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Identification of Protein-Protein Interactions of Amyotrophic Lateral Sclerosis Associated Protein TDP-43

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Identification of Protein-Protein Interactions of Amyotrophic Lateral Sclerosis Associated Protein TDP-43

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science

By

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Hanoor Sharma ENTITLED Identification of Protein-Protein Interactions of Amyotrophic Lateral Sclerosis Associated Protein TDP-43 BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

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Thank you.

ABBREVIATIONS

3-AT - 3-Amino-1, 2, 4-triazole

AD - Activation domain

ALS - Amyotrophic lateral sclerosis

APP - Amyloid precursor protein

BP sites - attB1, attB2 and attP1, attP2 sites

C9ORF72 - Chromosome 9 open reading frame 72

DAT - Dopamine transporters

DB - DNA binding domain

FTLD - Frontotemporal lobar degeneration

FUS - Fused in sarcoma

hnRNP - Heterogeneous nuclear ribonucleoprotein

LR sites – attL1, attL2 and attR1, attR2 sites

LT – Leucine and tryptophan

LTH – Leucine, tryptophan and histidine

NAC1 - Nucleus accumbens 1

NES - Nuclear export signal

NLS - Nuclear localization sequence

RRM1- RNA recognition motif 1

RRM2 – RNA recognition motif 2

ABSTRACT

Hanoor Sharma. M.S. Department of Microbiology and Immunology, Wright State University, 2016. Identification of Protein-Protein Interactions of Amyotrophic Lateral Sclerosis Associated Protein TDP-43.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease caused by progressive degeneration of upper and lower motor neurons in the brain and spinal cord. Multiple mutations are found in some of the proteins associated with ALS, including superoxide dismutase (SOD1), fused in sarcoma (FUS) and trans-activation response DNA-binding protein (TDP-43). TDP-43 is a DNA and RNA binding protein, well conserved, and ubiquitously expressed in all tissues. TDP-43 resides in the nucleus and sometimes shuttles between nucleus and cytoplasm. Mutations in TDP-43 leads to mislocalization of TDP-43 to the cytosol where it was ubiqutinated and hyperphosphsorylated, ultimately leading to neuronal cell death. The aim of this project is to identify and compare binding partners of both wild type (WT) and mutant TDP-43 using yeast two hybrid screening (Y2H). We identified PICK1 (Protein interacting with protein C-kinase) that binds to both wild type and disease causing mutant (M337V) TDP-43. Interestingly, PICK1 also interacts with other TDP-43 mutants (D169G, Q331K, G298S, and A315T), although the affinity of interaction is weaker.

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INTRODUCTION AND BACKGROUND

Amyotrophic Lateral Sclerosis (ALS)

ALS is a neurodegenerative disease resulting from the progressive degeneration of upper and lower motor neurons in the spinal cord, brainstem and motor cortex (Rothstein, 2009) and is particularly devastating due to its rapid rate of progression and lethality. ALS has two forms, sporadic and familial. Sporadic ALS is spontaneous and affects 90 percent of patients while familial form is hereditary and affects 10 percent of ALS patients. The main symptoms of ALS are muscle atrophy and spasticity, difficulty in speaking (dysarthria), swallowing (dysphagia), and breathing (dyspnea). Individuals affected by ALS ultimately lose the ability to initiate and control all voluntary movement, although bladder and bowel sphincters and the muscles responsible for eye movement are usually, but not always, spared until the final stages of the disease. Ultimately respiratory failure results in death of ALS patients within three to five years from the onset of symptoms (Johnston et al., 2006). ALS can affect people of any age but usually strikes late middle age people. A pathological hallmark of ALS is ubiquitin-positive inclusions composed of misfolded proteins found in neuronal and glial cells (Johnston et al., 2006), but it's still unclear if these inclusions cause the disease. Despite major research efforts, the cause of ALS is unknown and no cure currently exists. Several mechanism including cytoskeleton abnormalities, protein instability, protein aggregation, and impairment of proteasomes have been proposed but the exact mechanism and why cell death is specific to motor neurons remains elusive. Multiple mutations in a variety of proteins have been implicated in ALS including Superoxide dismutase 1 (SOD1), Fused in sarcoma (FUS),

Senataxin (SETX), chromosome 9 open reading frame 72 (C9ORF72) and Trans-activation response DNA-binding protein (TDP-43).

TDP- 43

TDP-43 is a 414-amino acid DNA and RNA binding protein that is highly conserved and ubiquitously expressed. It was identified as one of the proteins responsible for ALS (Neumann et al., 2006). TDP-43 is encoded by the *TARDBP* gene located on chromosome 1 (Neumann et al., 2006) and contains two RNA recognition motifs (RRM1 and RRM2), a nuclear export signal (NES) within RRM2, a nuclear localization sequence (NLS) in the N terminus and a C-terminal glycine-rich domain (Figure 1; Fuentealba, et al., 2010). The RRMs are involved in RNA stabilization and processing, while the NES allows the export of TDP-43 from the nucleus to the cytoplasm and the NLS is involved in the import of TDP-43 into the nucleus. The C-terminal is the region where most of the protein-protein interactions take place (Guo et al., 2011). Interestingly, this is also the region where most of the ALS-linked mutations were identified.

TDP-43 is an important component of the Drosha complex, a class II RNase enzyme responsible for initiating the processing of microRNA in the nucleus (Figure 2a) (Ling et al., 2010). TDP-43 functions in binding to heterogeneous nuclear ribonucleoprotein (hnRNP) and regulates splicing of pre-mRNA species (Figure 2b). TDP-43 also functions to silence the promoter sequence of single stranded DNA (ssDNA), resulting in transcriptional repression (Figure 2c). In cytoplasm, TDP-43 protects mRNAs from stress conditions by forming stress granules (Figure 2d) (Edwards et al., 2013).

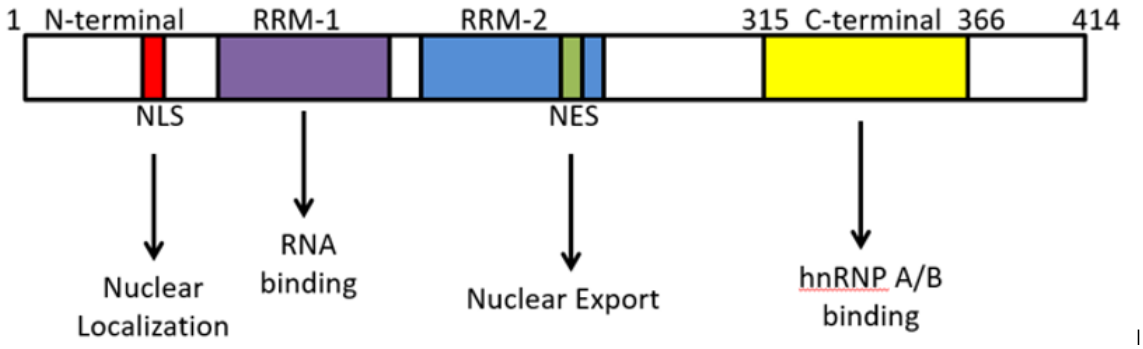

Figure 1: Structural domains of TDP-43. A schematic of TDP-43 protein shows the two RNA recognition motifs (RRM1 and RRM2, purple and blue respectively), NES (green), NLS (red) and glycine rich domain (yellow) (Adapted from Carlomagno et al., 2014).

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TDP-43 also plays a role in the translocation of mRNA granules from the nucleus to the cytoplasm (Figure 2e). TDP-43 binds to a 3′ untranslated region of mRNA that stabilizes the mRNA in the cytoplasm (Figure 2f). TDP-43 is essential for the early development of embryo. Studies have shown that the expression of the TDP-43 gene leads to healthy embryonic development of mice; whereas the absence of TDP-43 is lethal (Sephton et al., 2012).

Mislocalization of TDP-43

More than 30 mutations have been identified in TDP-43 associated with ALS (Herskowitz et al., 2012). Mutations, in most cases, lead to the protein mislocalization and aggregation in the cytosol, which is toxic to neuronal cells (Barmada et al., 2010, Guo et al., 2011, Benajiba et al., 2009, Chen-Plotkin et al., 2010; Figure 3). Although exact mechanism is still unknown, it has been proposed that loss of function of TDP-43 in nucleus and a gain of toxicity in cytosol may result in neurodegeneration in ALS patients (Kabashi et al., 2011).

TDP-43 interactions

TDP-43, shuttling between nucleus and cytosol, has been reported to interact with proteins both in nucleus and cytosol. One of the cytoplasmic proteins that interact with TDP-43 is polyadenylate binding protein-1 (PABPC-1), a RNA binding protein that stabilizes poly (A) mRNA and regulates protein translation (Bernstein et al., 1989). PABPC-1 strongly interacts with TDP-43 and this interaction is RNA dependent, because the binding between TDP-43 and PABPC-1 is diminished when the interaction is treated

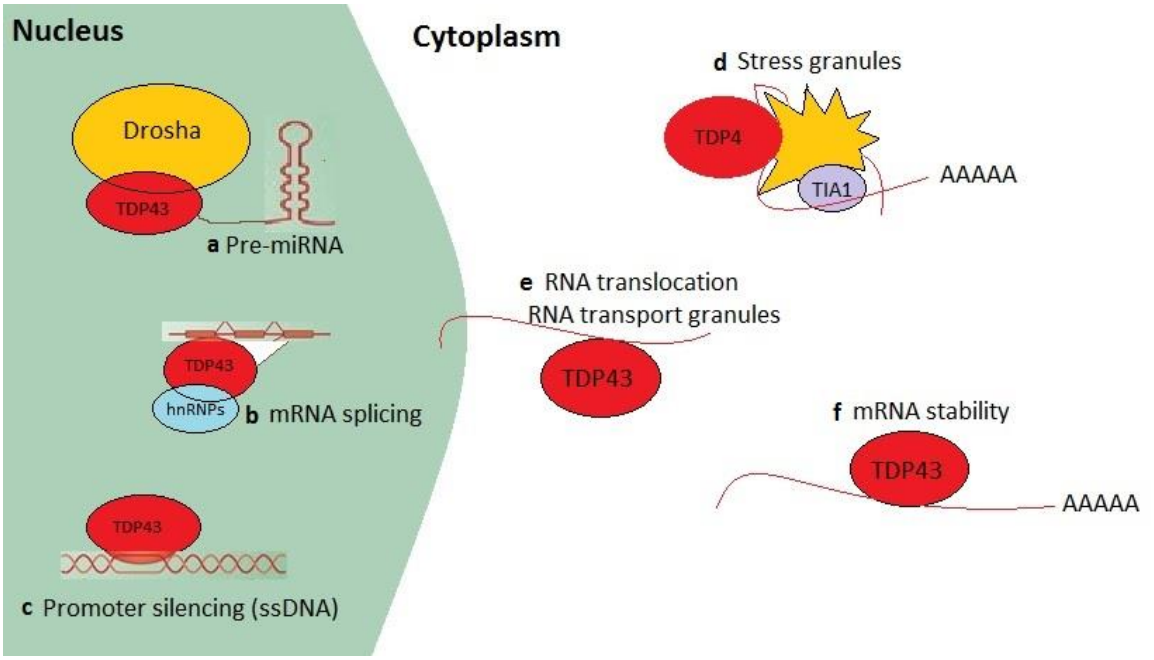

Figure 2: Biological functions of TDP-43 in nucleus and cytoplasm. In the nucleus, TDP-43 functions to a. process primary microRNA along with drosha b. regulate spicing of pre-mRNA species along with hnRNP. and c. act as a transcriptional repressor. In the cytoplasm, TDP-43 a. protects mRNA under condition of stress. b. stabilizes mRNA. and c. assists in nucleo-cytoplasmic shuttling of mRNA. (Adapted from Edwards et al., 2013).

