RNA interference based Screen to Identify Genetic Modifiers of dVAPB

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Abstract

Amyotrophic Lateral Sclerosis (ALS) is a progressive neuro-degenerative disease affecting motor neurons**.** VAPB- VAMP (vesicle associated membrane protein-associated protein) – associated protein B has been identified as gene involved in type 8 ALS. However, the exact mechanism by which mutation in VAPB causes neuro-degeneration is unknown. Here we present an RNAi based genetic screen to dissect out an integrated genetic network of VAPB function modifiers. We have screened 10% of *Drosophila melanogaster* genome and have identified 78 modifiers (27 enhancer and 51 suppressors). Modifiers detected in our screen include known ALS causing genes such as SOD1, Alsin2 and TDP-43. Modifiers also include genes that have been implicated in other neurodegenerative diseases such as Parkinson's disease and Huntington's disease. We have also identified proteins such as SNAMA, with a known role in apoptosis, and Derlin-1, a component of ER associated degrading machinery as VAPB modifiers.

Introduction

Amyotrophic lateral sclerosis disease (ALS), first discovered by a French neurologist Jean-Martin Charot in 1869, is a progressive motor neurodegenerative orphan disease, with median onset age of 55 years(1). A typical pathological hallmark of this disease is the dysfunction of specific upper and lower motor neurons leading to spasticity, hyper-reflexia of upper motor neurons, generalized weakness, muscle atrophy, and paralysis of the lower motor neurons. The symptom worsens with time and leads to the death of the person with respiratory function failure within a period of 5 year from onset of symptoms (2). A Majority (90%) of the ALS cases are of sporadic type and <10% are of familial type with a known genetic cause. But given the pathological similarity between both the cases, the most of the understanding of the disease is got from the genetic analysis of mutant proteins.

Till date thirteen genetic loci for ALS have been identified till date with SOD1 (ALS1) being the best studied (**Table1**) (3). Mutations in superoxide dismutase (SOD1) enzyme, which converts the reactive superoxide, a byproduct of glycolysis, to hydrogen peroxide, has been widely studied and characterized in the pathology of ALS (4). Experiments suggest that gain of toxicity because of the mutation is responsible for neuronal apoptosis. Misfolded mutant SOD1 forms ubiquitinated aggregates in the cell, which are directed towards proteosomal degradation, which in turn leads to the overloading of proteosome and ultimate failure of its function. Malfunction of proteosome then inhibits the degradation of many other proteins and causes motor neuron toxicity (5).

Recently a new locus for ALS (ALS8) at (20q13.3) was identified in a large Brazilian family (6). ALS8 is an autosomal dominant slow progressive disorder caused by mis-sense mutation in human Vesicle Associated Membrane Protein (VAMP)-Associated Protein (VAP). In the affected family, a conserved proline residue at position 56 was substituted with a serine (P56S). Residue 56 is a highly conserved residue in N terminal MSP domain of this protein (**Figure 1A**) (6). VAP was first identified in the *Aplysia californica* as VAP33, a protein of mass 33kDa that interacts with VAMP and was shown to be important for release of neurotransmitter (7). The VAP family protein consists of VAPA, VAPB & VAPC. VAPC is a splice variant of VAPB (8-

10). VAPB is Type 2 integral endoplasmic reticulum membrane protein found in all eukaryotic organisms (**Figure 1B**), ranging from yeast to humans. In humans VAPB has 63% identity at amino acid level with its paralogous protein VAPA. All VAP family proteins have a N-terminal MSP domain that shares a high (22%) sequence homology with nematode Major Sperm Protein (MSP)(11) and also contains a 16 amino acid consensus sequence conserved across the whole animal kingdom(12-13). Human homologue of VAP also contains a coiled coil trans-membrane domain with putative dimerization motif GxxxG (14) and a variable central coiled coil domain that resembles the CCD domain found in VAMPs and other proteins (Figure1A) (9). VAP proteins are involved in a variety of cellular functions including lipid metabolism and transport, membrane trafficking, neurotransmitter exocytosis, Unfolded Protein Response (UPR), cytoskeleton stability, etc (13).

