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Methods Paper

Exploring the selective vulnerability in Alzheimer disease using tissue specific variant analysis

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ABSTRACT

The selective vulnerability of distinct regions of the brain is a critical factor in neurodegenerative disorders. In Alzheimer's disease (AD), neurons in hippocampus situated in medial temporal lobe are immensely damaged. Identifying tissue-specific variants is essential in order to perceive the selective vulnerability in AD. In current work, we aligned mRNA-seq data with HG19/HG38 genomic assembly and identified specific variations present in temporal, frontal and other lobes of the AD using sequence alignment map tools. We compared the results with the genome-wide association and gene expression quantitative trait loci studies of the various neurological disorders. We also distinguished variants and epitranscriptomic modifications through the RNA-modification database and evaluated the variant effect in the coding/UTR regions. In addition, we developed genetic and functional interaction networks to understand the relationship between predicted vulnerable variations and differentially expressed genes. We found that genes involved in gliogenesis, intermediate filament organization are altered in the temporal lobe. Oxidative phosphorylation, and calcium ion homeostasis are modified in the frontal lobe, and protein degradation, apoptotic signaling are altered in other lobes. From this study, we propose that disruption of glial cell structural integrity, defective gliogenesis, and failure in glia-neuron communication are the primary factors for selective vulnerability.

1. Introduction

Alzheimer disease is one of the most prevalent neurodegenerative disorders [1]. This disease predominately impairs memory, cognitive skill, and language perception and results in motor imbalance [2, 3] at later stages. The selective vulnerability of hippocampal neurons located in temporal lobe followed by the progression of the disease to higher cortical areas is the most distinct phenomenon in neuronal cell death.

Various hypotheses have been proposed to understand the cause of vulnerability such as protein aggregation, mitochondrial stress and calcium load. Hirai et al., [4] showed the presence of abnormalities in cytochrome oxidases and mitochondrial morphology in AD hippocampal neuron. The gamma oscillation in the hippocampus neuron demands higher energy [5]. The elevated energy demand and higher respiration rate resulted in increased production of reactive oxygen species (ROS) response in mitochondria leading to neuronal oxidative stress [6] as well as an aberration in calcium homeostasis, which induce the ROS production [7]. All the above events revealed that hippocampus neurons require a greater amount of energy to maintain their functional integrity [8, 9]. Currently, our knowledge about the factors which mainly contribute to this vulnerability is very limited. The mRNA-seq analysis of different tissues would provide deep insights to understand the effect of variants in specific tissue gene functionality and contribute to disease. In addition, determination of tissue-specific gene variants [10] are necessary to understand the selective vulnerability and neuronal death. We performed tissue-specific gene network

Abbreviations: GWAS, Genome-wide association study; AD, Alzheimer's disease; PD, Parkinson's disease; AMD, Age-related macular degeneration; ROS, Reactive oxygen Species; SAM, Sequence Alignment/Map; eQTL, Expression quantitative trait loci; RMBase, RNA modification Database; MAG, Myelin-associated glycoprotein; MTURN, Maturin; GFAP, Glial fibrillary acidic protein; MBP, Myelin basic protein; EDIL3, EGF-Like Repeats AndDiscoidin Domains 3; ENO2, enolase-2; GAPDH, glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH); COX6A1, Cytochrome C oxidase subunit 6A1; UQCR, Ubiquinol-Cytochrome C Reductase, Complex III Subunit; NDUFAB1, NADH: ubiquinone oxidoreductase subunit AB1; PRNP, prion protein; JPH3, Junctophilin 3; PSMD2, 26S proteosome non-ATPase regulatory subunit 2; UBC, Ubiquitin-C; RAF1, Ras protein family kinase; WNK1, Without no lysine; MAPK, Mitogen associated protein kinase; CDK5R1, Cyclin-dependent kinase 5 regulatory subunit 1; GABRB3, gamma-aminobutyric acid type A receptor beta3 subunit (GABRB3),; ESCRT-III, Endosomal sorting complexes required for transport; DG, Differentially expressed gene; VG, Variation gene; LG, Linker gene

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analysis of predicted variants along with differentially expressed genes [11] from transcriptome data procured from AD patients.

We have identified gene variants from temporal, frontal, and other lobes of mRNA-seq data [11] and these variants are compared with gene expression quantitative trait loci (eQTL) of the various neurological disorder for assessing the effect of variant in gene expression. Further, we evaluated the involvement of these variants in epitranscriptomic modifications. We also determined the effect of variants in transcription factors (TF), miRNA binding sites and compared with differential expression studies. In pursuance of determining the relationship between predicted vulnerable variations genes (VG) and differentially expressed genes (DG) [11], we have built genetic, co-expression and functional interaction networks. We have also identified variations in coding/UTR region and analyzed the effect of variants inregulatory elements. From the above analysis, we propose that loss of glial cell structural integrity, dysregulation of gliogenesis and aberration in neuron-glia communication, which is essential to transport the metabolic products such as lactate, pyruvate and neurotrophic factors are the major factors contributing towards selective vulnerability followed by energy demand, protein aggregation, calcium load and eventually leading to neuronal death.

2. Materials and methods

2.1. Dataset

The Alzheimer transcriptome datasets were retrieved from the Sequence Retrieval Archive (SRA) public database (PRJNA79871) and this mRNA data was procured from temporal lobe, frontal lobe and total brain (Includes temporal, frontal and other lobes) tissues of AD patients and control subjects and tabulated in Supplementary Table S1. The age groups of AD patients are above 80 and those of control are 26–69. We have directly taken the data and did not make any adjustment due to age in the present analysis [11]. This dataset includes the sequence information encoded in FASTA format and quality of each base pair encrypted in the American standard code for information interchange (ASCII) format, also known as the FASTQ file. For further analysis, these FASTQ files were preprocessed in order to remove sequencing artifacts. The Supplementary Fig. S1 depicts the workflow for variant analysis using mRNA seq data.

We have used the mRNA-seq data because the genome data are not publically available. In addition, RNA-seq is widely used for isoform identification, fusion gene detection, and variant calling [12]. Other than the current data used for this analysis, we do not have publically available appropriate large size RNA seq data to study the vulnerability in the temporal region. In order to validate the predicted variants from this study we compared the variants with the eQTL and GWAS studies of various neurological disorders. In Supplementary Table S2 we tabulated eQTL and GWAS studies used for this analysis.

2.2. Preprocessing and quality check

Next generation sequencing approach generates an immense amount of raw data from the sequencing experiments. These raw data include sequence artifacts such as, poor quality reads and adapter contamination which have an impact on the downstream analysis. However, good quality of transcriptome data is very substantial in order to avoid counterfeit outcome. Pre-processing is performed using the NGSQC toolkit to assess the quality of the data, examine the distribution of reads, non-nucleotide composition, and adapter content. Adapter contaminations are eliminated before the spliced alignment [13].

2.3. Mapping of preprocessed data with human genome reference

Sequence alignment of the transcriptome is challenging and mapping algorithms should classify the splicing and coding region. TopHat2 [14] is a spliced aligner which allows the user to map the transcriptome to the human genome assembly. HG38 has been recently updated which includes updated noncoding gene information compared to HG19. The given dataset is mapped to these two genome assemblies with genomic annotation; including genomic annotation in the alignment protocol improves the mapping quality. Mapping rate was slightly increased with HG38 (96%) genomic assembly when compared to HG19 (93%) due to enhanced noncoding annotation.

2.4. Variant calling

The preprocessed input reads are mapped against HG19/HG38 with genomic annotations.