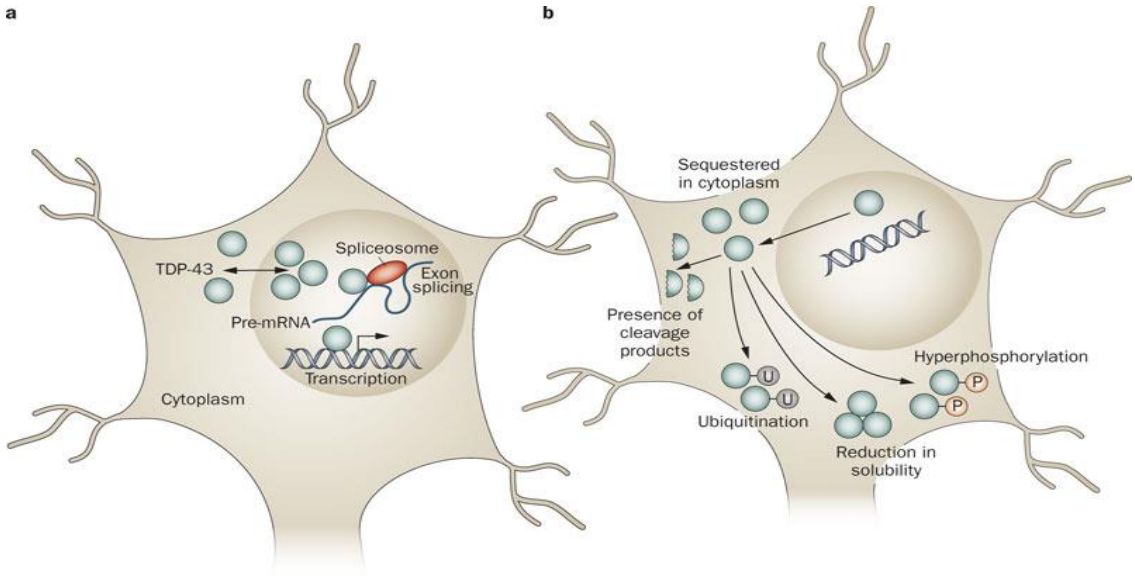

Figure 3: TDP-43 under normal and disease conditions. a.TDP-43 normally resides in the nucleus and regulates gene expression and pre-mRNA splicing b. Mutated TDP-43 leaves the nucleus, accumulates in the cytoplasm where it is ubiquitinated, hyperphosphorylated, cleaved (Chen-Plotkin et al., 2010)

with RNase enzyme (Freibaum et al., 2010; Table 1). In nucleus, TDP-43 interacts with heterogeneous nuclear ribonucleoprotein (hnRNP). hnRNP plays important function in regulating splicing activity and in transport of mRNA out of nucleus (Buratti, 2005) (Table 1).

There are other proteins that interact with TDP-43. Nucleus Accumbens 1 (NAC1), is a member of the family of repressor proteins, that are involved in transcriptional repression (Ahmad et al., 2003) and actin cytoskeleton regulation of neurons (Kang et al., 2004). The actin cytoskeleton regulation helps in morphological development of neurons (Luo et al., 2002). This study showed that NAC1 interacts with WT TDP-43 in the nucleus. When treated with glutamate, NAC1, TDP-43, and proteasome colocalizes together into the cytoplasm, resulting in death of motor neurons (Scofield et al., 2012) (Table 1). Therefore NAC-1 may play a role in cytoplasmic mislocalization and degradation of proteins in the neurons of patients suffering from ALS.

Amyloid precursor protein (APP), a type 1 membrane protein when treated with β secretase, APP is cleaved into toxic amyloid β peptide and intracellular domain of APP called AICD. WT TDP-43 was shown to interact with AICD in nucleus (Table 1). Overexpression of TDP-43 enhanced AICD-induced p53 transcription (a tumor suppressor protein) and apoptosis, ultimately resulting in death of Hela cells by arresting p53, demonstrating that abnormal TDP-43 and AICD interactions may lead to cell death (Wang et al., 2014). Fused in sarcoma (FUS) is another RNA binding protein that is involved in ALS (Verbeeck et al., 2012). Some of the mutant TDP-43 has been shown to have an increased interaction with FUS in the nucleus (Michaeline et al., 2013). Mutant TDP-43

also promotes posttranslational modifications such as phosphorylation and ubiquitination of FUS (Lagier-Tourenne et al., 2010). (Table 1).

Although the detailed mechanism of TDP-43 involvement with the disease remains unclear, much of TDP43 function has been learned from studying its binding partners. So far, protein interaction studies were overwhelmingly performed on the WT TDP-43, and little was dedicated to the disease causing mutant proteins**.**

Table 1: Known Protein-Protein Interactions with TDP-43. The table shows proteins that interact with TDP-43, their functions and the role of their interactions (Co-IP: co- immunoprecipitation)

Gene	Protein	Protein Function	Role of Interactions	Detection method	References
PABPC 1	Polyadenylate-binding protein -1	Stabilizes mRNA and RNA dependent	Unknown	Co-IP	Freibaum et al., 2010
hnRNP	Heterogeneous nuclear ribonucleoprotein	Splicing and Transport mRNA out of nucleus	Inhibits splicing activity	Pull-down	Buratti et al., 2005
NAC1	Nucleus Accumbens1	Repression of transcription factors	Degradation of protein in the presence of glutamate	Co-IP	Scofield et al., 2012
AICD	Amyloid Precursor Protein Intracellular Domain	Not known	Apoptosis	Co-IP	Wang et al., 2014
FUS	Fused in sarcoma	Regulates transcription, mRNA splicing and DNA repair	Formation of aggregates	Co-IP	Ling et al., 2010

HYPOTHESIS

I hypothesize that binding partners of WT and the mutant form of TDP-43 are different. The mutant protein's loss of normal interaction or gain of abnormal interaction may help to understand why the mutant protein is malignant to the cell.

SPECIFIC AIMS

Aim 1: To identify proteins that interact with WT TDP-43

Previous protein interaction studies on WT TDP-43 have been done using pull down assays followed by western blotting or mass spectrometry. Here I used Y2H, a genetic approach widely used to identify protein-protein interactions. Due to complementarity of the different methods, I expect to identify new interactions for TDP-43. The cDNA libraries of the brain and the fetal brain were used to check if the interactions of TDP-43 differ at various stages of life.

Aim 2: To identify proteins interacting with mutant TDP-43

My second aim will use the same Y2H method as above, with the focus on TDP-43 mutants commonly found in ALS patients, including D169G, G298S, A315T, Q331K and M337V. The rational for choosing these mutants are: firstly, these five mutants of TDP-43 are commonly found in ALS patients; secondly, these mutants cover both sporadic and familial cases (D169G and Q331K in sporadic while G298S, A315T and M337V in familial cases); Lastly, these mutants were selected from different parts of protein: D169G in RRM1 domain and G298S, A315T, Q331K and M337V from C- terminus of the protein, where the most mutations were identified (Figure 4).

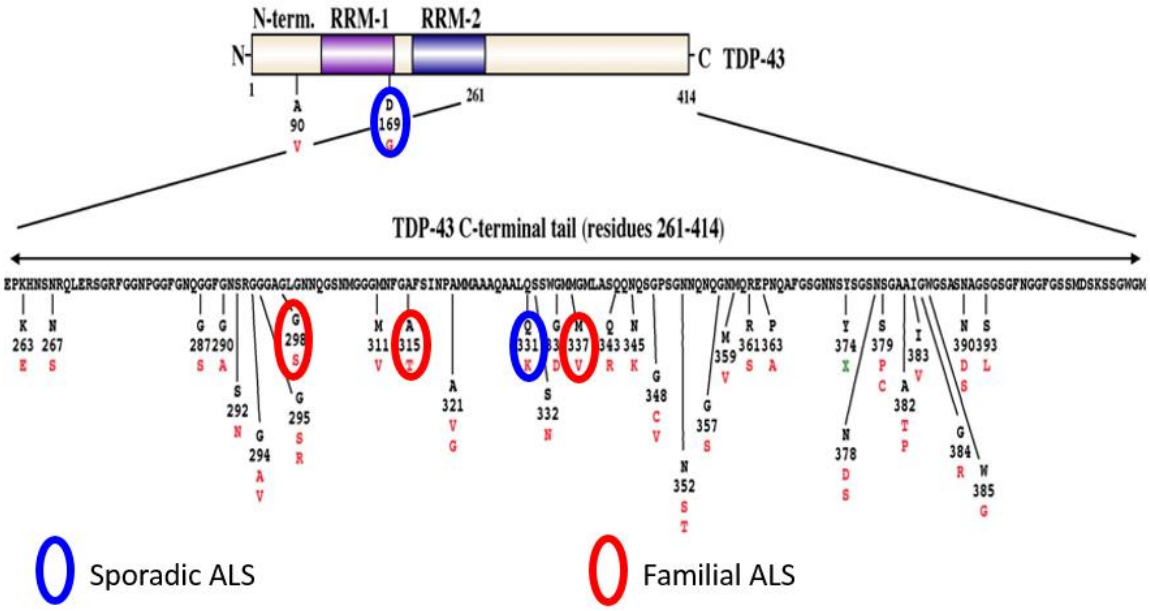

Figure 4: ALS associated TDP-43 mutations. Most of the TDP-43 mutations are found in C terminal region of TDP-43. Those highlighted in red are the mutated residues and X in green represents the truncated mutant. The mutants chosen for this project are circled in blue 0 (sporadic: D169G and Q331K) and in red 0 (familial: G298S, A315T and M337V) (Buratti et al., 2009).

METHODS AND MATERIALS

PCR amplification of TDP-43

Full length WT TDP-43 was amplified from pRS426 Gal1/TDP-43 by PCR using forward primer 5' GGGGACAACTTTGTACAAAAAAGTTGGC 3' and reverse primer 5' GGGGACAACTTTGTACAAGAAAGTTGA 3'. The forward and reverse primers include gateway cloning sites (attB1 and attB2) respectively. The amplification protocol consisted of an initial denaturing step at 95°C for 5 minutes, followed by 30 cycles of denaturing at 95°C for 30 seconds, annealing at 54°C for 30 seconds, and extension at 72°C for 90 seconds. A final extension step was done at 72°C for 10 minutes to complete any partial amplicons.

Construction of DB-TDP-43 by Gateway Cloning

Gateway cloning is an efficient method to transfer DNA between two plasmids. The cloning consists of two steps, BP and LR reaction. In the BP reaction, the full length TDP-43 gene (PCR product with attB sites) was inserted into the pDONR223 (Donor vector) in the presence of BP Clonase, to develop a TDP-43 entry clone. An entry clone is then used to transfer the gene into different destination vectors. In order to confirm that TDP-43 was successfully inserted into the pDONR223, restriction digestion was done using EcoRV and XhoI. In the LR reaction, the TDP-43 gene was transferred from entry clone to a destination vector (DB empty vector) in the presence of LR clonase, to develop a DB-TDP-43 clone (TDP-43 is fused with DNA binding domain). The clone was confirmed by restriction digestion (XcmI) and sequencing.

Transformation of Yeast

DB-TDP-43 was transformed into the yeast strain Y8930 (haploid mating type α) using a one-step transformation protocol. Briefly, freshly grown cells were resuspended with DNA in 100ul transformation buffer (100uM lithium acetate, 100uM DDT and 40% PEG), incubated at 42°C for 40 minutes, with a brief vortex every 10 minutes. The selective marker for DB-TDP-43 construct is Leu2, so the cells were plated onto Leucine dropout plates and grown at 30°C. Transformants usually appear in 3 days.

