Identification of RNA bound to the TDP-43 ribonucleoprotein complex in the adult mouse brain

Running Title: Identification of RNA bound to TDP-43

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Objectives. Cytoplasmic inclusions containing TDP-43 are a pathological hallmark of several neurodegenerative disorders, including amyotrophic lateral sclerosis (ALS) and frontotemporal dementia. TDP-43 is an RNA binding protein involved in gene regulation through control of RNA transcription, splicing and transport. However, the function of TDP-43 in the nervous system is largely unknown and its role in the pathogenesis of ALS is unclear. The aim of this study was to identify genes in the central nervous system that are regulated by TDP-43. **Methods**. RNA-immunoprecipitation with anti-TDP-43 antibody, followed by microarray analysis (RIP-chip), was used to isolate and identify RNA bound to TDP-43 protein from mouse brain. **Results**. This analysis produced a list of 1,839 potential TDP-43 gene targets, many of which overlap with previous studies and whose functions include RNA processing and synaptic function. Immunohistochemistry demonstrated that the TDP-43 protein could be found at the presynaptic membrane of axon terminals in the neuromuscular junction in mice. **Conclusions**. The finding that TDP-43 binds to RNA that codes for genes related to synaptic function, together with the localisation of TDP-43 protein at axon terminals, suggest a role for TDP-43 in the transport of synaptic mRNAs into distal processes.

Key words: TDP-43, amyotrophic lateral sclerosis, RNA processing

Introduction

Amyotrophic lateral sclerosis (ALS) is characterised by ubiquitinated cytoplasmic, nuclear, and neuritic inclusions in the motor neurons of the brain and spinal cord. The discovery that TDP-43 is a major component of these inclusions (1, 2) was a key breakthrough in the understanding of ALS as it suggests that TDP-43 functions in important neuronal activities, the impairment of which would lead to degeneration of specific neuronal populations. TDP-43 is a heterogeneous nuclear ribonucleoprotein (hnRNP) that is encoded by the *TARDBP* gene (3). The discovery of mutations within *TARDBP* in familial and sporadic ALS patients (4, 5) has established a clear pathogenic link between TDP-43 and ALS. Approximately 40 different *TARDBP* missense mutations have now been reported in ALS, including three cases of ALS plus concomitant frontotemporal lobar degeneration (6). However, although TDP-43 has been implicated in a diverse range of metabolic and physiological processes, including RNA transport and splicing, mutations have provided no insight into why motor neurons die in ALS patients.

TDP-43 binds to UG-rich repeats in target RNAs, facilitating mRNA splicing and transport. The ability of TDP-43 to bind RNA, the presence of a nuclear localisation signal, and a strong nuclear export sequence indicate that TDP-43 has a role in nuclear-cytoplasmic shuttling of mRNAs (7). The observation of TDP-43 within RNA granules in developing brain further suggests that TDP-43 may be involved in mRNA transport (8). As DNA is confined to the nucleus, appropriate subcellular trafficking of mRNA plays an important role in determining the biological activities of the transcripts, particularly in neurons where the distance between the nucleus and subcellular compartments can be significant. In addition to its role in RNA transport, TDP-43 also regulates splicing by binding to UG-rich sequences within pre-mRNA (9). Tissue-

specific alternative mRNA splicing is essential for the expression of functionally different gene products from a single gene, and various human diseases are known to be caused by disruption of the splicing machinery (10).

There is increasing evidence that ALS is a disorder of RNA metabolism. RNA oxidation precedes the development of overt motor neuron damage in transgenic mice expressing ALS-associated mutations in superoxide dismutase -1 (SOD1) (11). Mutations in FUS/TLS, an RNA binding protein with similar structure to TDP-43, have been associated with both frontotemporal dementia (FTD) and ALS (6, 12). Similarly, mutations in SETX, a DNA/RNA helicase involved in RNA transcription and translation, have been linked with cerebellar ataxia, progressive motor neuropathy and more recently with ALS (6, 13). Another well-established example of altered RNA processing in neurodegeneration is loss of the SMN (survival of motor neuron) RNA binding protein in spinal muscular atrophy, a form of motor neuron disease with earlier onset than ALS (14). Other ALS-associated genes involved in RNA processing pathways include the ribonuclease protein, angiogenin (ANG) (15), and elongator protein 3 (ELP3), a component of the RNA polymerase II complex (16).