VAP function in regulating vesicle trafficking; SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) are proteins that are involved in vesicle tethering, docking and fusion. V- SNAREs include the VAMP protein and are predominantly found on the vesicle membrane arising from the donor compartment and t- SNARE, includingSNAP-25 and Syntaxin family proteins are predominantly found on the target membrane (15).When v & t SNAREs comes in close proximity they heteromerize and this facilitates the fusion of the vesicle with the target compartment (16-17). VAP binds to VAMP and can regulate vesicle trafficking. Both the N and C terminal of VAPB play role in interaction with VAMP and CCD, a variable domain, is involved in interaction with SNARE proteins (18).

VAP function in lipid metabolism: The Yeast homologue of VAPB, SCS2 functions in lipid metabolism. SCS2 regulates the transcription of INO1 enzyme, a key enzyme catalyzing the rate limiting step in Inositol synthesis, by interaction with a FFAT motif containing protein called OPi1 (19). INO1 and other lipid metabolism related enzyme's transcription is regulated by the Ino2p and Ino4p transcription factor activator complex, which binds to UASINO (inositol sensitive upstream activating sequence) (20).Opi1P protein negatively regulates the transcription of INO1 by binding to the Ino2p –Ino4p activator complex in nucleus(21).SCS2 at ER membrane binds to the OPi1P and favors INO1 transcription. This translocation of Opi1P plays a critical role in the transcription of INO1, which in turns regulated by the level of PA (phosphatidic acid) in the cytosol. High PA levels at ER stabilize the SCS2 and Opi1p interaction

and thus favor the inositol synthesis whereas low level of PA facilitates the opi1P transport in nucleus and prevents the inositol synthesis (22-23)**.**

VAP function in lipid trafficking: VAP has been shown to have specific roles in lipid trafficking from ER to Golgi. The MSP domain of VAP binds to FFAT motif containing lipid-binding protein CERT and regulates its efficient and selective ceramide-transferring function from ER to Golgi (24) where in it is processed into sphingomyelin synthesis.

Involvement of VAP in ER stress: As in the case of SOD-1 protein, mutant VAPB in **Drosophila** (25) and also in mammals forms ubiquitin positive insoluble aggregates (26). These aggregates have been shown to be resistant to proteolysis (27). VAPB also known as positive regulator of ER stress signaling pathway (IRE/XBP pathway) and P56S mutation abrogates VAPB mediated unfolded protein response (UPR) activation (28-29). Similarly to the above study, VAPB was found to interact with ATF6 (a UPR mediated transcription factor) in yeast two hybrid screen and P56S mutation causes a strong binding and inhibition of ATF-6 results in misregulation of UPR mediated motor neuron death(30).

Regulation of ER structure through VAP-Nir protein interaction: VAPB is known to regulate ER structure through its interaction with Nir family proteins. Nir family proteins interact with VAPB through their FFAT motif. VAP B causes a rearrangement of ER through its interaction with Nir2 and Nir3 protein. VAPB-Nir2 interaction induces the stacked ER membrane array and VAP-Nir3 interaction causes gross remodeling of ER structure and causes bundling of ER along the altered ER structure. VAP interaction with Nir1 does not cause any significant change in the structure of ER. There are suggestions that VAP at the ER act as a receptor for many FFAT containing proteins that are expressed in cell –cell specific manner or may even be expressed in a specific physiological conditions, thus regulating ER structure by changing the conformation of VAP dimer (31).

VAPB's role as ligand: In *Drosophila*, the VAPB's MSP domain act as a ligand for Eph receptor and activate the downstream signaling pathways (32). VAPB over-expression results in excess cleaved VAP MSP domain in the organism and this is responsible for structural abnormalities in the flight muscle. In contrast to this VAPB P58S over-expression does not show any such phenotype as the mutant protein lacks the ability to get cleaved and act as a ligand.

Similarly over-expression of VAP at the neuromuscular junction causes a decrease in number of boutons and an increase in bouton size. VAPB (P56S) mutant protein over-expression does not show any such phenotype (32). This finding suggests VAPB has another role as ligand and malfunction of this process is a putative pathomechanism for ALS.

Three possible mechanisms by which VAPB could be involved in motor neuron disease:

(1) Specific mutations in VAPB form aggregates that incorporate wild type VAP in a dominant negative manner. This causes ER stress and induces the up-regulation of UPR.
(2) VAPB mutants lose the ability to anchor the FFAT motif proteins that are involved in lipid metabolism and transport. Perturbation of FFAT function is a possible cause of motor neuron death.
(3) VAPB mutants abrogate function of MSP domains role as a ligand for Ephrin receptors and this affects downstream signaling pathways(32).