Site directed mutagenesis

Site directed mutagenesis was used to introduce mutations to TDP-43 on entry vector of pDONR223. Sense and antisense primers for 5 different TDP-43 mutants (D169G, Q331K, G298S, A315T and M337V) are shown on table 2. The reaction consisted of an initial denaturing step at 95°C for 30 sec, followed by 18 cycles of denaturing at 95°C for 30 seconds, annealing at 55°C for 1 minute and elongation at 68°C for 3 minutes. The final extension step was done at 68 °C for 10 minutes. The methylated and non-mutated parental DNA was then digested using Dpn I. The newly synthesized DNA with TDP-43 mutations was then transformed into DH5α *Escherichia coli* cells and was plated on LB/spectinomycin plates. The colony was picked from each mutant and miniprep was performed (Qiagen). The mutations were confirmed by sequencing.

Table 2: Primers for site directed mutagenesis. Below is the table showing forward and reverse primers used in site directed mutagenesis.

Mutant	Forward primers	Reverse primers
D169G	5'GTCACACCATCGTCCACC TATCATATGTCGCTGT3’	5'ACAGCGACATATGATAGGTGGA CGATGGTGTGAC3'
Q331K	5'CCCCAACTGCTCTTTAG TGCTGCCTGGGC 3'	5'GCCCAGGCAGCACTAAAGAGCA GTTGGGG 3'
G298S	5'TGATTGTTTCCCAAACTA GCTCCACCCCCTCTG 3'	5'CAGAGGGGGTGGAGCTAGTTTG GGAAACAATCA 3'
A315T	5'GGATTAATGCTGAACGTA CCAAAGTTCATCCCACCA 3'	5'TGGTGGGATGAACTTTGGTACGT TCAGCATTAATCC 3'
M337V	5'GGCTAACATGCCCACCAT ACCCCAACTGCTC 3'	5'GAGCAGTTGGGGTATGGTGGGC ATGTTAGCC 3'

Y2H

Y2H is a widely used genetic method to identify protein-protein interactions. In Y2H, the transcription factor GAL4 is split into two domains, the activation domain (AD) and DNA binding domain (DB). The AD was fused with an unknown gene from cDNA library and transformed into the yeast strain Y8800 (haploid mating type a). The DB fused with TDP-43 plasmid was transformed into yeast strain Y8930 (haploid mating type α). When AD and DB are separate, transcription of the reporter gene does not occur (Figure 5a). However, when prey protein (Y) interacts with the protein of interest (TDP43), the interaction will bring AD and DB to close proximity, resulting in transcription of the reporter gene (Figure 5b). The reporter gene used in my screenings was *HIS3,* which promotes growth on plates lacking histidine. The two yeast (Y8800 and Y8930) were mated for four hours to bring AD and DB constructs together. A 1:10,000 dilution was plated on glucose plates lacking leucine and tryptophan (LT-) to determine the number of clones screened. The rest of the cells were plated on glucose plates lacking leucine, tryptophan and histidine (LTH-) to identify potentially positive interactions.

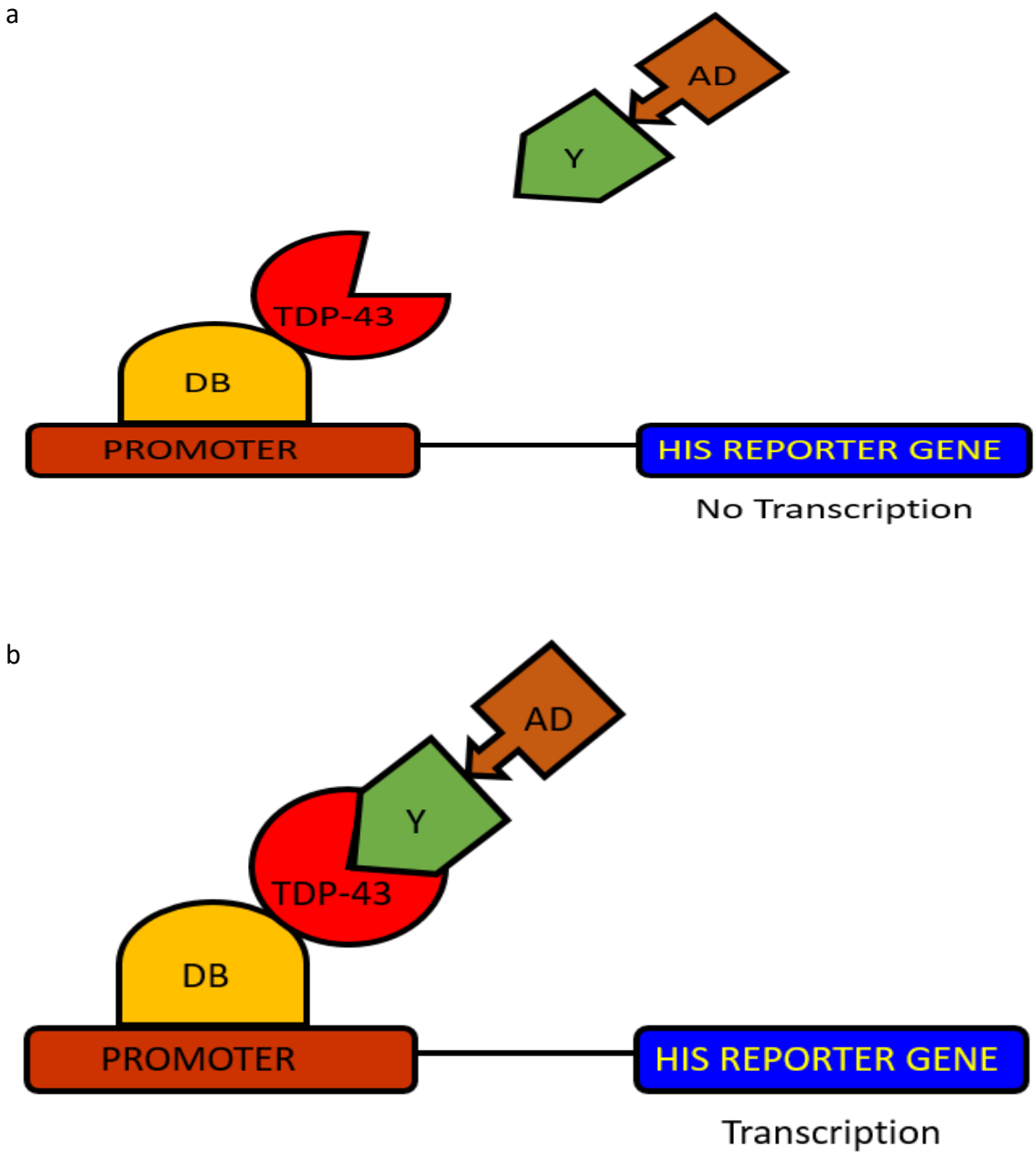

Figure 5: Y2H screening. The DNA binding domain is fused with TDP-43 (DB-TDP-43) and unknown protein (Y) from the cDNA library is fused with activation domain (Y-AD). a. When DB-TDP-43 are separate from AD-Y, *HIS* reporter gene is not activated and no transcription occurs. b. When DB-TDP-43 interacts with Y-AD, the transcription of the HIS reporter gene will be activated, so the strain will grow in the absence of histidine.

Colony PCR

Colony PCR is used to amplify unknown gene (Y) from AD constructs. In this method, colony that contains AD-Y was lysed and directly used as a template. Forward and reverse primers was designed based on the vector sequences that flank unknown gene Y (Figure 6). The colonies (positive hits) from Y2H were lysed in lysis buffer (0.1M NaPO4 buffer of pH 7.4 mixed with 2.5 mg/ml Zymolase) for 30 minutes. PCR was performed using forward primer (ADF) 5' CGCGTTTGGAATCACTAC AGGG-3' and reverse primer (ADDB reverse) 5' CTGGCAAGGTAGACAAGCC 3'. The conditions for PCR include an initial denaturing step at 95°C for 5 minutes, followed by 35 cycles of denaturing at 95°C for 30 seconds, annealing at 54°C for 30 seconds and extension at 72°C for 5 minutes. The final extension step was done at 72°C for 10 minutes to complete any partial amplicons. Finally, gel electrophoresis was performed to detect the amplified DNA. The DNA was purified using gel DNA recovery kit (Zymoclean).

Gap Repair

Gap repair is used to remake the AD constructs identified from Y2H screening. When the linearized AD vector and colony PCR product (Y) (that was amplified based on the sequences of the AD vector) were transformed into yeast Y8800, homologous recombination will happen and the AD construct will be remade with the amplified gene inserted into it (Figure 7). The circular AD vector was linearized by Sma I restriction enzyme at 25°C for 3 hours. *Saccharomyces* (Y8800) was grown overnight in YPD medium and the PCR product obtained from colony PCR was transformed with linearized AD into Y8800. The transformed cells were plated on tryptophan dropout plates.

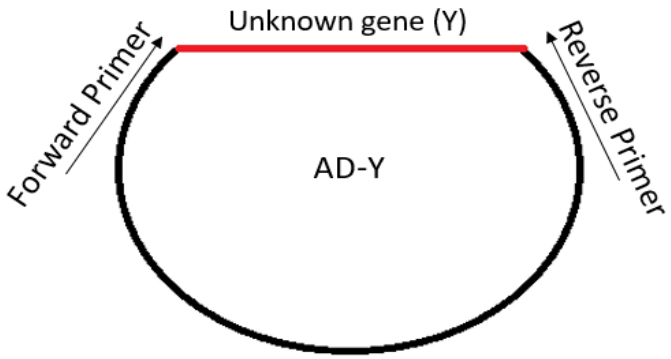

Figure 6: Colony PCR. A figure shows the amplification of unknown gene (Y) from a yeast colony using forward and reverse primers that are complementary to the AD vector.

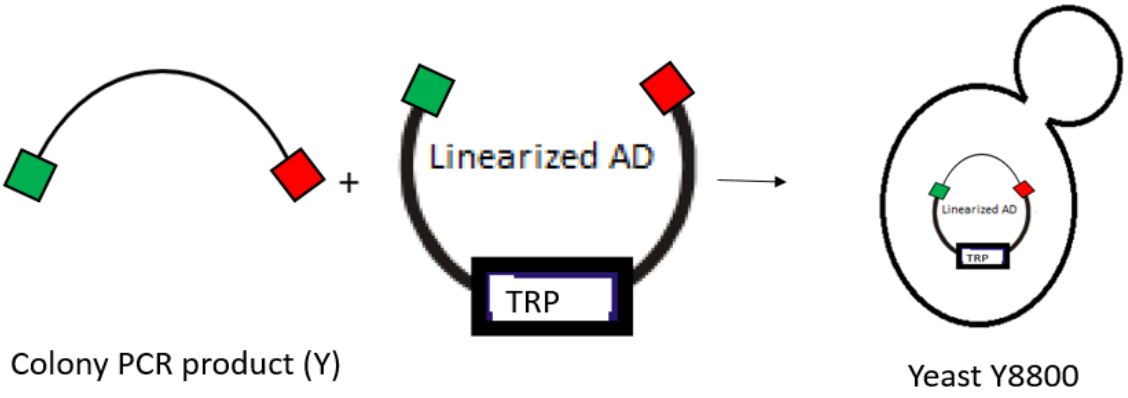

Figure 7: Gap repair. A schematic diagram indicates that when the colony PCR product (Y) and linearized AD vector are transformed into yeast Y8800, since the two share the same sequences (as indicated in green and red) at the ends, homologous recombination will happen and AD vector will be successfully remade with the gene (Y) inserted into it.