Even though most ALS patients do not have mutations in TDP-43 (but TDP-43 still forms aggregates) the loss of TDP-43 function and resulting disruption of RNA processing is likely to be a major contributor to the disease. This is supported by the fact that a transgenic mouse overexpressing mutant human TDP-43 does not develop cytoplasmic aggregates and yet motor neurons still degenerate, suggesting that aggregates are not the primary cause of degeneration (17). Furthermore, knockdown of TDP-43 in *Drosophila* (18) and zebrafish (19) leads to behavioural motor deficits and abnormal motor neuron morphology. We therefore hypothesise

that any dysfunction of TDP-43 (aggregation or mutation) will affect the RNA-binding ability of the protein and the subsequent regulation and processing of RNA transcripts needed for motor neuron function.

An important step in understanding how TDP-43 causes ALS is identifying the RNA transcripts that TDP-43 regulates, followed by the production of a comprehensive map of RNA targets in normal nerve tissue. Recent reports have begun to identify the RNA targets of TDP-43 in the rodent brain, using a combination of RNA-immunoprecipitation followed by either direct sequencing (20-23) or microarray analysis (24). In the first report, cultured embryonic rat cortical neurons were used and the 4,352 genes identified were enriched for Gene Ontology terms related to synaptic function, RNA metabolism and neuronal development (20). In the second study, over 6,300 potential TDP-43 RNA targets were identified in the adult mouse brain (21). The main functional categories enriched in this second study also included synaptic function, as well as ion channel activity and transmembrane transporter activity. The study by Tollervey et al. (22) included a comparison between healthy and FTD post-mortem brain samples, which identified 59 transcripts with altered TDP-43 binding. In the final two studies, human neuroblastoma cell lines (23) and mouse motoneuronal cell lines (24) were analysed, with both studies identifying less than 200 RNA targets of TDP-43. In the current study, genome-wide analysis was performed to identify RNA bound to TDP-43 protein from total mouse brain tissue. Localisation of TDP-43 within axon terminals of the mouse neuromuscular junction was also examined.

Material and Methods

Animals and tissue preparation

Four 10-week-old C57BL/6J mice were euthanased and brain tissue harvested. Mouse brain

tissue was homogenised in polysome lysis buffer supplemented with RNase and protease

inhibitors as previously described (25). Following centrifugation at 15,000 x g for 15 minutes at

4˚C to pellet large particles, the clear supernatant was used in subsequent IP experiments. Three

samples were used in the microarray analysis and the fourth was used for TDP-43 target

validation by RT-PCR. The University of Queensland Animal Ethics Committee approved all

experiments.

RNA immunoprecipitation (RIP) and western blotting

250 µg of protein lysate from each mouse brain was used for IP with 5µg of either rabbit anti-

TDP-43 antibody (Abcam, Waterloo, Australia) or normal rabbit IgG (Sigma Aldrich, Sydney,

Australia). The antibodies were incubated with the lysate overnight at 4˚C, after which 50 µL of

protein G Dynabeads (Invitrogen, Mulgrave, Australia) were added and the solution incubated

for 1 hour at 4°C with rotation. Following several washes with washing buffer (Invitrogen) 20

µL of the protein-bead complex was eluted using 10 µL of elution buffer (Invitrogen) and

separated on a 10% NuPage Bis-Tris gel (Invitrogen). RNA was isolated from the remaining 30

µL of protein-bead complex using TRIzol reagent (Invitrogen) followed by DNase I treatment

(Ambion, Mulgrave, Australia).

Microarray analysis

The TDP-43 and IgG immunoprecipitated RNA from three mice was converted to cDNA,

fragmented, then biotin labelled using the WT cDNA Synthesis & Amplification Kit and

Terminal Labeling Kit (Affymetrix, Santa Clara, CA) for Affymetrix GeneChip Mouse Gene 1.0

ST arrays and the 3'-IVT Expression Analysis Kit (Affymetrix) for GeneChip Mouse 430 arrays.