A number of VAPB physical interactors are known, but as of today the exact mechanism(s) that lead to neuronal cell death are unknown. It is important to dissect out a comprehensive VAPB genetic interaction map to understand the precise cellular function of VAPB in a holistic way. This interaction map, which integrates physical and genetic interactor data will assist in developing mechanisms the explain VAP mutations to motor neuron-degeneration. Recently a *Drosophila* model of ALS8 has been developed by Ratnaparkhi *et. al.* (2008); the authors showed that mutant VAPB protein recruits the wild type protein and affect its activity in a dominant negative mechanism (25).They have also found that mutant VAP interfere with BMP signaling at neuromuscular junction using as a ALS8 model.

We have used the *Drosophila* genetic model developed by Ratnaparkhi *et. al.* to do a Genome wide RNAi screen. Currently, we have screened 10% of *Drosophila* genome using RNAi lines from the National Institute of Genetics, Japan to identify genetic modifiers of VAPB function and have, till date, identified 78 modifiers. The starting line for our screen is a recombinant line where VAPB expression using *sca*-GAL 4 driver leads to the loss of approximately half the macrochaetae lat 25 °C and all Macrochaetae at 28 °C (**Figure 2**(A-B). We have used this VAP overexpression phenotype to identify enhancers (which enhance the loss of macrochaetae phenotype) and also suppressors (which suppress the loss of macrochaetae phenotype) of VAP

function. We reduce the transcript level of a known gene using RNAi in ScaGal4>VAPB over expression background and observed the effect of this knockdown on macrochaetae number.

Materials and methods

Drosophila lines were maintained on corn meal agar medium at 25 °C. RNAi lines for primary and secondary screens were obtained from the National Institute of Genetics (NIG) stock center, Japan. For further validation fly lines were obtained from Bloomington Stock Center, Indiana, and Vienna Drosophila Stock Centre (VDRC), Austria. *sca*-Gal4, UAS-VAPB lines have been described earlier (25).

Primary screening; Primary screening was carried out at 25 °C and 29 °C by Senthilkuamr D (33) at the National Institute of Genetics, Japan. Primary Screening involved screening for Enhancers at 25 °C and Suppressors at 29 °C, without correcting for the effect of the RNAi line on macrochaetae morphology. The starting line for the screen was *sca*-Gal4,-UAS-VAPB/ Cyo recombinant line. This line showed, on average, a decrease in macrochaetae to 5.5 ±1 from the usual wild type 10. *sca*-Gal4,-UAS-VAPB virgins were crossed with males from individual RNAi lines. After egg laying the flies was transferred to a new vial and the vials with embryos were incubated at 25 °C and also at 29 °C. F1 progeny were screened for qualitative change in macrochaetae from the initial 5-6 Macrochaetae (33).

Secondary screen: The secondary screen was carried out in-house (at IISER). The secondary screen was carried out on the positive 'hits', both enhancers and suppressors identified in the primary screen. The goals of the secondary screen were twofold. First, the secondary screen was quantitative, with macrochaetae numbers of 10 females from each cross being noted (supplementary Table, S1). This allowed rejection of many primary candidate genes based on statistical analysis. Specifically, any experiment that did not show macrochaetae that were significantly different from the starting 5.5 ±1, were rejected.

Second, phenotypes of RNAi lines themselves were taken into account. If *sca*-Gal4,-UAS-RNAi lines by themselves showed Macrochaetae phenotypes, they were considered for rejection, based in the severity of the phenotype and its relationship to the experimental (*sca*-Gal4,-UAS-VAP, UAS-RNAi) phenotype. In general, any RNAi line showing deviation of average macrochaetae phenotype (macrochaetae no >7) in experimental cross from master control average phenotype (macrochaetae no = 5.5) was qualified as suppressor of VAP. And Any RNAi line showing

deviation of average macrochaetae phenotype (macrochaetae no <4) in experimental cross from master control average phenotype (macrochaetae no = 5.5) was qualified as suppressor of VAP.

Secondary Screening Statistical methodology (figure 3)

Data analysis and network construction: We have used following bioinformatics software and online databases for analysis of the data from the secondary screen.

(1) **Cytoscape**. This open source bioinformatics software was used to visualize the VAPB interaction network (34-35).

(2) **STRING**. This database of known and predicted protein-protein interaction and Flybase was used to dissect out direct or indirect VAPB interaction with other proteins (36-37).