Hits Verification

The colonies obtained from gap repair were mated with DB-TDP-43 on YPD plates for 4 hours. The mated colonies were spotted on LT- and LTH- plates. The growth on LTH- plates confirms the positive interactions. The corresponding PCR product were sent for sequencing, and the results were subjected to a BLAST search against the NCBI database

RESULTS

Amplification of TDP-43 template

TDP-43 is a 414 amino acid DNA/RNA binding protein with a molecular mass of 43 KDa. Before performing Y2H and analyzing the putative interactions with TDP-43, I amplified the template of TDP-43 in order to make the DB-TDP-43 construct. The template was amplified with a PCR reaction and was electrophoresed on a 1% agarose gel. As shown in Figure 8, PCR product is ~ 1.3 Kb, the expected size of TDP-43.

Construction of DB-TDP-43 by Gateway Cloning (BP reaction)

In order to make DB-TDP-43, gateway cloning was performed. It consists of two step reactions, a BP and a LR reaction. In the BP reaction, the DNA template (TDP-43) was successfully transferred into the entry vector (pDONR223) (Figure 9). The entry clone was verified by restriction digestion using EcoRV and XhoI. The expected 1.2 kb and 2.7 kb confirms that TDP-43 was successfully inserted into the Entry clone.

In the LR reaction, TDP-43 was successfully transferred into the destination vector (DB empty vector) from the entry clone in the presence of LR clonase (Figure 10). In order to confirm that the gene was successfully inserted into DB vector, restriction digestion was performed using a XcmI restriction enzyme. On gel electrophoresis the size of the bands observed was 3.6kb and 6kb as expected.

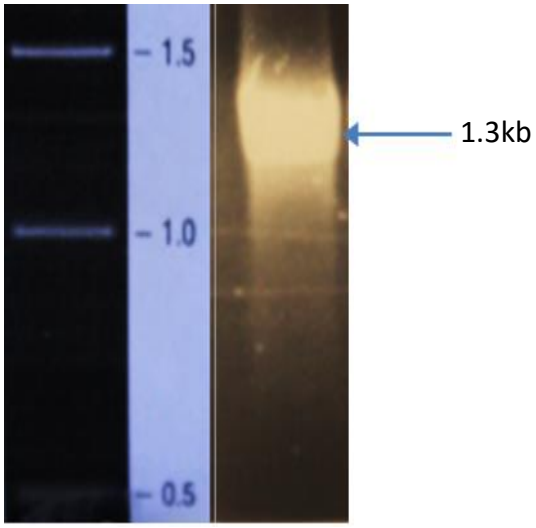

Figure 8: Gel electrophoresis of PCR product of TDP-43. Gel Electrophoresis of the TDP-43 showing the band size of ~1.3 kb. On the left is the ladder showing 0.5 to 1.5 kb sizes.

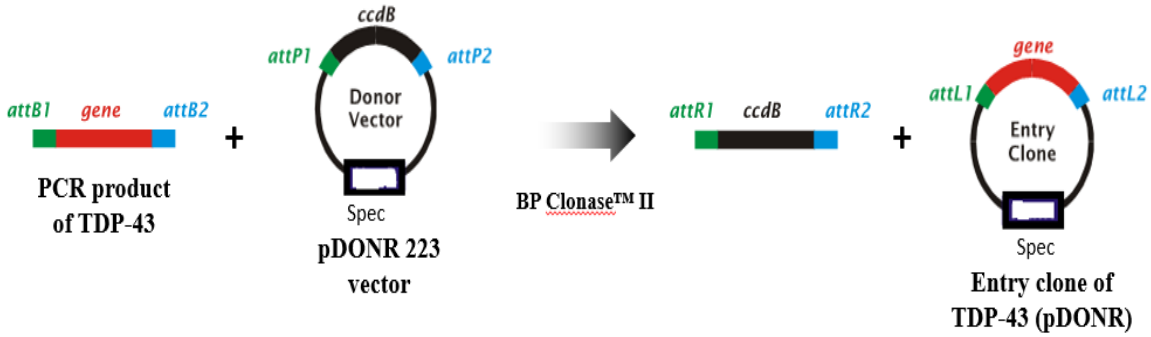

Figure 9: BP reaction (Gateway cloning). The PCR product of TDP-43 flanked with attB sites was inserted into the pDONR223 vector in presence of BP Clonase II enzyme with two flanking recombination sequences called “attP 1” and “attP 2”, to construct “TDP-43 Entry clone”.

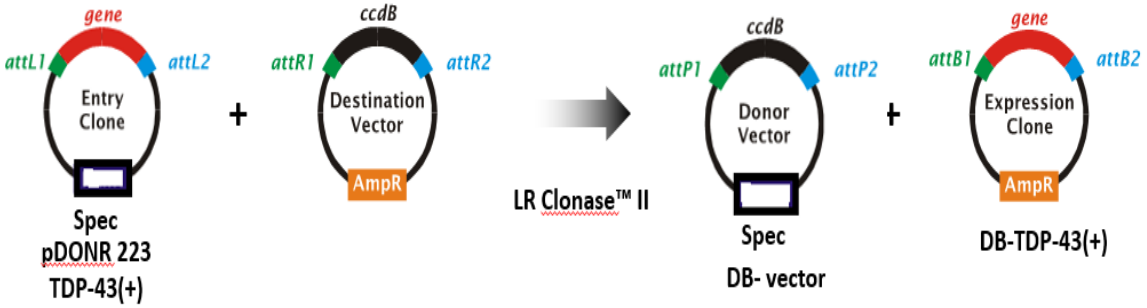

Figure 10: LR reaction (Gateway cloning) - The TDP-43 DNA fragment was transferred from entry vector to destination vector (DB empty vector) in the presence of LR clonase enzyme to construct TDP-43 destination vector (DB-TDP-43).

Construction of mutant TDP-43 by site directed mutagenesis

Five mutants of TDP-43 chosen for this project are D169G, Q331K (sporadic), G298S, A315T and M337V (Familial). To identify the binding partners of mutated TDP-43, we introduced each of these mutations into TDP-43 on the entry vector pDONR223 by site-directed mutagenesis. All the mutated gene of TDP-43 was later transferred to the destination vector by LR reaction of gateway cloning. The constructed expression clone of each mutant (DB- DI69G, DB-Q331K, DB-G298S, DB-A315T and DB-M337V) was then confirmed by restriction digestion with PvuII. On gel electrophoresis, 4.1 and 5.5 kb was observed as expected. All DB constructs of mutant TDP-43 were ultimately confirmed by sequencing.

Y2H screening

Y2H is a genetic approach to identify protein-protein interactions. In Y2H, when WT DB-TDP-43 interacted with the AD-cDNA libraries of brain and fetal brain proteins, colony growth was observed on LTH. The colonies appeared on LT- indicates the number of genes screened. Y2H was also repeated for each of the five missense mutants of TDP-43 (D169G, Q331K, G298S, A315T and M337V) using libraries from both human brain and human fetal brain. The results from sreening using cDNA library of human brain were shown in Figure 11 and summarized in Table 3. The plate pictures from screening using cDNA library of fetal brain looked similar to Figure 11 (data not shown). The results were summarized in Table 3

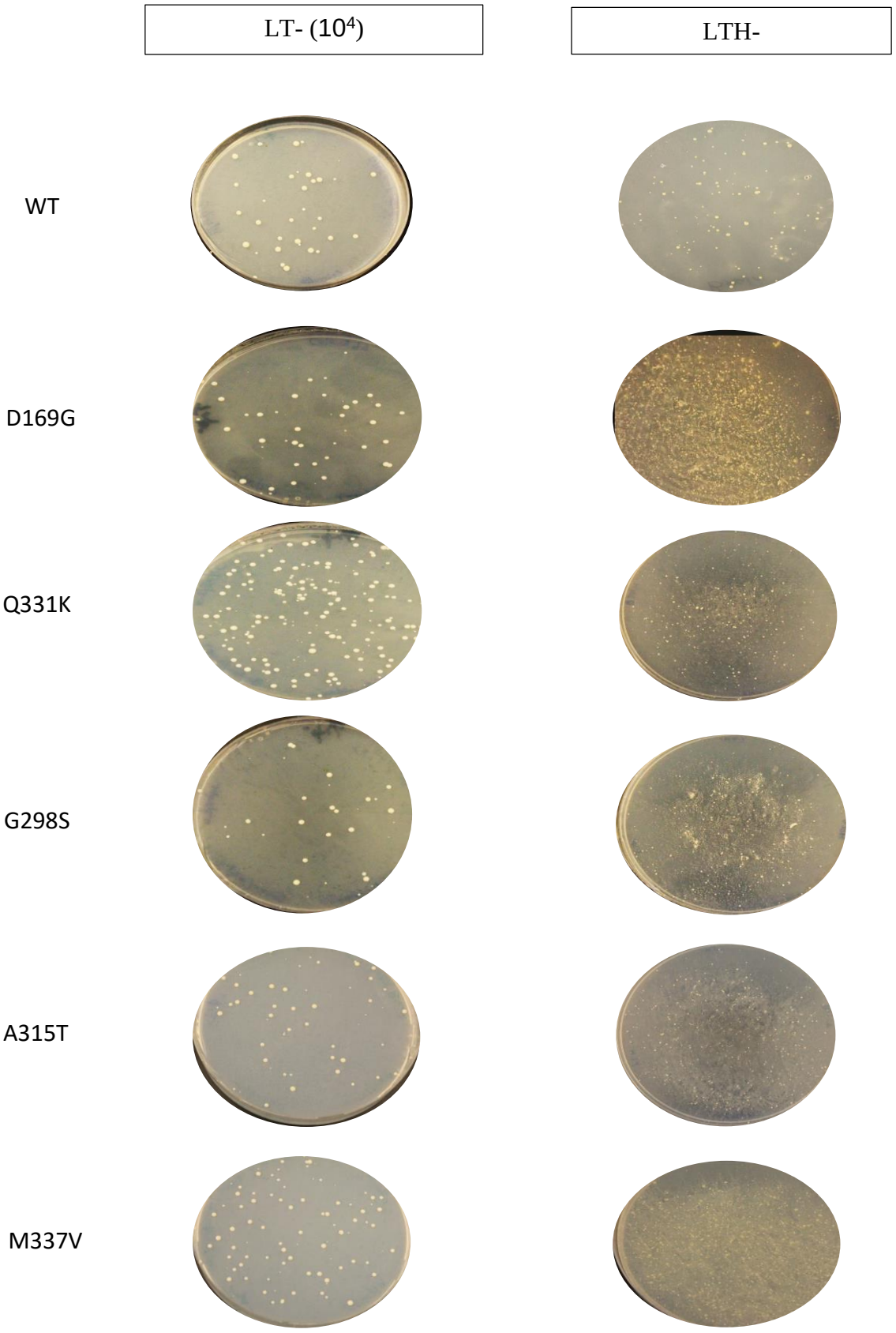

Figure 11: Y2H screening using cDNA library from human brain. The figure shows colony growth on LT- plates (left) and LTH- (right) when Y2H was performed with DB-WT and five mutants of TDP-43 (D169G, Q331K, G298S, A315T and M337V) as baits. The number of colonies on LT- plates indicates total number of genes screened. as indicated, ~1 million clones was screened from each WT and mutant TDP-43. The number of colonies on LTH- plates probably indicates total number of positive hits.