Labelled RNA was hybridised to arrays overnight, washed then scanned using the Affymetrix GeneChip Scanner. Successful hybridisation to the microarray was determined using Expression Console software (Affymetrix) and the data (.CEL files) transferred to Partek Genomics Suite for statistical analysis. TDP-43 and IgG immunoprecipitated RNA samples were each hybridised to 3 GeneChip Mouse Gene 1.0 ST and 3 GeneChip Mouse 430 (n = 6 for each group). Raw data files have been submitted to ArrayExpress (accession: E-MEXP-3500).

Bioinformatic analysis

Data analysis was performed using Partek Genomics Suite (Partek Inc. USA) in strict adherence to the MIAME (Minimum Information on Microarray Experiment) standards. Steps performed in Partek included 1) robust microarray averaging (RMA) to normalise the data, 2) principal component analysis (PCA) to check for sample outliers, 3) analysis of variance (ANOVA) to generate p values and finally, 3) generation of gene lists using the conditions $p < 0.05$ and fold change > 2. The gene lists generated by Partek were further analysed using Ingenuity Pathway Analysis (IPA) software (Ingenuity Systems, Redwood City, CA). IPA was used to assess the signalling and metabolic pathways, molecular networks, and biological processes that were most significantly represented in the microarray dataset. The MEME suite was used to identify common sequence motifs shared among the binding partners (26). Gene Ontology (GO) enrichment analysis of the significant transcripts was carried out using FatiGO (27).

TDP-43 target validation using RT (reverse transcriptase) PCR

TDP-43 and IgG immunoprecipitated RNA (100 ng) was reverse transcribed using the SuperScript III kit (Invitrogen) to produce cDNA. Primers used for amplification are listed in Supplemental file 1. PCR conditions for the experiment were: denaturation at 94˚C for 1 min; 10

cycles of denaturation at 94˚C for 30 s, annealing at 60˚C for 30 s, extension at 72 ˚C for 30 s; 25

cycles of denaturation at 94˚C for 30 s, annealing at 55˚C for 30 s and extension at 72˚C for 30 s;

final extension at 72˚C for 2 min. PCR products were visualised on 2% agarose gel with SYBR

safe gel stain (Invitrogen).

Immunohistochemistry

Extensor digitorum muscles were dissected from postnatal day 18 mice and fixed by immersion

in 4% paraformaldehyde in phosphate buffer for 20 min at room temperature then washed 3

times for 10 min in phosphate buffered saline pH 7.4 (PBS) containing 0.1 M glycine. Muscles

were then incubated in Alexa-488 α-bungarotoxin (α-BTX; Sigma, St. Louis, MO), diluted at

1:500 in 2% bovine serum album (BSA) with 0.2% Triton X-100 in PBS, for 30 min at room

temperature, followed by 4 by 10 min washes in PBS. Muscles were then post-fixed in -20℃

methanol for 5 minutes and then washed twice in PBS. Next, the muscles were incubated

with rabbit anti-TDP-43 antibody (Abcam) diluted 1:100 in 2% BSA with 0.2% Triton X-100 in

PBS overnight at 4℃. They were then washed in PBS, followed by overnight incubation in

Alexa-568 goat anti-rabbit secondary antibody (Invitrogen) diluted 1:500 in 2% BSA and 0.2%

Triton X-100 in PBS at 4C. Next day the muscles were washed in PBS and mounted in anti-fade

(Flurogold[TM], Hercules, BioRad, CA). Muscles were viewed and imaged using a Zeiss

AxioImager Z2 fluorescence microscope.

Results

TDP-43/RNA complexes were immunoprecipitated from whole mouse brain lysates using a

rabbit anti-TDP-43 antibody. TDP-43 immunoprecipitation (IP) was validated by western blot

that showed TDP-43 was specifically pulled down from mouse brain lysates using the anti-TDP-

43 antibody but not with IgG (Supplemental file 2). Purification of RNA from the isolated TDP-43/RNA complex recovered a total of 250-350 ng of RNA from each RNA IP (RIP) experiment. The RIP-chip analysis of mouse brain tissue produced a list of 1,839 potential TDP-43 RNA targets with significant p-values (<0.05) and fold change (>2) (Supplemental file 3). The data from the present study was then compared to previous publications (where gene lists were available) to determine the extent of overlap between the TDP-43 target genes identified (Fig 1). The genes identified in the present study represent 17.2% of the Sephton et al. study, 17.9% of the Colombrita et al. study and 11.8% of the Xiao et al. study. The *Arf3* gene was the only sequence common to all four studies.