(3) **GOToolBox.** This program was used to investigate the gene ontology of modifiers identified in screen (38-39).

Results

Validation of the *sca*-Gal4; UAS-VAPB recombinant line: A genetic recombinant line over-expressing wild type form of VAPB using a *scabrous* GAL4 driver was generated. This line shows loss of macrochaetae, with an average of 5 to 6 macrochaetae per female animal as compared to wild type 10 macrochaetae. Before starting the screen, we validated functionality and utility of the line as follows:

Wild VAP over expression at 28 °C using *scabrous* Gal-4 driver (*sca-GAL4*; UAS-VAPB) gives an average of 0 macrochaetae. Knockdown of VAP in the same genetic background using VAP RNAi (***sca-GAL 4*; UAS-VAP-33xUAS-VAP-33 RNAi)** lines gives an average of 10 macrochaetae. This clearly depicts that, in our experiment, macrochaetae number depends on VAPB function and can be *reversed* by reduction of VAPB (Figure 2 (C-D)). Over-expression of VAP P56S mutant does not reduce number of macrochaetae at 25 °C (Figure not shown), again indicating that macrochaetae number is a readout of VAPB function.

Primary screen: We screened 2600 lines (1435 genes) for qualitative change in macrochaetae phenotype in individuals with genotype *sca*-GAL4, UAS-VAPB/+ x UAS –RNAi/+ in comparison to individuals with genotype *sca*-GAL4, UAS-VAPB/+, at two temperatures, 25 and 29 °C. At 29 °C suppressors were easy to pick as VAPB over-expression causes a reduction in macrochaetae number (~0 macrochaetae) and any RNAi lines which causes a rescue of macrochaetae from 0 to > 3 was identified as suppressor (**Figure4(A-C)**). Similarly enhancers was easily identified at 25 °C, because at 25 °C the average macrochaetae score is between 5 and 6 and any RNAi line that causes a reduction of macrochaetae number to < 4 were classified as enhancers (Figure4 (D-F)). We identified 435 genes as modifiers of VAP mediated loss of bristle phenotype. The Primary screen was carried out at the National Institute of Genetics, Japan. The positive hits in the primary screen were further validated by a Secondary Screen that was more quantitative in nature and had controls in place.

Secondary screen: A quantitative analysis of 395 lines, each representing a gene identified in the primary screen was done at 25 °C. The quantitative part involved the counting of Macrochaetae from ten F1 females for each cross. Macrochaetae numbers from *sca*-GAL 4, UAS-VAP/+ x UAS-RNAi/+ (experimental crosses), were compared with *sca*-GAL4; UAS-

VAP/+ (master control) by doing an independent t-test. A significant difference in phenotype with p value<=0.01 and average Macrochaetae number of >6.5 was considered as suppressor of VAPB. For experimental crosses with average phenotype <4 macrochaetae, we have done independent t-test between normalized data score of *sca*-GAL4, UAS-VAP/+ x UAS-RNAi/+ against normalized data from *sca*-GAL4/+ x RNAi/+. Lines showing a statistical significance of p value <=0.01 was considered as enhancer of VAPB. Using the above criteria, we identified 78 modifiers of VAPB. Among these 78 modifiers, 27 are enhancers (1.8% of total lines screened) and 51are suppressors (3.5% of total lines screened). Modifiers were ranked on the basis of statistical significance **Table (2-4).**

Gene ontology studies analysis of identified VAPB modifiers using GoToolBox and Flybase online databases shows diverse roles of modifiers, including roles in energy homeostasis, nuclear import/export, protein biogenesis, cytoskeleton trafficking, lipid biosynthesis, stress response, RNA processing, ion transport, chromatin modifier (**Figure 5).**

Modifiers also contains some novel interactor namely SNAMA (physical interactor of VAPB) which have known role in cell cycle regulation(41), Derlin-1 and ATF-6 which have known role in UPR signaling pathway(42), mTOR which have known role in protein translation initiation control (61), TDP-43 –aggregation of this protein is a prominent hallmark of ALS pathology. Some of the modifiers are ER resident protein and are physical interactor of VAPB (data not shown). Modifiers list also contains known ALS loci including Alsin-2, SOD1, TDP-43.