Commonly, mRNA-seq data consists of amplified reads from polymerase chain reaction (PCR) and these reads contribute to erroneous and greater read depth during variant discovery. Therefore, amplified reads should be removed before variant calling [15]. The duplicates from the alignment file are discarded using Rmdup and PICARD toolkit and the alignment files are subjected to SNP calling using Mpileup and Varscan from Sequence alignment map (SAM) tools [16, 17]. We used the following criteria to detect the variants [15],

(i) Minimum SNP calling base Read depth ≥ 10
(ii) Minimum supporting reads ≥ 4
(iii) Minimum base quality of variant calling ≥ 30
(iv) Redo base alignment quality
(v) Transition/Transversion ratio > 2.5 (In order to avoid sequencing error)
(vi) p-value threshold for variant calling < 0.05

The variants are annotated using the ANNOVAR tool [18] which includes the details such as, gene name, genomic locations and known SNP information from dbsnp and catalog of somatic mutations in cancer (COSMIC) database. The structured query language (SQL) is used to retrieve SNP information unique to AD samples (these variants are not included in control samples).

2.5. Identifying epitranscriptomic modifications

The epitranscriptomic modifications such as N^6-methyl adenosine, 2′-O-dimethyl adenosine, 5-methyl cytidine, pseudo uridine, 5-hydroxyl methyl cytidine and N^1-methyl adenosine play an essential role in gene regulation. These types of RNA modifications are curated for human and also deposited in RMbase [19]. By comparing this database, we identified the variants, which influence RNA modifications.

2.6. Predicting the effect of variants present in the coding/UTR region

The effect of variations present in the different tissues from the AD sample is predicted using different tools such as using SIFT, PolyPhen2, Mutation Taster, Condel, Provean, MetaSVM, LRT_pred, Human splice finder and FATHMM [20–25]. The influences of structural features have been analyzed with CUPSAT [26], FOLD-X [27], I-stable [28], MU-pro [29] databases using the three dimensional structure of variants obtained with I-TASSER [30].

Identifying the functional effect of variants present in the regulatory region provides deep insights into the regulation of gene expression. Jion zhou et al., developed a method DeepSEA, deep learning based sequence analyzer based on various chromatin feature and evolutionary based conservation score for classifying the effect of variants in terms of eQTL and association of variants with disorder. The effect of identified UTR variants is predicted using GWAVA score, DeepSea score, eQTL probability, GWAS probability and HGMD probability [31, 32].

(i) DeepSea score: Deep learning network-based prediction, which

uses various chromatin features such as evolutionary information, genomic/chromatin annotation, and regulatory sequence. (DeepSea Score < 0.01 shows there is a significant functional change due to variation).

(ii) eQTL probability: Deep learning network predicts the effect of variants in terms of change in gene expression level using boosted logistic regression classifier (Higher probability (> 0.5) indicates that there is a change in expression profile due to variant).

(iii) GWAS and HGMD probability: This Deep learning network measure incorporates the effect of variants in genome wide disease traits and also predicts the consequence of variants in evolutionary conserved regulatory elements (Higher probability (> 0.5) shows that variants may affect the regulatory elements).

(iv) GWAVA score: Segregates the effect of variants based on epigenomic and genomic annotation. Score > 0.5 indicates the damaging effect of variants. (This method specific for known reported variants).

In addition, the association of predicted vulnerable variants with AD [33] and other neurological disorder has been analyzed using GWAS central [34] and PD gene database [35]. GRASP database [36] was used to identify the effect of variants with respect to triat (eQTL) of the various neurological disorders.

2.7. Predicting the effect of variants in transcription factor binding sites and miRNA binding

We used the tools, SNP2TFBS [37], Haploreg4.1 [38], MotifbreakerR [39] and rSNPbase [40] for predicting the effect of variants in regulatory elements. We obtained similar results with Haploreg and SNP2TFBS. In addition, Haploreg4.1 is updated frequently and the performance is better than others. Hence, we used Haploreg4.1 for further analysis. By employing evolutionary information we predicted the effect of variant in miRNA conserved binding motif using polymiRTS database [41], miRNASNP [42], and miRVAR [43]. The miRNASNP and polymiRTS databases are frequently updated and therefore, we used these two databases for the annotation. In order to improve the quality of prediction, we compared the predicted regulatory elements and miRNA with differential expression studies of Alzheimer's disease.

2.8. Network analysis and classification of biological function

To identify the interaction between predicted variants and reported differentially expressed genes [11], we obtained the genetic and co-expression based interaction network from the genemania cytoscape app [44]. Genemania retrieves the network from the published literature. Properties of this network are interpreted using the network analyzer. We performed community structure network analysis of co-expression networks using community cluster (GLay) cytoscape app [45] to understand the interactions between different cluster modules. In order to identify the functional relationship between these genes, we implemented functional interaction network through Reactome FI cytoscape app [46]. Biological functional classification of this interaction network is elucidated by the ClueGo cytoscape app [47].

3. Results and discussion

The transcriptome data acquired from the temporal lobe, frontal lobe and total brain (includes other lobes) tissues of AD patients and control samples (Supplementary information Table S1) (SRP004879) are subjected to variant calling protocol. Fig. 1 shows that the numbers of unique variations exclusively present in the AD sample and also located in HG19/HG38 genomic assembly.

From the above variant analysis, we found that higher numbers of variations are present in the UTR compared to the coding region. The variants associated with this region disrupt the miRNA binding and transcription factor binding. These regulatory segments are evolutionarily conserved [48]. In addition, UTR variants located in the amyloid precursor protein (APP) disrupt the miRNA binding and dysregulate the gene expression in AD [49, 50]. In dementia, 3′UTR variants present in the TAR DNA Binding Protein (TARDBP) upregulates the gene expression [51]. These reports showed that UTR variants play a major role in gene regulation and disease pathogenesis.

3.1. Temporal lobe

Temporal lobe plays a significant role in cognitive, auditory processing, verbal recognition, emotional processing, learning, spatial memory, and behavior [52]. The damaging effect of nonsynonymous variations has been predicted by different tools and tabulated in Table 1.

We observed that glial fibrillary acidic protein (GFAP) plays an essential role in preserving filament integrity. Structural and sequence analysis revealed that D295 in wild type forms a salt bridge with conserved residue K228 (Supplementary Figs. S2, S3). The mutant D295N disrupts salt bridge and hence destabilizes the protein up to 1 kcal/mol [26–29] and also this variant is associated with eQTL studies of AD [36]. Table 2 illustrates the predicted 3′UTR variants effect in transcription factor and miRNA binding using different tools and HG19/HG38 genomic assembly.

We identified Maturin (MTURN) and Myelin-associated glycoprotein (MAG) gene variants, which have not been reported in eQTL and genome wide association (GWAS) studies and these genes play an important role in glia-neuron communication. Dysregulation of this gene may interrupt the cross-talk. The predicted expression quantitative trait loci (eQTL) probability (~0.7) from the deep learning network shows that myelin basic protein (MBP), and EGF-like repeats and discoidin domains-3 (EDIL3) variants may affect the gene expression by affecting the regulatory elements (Table 2). GWAS revealed that MBP variant is also associated with Parkinson's disease (PD) and AD.

In Table 2, we listed the importance of variant in TF and miRNA binding. Novel gene variant MAG affects the transcription factors (TF) such as myocyte enhancer factor-2 (MEF2D) and SMAD family member 3 (smad3). MEF2D plays an essential role in neurogenesis and neuronal survival by activating anti-apoptotic signal whereas SMAD is involved in microglial regulation which is implicated in neuronal injury. These TFs are downregulated in AD. MAG variant disrupts the binding of miRNA hsa-miR-21-3p and miRNA signature studies showed that this miRNA is dysregulated in the AD. These observations (Table 2) demonstrate that identified UTR variants may interrupt the miRNA and TF binding.

From the genetic interaction and co-expression networks between differentially expressed genes and variation genes, we identified vulnerable variants (hub- degree ≥ 5) present in the temporal lobe depicted in Fig. 2a and details are given in the Supplementary information Tables S3 and S4.