The MEME Suite program (26) was then used to identify common sequence motifs from the 150 most highly enriched (based on fold change) TDP-43 target RNAs. The most common motif identified from the TDP-43 RIP-chip was (TG)n, corresponding to the known (UG)n RNA binding motif of TDP-43 (Fig 2A). As (TG)n is a common repeat, we also analysed RNA from the IgG control RIP-chip experiment (background "noise" from non-specific binding). The (TG)n repeat motif was not enriched among the RNAs recovered from the IgG control RIP-chip experiment; only an (A)n motif was identified, which corresponds to the mRNA polyA site (Fig 2B). The frequency of the (TG)n motif within cDNA sequences was 63%. Of those genes containing a (TG)n motif, the location of the motif was predominantly in the 3'UTR (92%). The second most common motif identified was a CG-rich sequence (Fig 2C), which was primarily found in the 5'UTR (79%) and may simply reflect the high GC content of the promoter region. The motif analysis confirms the RIP-chip method is detecting true RNA targets of TDP-43, as the known binding motif (UG)n was the most common sequence detected.

Ingenuity Pathway Analysis of the TDP-43 RNA targets was used to identify biological functions that are potentially regulated by TDP-43. Multiple genes were related to the expected biological functions associated with ALS, including nervous system development and function and neurological disease (Table 1). Molecular and cellular functions included RNA post-transcriptional modification and gene expression. Analysis of Gene Ontology (GO) terms enriched among TDP-43 RNA binding partners revealed the top terms in each of the three GO term categories of cellular component, molecular function and biological process were synaptosome (P = 1.18 x 10^{-9}), RNA binding (P = 6.27 x 10^{-21}), and modification-dependent protein catabolic process (P = 4.46 x 10^{-13}), respectively (see Supplemental File 4 for complete list). The enrichment of GO terms related to the synaptosome, suggested that TDP-43 may be involved in transport of mRNA and regulation of translation within presynaptic compartments. If TDP-43 were involved in transport of mRNA to the synapse it would be expected that TDP-43 protein would be appropriately localised there. In order to investigate this, localisation of the TDP-43 protein at the mouse neuromuscular junction was examined by immunohistochemistry. Analysis of the immunolabelling revealed that TDP-43 was concentrated presynaptically within the motor nerve terminal, directly opposite postsynaptic acetylcholine receptors (Fig 3).

The TDP-43 target RNAs related to synaptic function identified in this study are shown in Table 2, where they have been grouped into seven broad functional categories and overlap with the Sephton et al. (20) study has been highlighted. Thirteen genes from Table 2 were randomly selected for validation in an independent RIP experiment using RT-PCR (Fig 4 and Supplemental file 1), including seven genes associated with synaptic vesicle trafficking (top row of Fig 3). The gene encoding TDP-43 (*Tardbp*) was also included, as there is evidence that TDP-

43 may be self-regulated. The RT-PCR results confirmed that these RNA targets were enriched in the TDP-43 IP RNA preparation.

Discussion

The RIP-chip analysis of mouse brain tissue provided a list of 1,839 putative RNA targets of TDP-43. The most common sequence detected in the 150 most abundant RNAs was the known TDP-43 binding motif (UG)n (Fig 2) confirming the RIP-chip method was detecting true RNA targets of TDP-43. Of the 1,839 genes identified in mouse brain in this study, 699 (38%) overlapped with those found in rat embryonic cortical neuron cultures (20). Comparison with other published reports of TDP-43 RNA targets revealed a single gene was common amongst the four studies (Fig 1). The *Arf3* gene encodes a small guanine nucleotide-binding protein that stimulates ADP-ribosyltransferase activity and which also plays a role in vesicular trafficking (28). The fact that only one gene was common to all studies is most likely due to the different cell/tissue types studied, as well as the fact that the two studies using neuronal cell lines (23, 24) only identified a relatively small number of TDP-43 targets. The *Arf3* gene contains a large $(TG)_{87}$ repeat in the 3'UTR and the corresponding mRNA has been isolated from cortical axons (29). Therefore, further investigation of the ARF3 gene and it's protein in ALS and FTD is warranted.