Table 3: Y2H Screening of WT and mutant TDP-43. This table shows the results of Y2H screening of wild type and mutants of TDP-43 i.e. D169G, Q331K, G298S, A315T and M337V, using cDNA libraries of human brain and fetal brain. The number of colonies on LT- plates indicated the number of genes screened. The number of colonies on LTH- plates were the number of potential interactions. The number of picked colonies indicated the number of interactions (big colonies) selected for further analysis.

cDNA libraries	Mutants	Screened (10^4) (LT-)	Interactions (LTH-)	Picked colonies
1. Brain cDNA library	Wild Type	100	400	127
	D169G	122	62	45
	Q331K	96	50	30
	G298S	118	58	45
	A315T	95	45	30
	M337V	76	40	15
2. Fetal Brain cDNA library	Wild Type	80	250	90
	D169G	136	50	15
	Q331K	154	33	5
	G298S	160	30	10
	A315T	122	45	15
	M337V	120	70	40

Identification of AD-Y interactors by Colony PCR

To confirm that the colonies contained genes encoding proteins that interact with TDP-43, colony PCR was performed to identify all the interactors of wild type and mutant TDP-43. The encoding sequence of unknown gene 'Y' was retrieved by PCR amplification directly from yeast colonies. The amplified sequence was electrophoresed on agarose gel. Several PCR products showed identical sizes, indicating that they might represent same sequences. PCR products of unique sizes were selected for next step (Figure 12).

Gap Repair to re-make AD-Y constructs identified from Y2H screening

Gap repair was performed to reconstruct each AD-Y plasmid. The unique PCR product from colony PCR above has the end sequences matching with AD vector. When it's transformed together with linearized AD vector into Y8800 (mating type a), homologous recombination happens, bringing the gene and AD vector together to remake the AD constructs. Colony growth on Trp- plates indicates successful recombination. Figure 13 represents gap repair of one hit from Y2H screening of wild type and mutant (D169G, Q331K, G298S, A315T, and M337V) TDP-43. The same procedure was repeated for all other hits.

Hits verification

Y8800 containing AD-Y reconstructed from gap repair was mated with Y8930 containing DB-TDP-43. i.e. Y8800 containing AD-Y from Y2H screening for WT TDP-43 were mated with Y8930 containing DB-TDP-43 (Figure 14). Similarly Y8800 containing AD-Y from Y2H screening for mutant TDP-43 were mated with Y8930 containing its corresponding DB mutants. The mated strains were then spotted onto LT- and LTH- plates.

The growth on indicating the successful mating to bring the two constructs together. The growth on LTH- plates confirms positive interactions. Figure 14 shows representative results from WT TDP-43 and its mutant A315T and M337V.

Assembling and Verification of hits

All the verified interactors from WT and mutant TDP-43 were assembled and summarized in Table 4. A total of 131 proteins were identified from Y2H screenings of WT and mutant TDP-43 together. The summary of the hits are shown in table 5.

To determine similarity and differences of binding partners of WT and mutant TDP-43, the positive hits identified from Y2H of one bait protein (i.e. WT TDP-43) will be crosschecked for their potential interactions with other bait protein (i.e. mutant TDP-43). Before doing this, I checked the interactions of the hits with their corresponding baits first. Only one hit from Y2H screening of WT TDP-43 still showed interaction with TDP-43. All other hits (130 out 131) did not show interactions with their corresponding baits any more (Figure 15). Since all of 131 hits were previously confirmed, this result is completely unexpected.

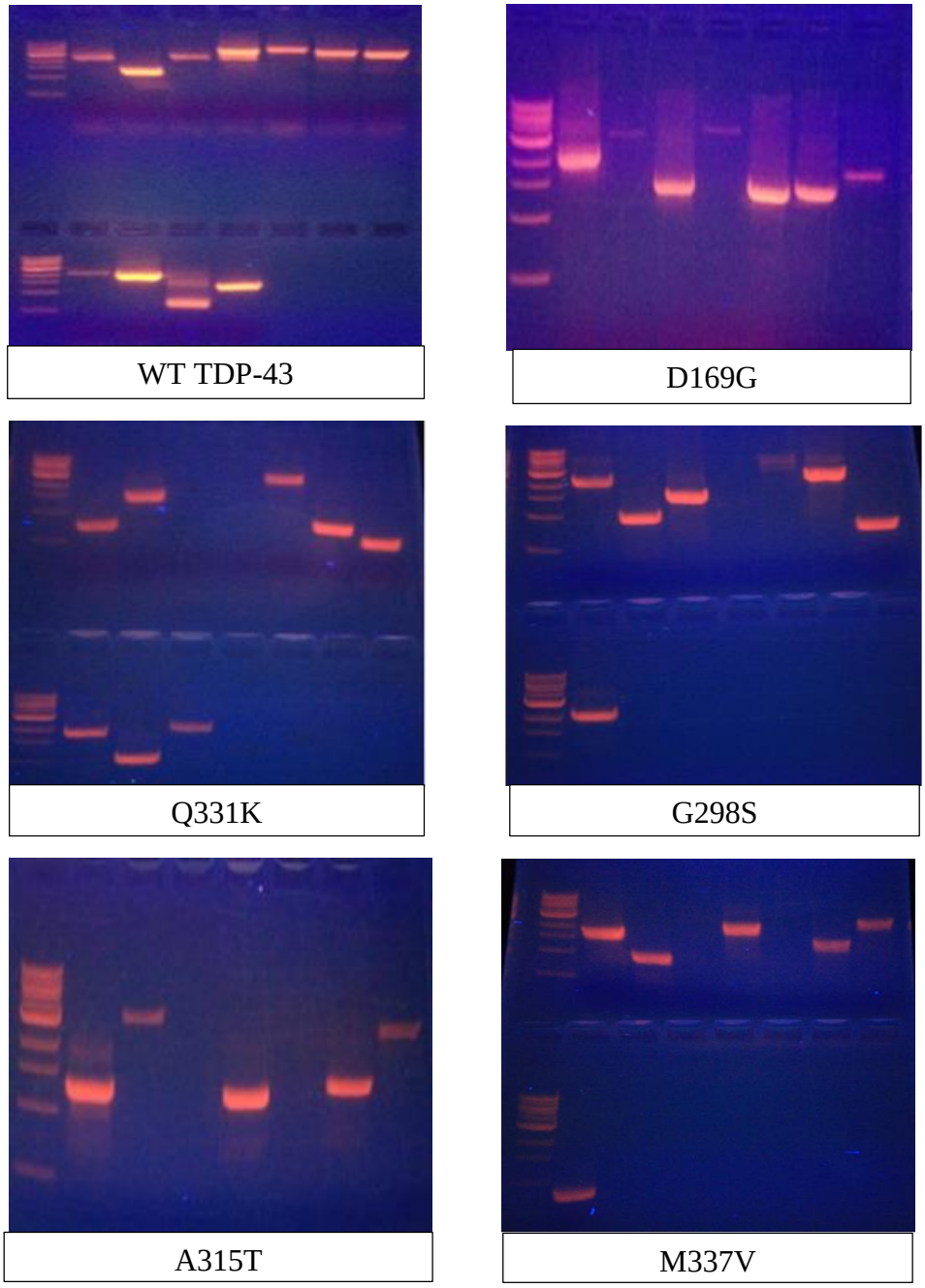

Figure 12: Colony PCR product of hits identified from Y2H screenings of wild type and mutant TDP-43. Total 467 hits from Y2H screening of WT and mutant TDP-43 (Table 3) were selected for colony PCR. These gel pictures illustrate some of the colony PCR products from Y2H screening of wild type TDP-43 and five different mutants (D169G, Q331K, G298S, A315T, M337V). Size of the DNA bands was determined by 1 kb ladder (NEB) on the left.

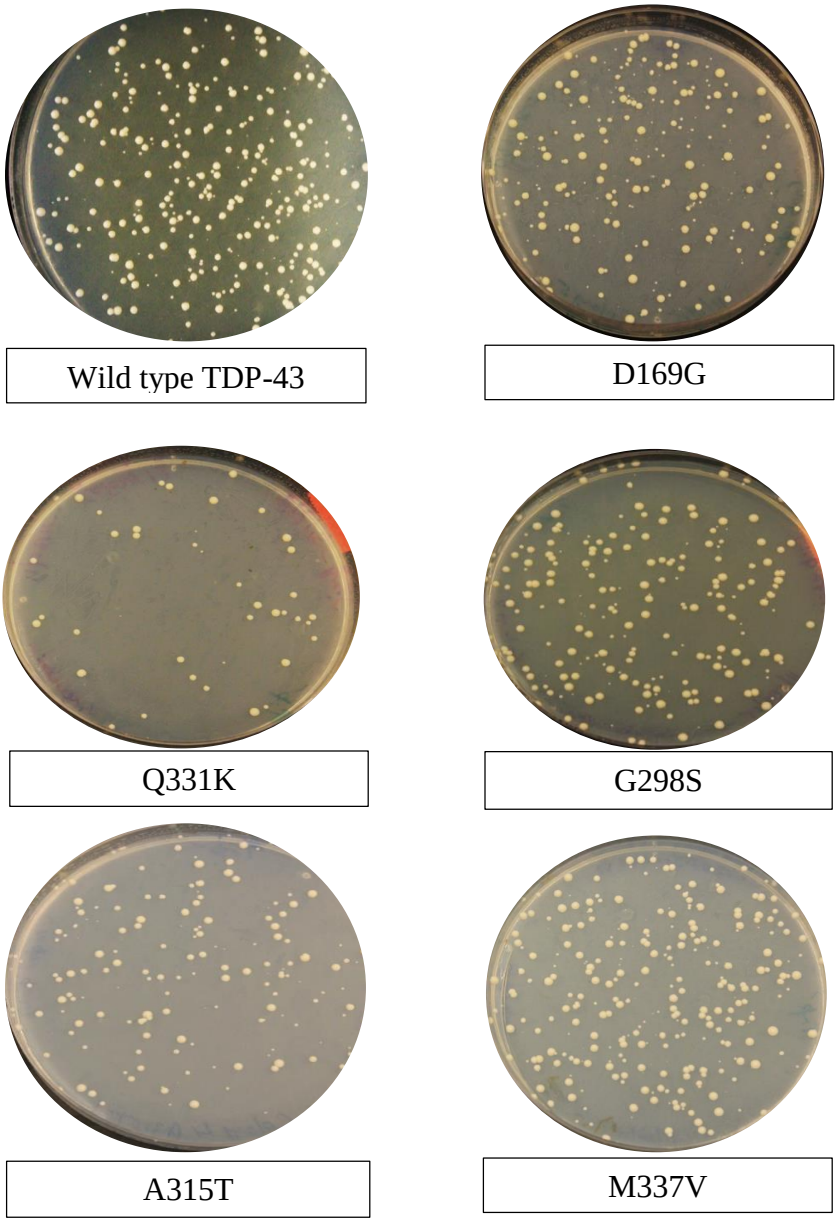

Figure 13: AD constructs remade by gap repair. The figure above shows yeast strain Y8800 that contain AD constructs re-made through gap repair. One representative hit from Y2H screening of WT and mutant TDP-43 (D169G, Q331K, G298S, A315T and M337V) was shown.