Discussion

In this study, based on a reverse genetics screen, we have identified VAPB modifiers (enhancer/ suppressor) that have roles in various biological processes energy homeostasis, nuclear import/export, protein biogenesis, cytoskeleton trafficking, lipid biosynthesis, stress response, RNA processing, ion transport, chromatin modifier. If valid, the modifiers identified are genetics interactors of VAPB. A recent study has shown that VAPB mRNA level decreases in a large cohort of sporadic ALS(SALS) patients (40).This along with earlier study showing a decrease of VAPB levels in G93A-SOD1 mouse ALS model suggest a fundamental role of VAPB in pathogenesis of ALS(26). We have also identified SOD1 as suppressor of VAPB figure 6(E) wherein depletion of SOD1 using TRIP RNAi line results in increase in number of macrochaetae. Interestingly overexpression of SOD1 along with VAP also behaves like VAPB suppressor or causes an increase in number of macrochaetae **figure 6(**D).

We have also identified known ALS genes (ALS: Alsin2; ALS10: TDP-43) (Table1) other than ALS1: SOD1 in our screen as VAPB modifier which suggest a common mechanism of ALS pathology. This also suggests a possible network operating between these proteins. Moreover SOD1 ,TDP-43 and VAPB P56Smutant proteins follows similar mechanism wherein mutant protein undergo conformational changes causing misfolded aggregate at ER lumen eliciting ER stress linked specifically to neuronal cell death. Modifiers also include genes, which are known as neuro-degeneration modifiers in diseases such as ALS, Parkinson's disease and Huntington's disease (Table 5). We have also identified SNAMA (Something that sticks like glue) with known role in apoptosis (41), and Derlin-1, a component of ER associated degrading machinery as VAPB modifier (42) .

TDP43 - which is a TAR DNA binding protein has been identified as a major component in ubiquitin positive inclusion in neuron in ALS and FTD as well several other neurodegenerative disorder (43).TDP-43 is highly conserved, non-redundant nuclear protein in mammalian species and essential gene as its deletion leads to the early embryonic lethality (43-45). It interacts with many other proteins in the cell and forms protein complex involved in important cellular functions (46-48). Experimental evidence shows that its expression is tightly regulated, as heterozygote mutant also shows normal mRNA and protein levels (44-46). Moreover either lack of or over abundance of TDP-43 could lead to the dysfunctional multi- protein complex

formation ensuing abnormality or a possible cause of neuron-degeneration. In our screen down regulation of TDP-43 using RNAi causes rescue of macrochaetae caused by VAPB over-expression figure 6(B). This suggests that TDP-43 might be functioning upstream in the pathway. Recent studies using RNA sequencing, performed between wild-type and mutant form of TDP-43 shows no change in level of VAPB mRNA bound by these proteins. This tells that the interaction between VAPB and TDP-43 is not at the level of transcription (49). TDP-43 generally resides in nucleus (50)and has been reported to mislocalise and form cytoplasmic aggregates in many sporadic ALS cases. Additionally VAPB P56S but not VAPB wild-type mice model develop cytoplasmic ubiquitin positive TDP-43 aggregates suggesting a link between abnormal VAPB P56S function and TDP-43 mislocalization(51). Similarly, studies in SOD1 familial cases and in mutant SOD1 transgeneic mouse also results in TDP-43 mislocalization (52-53).

Another protein identified is Ataxin -2, a reported modulator of TDP-43 mediated toxicity and ultimately motor-neuron-degeneration. The expansion of PolyQ tract of ataxin-2 from its normal 22-23 length is implicated to have its role in much neurodegenerative disease. Recent study has shown that intermediate expansion of polyQ-tract length as a risk factor in ALS disease (54). The authors have also shown that ataxin-2 and TDP-43 binds interacts in an RNA dependent manner (54). Our experimental evidence of VAP and TDP-43 genetic interaction indirectly/directly suggests a possible role of VAP in TDP-43 aggregation mediated neuron death. It will be interesting to confirm the physical interaction of VAP and TDP-43 or their affect on each other in aggregate formation, which will lead us get an insight into ALS disease pathology.

ER stress and VAP: Mutation in SOD1 causes it to remain in unfolded state and causes the up regulation of ER associated chaperone (BIP, PDI) (55). BIP chaperone then binds to the unfolded SOD1 proteins, ensuing an increase in free and active stress sensors (ATF6, IRE1, and PERK) which triggers the downstream signal transduction pathway of UPR(56-57), ultimately leading to motor neuron death . In the similar way VAP P56S mutant misfolds and forms aggregate(26) and causes ER stress, thus recruits the ER chaperone, which otherwise binds to the stress sensor proteins and keep them in inactive form, resulting in free (IRE1, ATF6,and PERK) which activates the UPR transduction pathway and causes neuronal death(28-30). VAPB in yeast is known to interact with ATF6 and act as a positive regulator of UPR and VAPB P56S mutation

and abrogates ATF6 mediated UPR activation(30). We have also identified ATF6 as suppressor of VAP in our screen Figure 6, where it rescues the macrochaetae loss caused by VAP overexpression.