We hypothesise that RNPs such as TDP-43 may be playing a major role in transport of mRNAs along the axonal length for site-specific translation at the synapses, such as the neuromuscular synapse, and are crucial for fine-tuning of synaptic transmission. This proposed role for TDP-43 regulation of local translation is supported by immunohistochemistry demonstrating that TDP-43 is localised to the presynaptic neuromuscular junction in mouse muscle (Fig 3). In human motor

neurons, TDP-43 is found in the nucleus, rough endoplasmic reticulum, mitochondria, and of relevance to this study, in synaptic vesicles of presynaptic terminals of anterior horn neurons (30). A recent report has also demonstrated that TDP-43 is actively transported in motor neuron axons (31), lending further support to the hypothesis that TDP-43 is involved in transport of RNA for local translation at presynaptic terminals.

The network and gene ontology enrichment analyses conducted in this study, together with the localisation of TDP-43 at the presynaptic membranes (Fig 3), strongly suggest that TDP-43 may regulate the RNA of genes involved in synaptic transmission. Of particular interest were the number of genes related to synaptic vesicle docking, fusion and exocytosis (Table 2), such as syntaxins (*Stx1b*, *Stx12*), syntaxin binding proteins (*Stxbp5*, *Stxbp6*), synapsin (*Syn2*) and synaptophysins (*Syp*, *Sypl1*). Previous reports have shown that synaptophysin (32-37) and synapsin (34) both decrease in the anterior horn region of the spinal cord in ALS patients. Syntaxin is also decreased in the anterior horn, though to a lesser extent than synaptophysin and synapsin (34). The decrease in expression of synaptic vesicle proteins in the spinal cord of ALS patients may be due to loss of TDP-43-regulated transport of the RNA coding for these proteins. TDP-43 has previously been shown to be localised to RNA granules in dendrites of rat hippocampal neurons, and these granules also contain mRNA of known TDP-43 targets (38). Axons also contain mRNAs and ribosomes, and are metabolically active in synthesising proteins locally (39). Therefore, loss of TDP-43 mediated transport of synaptic vesicle mRNAs could disrupt both dendritic synapses and the neuromuscular junction.

Another well-established example of altered RNA processing in motor neuron disease is loss of SMN protein in spinal muscular atrophy (SMA) (40). SMN plays a multifunctional role in

ribonucleoprotein metabolism, pre-messenger RNA splicing and RNA transport. In SMA, expression of presynaptic vesicle proteins such as synaptophysin, VAMP and synaptotagmin are decreased (41) and mouse models of SMA show evidence of impaired synaptic vesicle release at neuromuscular junctions (42). Similarly, impaired synaptic vesicle release, due to loss of TDP-43-mediated transport of synaptic vesicle RNAs, may also result in muscle denervation and loss of motor neuron function in ALS.

Mutations in ubiquitously expressed proteins like TDP-43 and SMN lead to degeneration of motor neurons in the CNS but not other tissues in which these proteins are expressed. Motor neurons may be particularly sensitive to disruptions in mRNA transport, due to their long axon length. Recent work from *C. elegans* suggests that local presynaptic translation takes place in the mature nervous system, and that regulating this capacity is required for the maintenance of synapse and axon morphology (43). In human motor neurons, synaptic terminals can be more than a metre removed from the nucleus. Decreased capacity to transport mRNAs into distal processes could jeopardise the ability to respond to injury or local stimuli within axons. In ALS, loss of synapse proteins may be what ultimately leads to motor neuron death.

In this study we have shown that TDP-43 is localised at the presynaptic membrane of axon terminals of the mouse neuromuscular junction, and that over 100 RNA binding targets of TDP-43 are associated with synaptic function. Decreased capacity to transport synaptic mRNAs into distal processes may be one possible mechanism that ultimately leads to motor neuron death in ALS. Future experiments will be necessary to demonstrate co-localisation of identified RNA targets with the TDP-43 protein, within presynaptic terminals of motor neurons.