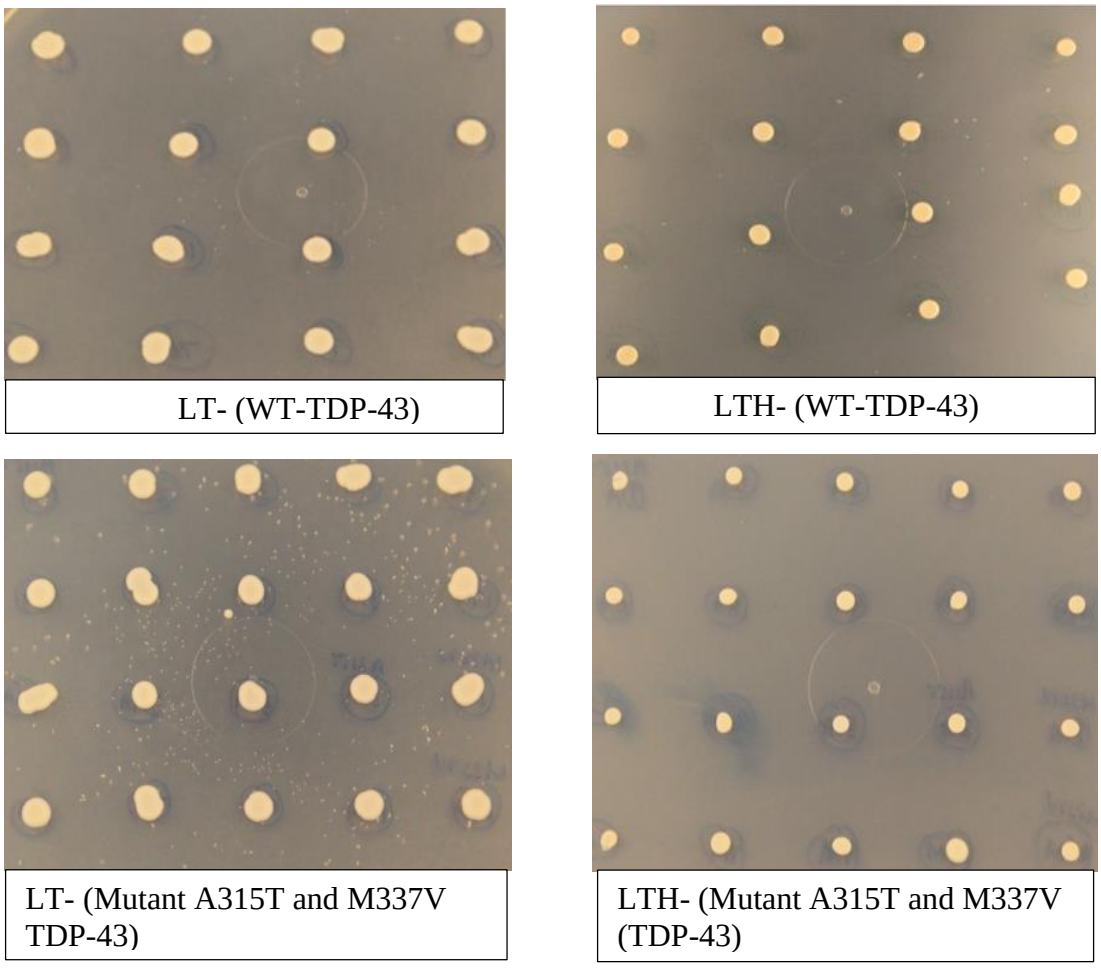

Figure 14: Verification of Y2H hits from WT and mutant (A315T and M337V) TDP-43. Yeast strains with DB-WT and mutant DB-TDP-43 (A315T and M337V) were mated with strain containing AD constructs, re-made by gap repair. Cells after mating were spotted onto LT- and LTH- plates. Growth on LT- plates indicates successful mating to bring two constructs together. Growth on LTH- plates indicates positive interactions.

Table 4: Summary of Hits. The table below shows the verified hits from both wild type and mutant TDP-43. The name of the colony (in my stock plates for my own record), cDNA library used, bait of WT or mutant TDP-43, size of colony PCR product is indicated for each of the hits.

Name of the colony	cDNA library	Wild type or Mutant	Band sizes
B1	Brain	Wild type (WT)	1.7 kb
B3	Brain	Wild type (WT)	3.0kb
B4	Brain	Wild type (WT)	3.2kb
B5	Brain	Wild type (WT)	3.0kb
B7	Brain	Wild type (WT)	2.0kb
B8	Brain	Wild type (WT)	1.5kb
B9	Brain	Wild type (WT)	0.7kb
B10	Brain	Wild type (WT)	1.0kb
B15	Brain	Wild type (WT)	0.8kb
B17	Brain	Wild type (WT)	1.6kb
B63	Brain	Wild type (WT)	1.7kb
B64	Brain	Wild type (WT)	2.3kb
B76A	Brain	Wild type (WT)	0.9kb
B76B	Brain	Wild type (WT)	2.3kb
B78	Brain	Wild type (WT)	5kb
B91	Brain	Wild type (WT)	4kb
B93	Brain	Wild type (WT)	1.2kb
B97	Brain	Wild type (WT)	0.6kb
B99	Brain	Wild type (WT)	0.4kb
FB1	Fetal brain	Wild type (WT)	2.1kb
FB3	Fetal brain	Wild type (WT)	1.7kb
FB5	Fetal brain	Wild type (WT)	1.3kb
FB11	Fetal brain	Wild type (WT)	0.9kb
FB12	Fetal brain	Wild type (WT)	0.8kb
FB13	Fetal brain	Wild type (WT)	1.5kb
FB16	Fetal brain	Wild type (WT)	0.5kb
FB17	Fetal brain	Wild type (WT)	2.0kb
FB18	Fetal brain	Wild type (WT)	1.0kb
FB35	Fetal brain	Wild type (WT)	1.2kb
FB36	Fetal brain	Wild type (WT)	2.3kb
FB53	Fetal brain	Wild type (WT)	0.7kb
FB59	Fetal brain	Wild type (WT)	1.3kb
DB1	Brain	Mutant D169G	2kb
DB3	Brain	Mutant D169G	1.5kb
DB5	Brain	Mutant D169G	1.4kb
DB7	Brain	Mutant D169G	1.8kb
DB11	Brain	Mutant D169G	0.9kb
DB13	Brain	Mutant D169G	2.5kb
DB17	Brain	Mutant D169G	1.3kb
DB18	Brain	Mutant D169G	2.2kb
DB19a	Brain	Mutant D169G	2.3kb
DB21	Brain	Mutant D169G	1.6kb
DB24	Brain	Mutant D169G	5kb
DB26	Brain	Mutant D169G	3kb
DB28	Brain	Mutant D169G	4kb
DB29	Brain	Mutant D169G	0.7kb
DFB2	Fetal brain	Mutant D169G	0.4kb
DFB3	Fetal brain	Mutant D169G	3kb
DFB4	Fetal brain	Mutant D169G	2.8kb
DFB8	Fetal brain	Mutant D169G	2kb
DFB11	Fetal brain	Mutant D169G	0.6kb
DFB12	Fetal brain	Mutant D169G	0.3kb
QB1	Brain	Mutant Q331K	1.6kb
QB4	Brain	Mutant Q331K	2kb
QB5	Brain	Mutant Q331K	5kb
QB7	Brain	Mutant Q331K	2.1kb
QB11	Brain	Mutant Q331K	1.7kb
QB12	Brain	Mutant Q331K	2.4kb
QB13	Brain	Mutant Q331K	2.3kb
QB14	Brain	Mutant Q331K	2.6kb
QB17	Brain	Mutant Q331K	0.7kb
QB20	Brain	Mutant Q331K	1.5kb
QB22	Brain	Mutant Q331K	0.8kb

QB23	Brain	Mutant Q331K	1.8kb
QB27	Brain	Mutant Q331K	0.9kb
QB28	Brain	Mutant Q331K	0.5kb
QB29	Brain	Mutant Q331K	2.7kb
QFB2	Fetal brain	Mutant Q331K	1.5kb
QFB3	Fetal brain	Mutant Q331K	1.7kb
QFB4a	Fetal brain	Mutant Q331K	0.6kb
QFB5	Fetal brain	Mutant Q331K	2kb
GB2	Brain	Mutant G298S	0.5kb
GB4	Brain	Mutant G298S	1.2kb
GB6	Brain	Mutant G298S	2.0kb
GB8	Brain	Mutant G298S	2.5kb
GB12	Brain	Mutant G298S	1.6kb
GB13	Brain	Mutant G298S	0.8kb
GB16	Brain	Mutant G298S	2.0kb
GB17	Brain	Mutant G298S	0.7kb
GB19	Brain	Mutant G298S	1.3kb
GB28	Brain	Mutant G298S	3.0kb
GB30	Brain	Mutant G298S	1.3kb
GFB1	Fetal brain	Mutant G298S	0.7kb
GFB2	Fetal brain	Mutant G298S	1.8kb
GFB3a	Fetal brain	Mutant G298S	4.0kb
GFB3b	Fetal brain	Mutant G298S	1.2kb
GFB4	Fetal brain	Mutant G298S	2.0kb
GFB7	Fetal brain	Mutant G298S	3.0kb
GFB8	Fetal brain	Mutant G298S	0.8kb
AB1	Brain	Mutant A315T	1.1kb
AB2	Brain	Mutant A315T	0.8kb
AB3	Brain	Mutant A315T	2.0kb
AB4	Brain	Mutant A315T	1.0kb
AB5	Brain	Mutant A315T	1.5kb
AB6	Brain	Mutant A315T	0.9kb
AB8	Brain	Mutant A315T	3.0kb
AB9	Brain	Mutant A315T	1.6kb
AB15	Brain	Mutant A315T	0.5kb
AB19	Brain	Mutant A315T	2.7kb
AB23	Brain	Mutant A315T	1.3kb
AB24	Brain	Mutant A315T	1.9kb
AB35	Brain	Mutant A315T	1.4kb
AB36	Brain	Mutant A315T	2.2kb
AFB1b	Fetal brain	Mutant A315T	1.3kb
AFB2	Fetal brain	Mutant A315T	5kb
AFB4	Fetal brain	Mutant A315T	1.3kb
AFB6	Fetal brain	Mutant A315T	1.4kb
AFB7	Fetal brain	Mutant A315T	3.0kb
AFB8	Fetal brain	Mutant A315T	0.4kb
AFB9	Fetal brain	Mutant A315T	4.0kb
AFB12	Fetal brain	Mutant A315T	2.0kb
AFB14	Fetal brain	Mutant A315T	2.2kb
MB1	Brain	Mutant M337V	1.3kb
MB6	Brain	Mutant M337V	0.7kb
MB7	Brain	Mutant M337V	2.0kb
MB9	Brain	Mutant M337V	0.8kb
MB14	Brain	Mutant M337V	3.0kb
MB15	Brain	Mutant M337V	0.4kb
MFB1	Fetal brain	Mutant M337V	3.0kb
MFB3	Fetal brain	Mutant M337V	1.3kb
MFB4	Fetal brain	Mutant M337V	2.8kb
MFB5	Fetal brain	Mutant M337V	1.4kb
MFB6	Fetal brain	Mutant M337V	2.0kb
MFB10	Fetal brain	Mutant M337V	0.9kb
MFB11	Fetal brain	Mutant M337V	1.8kb
MFB16	Fetal brain	Mutant M337V	1.5kb
MFB17a	Fetal brain	Mutant M337V	4.0kb
MFB21	Fetal brain	Mutant M337V	5kb
MFB25	Fetal brain	Mutant M337V	1.7kb
MFB27	Fetal brain	Mutant M337V	2.1kb
MFB31	Fetal brain	Mutant M337V	1.0kb

Table 5: Number of confirmed hits. These are the number of positive interactions of WT and mutant TDP-43 screened from cDNA libraries of brain and fetal brain. All these interactions have been confirmed by gap repair. Out of the total 131 interactions of both WT and mutant TDP-43, the number identified from brain vs fetal brain is: 19 vs 13 for WT, 14 vs 6 for D169G, 15 vs 4 for Q311K, 11 vs 7 for G298S, 14 vs 9 for A315T, and 6 vs 13 for M337V respectively.

