Integrative functional analyses of the neurodegenerative disease-associated *TECPR2* gene reveal its diverse roles

Ido Shalev
Ben-Gurion University of the Negev
Judith Somekh
University of Haifa
Alal Eran (alal@bgu.ac.il)
Ben-Gurion University of the Negev https://orcid.org/0000-0001-6784-7597

Research article

Keywords: Integrative functional analysis, neurodegenerative disorders, autophagy, ribosome, TECPR2

Posted Date: January 30th, 2020

DOI: https://doi.org/10.21203/rs.2.22274/v1

Abstract

Background

Loss of tectonin β-propeller repeat-containing 2 (*TECPR2*) function has been implicated in an array of neurodegenerative disorders, yet its physiological function remains largely unknown. Understanding *TECPR2* function is essential for developing much needed precision therapeutics for TECPR2-related diseases.

Methods

We leveraged the considerable amounts of functional data to obtain a comprehensive perspective of the role of *TECPR2* in health and disease. We integrated expression patterns, population variation, phylogenetic profiling, protein-protein interactions, and regulatory network data for a minimally biased multimodal functional analysis. Genes and proteins linked to *TECPR2* via multiple lines of evidence were subject to functional enrichment analyses to identify molecular mechanisms involving TECPR2.

Results

TECPR2 was found to be part of a tight neurodevelopmental gene expression program that includes *KIF1A*, *ATXN1*, *TOM1L2*, and *FA2H*, all implicated in neurological diseases. Functional enrichment analyses of *TECPR2*-related genes converged on a role in late autophagy and ribosomal processes. Large-scale population variation data demonstrated that this role is nonredundant.

Conclusions

TECPR2 might serve as an indicator for the energy balance between protein synthesis and autophagy, and a marker for diseases associated with their imbalance, such as Alzheimer's disease, Huntington's disease, and various cancers. Our work further suggests that TECPR2 plays a role as a synaptic proteostasis regulator during synaptogenesis, highlighting its importance in developing neurons. By advancing our understanding of TECPR2 function, this work provides an essential stepping stone towards the development of precision diagnostics and targeted treatment options for TECPR2-related disorders.

Background

The product of the TECPR2 (tectonin beta-propeller repeat containing 2) gene on chromosome 14q32.31 belongs to the tectonin β-propeller repeat-containing protein family, and contains mammalian-specific WD (tryptophan-aspartic acid repeat) and TECPR domains [1, 2]. TECPR2 dysfunction has been reproducibly implicated in severe neurodegenerative disorders (MIM: 615031). Individuals with TECPR2 loss of function (LoF) variants suffer from familial dysautonomia-like syndromes characterized by hereditary sensory autonomic neuropathy with intellectual disability (ID) [3, 4]. It was therefore suggested that TECPR2 LoF variants might serve as a "point diagnosis" for patients with ID [3, 4]. Other missense

TECPR2 variants are considered causative of progressive motor neuron disease and late-onset hereditary spastic paraplegia (HSP) [5, 6]. TECPR2 was also suggested to be a candidate gene for human neuroaxonal dystrophies, a set of rare, heterogeneous inherited neurodegenerative conditions characterized by various sensory-motor deficits [7]. As of 2020, only a handful of families with TECPR2-related disorders have been described, however it has been suggested that these reports underestimate the true prevalence of such disorders [3]. Moreover, although TECPR2 dysfunction plays a well-established role in neurodegenerative disorders, its exact function remains largely unknown. Understanding TECPR2 function is essential for developing rational treatment options for TECPR2-related disorders, which are currently lacking.

TECPR2 has been implicated in autophagy, an evolutionarily conserved intracellular degradation system by which cytoplasmic materials are delivered to and degraded in the lysosome [8]. This process consists of four stages: initiation, nucleation, elongation, and maturation. Autophagy starts with the nucleation of a newly formed vesicle known as a phagophore. During elongation, the phagophore forms a double membrane vesicle known as an autophagosome. The autophagosome later fuses with the lysosome, forming an autolysosome, whose contents are then degraded. Each stage involves the function of multiprotein complexes. The selectivity of this process is controlled by autophagy receptors that specifically degrade intracellular ubiquitinated aggregates, bacteria, specific organelles, or nucleic acids. TECPR2 was found to interact with several autophagy-related proteins, including GABARAP and MAPLC3 (LC3) [9, 10], which are required for proper elongation and expansion of the forming autophagosomes [11]. Furthermore, HEK293 cells with truncated TECPR2 proteins demonstrated reduced levels of the autophagosomal marker LC3II and attenuated delivery of LC3II and the cargo recruiting protein p62 for lysosomal degradation. This evidence is the basis for the current view of TECPR2 as a positive regulator of autophagy in humans [1], a function thought to play a key role in typical brain ageing [12]. However, the precise role of TECPR2 in autophagy remains to be defined. Such definition is essential for elucidating the link between autophagy and TECPR2-mediated neurodegenerative disease.