Acknowledgements

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Figure Legends

Figure 1. Venn diagram illustrating the number of genes in common between the present study and previously reported TDP-43 RNA targets. Numbers in brackets indicate the total number of TDP-43 targets identified by Sephton et al. (20), Xiao et al. (23), Colombrita et al. (24) and the present study (Narayanan). ==The full list of TDP-43 binding targets identified by Polymenidou et al. (21) was not available for comparison.==

Figure 2. TDP-43 RNA targets are enriched for (UG)n repeats. The cDNA corresponding to RNA targets identified from TDP and control pull down were submitted to the MEME Suite. **A)** The most common motif identified from the TDP-43 RIP-chip was (TG)n, corresponding to the known (UG)n RNA binding motif of TDP-43. No (TG)n repeats were identified in genes from the IgG control RIP-chip experiment (**B**). An (A)n motif was identified, which corresponds to the mRNA polyA site. **C)** The second most common motif identified from the TDP-43 RIP-chip was a GC-rich sequence.

Figure 3. Presynaptic localisation of TDP-43 protein at the mouse neuromuscular junction. **A.** Schematic showing the nerve terminal and muscle with presynaptic TDP-43 and postsynaptic acetylcholine receptors (AChRs) as in image B. Immunostaining of the extensor digitorum longus neuromuscular junction in C57BL/6J mice showed the presence of TDP-43 (red) in the presynaptic terminal and opposing acetylcholine receptors on the postsynaptic muscle (green). Scale bar 5 μm.

Figure 4. Confirmation of TDP-43 RNA targets by reverse transcription (RT) PCR. RT-PCR was used to validate 14 TDP-43 targets using cDNA generated from an independent RIP

experiment. T = cDNA from TDP-43 RIP; C = cDNA from control IgG RIP. Genes in the top

panel are all associated with synaptic vesicles.

Table 1. Top biological functions associated with TDP-43 target RNAs, identified using

Ingenuity Pathway Analysis.

Function	p-value	# of genes
Disease and disorders		
Neurological disease	3.38×10^{-13}	492
Genetic disorder	1.31×10^{-12}	767
Skeletal and muscular disorders	1.31×10^{-12}	356
Molecular and cellular functions		
RNA post-transcriptional modification	3.74×10^{-21}	96
Gene expression	1.57×10^{-17}	355
Post-translational modification	2.74×10^{-14}	187
Physiological and developmental function		
Nervous system development and function	5.88×10^{-6}	162
Connective tissue development and function	1.91×10^{-5}	108
Tissue development	6.10×10^{-5}	128

Table 2. TDP-43 target genes associated with synaptic function. Symbols in bold text represent TDP-43 target genes also reported by Sephton et al. (20).