The genetic/co-expression networks showed that the variant possessing genes such as MBP, GFAP, MAG, EDIL3, and MTURN interacts with at least five differentially expressed genes. Therefore, these genes are considered to be potential hubs. MBP gene showed a higher number of connections with increased betweenness centrality measure. MBP, MAG and MTURN genes possess increased closeness centrality measures which denote these nodes are essential for interaction. The community analysis of co-expression network (Supplementary Fig. S4a) revealed that MBP, GFAP and MTURN genes are highly interconnected within the cluster of differentially expressed genes. GFAP, EDIL3 and MAG genes interact with other cluster modules which in turn, act as bridging components.

To identify the biological functional role of detrimental variations, we implemented a functional interaction network obtained from deleterious variations predicted by current study and reported differentially expressed genes [11]. Fig. 3a illustrates functional interaction network

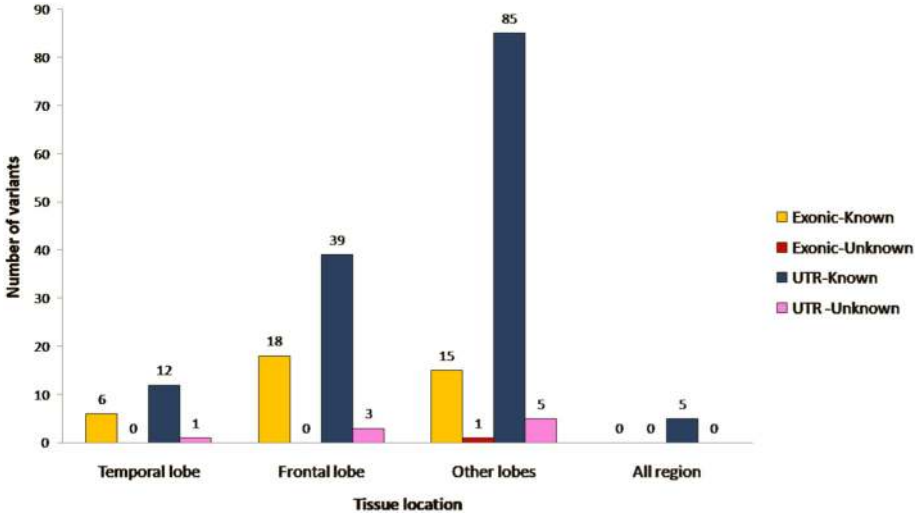

Fig. 1. Common variations present in the AD samples procured from two different genomic assemblies (HG19/HG38).

Table 1
The functional effect of non-synonymous variants predicted by various tools from the AD sample and classified based on the tissue location.

Tissue	Gene name	Amino_acids	SNP_ID	Score (out of 8)	SIFT	Poly phen	FATHMM	LRT	Meta SVM	Mutation taster	PROVEAN	Condel
Temporal	GFAP	D295N	rs1126642	6	1	1	1	1	0	0	1	1
Temporal	SEPT4	E212V	rs17741424	5	1	1	0	1	0	0	1	1
Temporal	CIRBP	R81C	rs111547108	1	1	0	NA	NA	NA	0	NA	0
Frontal	ARL8A	H147Y	rs150397242	1	NA	0	0	NA	NA	1	NA	–
Frontal	ANAPC5	A630V	rs13141	2	0	0	0	1	0	1	0	0
Frontal_Other lobes	SCAF1	K1231R	rs61743199	4	0	1	0	0	0	1	1	1
Frontal_Other lobes	SPARCL1	T419A	rs1130643	1	0	0	1	0	0	0	0	0
Frontal_Other lobes	COX6A1	H76Y	rs140243339	3	0	0	0	1	0	1	1	0
Other lobes	IFITM2	I121V	rs1059091	1	0	0	1	NA	0	0	0	0
Other lobes	PHB	R43L	rs2233665	5	0	0	1	1	1	1	1	0

1: Damaging effect; 0: Neutral effect; NA- Not Available.

of the temporal lobe and details are given in Supplementary information Table S5. Some of the important interactions are discussed below.

Differentially expressed genes (cyan), variant genes (Pink), Linker genes (Orange). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 3a showed that GFAP variation gene (VG) interacts with differentially expressed gene (DG) desmin (DES) through linker gene (LG) vimentin (VIM). These genes belong to the family of intermediate filaments. GFAP and VIM genes are essential for the stability of astrocytes and disruption of these genes resulted in improper myelination of axons, diminished glial scar formation and lead to the abnormal structural integrity of the white matter [59] GFAP, vimentin, and desmin are necessary for axonal lysosome mobility [60]. Hence, variation in GFAP may affect the structural integrity of astrocytes. MAG (VG) catalyzes myosin light chain (MYL9) (DG) through activating Ras Homolog Family Member A (RHOA) (LG). EDIL3 (VG) also activates RHOA by triggering protein tyrosine kinase (PTK2) (LG). Earlier studies showed that MYL9 interacts with N-methyl-D-aspartate (NMDA) receptors and regulates hippocampus neuron [61]. Therefore, defects in MAG and EDIL3 genes may affect the myosin motor regulation.

Biological classification of the functional interaction network of this region revealed that genes responsible for gliogenesis, sleep regulation, long-term synaptic potentiation, intermediate filament organization, G-protein coupled receptor signaling and nucleotide metabolism are altered. Supplementary Fig. S5a depicts the functional classification of variants and differentially expressed genes. The critical biological functions of predicted vulnerable variants present in the temporal lobe of AD patient are discussed below.

3.1.1. Gliogenesis and axonogenesis

Glial cells play an essential role in brain function, which helps neuron by providing energy supplements, structural stability, support, guarding neurons against pathogenic invaders and also regulates neurotransmission. Unlike neurons, glial cells can regenerate and the development process of glial cell is known as gliogenesis. Disruption of gliogenesis may result in loss of tissue repair during injury. MAG gene is involved in glia and axon signaling and also performs a bidirectional role in axon protection and inhibition during the injury by interacting with RHOA (small GTP-Binding Protein RhoA) [62]. Prior reported studies exhibit that MAG is involved in axonal protection and enhances the stability of myelin and axonal interaction [63]. MTURN gene is essential for neurogenesis and abundantly present in the hippocampus, amygdala, and cerebellum [64]. MTURN gene variant has not been reported in dbsnp. eQTL probability (Table 2) predicts that significant functional changes due to variations in the 3′ untranslated region of these genes may influence the regulation of transcript which is crucial for glia and neuron communication. Therefore, variations in these genes may disrupt the gliogenesis.

3.1.2. Intermediate filament organization

Intermediate filaments play a major role in maintaining axon stability. GFAP belongs to the class of intermediate filaments which mainly contributes to the structural integrity of astrocytes. During tissue damage/injury, these glial cells undergo reactive gliosis (increased amount of glial cells recruited in the injured area and shield the injured area from toxic substituents). GFAP also plays a crucial role in glia-neuron communication and serves as a glial cell biomarker [65]. However, variation in the coding region of GFAP (Table 1) (D295N)

Table 2
The effect of predicted 3′UTR variants in TF and miRNA binding.

