genome. Currently endocytic trafficking and axonal outgrowth has emerged as potential functions of alsin, and there appears to be a dynamic relationship between the different protein domains. There is some evidence that *ALS2* and *SOD1* share some functional characteristics that may explain why mutations in either gene have such detrimental effects in humans. Both *ALS2* and *SOD1* mouse knockouts are susceptible to toxicity from mitochondrial complex I inhibitors, such as paraquat, as a result of the likely proliferation of free radical oxygen species [29,41]. It also appears that in cell lines, SOD1 toxicity from overexpression of disease causing mutants can be suppressed by alsin overexpression and that this protection is likely caused by RhoGEF properties of the DH/PH domains [42,43]. Deficiency in the *ALS2* gene, however, does not affect the progression of motor neuron degeneration of SOD1-G93A transgenic mice [44].

While the precise etiology of motor neuron disease remains elusive, it appears that perturbations in molecular and vesicular trafficking have emerged as a major pathogenic mechanism in motor neuron disease. Aberrant axonal transport in motor neurons of *SOD1* transgenic mice has been reported recently [45], and *ALS2* knockout neurons exhibit abnormal Rab5-dependent endocytosis [30,31]. In the last few years, several genes associated with proteins integral to motor proteins, and the secretory pathway have been shown to have mutations leading to various types of motor dysfunction. Mutations in vesicle-associated membrane protein/synaptobrevin-associated membrane protein B (VAPB), which is involved in ER-Golgi transport and secretion, causes a dominant form of familial ALS, ALS8 [46]. A missense mutation in VPS54, a component of the Golgi-associated retrograde protein complex, is believed to contribute to the motor neuron disease phenotype observed in the Wobbler mouse [47]. In addition, mutations in components of motor protein complexes such as dynein, dynactin p150^{glued}, and the kinesin family protein KIF5A are also linked to motor neuron disease [48-51].

Recently, we provided a direct link between alsin and GluR2 synaptic trafficking in motor neurons [27]. We found that alsin interacts with GRIP1, a key regulator of synaptic targeting of GluR2, making neurons more susceptible to glutamate-mediated excitotoxicity, a mechanism implicated in the onset of sporadic ALS. Further identification of disease causing mutations may help clarify the molecular mechanisms of ALS and provide a better understanding of how the sporadic and familial forms of the disease are linked.

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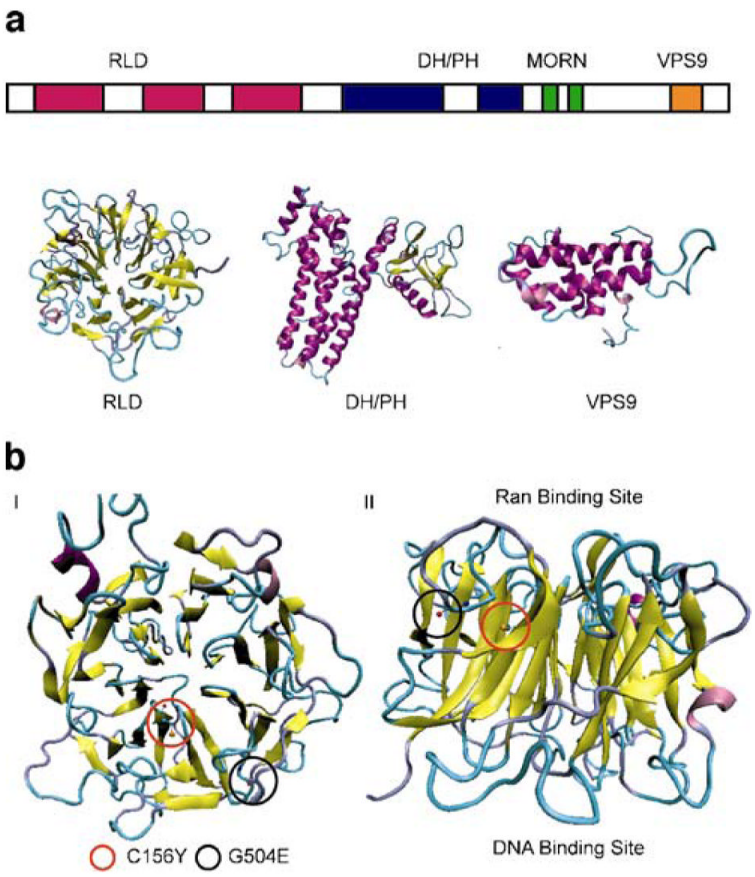

Fig. 1.

Domain structures of alsin. **a** A schematic presentation of linear structure of full-length alsin and 3D structure models of predicated alsin functional domains. **b** 3D models of alsin RLD domain marked with two missense mutations, C156Y and G540E (*I* a top view, and *II* a side view). The mutation C156Y is located on a beta-strand in one of blade repeats close to the Ran-binding site. C156Y may break the blade and beta-propeller structure due to the bigger size of Tyr side-chain compared with the side-chain of Cys. That may cause the instability of mutant protein and may weaken or lose the binding between RCC1 and Ran. In addition to the side-chain collision, change of the non-polar Cys to polar Tyr may also cause the some polar changes in the hydrophobic core. The mutation G540E is located at the molecular surface. Glu is an acidic polar amino acid with big side-chain. Gly is a hydrophobic non-polar amino acid without side-chain. This change may affect the water network around the molecular surface. This mutation is not on the two binding sites, so it may not affect the interaction of alsin RLD with other molecules

Table 1

Genetic mutations in the *ALS2* gene

Base mutation	AA mutation	Exon	Type	Clinical	Features	References
3742_delA	M1207	22	STOP/NS	IAHSP	U	[11]
1471_delGTTTCCCCCA	V491	6	FS	IAHSP	U	[11]
2660_delAT	N846	13	FS	IAHSP	U	[11]
1130_delAT	I336	4	FS	IAHSP	U	[11]
G1619A	G540E	6	MS	JPLS	U	[7]
261_delA	A46	3	FS	JALS	U/L	[5]
1548_delAG	T475	5	FS	JPLS	U	[5]
1867_delCT	L623	9	FS	JPLS	U	[6]
G669A	C156Y	4	MS	IAHSP	U	[8]
C3115T	R998	18	STOP/NS	IAHSP	U	[52]
4844_delT	V1574	32	FS	IAHSP	U	[53]
138_delA	A46	3	FS	JALS	U/L	[6]
553_delA	T185	4	FS	JALS	U/L	[10]

FS Frameshift, *MS* missense, *NS* nonsense, *U* upper motor neuron, *L* lower motor neuron