Gene Symbol	Gene Name	Gene Symbol	Gene Name
SYNAPTIC VESICLE ASSOCIATED PROTEINS		**ION CHANNELS & TRANSPORTERS**	
Cadm1	Cell adhesion molecule 1	**Atp1a2**	ATPase, Na+/K+ transporting, alpha 2 polypeptide
Cadps	Calcium-dependent secretion activator	Atp2b2	ATPase calcium-transporting plasma membrane 2
Dmxl2	Dmx-like 2	**Cacnb4**	Calcium channel voltage-dependent beta-4 subunit
Esyt2	Extended synaptotagmin-like protein 2	**Kcnd2**	Potassium voltage-gated channel Shal-related subfamily member 2
Napb	NSF attachment protein beta	Gabra1	Gamma-aminobutyric acid receptor subunit alpha-1
Snapin	SNAP-associated protein	Gabra2	Gamma-aminobutyric acid receptor subunit alpha-2
Snca	Alpha synuclein	Gabra5	Gamma-aminobutyric acid receptor subunit alpha-5
Stx12	Syntaxin 12	**Gabrb2**	Gamma-aminobutyric acid receptor subunit beta-2
Stx1b	Syntaxin 1B	**Gabrb3**	Gamma-aminobutyric acid receptor subunit beta-3
Stxbp5	Syntaxin-binding protein 5	Glra2	Glycine receptor subunit alpha-2
Stxbp6	Syntaxin-binding protein 6	**Glrb**	Glycine receptor subunit beta
Syap1	Synapse-associated protein 1	**Gria2**	Glutamate receptor 2
Sybu	Syntabulin (syntaxin-interacting)	**Gria3**	Glutamate receptor 3
Syn2	Synapsin-2	**Gria4**	Glutamate receptor 4
Syp	Synaptophysin	**STRUCTURAL PROTEINS**	
Sypl1	Synaptophysin-like protein 1	**Actr3**	Actin-related protein 3
Vamp3	Vesicle-associated membrane protein 3	**Ank3**	Ankyrin 3
SIGNAL TRANSDUCTION		Apc	Adenomatous polyposis coli
Anks1b	Ankyrin repeat and sterile alpha motif domain-containing protein 1B	**Bsn**	Bassoon
Bdnf	Brain-derived neurotrophic factor	Cald1	Caldesmon 1
Cav2	Caveolin-2	**Dlg1**	Disks large homolog 1
Cbln4	Cerebellin-4	Dmd	Dystrophin
Fbxo45	F-box/SPRY domain-containing protein 1	Gphn	Gephyrin
Gap43	Neuromodulin	**Kif3a**	Kinesin family member 3A
Homer1	Homer protein homolog 1	Lin7a	Lin-7 homolog A
Lphn2	Latrophilin-2	**Lin7c**	Lin-7 homolog C
Lrrtm2	Leucine-rich repeat transmembrane neuronal protein 2	**Myo5a**	Myosin Va
Pcdh8	Protocadherin-8	Nefl	Neurofilament light polypeptide
Pdzrn3	PDZ domain-containing RING finger protein 3	**Sept3**	Septin 3
Strn	Striatin, calmodulin binding protein	Sept7	Septin 7
Sumo1	SMT3 suppressor of mif two 3 homolog 1	**Sept11**	Septin 11
Wnt5a	Protein Wnt-5a	Syne1	Synaptic nuclear envelope 1
Ywhaz	14-3-3 protein zeta/delta	**GTPASE SIGNALLING**	
ENZYME ACTIVITY		Abi1	Abl interactor 1

Akap5	A-kinase anchor protein 5	*Chn2*	Chimerin 2
Camk2d	Calcium/calmodulin-dependent protein kinase type II delta chain	*Erc2*	ERC protein 2
Cask	Calcium/calmodulin-dependent serine protein kinase	*Gopc*	Golgi-associated PDZ and coiled-coil motif-containing protein
Epha4	Ephrin type-A receptor 4	*Gnai1*	Guanine nucleotide-binding protein G(i), alpha-1 subunit
Epha7	Ephrin type-A receptor 7	*Gnas*	Guanine nucleotide-binding protein alpha subunit
Gad2	Glutamate decarboxylase 2	*Kras*	GTPase Kras
Gls	Glutaminase	*Rab11a*	Ras-related protein Rab-11A
Gsk3b	Glycogen synthase kinase-3 beta	*Rab14*	Ras-related protein Rab-14
Hnmt	Histamine N-methyltransferase	*Rab5a*	Ras-related protein Rab-5A
Lnpep	Leucyl-cystinyl aminopeptidase	*Rasgrp2*	RAS guanyl-releasing protein 2
Mdm2	E3 ubiquitin-protein ligase Mdm2	*Sos1*	Son of sevenless homolog 1
Pik3r1	phosphoinositide-3-kinase, regulatory subunit 1	**RNA PROCESSING**	
Ppfia2	Protein tyrosine phosphatase, receptor type, f polypeptide interacting protein (liprin), alpha 2	*Cpeb1*	Cytoplasmic polyadenylation element-binding protein 1
Ppp3ca	Protein phosphatase 3 catalytic subunit alpha	*Cript*	Cysteine-rich PDZ-binding protein
Ptgs2	Prostaglandin G/H synthase 2	*Egr1*	Early growth response protein 1
Rps6kb1	Ribosomal protein S6 kinase beta-1	*Fos*	FBJ osteosarcoma oncogene
Sh3kbp1	SH3 domain-containing kinase-binding protein 1	*Mef2c*	Myocyte enhancer factor 2C
Ube2v2	Ubiquitin-conjugating enzyme E2 variant 2	*Neurod2*	Neurogenic differentiation 2
Usp14	Ubiquitin carboxyl-terminal hydrolase 14	*Pja2*	Praja ring finger 2
Usp46	Ubiquitin carboxyl-terminal hydrolase 46	*Syncrip*	Synaptotagmin binding, cytoplasmic RNA interacting protein