Tissue location	Gene name	GWAS	Deep sea, GWAVA score	eQTL, GWAS, HGMD probability (%)	Predicted regulatory motifs/TFBS	Function & gene expression studies of predicted TF involved in AD	Predicted miRNA targets & gene expression studies of predicted miRNA in AD	SNP ID
Temporal lobe	EDIL3	PD	0.002,0.72	71.78,78.38,51.35	FOXd3, FOXO	ROS regulation, Mitochondrial permeability, neuronal survival, Upregulated [53]	NA	rs148788452
	MAG	NA	0.007,0.58	57.93,66.07,51.72	MEF2D, SMAD	Neurogenesis, Neuronal survival and regulation of microglial activation, Downregulated [54, 55]	hsa-miR-21-3p, Downregulated [56]	rs73031737
	MTURN	NA	0.007,NA	93.05,87.59,58.24	NA	NA	NA	NA
	MBP	PD,AD	0.673,0.41	80.91,56.84,15	HOXD10	Apoptosis, Upregulated [57]	hsa-miR-124-3p,hsa-miR-34b-5p, downregulated [58]	rs9199
Temporal, frontal and total brain	VPS4A	PD, Prion	0.003,0.58	90.17,87.65,56.94	NA	NA	NA	rs12258
	ACTR1A	Bipolar	0.039,0.64	80.46,74.89,50.92	NA	NA	hsa-miR-487a-5p, Dysregulated	rs5870
	PTGDS	AD	0.014,0.76	58.19,60.87,58.51	EGR1	Regulates APP processing, Up regulated	NA	rs6926

PD- Parkinson disease, AD-Alzheimer's disease, AMD-Age related macular degeneration, NA- Not Available.

may affect the functional and structural integrity of cytoskeleton assembly. The same variation has been reported for Alexander disease [66].

Myelination is essential for high-speed electrical conduction over the long destination. MBP plays a crucial role in maintaining the structural integrity of myelin sheath and it is also important for the development of oligodendrocytes. It supports neuron by providing neurotrophic factor and energy supplements such as lactate [67, 68]. Earlier studies reported that MBP degrades the beta amyloid through autocatalytic cleavage mechanism and inhibits fibril formation [69]. The predicted eQTL probability (Table 2) revealed that variation in this gene may alter the regulatory features responsible for transcript formation which in turn affect the structural integrity of myelin sheath.

3.2. Frontal lobe

The frontal lobe plays a crucial role in decision making, language, processing short-term and long-term memory [70]. Table 3 illustrates the predicted 3′UTR variants effect in TF and miRNA binding using different tools (frontal lobe). The predicted eQTL and human gene mutation data (HGMD) probability revealed that several variants listed in Table 3 may alter the gene expression. Specifically, gene variants such as, ATPase H+ transporting V0 subunit-D1 (ATP6V0D1) and dynactin (DCTN1) showed higher predicted eQTL probability (~0.6) and have not been reported in GWAS of the AD. Gene expression profile studies showed that ATP6V0D1 and DCTN1 genes are differentially expressed in dementiated AD brain [71–76]. This shows that the predicted variants may modulate the gene expression. RAP1-GTPase activating protein-2 (RAP1GAP2) gene is involved in the regulation of axonogenesis participates in neurotrophin signaling and associated with amyotrophic lateral sclerosis (ALS). Furthermore, we identified gene variants such as DCTN1 and ATP6V0D1 play a crucial role in lysosomal/endosomal transport. From the predicted eQTL probability the above discussed gene variants may alter the gene expression pattern.

We showed that these gene variants interrupt the binding of TF, miRNA binding and implicated in AD, which play an essential role in mitochondrial energy metabolism and cholesterol regulation. Disruption of these TFs may lead to aberrant energy production which in turn damages neuronal functionality. Fig. 2b illustrates the genetic and co-expression network between differentially expressed genes and variation genes in the frontal lobe.

The genetic/co-expression networks illustrates variation genes such as enolase-2 (ENO2), glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH), Junctophilin-3 (JPH3), NDUFAB1, prion protein (PRNP), and COX6A1 interact at least five differentially expressed genes with increased closeness centrality (~0.4) and neighborhood connectivity (~8) so that these genes are crucial for interaction and act as potential hubs. The community analysis showed that ENO2 gene interacts with five differentially expressed genes within a cluster and this gene interacts with other clustering modules also (Supplementary Fig. S4b). This results show that ENO2 is essential for network communication.

Fig. 3b illustrates the functional interaction network of the frontal lobe, which depicts the relationship between the differentially expressed genes and vulnerable variations. Some of the important interactions are discussed below: Fig. 3b showed that ENO2, GAPDH (VG) regulated by E1A Binding Protein-P300 (EP300) (LG) and cAMP-response element binding protein (CREBBP) (LG). ENO2 and GAPDH interact with phosphoryl enol pyruvate carboxylase (PCK1) (DG). These genes are essential for producing secondary metabolites in glycolytic/gluconeogenesis pathways and these variations may result in abnormal energy metabolism. COX6A1 (VG) gene associated with complex IV subunit of electron transport chain. Functional interaction network revealed that impairment of this gene activates cytochrome-c (CYCS) (LG) that triggers caspase-3 (CASP3) (LG) which plays a decisive role in cell death and synaptic plasticity [90]. JPH3 (VG) functionally interacts with inositol 1,4,5-trisphosphate receptor type-3 (ITPR3) (DG) which