Bait / **cDNA library**	**WT**	**D169G**	**Q331K**	**G298S**	**A315T**	**M337V**
Brain	19	14	15	11	14	6
Fetal Brain	13	6	4	7	9	13

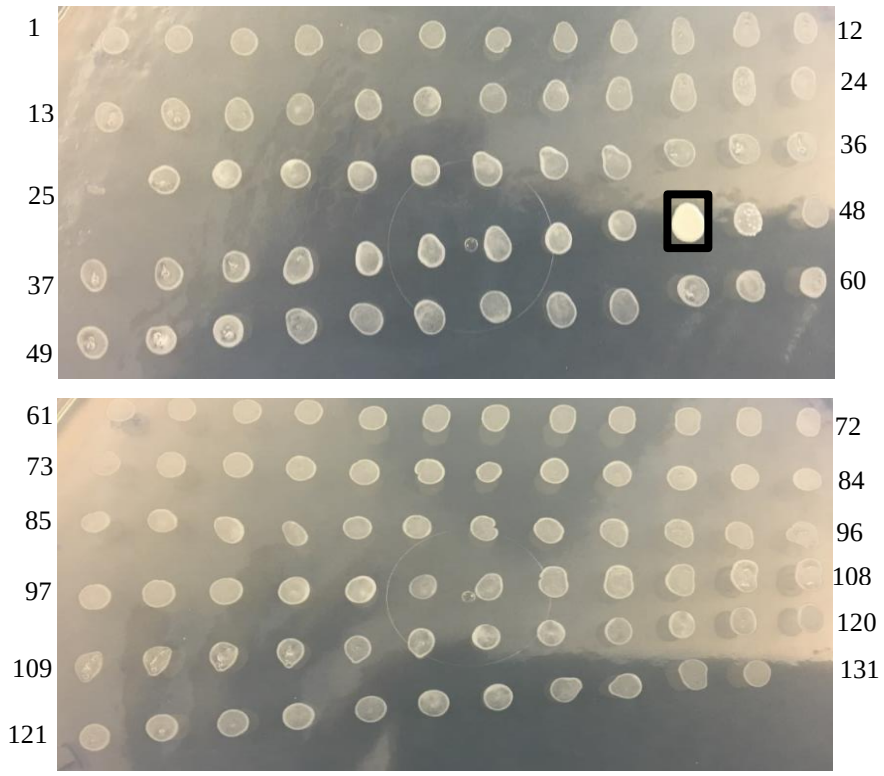

Figure 15: Verification of hits. Strains containing 131 AD constructs (confirmed hits) were mated with yeast strain containing the corresponding DB-TDP-43 constructs, of which the hits were initially identified. Cells after mating were spotted onto LT- plates (not shown) and LTH- plates. only one strong growth was observed on LTH- plates (highlighted),indicating a postive interaction. Unexpectedly, 130 out 131 prevously confirmed hits did not show intereaction with their corresponding bait: 1-18 G298S, 19-41 A315T, 42-60 M337V, 61-92 WT TDP-43, 93-112 D169G, 113- 131 Q331K.

Reverification of hits

Surprisingly, upon verification, only one hit showed a positive interaction, as shown in Figure 15. To figure out possible reason, I revived all the hits from glycerol stock (stored after Y2H screening), and retested the growth. Besides the LT- and LTH- plates previouly used, I added 3AT to LTH- plates. 3-AT, an inhibitor of HIS3 reporter gene activity, prevents growth from background activity (no interaction between bait and prey), so that it significantly decrease false positives. In addition, three control strains were added to the plates to indicate strong, weak and no interaction respectively. After this test, only 17 out of 131 hits previouly identified still showed growth, as highlighted on LTH-+3AT plates in Figure 16, indicating most of the growth previously seen on LTH- plates may be from background activity of reporter gene, not actual interaction between prey and my bait (WT and mutant TDP-43). 8 out of the 178 can further be validated through gap repair. Among these 8, 4 are from the screening using WT TDP-43 as bait (3 from library of fetal brain, and 1 from that of brain). Other four is from cDNA library of brain, one each using D169G, G298S, A315T and M337V as bait. Interestingly, none of hits for mutant TDP-43 are from cDNA library of fetal brain.

Crosscheck (Comparison)

In order to find similarities and differences between WT and mutant TDP-43 interactions, yeast strains (Y8800) containing 8 hits (1-8 in Figure 17) were mated with a yeast strain 8930 containing DB constructs of WT and mutant TDP-43 (D169G, Q331K, G298S, A315T and M337V). As shown in Figure 17, hit #2 strongly interacts with WT and mutant TDP-43. The other 7 hits showed strong interactions with WT and M337V, weak interactions with G298S and A315T, and very weak interactions with D169G and Q331K.

Sequencing of hits

The colony PCR product from 8 confirmed hits was run on gel (Figure 18). the same size of DNA bands suggests that all 8 hits may be the same gene. Sequencing confirmed it was the case. The sequences in hit #1-8 are all from *PICK1* gene, encoding a protein named as protein interacting with C-kinase 1.

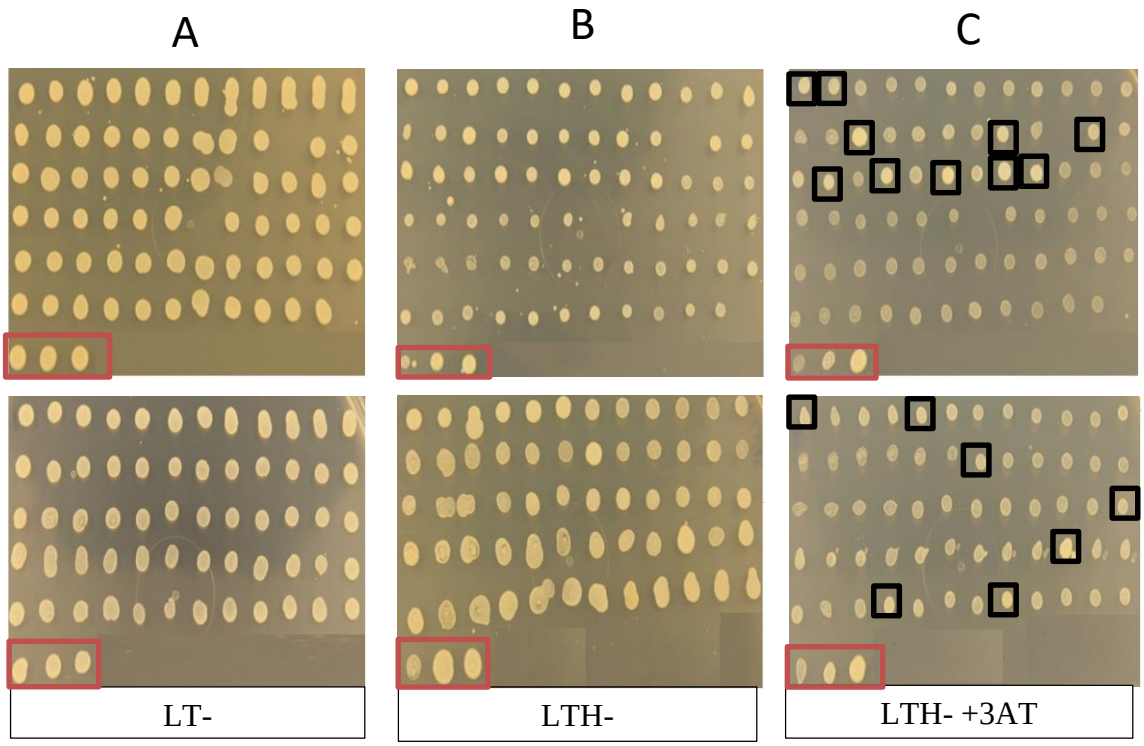

Figure 16: Reverification of Hits. Total 131 hits identified from Y2H screening of WT and mutant TDP-43 were revived from glycerol stock, and spotted onto A. LT- plates. Cell growth on LT- indicate it contains contructs of both bait and prey. B. LTH- plates. Cell growth on LTH- plates indicates potentially the positive interactions between the bait and prey. C. LTH- +3AT plates. 3AT acts as an inhibitor for *HIS* repoter gene, decreasing cell growth from background activity of report gene, so in this condition, strong and weak interactions are usually easier to distinguish. 17 colonies showed growth on LTH- +3AT plates indicating positive interaction (black boxes). The bottom row marked with pink box are the 3 control strains indicating no interaction, weak interaction and strong interaction respectively (from left to right).

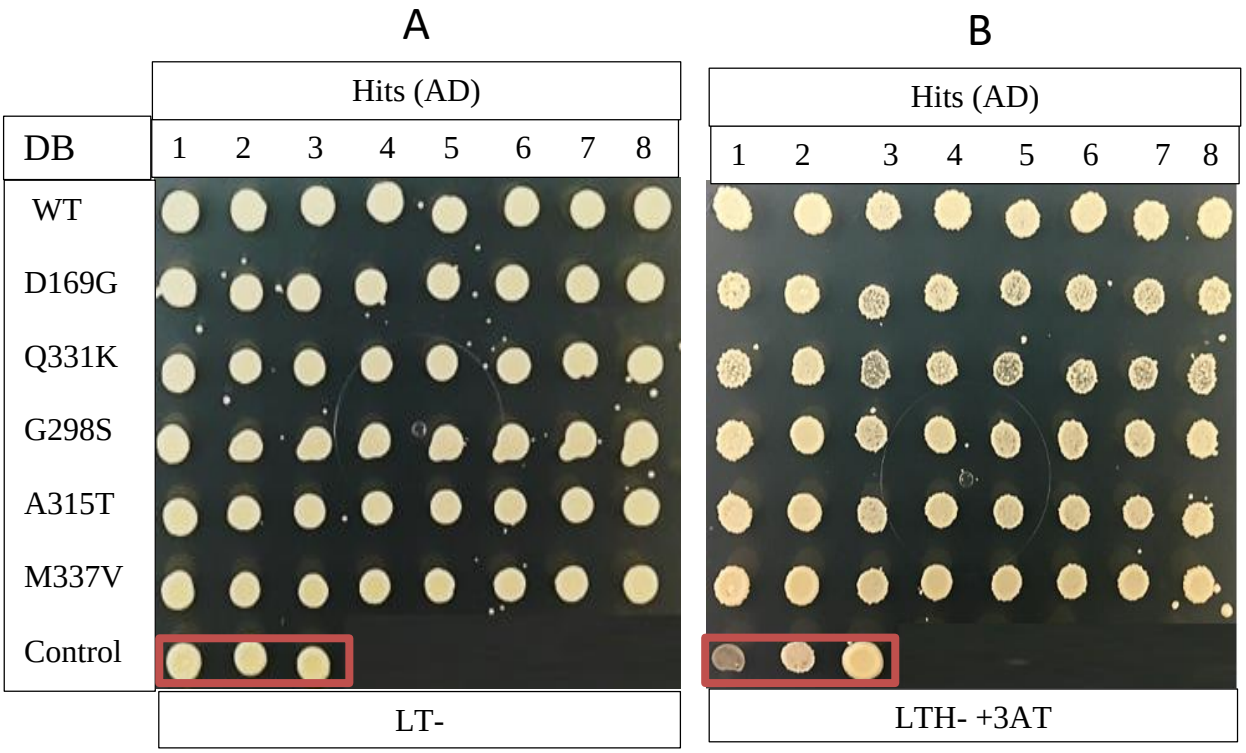

Figure 17: Crosscheck (Comparison). The 8 hits (yeast strain with AD construct re-made through gap repair) were mated with yeast strains containing DB construct of WT and five different mutants. Cells were then spotted onto LT- plate (A) and LTH-+3AT plate (B) Growth on LT- plates indicates successful mating to bring the bait and prey together. Growth on LTH-+3AT indicates positive interactions. Three control strains indicating no interaction, weak interaction and strong interaction (from left to right), were also included on each plate (marked by pink boxes).