We have found Derlin-1 as a suppressor in the screen (Figure6). Derlin-1 is one of the components required for retro-translocation of misfolded protein from ER lumen to cytoplasm (58-59). The misfolded protein is first recognized by the ER-resident chaperon and receptors and then targeted for retro translocon, and then Derlin-1 recruits a membrane complex containing VIMP, the p97 complex, and E3 ligase. Once the substrate emerges in to cytoplasm it is captured by p97 and then polyubiquitinated by E3, subsequently directed for proteosomal degradation (60). In presence of mutant SOD-1 protein but not wild type protein binds with Derlin-1 protein and impairs the ERDA (ER-associated degradation) and activates the alternative apoptotic pathway. mSOD1: Derlin-1 interaction is very important for the mSOD1 induced ER stress activation of ASK1 apoptotic pathway (42). Moreover perturbation of binding between mSOD1 and Derlin-1 suppress the SOD1 induced ER stress and, ASK1 Activation, and motor neuron-degeneration. mSOD1 causes an accumulation of misfolded protein at ER lumen and activates ER-stress signaling pathway, by activating IRE1 and PERK pathway (42). IER1 further recruits TRAF2 and ASK1 (apoptotic signal-regulating kninase1) on the ER membrane and thus activate the ASK1Dependent apoptotic pathway (42).

In contrary to the perturbation of mSOD1-Derlin protein-protein interaction favoring ERDA restoration our experiments suggest that functional ERDA is critical for ER homeostasis. It is also possible that VAPB: Derlin-1 interaction at ER is important for ER stress mediated apoptosis.

VAP in cell cycle and apoptosis regulation: We have found SNAMA, a physical interactor of VAPB as a suppressor in screen (Figure6C). SNAMA contains a DWNN domain with a ubiquitin-like fold, a zinc finger, a RING finger like domain, a probable P53 binding region, and glutamic acid-rich and lysine –rich regions, and has been found role in RNA processing and apoptosis (41). It interacts with Dmp53 and RBF, which are Drosophila homologue of P53 and RB, as a negative regulator of apoptosis (41). SNAMA function like mammalian Mdm2 in regulating DmP53 level in cell and thus regulate cell cycle. SNAMA as a modifier of VAPB function gives rise to a possible role of VAPB in cell cycle and apoptosis regulation. And also

provides a possibility of VAPB mutation (VAP P58S) disrupting VAPB-SNAMA protein – protein interaction; leading to neuro-degeneration.

mTOR interaction role in translation control : mTOR (mammalian Target Of Rapamycin) forms a multiprotein complex(mTORC1 and mTORC2) and couples receptors to the translation machinery via these multiprotein complex(mTORCs) - known to have role in translation control ,long-lasting memory(61). mTOR in our screen, acts as a enhancer of VAP function (Figure 6G), overexpressed TED domain of mTOR results in loss of macrochaetae, suggesting a possible mechanism wherein TED domain sequester other component proteins and form of non functional protein complex. Also dysregulated function of mTOR signaling is known to cause numerous neurological disorders, such as Alzheimer's disease(62-64), Austin spectrum disorder (65) and Huntington's disease(66-67). VAP P56S mutation may causes abnormal VAP P56S-mTOR interaction causing a dysregulated translation and thus ER stress linked neuro-degeneration.all these are possible implication which further need to be established experimentally.

In summary, a reverse genetic screen, encompassing 10% of the *Drosophila* genome, has been utilized to discover genetic modifiers of *Drosophila* VAPB. An analysis of this data set both at the level of individual genes, and at the level of genetic networks has given us insight into VAPB function. We have integrated the modifiers identified with know VAPB physical interactors in *Drosophila* and also other animals to get a overall view of genetic networks and pathways interacting with VAPB. We expect that this information will give us insight not only into VAP function, but will also help us understand mechanisms of neuronal cell death and also factors contributing to neurodegenerative diseases such as ALS.

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