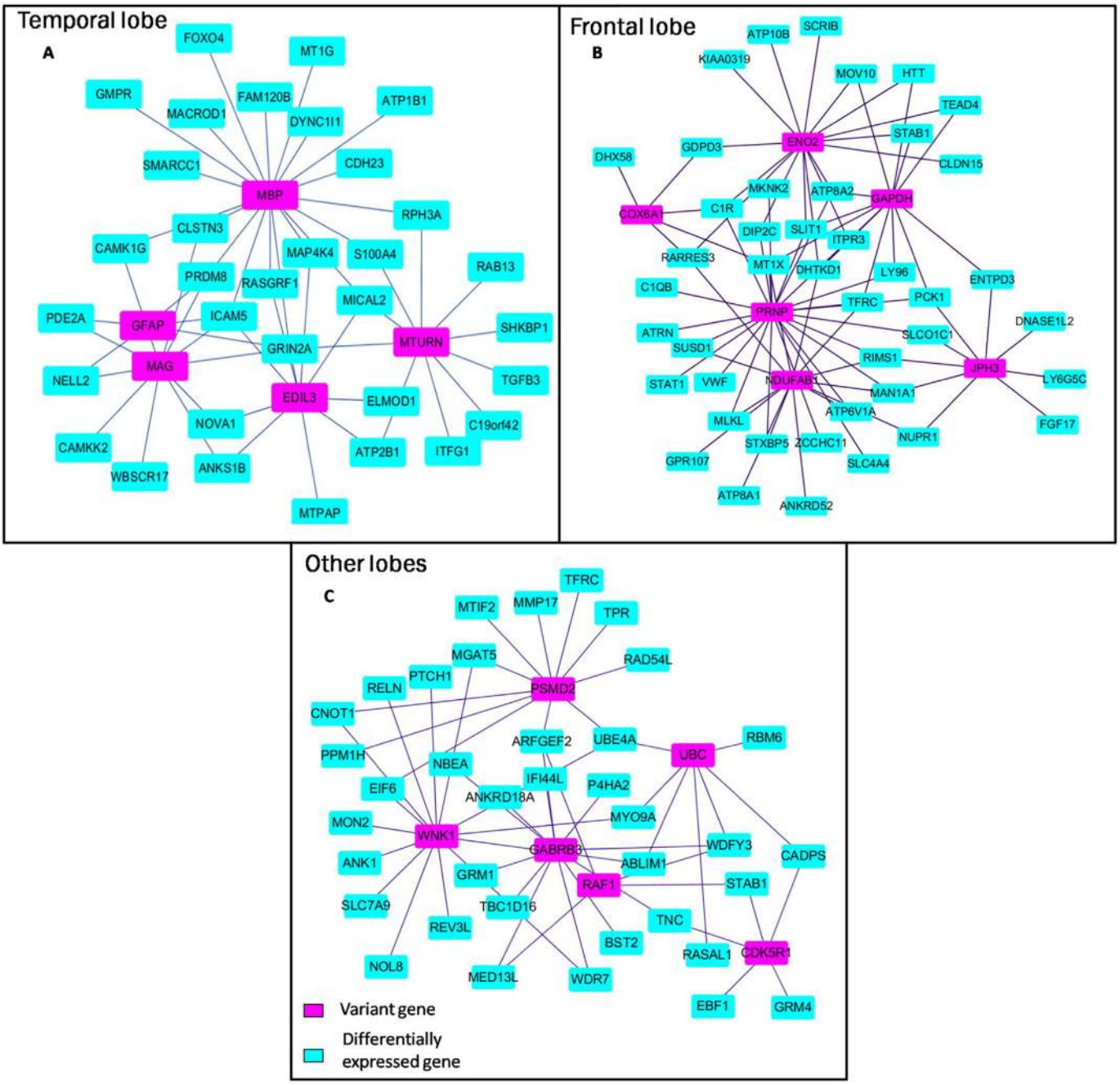

Fig. 2. Co-expression and genetic interaction network between differentially expressed genes and variant genes A) Temporal lobe B) Frontal lobe C) Other lobes. Differentially expressed genes (cyan), variant genes (Pink). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

regulates the intracellular calcium release via activating transient receptor potential cation channel subfamily C member-3 (TRPC3) (LG). Earlier studies reported that junctophilin knockout mice in hippocampal neurons lack calcium ion regulation and synaptic plasticity [91]. Therefore, variation in junctophilin may impair the intracellular calcium signaling.

Biological classification of the functional interaction network explained that genes involved in the precursor of metabolite and energy production, regulation of protein localization of membrane, ion homeostasis, neuron death, synaptic transmission and genes implicated in learning and memory are affected in the frontal lobe. Supplementary Fig. S5b illustrates the functional role of variations and differentially expressed genes. The critical biological functions of the predicted vulnerable variations present in the frontal lobe of AD patient are discussed below.

3.2.1. *Pyruvate metabolic process*

Pyruvate is an important secondary metabolite in glycolysis cycle and also acts as a precursor of lactate. Glial cells predominately supply lactate to energy demanding neurons, which is the primary resource for oxidative phosphorylation (OXPHOS) [92]. The gene expression profile studies illustrate that metabolic gene such as ENO2 and GAPDH are downregulated in AD patients [93]. The predicted eQTL probability showed that changes in the housekeeping genes such as GAPDH and ENO2 diminish the energy metabolism. ENO2 is crucial for catalyzing the phosphoenol pyruvate production and predominately present in the

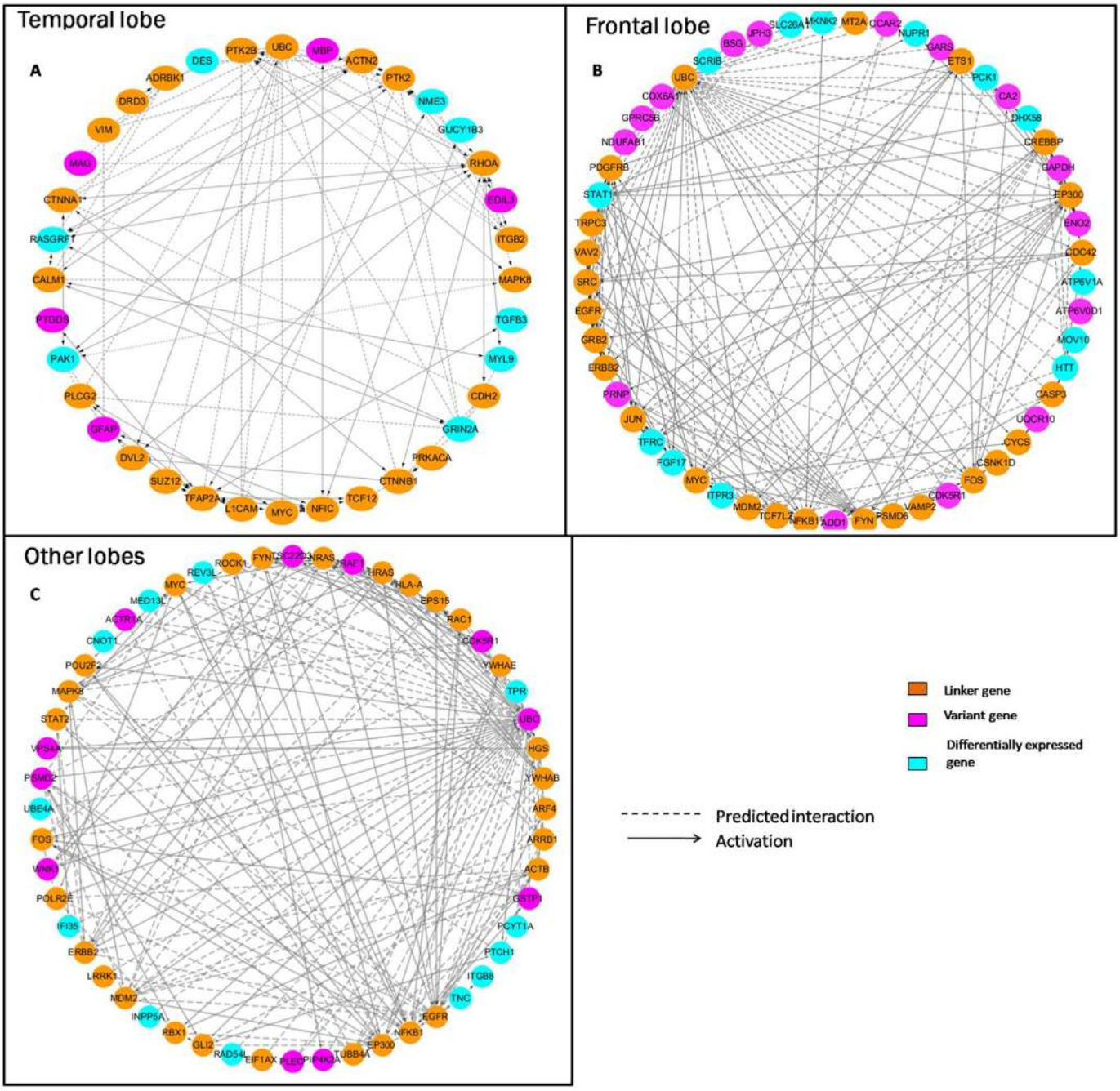

Fig. 3. Functional interaction network between differentially expressed genes and variant genes A) Temporal lobe B) Frontal lobe C) Other lobes.

glial, neuronal cell population. GAPDH is pivotal for the glycolytic cycle and also associated with cell survival by preserving the adenosine triphosphate (ATP) level and GAPDH participates in cell death through various mechanisms such as it induces caspase-independent cell death due to ATP decline. In addition to that, this gene forms a stabilized complex with E3 ubiquitin ligase and this complex interacts with CREB binding protein and induces apoptosis [94]. Itakura et al., [95] demonstrated that GAPDH aggregation stimulates the amyloidogenesis which results in mitochondrial dysfunction and also this variant was reported in GWAS of the AD. eQTL probability and genome wide annotation of variants (gwava score) (Table 3) predicted that variation in the UTR region of these genes may hinder the transcripts which are essential for energy production process.