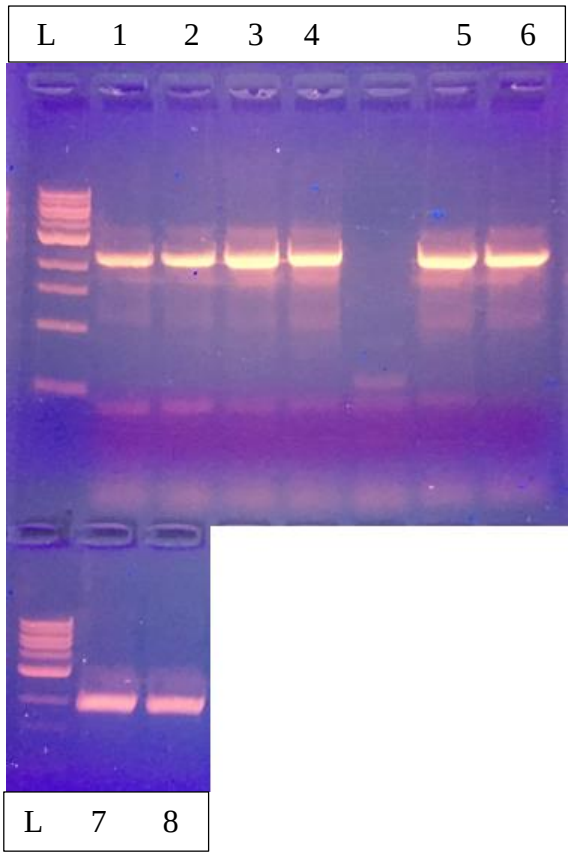

Figure 18: Electrophoresis of colony PCR products from 8 hits. The gel picture above shows DNA amplified from 8 hits. All of them showed the same band size of ~ 2kb, indicating they are might be from the same gene. L: 1 kb ladder.

DISCUSSION

The goal of this project was to identify the novel interactions with WT and mutant TDP-43 and to compare their binding partners. PICK1 was the only protein that was identified. Although PICK1 interacts with both WT and mutant TDP43, interestingly, it has weaker binding affinity to most mutant TDP-43.

Advantages and limitations of Y2H

Y2H is powerful genetic method for analysis of known, as well as identification of unknown, interactions within a living yeast cell (Brückner et al., 2009). Moreover weak and strong interactions can be easily detected by this method efficiently. The aim of this study was to screen human adult brain and human fetal brain cDNA libraries for interactors of WT and mutant TDP-43 using Y2H. I initially identified 131 potential interactors from cDNA libraries of adult human brain and fetal brain. As mentioned in table 5, out of 131 interactions, 19 interactions of WT TDP-43 were from the cDNA library of brain and 13 hits were from the cDNA library of fetal brain. The potentially positive hits of all the five mutants from cDNA library of brain were 60 and from the cDNA library of fetal brain was 39, respectively. Usually cDNA libraries consists of both highly expressed genes and low expressed genes. In order to decrease and equalize the expression of highly expressed genes, the cDNA libraries are normalized (Shcheglov et al., 2007). The cDNA libraries used in this project were not normalized. Instead I made sure ~1 million clones were screened, statistically that covers the whole genome.

Only 8 hits were considered real at the end from 131 hits initially identified. Because all initial 131 hits were supposed to be confirmed already by gap repair, this result

was very surprising and completely unexpected to us. The possible reason is that the growth initially seen on LTH- and later after gap repair was not from true activation of the reporter gene through interaction of bait and prey, instead it may have been from background activity of the reporter gene (Sobhanifar, 2003; Walhout and Mark, 2001). This is particularly a problem when the plates are incubated too long, leading to the growth of false positives. This issue, however, can be easily corrected if control strains are included to indicate the growth of no interactions, weak interactions, and strong interactions. Unfortunately, it was not what I did in my initial experiments. This is a good lesson to learn.

When all the 8 hits in figure 16 were crosschecked with DB constructs of WT and mutant TDP-43 (D169G, Q331K, G298S, A315T and M337V), although sequences of all the hits corresponded to the same gene PICK1, variability was observed in Hit 2. While other 7 hits showed weak interaction with mutant G298S and A315T, and even weaker or no interaction with mutant D169G and Q331K, hit #2 interacted strongly with WT and all mutant TDP43. The possible reason for this variablity can be that fragment of the gene in hit 2 is different from that of other hits. The weaker interactions of mutant TDP-43 also indicate that these regions of TDP-43 are important for its binding to PICK1. Further studies are needed to determine whether the weaker interaction of mutant TDP-43 with PICK1 is physiologically relevant.

It is worthy to note that my Y2H screening of TDP-43 did not identify any binding partners previously studied using other methods. This may not be necessarily surprising. Although different methods are usually complementary, they are uniquely able to identify only certain protein-protein interactions. It is interesting to know that only 20% of the

interactions are similar with different methods of screening (Ito et al., 2001). Of course, another possibility is that I did not saturate my screening, though this was not expected statistically.

PICK1 structure and function

PICK1 is a membrane protein and is ubiquitously expressed in all the tissues, especially in brain and testis (Xu and Xia, 2006). PICK1 is a mutli-domain protein, including a N-terminal acidic region (NAR), PSD-95/DlgA/ZO-1 (PDZ), Bin-Amphiphysin-Rvs (BAR) domain and C-terminal acidic amino acid region (CAR) (Li et al., 2016). NAR binds with Ca2+ and regulates the interaction of GluA2 subunit of AMPA (α amino- 3- hydroxyl-5-methylisoxazole- 4 propionic acid) with PICK1 during long term depression (Hanely and Hanely, 2005). The PDZ domain of PICK1 interacts with several membrane proteins such as acid sensing ion channels (ASICs). ASICs are involved in neuromodulation and pain perception in sensory neurons. Phosphorylation of protein kinase and Interaction of PICK1 regulates ASICs activity (Baron et al, 2002). Interestingly, the PDZ domain of PICK1 also interacts with PDZ binding domain of the coxsackie B virus and adenovirus receptor (Excoffon et al., 2004). BAR domain interacts with lipid molecules and regulates expression of PICK1 binding partners (Xu and Xia, 2006). The CAR domain, a negatively charged acidic region, hinders the lipid binding activity of BAR domain by decreasing positive charges of the BAR domain (Jin, et al., 2006).

Role of PICK1 in diseases

PICK1 is known to interact with many proteins and is involved in various diseases (Figure 19) (Li et al., 2016). The Table 6 shows some of the binding partners of PICK1. These interactions help in regulating activity of enzymes and ion channels, and the trafficking of various transporters.

Interaction between PICK1 and TDP-43

According to previous studies, the glycine rich domain of TDP-43 (Sephton et al., 2013) and PDZ domain of PICK1 are commonly involved in protein-protein interactions (Bolia et al., 2012). Are these two domains involved in their interaction with each other? I compared sequences of the 8 hits (Figure 16) from my screening, 1 to 700 nucleotides of PICK1 were the common sequences in all the hits that showed interaction with TDP-43. These 700 nucleotides correspond to the NAR domain and more than half of the PDZ domain of PICK1. Since TDP-43 does not contain any known PDZ binding motif, it is less likely that PDZ domain of PICK1 is directly involved. Thus I speculate that PICK1 interacts with TDP-43 through its NAR domain, probably with C terminal glycine rich domain of TDP-43. A future experiment can be performed to identify the specific domains that are involved in the interaction.

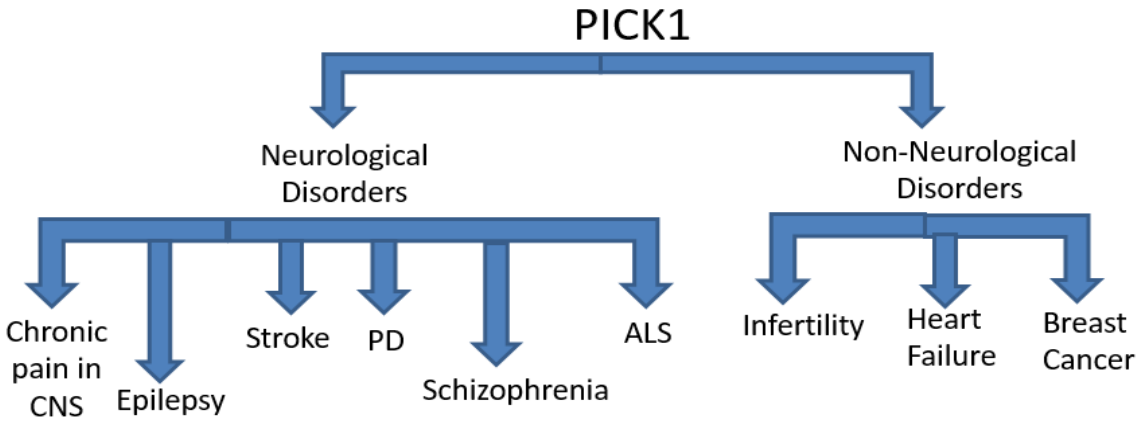

Figure 19: Role of PICK1 in various disorders. This is the schematic diagram showing the number of neurological and non-neurological disorders in which PICK1 is involved (Li et. al, 2016).

Table 6: Known protein interactions of PICK1. The table below shows the list of some of the proteins that interact with PICK1.

Gene	Protein	References
αPKC	Protein kinase Cα	Staudinger et al., 1995
EphR	Ephrin receptors	Torres et al., 1998
mGluR7a	Metabotropic glutamate receptors	Boudin et al., 2000
Arf1	Arfaptin1	Takeya et al., 2000
ErbB2	Erythroblastic leukemia viral oncogene	Jaulin et al., 2001
PrRP	Prolactin-releasing peptide receptor	Lin et al., 2001
DAT	Dopamine transporter	Torres et al., 2001
Kalirin-7	Oncoprotein	Penzes et al., 2001
ASICs	Acid-sensing ion channels	Baron et al., 2002
BNaC1	Ion channel family	Duggan et al., 2002
Netrin1	Netrin receptor	Williams et al., 2003
CAR	Coxsackie B virus and adenovirus receptor	Excoffon et al., 2004
Phosphoinositide	Lipid molecule	Jin et al., 2006

Role of PICK1 in ALS

PICK1 involvement in various neurodegenerative diseases is particularly interesting. The focus of my project is ALS, a neurodegenerative disease caused by loss of motor neurons (Rothstein, 2009). The cause of ALS is not yet known but glutamate toxicity is the common feature of this disease in human and animal models (Bosch et al., 2006). The three binding partners of PICK1: AMPA receptor, GLT-1b and serine racemase (SRR) act as key factors of glutamate mediated toxicity (Bassan et al., 2009; Dev et al., 1999; Fujii et al., 2006). And these binding partners are potentially involved in ALS (Table 7). In 2013, Focant et al. demonstrated that abnormal expression of PICK1 in astrocytes leads to development of ALS in rats. These data indicate how the interaction of TDP-43 and PICK1 may play an important role in pathogenesis of ALS.

Conclusion

PICK1 and TDP-43 are both reported to be involved in ALS, the interaction between the two may play an important role in normal and diseased conditions. Further studies need to be proceeded to determine the biological functions of this interaction, particularly whether the weaker interaction of mutant TDP43 with PICK1 is physiologically relevant.

Table 7: Known protein interactions of PICK1 involved in ALS. This table shows the three binding partners of PICK1 and their implications in ALS

PICK1 Partners	Role in ALS	References
AMPA receptor subunit GluR2	Calcium permeability – defect in mRNA process in sporadic ALS	Gardner et al., 2005
Glutamate receptor(GLT-1b)	Increased expression of GLT-1b in sporadic and familial ALS	Howland et al., 2002
Serine Racemase	D serine production in sporadic and familial ALS	Sasabe et al.,2007

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