3.2.2. *Oxidative phosphorylation*

In neurons, an abundant amount of energy is produced by oxidative phosphorylation which involves four different complexes. The variation study demonstrates that gene associated with complex I and IV is impaired in the frontal lobe. The final enzyme in OXPHOS is Cytochrome-C oxidase subunit-6A1 (COX6A1) is also known as complex IV involved in energy production by creating a proton electrochemical gradient across the mitochondrial membrane which stimulates ATP synthase in pursuance of ATP production [96]. The current study showed that there is a nonsynonymous variation (H76Y) exists in this gene. NADH: ubiquinone oxidoreductase subunit-AB1 (NDUFAB1) denoted as complex I implicated in transferring the electron from NADH to ubiquitin-c in the electron transport chain. The gene expression studies demonstrate that genes responsible for oxidative phosphorylation are downregulated in AD patients [97]. Analysis using the mutation taster tool and human

Table 3
The effect of predicted 3′UTR variants in TF and miRNA binding (Frontal lobe).

Gene name	GWAS	Deep sea, GWAVA score	eQTL, GWAS, HGMD probability (%)	Predicted regulatory motifs/TFBS	Function & gene expression studies of predicted TF involved in AD	Predicted miRNA targets & gene expression studies of predicted miRNA in AD	SNP ID
DCTN1	NA	0.013,0.71	59.94,61.81,48.98	NR2C2	Mitochondrial energy production, Downregulated [77]	NA	rs11555696
ATP6V0D1	PD	0.081,0.52	66,65.9,49.6	NRSF	Neuroprotection, Downregulated [78]	NA	rs72650132
CSRP1	AD	0.045,0.63	59.7,79.78,48.06	VDR	amyloid protein regulation, Downregulated [79]	hsa-miR-4701-3p Downregulated in ALS [80]	rs138467738
NAPB	Prion	0.002,0.56	69.99,77.74,55.87	AP1	Programmed cell death, Upregulated [81]	NA	rs8615
				ATF3	Regeneration of axon after injury, Dysregulated [82]		
TPP1	PD,AD	0.013,0.34	53.95,57.91,48.14	CEBPD, NRF1, NRSF, VDR	regulates neuronal inflammation, mitochondrial energy metabolism and neuroprotection, Downregulated [78, 83, 84]	hsa-miR-301a-3p,hsa-miR-301b, hsa-miR-130b-3p Dysregulated in AD [85–87]	rs7487
RANGAP1	AD	0.006,0.48	61.48,71.86,51.14	SMAD3	microglial activation, Downregulated [55]	NA	rs9611526
JPH3	AD	0.102,0.32	69.59,79.1,52.02	CTCF	Regulates APP, Dysregulated [88]	NA	rs11859208
PRNP	AD	0.044,0.51	70.15,67.66,45.88	NA	NA	NA	rs7274758
GAPDH	AD	0.027,0.63	46.51,5.46,50.99	NA	NA	NA	rs1136666
ENO2	AD	0.116,0.7	70.5,74.35,45.7	NA	NA	NA	rs1061223
RAP1GAP2	ALS	0.008,0.25	75.86,71.89,52.94	NA	NA	NA	rs1133326
BACE1	PD, AD, Prion	0.088,0.56	66.16,77.09,45	SREBP2	Cerebral Cholesterol homeostasis, Dysregulated [89]	NA	rs7083
CCAR2	PD,AD	0.073,0.43	76.57,80.02,47.38			NA	rs1059592

PD- Parkinson disease, AD-Alzheimer's disease AMD-Age related macular degeneration, NA- Not Available.

splice finder [25] predicts that this variations affect the exonic splicing enhancer and hence it may affect the splicing event.

3.2.3. Calcium ion homeostasis

Regulation of calcium plays a vital role in synaptic transmission and second messenger signaling. The variation analysis showed that there is a dysregulation of genes involved in the calcium ion regulation. Cellular prion protein (PRNP) participates in preserving the calcium ion homeostasis. PRNP deficient mice exhibits loss of intracellular calcium ion equilibrium [98]. BACE1 plays a significant role in reducing beta-amyloid formation inhibiting via beta-secretase cleavage (BACE1) of the amyloid precursor protein. However, cellular prion protein is reduced in sporadic AD patient [99, 100]. BACE1 (Table 3) variant associated with eQTL studies of AD [36] disrupts the regulatory motif (Table 3) of sterol regulatory element-binding transcription factor (SREBP) which plays an essential role in cholesterol regulation in glia. Previous studies showed that in AD, impairment of SREBP dysregulates the gene expression of BACE1. JPH3 gene is specifically abundant in the brain region and plays a decisive role in communication between the cell surface and calcium ion channel. Mice lacking junctophilin resulted in aberrant neuronal excitability and disruption in motor coordination [101]. This variant showed high GWAS probability as well as associated with eQTL studies of ALS [36]. The above discussed genes impair the calcium ion equilibrium that serves as essential messenger for neuronal survival and death.

3.3. Other lobes

The predicted UTR variants effect in transcription factor and miRNA binding using different tools from the other lobes of AD sample using HG19/HG38 genomic assembly are listed in Table 4. We identified novel gene variants such as cyclin-dependent kinase 5 regulatory subunit-1 (CDK5R1), synaptosome associated protein-91 (SNAP91) and G-Protein subunit alpha-Z (GNAZ) present in other lobes, which are not reported in eQTL or GWAS of AD. The gene expression studies revealed that these genes are differentially expressed in AD and cognitively impaired subjects [102–104]. This shows these gene variants may disrupt the gene expression pattern. GNAZ gene plays a critical role in preserving ion equilibrium, and chaperone mediated folding. SNAP91 plays an essential role in synaptic vesicle transport and dynamics. Dysregulation of this gene may interrupt synaptic transmission and also these variants shows higher eQTL probability.

The data showed (Table 4) that the predicted variants disrupt the transcription regulatory elements which are essential for regulation of amyloid protein aggregation, cell survival and neuronal death. Chaperonin Containing TCP1 Subunit-5 (CCT5), and Tropomyosin-3 (TPM3) gene variants exhibit increased eQTL and GWAS probability. The gene expression studies showed that these genes are differentially expressed in AD samples [71, 109]. Hence the predicted variants may affect the gene expression profile pattern. Golgi Autoantigen, Golgin Subfamily-B1 (GOLGB1) plays an essential role in vesicle transport, golgi organization and cilia assembly. TPM3 is important for stabilizing the actin filaments which is crucial for axonal transport also this variant is present in GWAS of AD and PD. CCT5 acts as a molecular chaperone and facilitates folding of actin and tubulin, pivotal for vesicle transport and also this variant is associated with the AD and age-related macular degeneration (AMD). The eQTL association studies revealed that CCT5, UBC variants are associated with ALS [36]. Additionally, by comparing RNA-modification database (RMbase) we found that SLAIN1 gene variant may disrupt N^6- adenosine methylation, which in turn may affect transcript regulation. SLAIN1 gene abundantly present in brain and spinal cord tissues, but the functional implication of this gene is still uncovered.

By constructing, the genetic interaction and co-expression networks from this region, we identified essential variants. Fig. 2c illustrates the genetic and co-expression network between differentially expressed

Table 4
The effect of predicted UTR variants in TF and miRNA binding (other lobes).

Gene name	GWAS	Deep sea, GWAVA score	eQTL, GWAS, HGMD probability (%)	Predicted regulatory motifs/TFBS	Function & gene expression studies of predicted TF involved in AD	Predicted miRNA targets & gene expression studies of predicted miRNA in AD	SNP ID
GNAZ	NA	0.115,0.56	76.79,75.63,54	EWSR NRSF	Controls protein aggregation, Upregulated [100] Neuroprotection, Downregulated [78]	hsa-miR-185-5p, Upregulated [50]	rs13407
SNAP91	NA	0.015,0.59	80.33,78.87,55.69	FOXA, FOXO	ROS regulation, Mitochondrial permeability, neuronal survival, Upregulated [53]	hsa-miR-487b-3p, Downregulated [50]	rs141242512
GOLGB1	Dyskinesia	0.121,0.6	59.93,76.02,47.93	HIF1	Neuroprotection, Dysregulated [105]	NA	rs1169
SLAIN1	AMD, Prion	0.005,0.4	58.43,75.2,54.92	SMAD3, SMAD	microglial activation, Downregulated [55]	NA	rs9600927
GPM6A	AD	0.002,0.66	76.75,77.26,50.07	NA	NA	hsa-miR-187-5p, Dysregulated [58]	rs17061725
EPHA4	AD	rs8508	0.045,0.48	NA	NA	hsa-miR-520 g-3p,hsa-miR-520 h, Upregulated [56]	rs8508
PSMD2	Bipolar	0.035,0.58	77.95,78.97,49.16	AP-4 RXRA	regulates Protein aggregation, Dysregulated [106] cholesterol metabolism, Upregulated [107]	hsa-miR-582-5p, Upregulated [58]	rs6845
RAF1	PD, AMD	0.146,0.39	75.72,79.23,49.65	NRF1	mitochondrial energy metabolism, Downregulated [78]	hsa-let-7i-3p, Upregulated [58]	rs1051208
WNK1	AD	0.008,0.59	79.52,84.42,50.87	FOXA, FOXF	ROS regulation, Mitochondrial permeability, neuronal survival, Upregulated [58]	NA	rs1060499
GABRB3	AD	0.084,0.49	75.4,72.79,47.52	NF-kB, RXRA	Cell survial/death, cholesterol metabolism, Upregulated [107, 108]	NA	rs2017247
UBC	AD	0.003,0.51	54.5,9.81,59.61	NA	NA	NA	rs41276688
CCT5	AMD, AD	0.061,0.73	77.79,65.93,44.43	NA	NA	NA	rs544
TPM3	PD, AD	0.027,0.54	72.4,76.1,47.75	NA	NA	NA	rs1051370
CDK5R1	NA	0.003,NA	70.19,75.95,51.99	NA	NA	NA	rs780753296
RNF220	AD	0.031,0.41	78.53,80.84,48.59	SREBF2	cholesterol metabolism, Dysregulated [89]	NA	rs2822

PD- Parkinson disease, AD-Alzheimer's disease AMD-Age related macular degeneration, NA- Not Available.

genes and variation genes in the other lobes.

The genetic and co-expression network showed that 26S-proteasome non-ATPase regulatory subunit-2 (PSMD2), Ubiquitin-C (UBC), Without no-lysine (WNK1), gamma-aminobutyric acid type-A receptor beta-3 subunit (GABRB3), Ras protein family kinase-1 (RAF1), and cyclin-dependent kinase-5 regulatory subunit-1 (CDK5R1) are considered to be potential hubs. These genes interact at least with five differentially expressed genes. However, these genes possess increased neighborhood connectivity (~8) in the network, which plays an essential role in cross-talk signaling. The community network analysis demonstrate that PSMD2 and RAF1 genes interact with at least five differentially expressed genes and serve as bridging nodes between different clusters (Supplementary Fig. S4c).

Fig. 3c illustrates the functional interaction network, which represents the association between the differentially expressed genes and predicted vulnerable variations. Some of the crucial interactions are discussed below. Fig. 3c explains that PSMD2 (VG) catalyzes the TF glioma-associated oncogene family zinc finger 2 (GLI2) (LG) which in turn regulates the expression of patched1 (PTCH1) (DG). These types of interaction are essential for ciliary assembly and hedgehog signaling [110, 111]. During tissue injury, this signaling is crucial for tissue repair and adult neurogenesis. Differential expression studies showed that PTCH1 gene is downregulated in AD patients [11]. Therefore variation in PSMD2 may result in aberrant hedgehog signaling. PSMD2 (VG), WNK1 (VG) and RAF1 (VG) genes activate the transcriptional regulator nuclear factor kappa-B subunit-1 (NFKB1) (LG) by phosphorylating inhibitors of NF-kB [112]. Activation of NF-kB is a critical event which decides the cell survival or cell death by interacting with p53 [113]. NF-kB regulates tenascin-C (TNC) (DG) and REV3-Like, DNA Directed Polymerase-Zeta Catalytic Subunit (REV3L) (DG). TNC plays a critical role in the production of inflammatory cytokines during tissue injury [114] and REV3L is crucial for DNA synthesis and repair at the time of cell stress [115]. RAF1 and WNK1 catalyze UBC (VG) which in turn functionally interacts with ubiquitination Factor E4A (UBE4A) (DG) which is an essential element for protein degradation mechanism. Differential expression studies revealed that above discussed genes are downregulated in AD patients [11]. PSMD2, WNK1, and RAF1 exhibit higher eQTL probability this results shows that these variants may affect the gene expression and may dysregulate defense machinery.

Functional interaction network between vulnerable predicted variants and differentially expressed genes in this region suggests that there is impairment in gene involved in the apoptotic process, regulation of neuron projection development, regulation of defense response, protein targeting to membrane, and regulation of the catabolic process. Supplementary Fig. S5c illustrates the functional role of predicted variants and differentially expressed genes. The essential biological functions of the predicted vulnerable variants present in this region are discussed below.

3.3.1. Regulation of defense response

The damaged or misfolded proteins degraded by proteasomal/lysosomal-mediated mechanism, which is an essential factor in the quality control of various organelles. UBC conserved protein participates in ATP-dependent proteasomal degradation mechanism through two different aspects: (i) Forming a covalent bond between poly-ubiquitin and misfolded protein and (ii) Poly-ubiquitin chain recognized by the 26S-proteasome and subjected to degradation. Previous studies addressed that ubiquitin is assembled in senile plaque/tangles of AD brains, but there is an impairment in degradation mechanism [116, 117]. This suggests that there may be abnormalities in the proteasomal degradation system due to energy dysfunction. PSMD2 is involved in degrading the misfolded proteins through an ATP-mediated degradation mechanism [118].The eQTL, HGMD probability and GWAVA score (Table 4) predicted that there is a significant functional change due to this variation in the UTR regions which may disrupt the transcript responsible for degradation mechanism.

3.3.2. Positive regulation of kinase activity

A protein kinase is the key regulator of various signaling pathways by activating/inactivating effector proteins. RAF1 and WNK1 participate in mitogen-activated protein kinases (MAPK) cascade during the oxidative, and proteolytic stress which in turn triggers MAPK signaling resulting in activation of NF-kB which plays an important role in cell survival and neuronal death [119]. Earlier studies reported that RAF1 and WNK1 genes are differentially expressed in the AD patients [120]. DeepSea network (Table 4) predicted that these genes showed a higher eQTL probability due to variations in the untranslated region. This type of variation may dysregulate the kinase activity and also associated with AD, PD and AMD.

3.3.3. Apoptosis

Apoptosis is the most critical regulatory process which controls the cellular population. CDK5R1 gene plays a bi-directional role in neuronal cell survival and death. The neuronal survival mechanism is mediated through neuregulin which in turn phosphorylates AKT, and this sort of activation downregulates the apoptotic process [121]. Furthermore, during the tissue damage, CDK5R1 (p35) phosphorylates p53 (tumor suppressor) and activates the caspase cascade leads to apoptotic process [122]. DeepSea score, eQTL probability and GWAS probability (Table 4) showed that UTR variation may lead to functional disruption of the transcript crucial for neuronal survival. GABRB3 is involved in ceramide-dependent neuronal apoptosis and also showed that mice lacking this gene exhibit diminished learning, memory, and motor activity [123]. The increased eQTL and GWAS probabilities (Table 4) suggest that changes in the regulatory elements may disrupt the transcript responsible for apoptosis.

The variants present in the temporal, frontal and total brain tissues are listed in Table 2. The important function of these gene variants are discussed below.

3.3.4. Disruption of motor protein function

Generally, motor proteins act like cargo. Microtubule-associated motor protein dynactin composed of actin-related protein-1 (ACTR1A) is essential for vesicle, exosome, lysosome transport and retrograde axonal transport, which carries the damaged mitochondria to soma for the cell repair or degradation. Inhibition of the dynactin resulted in blockage of cargo motility in axoplasm [124]. This type of cargo requires ATP for their transport. This variant interferes the binding of hsa-miR-487a-5p and also involved in AD [85]. Furthermore, DeepSea network and GWAVA score (Table 2) predicted that there may be expression level change due to UTR variant. By comparing RMbase the ACTR1A variant may hinder the N^6- adenosine methylation, which may inhibit cargo activity and also associated with eQTL studies of AMD [36].

3.3.5. Disruption of lysosomal/endosomal function

The misfolded protein degradation mediated by endosomal sorting complexes required for transport (ESCRT-III). It interacts with evolutionary conserved vacuolar protein sorting 4-homolog-A (VPS4A), which is a major regulator of vesicle trafficking, by forming heptamer and this complex catalyzed by ATP. Functionally impaired protein tagged using ubiquitin perceived by ESCRT-III and it transports the tagged complex for lysosomal degradation [125]. Higher eQTL probability and GWAVA score (Table 2) predicted that there may be a detrimental effect due to this UTR variant also identified in GWAS of PD and prion disease.

3.3.6. Inhibition of astrocyte proliferation

The inflammatory response modulated by prostaglandin D synthase (PTGDS) during tissue injury, which is abundantly present in the brain and CSF. This gene plays an important role in reactive gliosis and acts as an endogenous beta amyloid (Aβ) chaperone [126]. PTGDS variant interrupts the binding of early growth response 1 (EGR1) that regulates

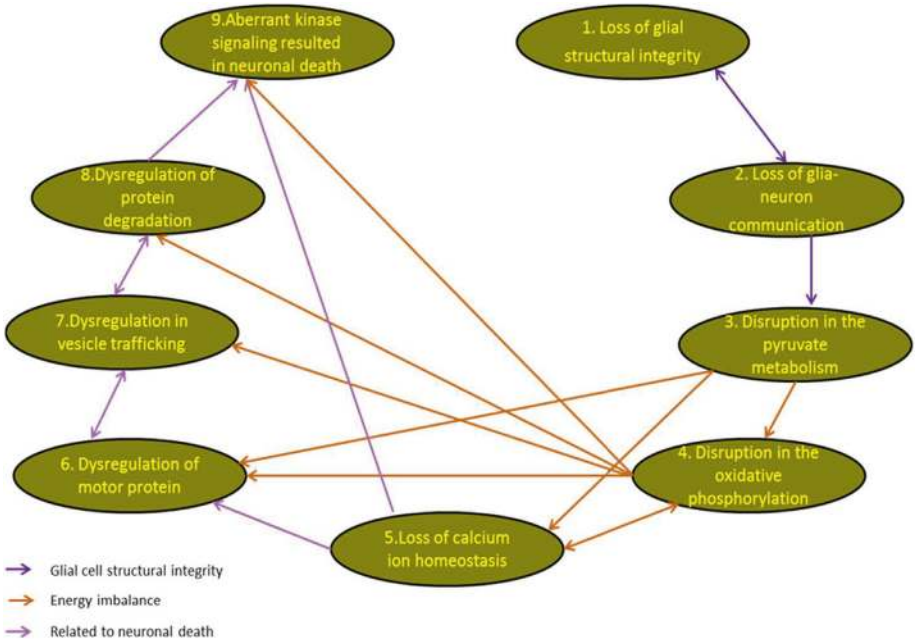

Fig. 4. Plausible mechanism for neurodegeneration and their functional relation.

presenilin-2 involved in APP processing and amyloid production. Studies reported that EGR1 is upregulated in AD [127]. The recent studies showed that higher concentration of PTGDS is capable of inhibiting mitochondrial ATP production of astrocytes and leads to apoptosis [128]. DeepSea score and GWAVA score (Table 2) predicted that there may be a significant functional change through this type of variation, this may dysregulate the astrocyte integrity and molecular chaperone activity.

3.3.6.1. Proposed plausible mechanism for selective vulnerability. The neuronal vulnerability is most common phenomena in degenerative disorder. The major cause of degeneration in the specific brain region is still unknown. From the transcriptome and functional network analysis, we hypothesized a conceivable mechanism for neuronal degeneration. Fig. 4 depicts hypothesized the plausible mechanism for vulnerability.

The structural and functional integrity of astrocytes is essential in order to preserve energy demanding neuron through providing glio transmitter and neurotrophic factors. Therefore, loss of glial cell integrity results in disruption of glia-neuron communication. Primarily, neurons, which need higher energy requirement, depend on the oligodendrocytes for their bio-energy [129]. However, energy craving neurons are unable to meet their energy demand for securing the functional homeostasis due to impairment of the glial function. Ultimately, this leads to energy imbalance. This type of metabolic stress causes an aberration in motor protein transport, calcium ion homeostasis, vesicle trafficking and protein degradation; all these above events require ATP for their specific functionality and this type of metabolic stress resulted in neuronal apoptosis. However, loss of communication between the glia and higher energy craving neuron is the primary cause of energy demand and degeneration.

3.3.6.2. Applications of the present study. This study provides insights to understand the underlying mechanism of selective vulnerability, which in turn helps to identify potential cellular signaling pathways involved in disease implication. In addition, we have identified aberrations in the structural functionality of glial cells, which result in crosstalk failure between glia and energy craving neurons. During energy crisis, restoration of glial function would provide neurotropic factors and secondary metabolites to save the surviving neuronal population against various stress stimuli. These results will be helpful to develop potential therapeutic strategies related with neurodegenerative diseases.

3.3.6.3. Limitations of the present study. The major limitation of the present study is size of the data due to the availability of RNA seq data only for 3 samples to public. However, most of the variants observed in this analysis agree well with other eQTL and GWAS studies of various neurological disorders using large datasets. In addition, we identified several novel variants and the pathways associated with them. The comprehensive analysis revealed the important factors for selective vulnerability of Alzheimer disease. The results obtained in this work could be strengthened upon the availability of large amount of data.

4. Conclusion

The variant analysis of transcriptome data is the most proficient way in finding the deleterious variation present in the coding/UTR regions. In this study, we have identified variations in the AD patients using mRNA-seq data retrieved from the different brain regions. In the temporal lobe we determined tissue specific vulnerable variants, which disrupt gliogenesis, myelination, glia-neuron communication, and astrocyte differentiation. In the frontal lobe, we found the variants which dysregulate pyruvate metabolism, oxidative phosphorylation, and calcium ion equilibrium. Aberrations in the protein degradation, protein kinase signaling, and apoptotic signaling were observed in other lobes. Genes involved in the regulation of motor proteins, astrocyte proliferation, and vesicle trafficking were altered in all AD brain tissues. We revealed that some of the predicted UTR variants interrupt the transcription factors and miRNA binding, which are dysregulated in AD. Disrupted TFs play an imperative role in microglial activation, apoptosis, protein degradation, neuronal survival and mitochondrial bioenergetics. From the above results, we observed that abnormalities in astrocyte structural integrity and glia-neuron communication affects the energy craving neuron and it is well known that these neurons depend on the glial cell for their energy requirement. Furthermore, these events lead to energy imbalance followed by disruption of protein degradation, calcium ion equilibrium and vesicle transport ultimately resulting in cell death. Extending this study to a larger population will be useful to understand the role of glial cell in selective neuronal vulnerability.

Author contributions

MMG and SAPD conceived the project. SAPD carried out the computations. SAPD, YT and MMG contributed toward discussions and manuscript preparation. All authors read and finalized the manuscript.

Competing financial interests

The authors declare no competing financial interests.

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Appendix A. Supplementary data

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