Moreover, TECPR2 was found to serve a crucial function in the maintenance of endoplasmic reticulum (ER) exit sites through the stabilization of SEC24D [10]. It was therefore suggested that TECPR2 might also function as a molecular scaffold linking the early secretion pathway and autophagy [10].

In light of these results, our current view of TECPR2 remains in the context of autophagy. Yet the focus on a priori knowledge of TECPR2 function limits our understanding of it to a small number of mechanisms. Thus, if we wish to gain a comprehensive understanding of the cellular roles of TECPR2 and their relations to neurodegenerative disorders, a broad, unbiased analyses of TECPR2 function is required.

Toward that goal, we exploited available human transcriptome and proteome data for a first comprehensive functional analysis of TECPR2. We compiled and integrated various sources of functional measures, including coexpression patterns, protein-protein interactions (PPI), transcription factor binding, population genomic variation, and phylogenetic profiling. We found that TECPR2 is tightly coexpressed with neurodevelopmental genes in a pattern that is consistent with a neurodevelopmental function.

TECPR2 expression is also downregulated in postmortem prefrontal cortex from individuals with Alzheimer's disease (AD) and Huntington's disease (HD) as compared to matched controls, consistent with autophagy-related alterations seen in AD [13]. We further found that TECPR2 expression is tightly negatively correlated with that of ribosomal proteins, suggesting that TECPR2 might play a role in maintaining a balance between autophagy and protein synthesis. Finally, massive population variation data demonstrated that TECPR2 serves a nonredundant function, as supported by its significant intolerance to LoF variation among diverse human populations. Our findings thus advance our understanding of TECPR2, ultimately enabling the development of much-needed therapeutic options for TECPR2-related neurodegenerative disorders.

Methods

For a comprehensive understanding of TECPR2 function, we integrated multiple independent layers of genome-scale functional measures (Fig. 1). These include neurodevelopmental expression patterns, coexpression partners, transcription factor binding, protein interaction partners, phylogenetic profiles, curated gene sets, and population variation.

Analysis of neurodevelopmental expression patterns

TECPR2 expression during human neurodevelopment was examined using the Allen Institute for Brain Science BrainSpan Atlas of the Developing Human Brain [14]. This dataset includes transcriptome-wide expression data from 237 prenatal and 287 postnatal tissue samples from different brain regions, individuals, ages, and sexes. Two multivariate generalized mixed linear models for prenatal and postnatal expression were tested with TECPR2 expression as the dependent variable and age as the independent variable. Sex and brain structure were used as covariates. A block design was used to account for the correlation of samples from different brain regions of the same individual.

TECPR2 coexpression analyses

The BrainSpan data detailed above was also used for identifying TECPR2 co-expressed gene modules. Only genes with correlation coefficient (r) > |0.7| across human brain development were included. We also mined GeneCards [15] for TECPR2 tissue co-expression partners, defined as gene pairs with expression correlation coefficient of > 0.7 across the Human Integrated Protein Expression Database (HIPED) or Genotype Tissue Expression (GTEx) data.

TECPR2 expression analysis across human tissues

We analyzed GTEx version 6 RNAseq data of 6,669 samples spanning 31 tissues from 246 individuals. Fastq data was obtained from dbGaP (accession phs000424.v6.p1) under authorized usage. Reads were quality-trimmed using Trimmomatic [16], and mapped to the reference human genome GRCh38 using

STAR 2.5.3 [17], following the ENCODE long mRNA mapping protocol [18]. Uniquely mapping reads were quantified using HTSeq [19], based on Gencode [20] V26 genes. EdgeR [21] was then used for filtering lowly expressed genes and normalizing the data using trimmed mean of M-values (TMM) normalization. Finally, Limma [22] was used for fitting age and gender adjusted models.

TECPR2 expression analysis in AD and HD

We compared TECPR2 gene expression in postmortem prefrontal cortex of 310 AD patients, 157 HD patients, and 157 non-demented controls, using Gene Expression Omnibus (GEO) dataset GSE33000 [23]. An age and sex-adjusted generalized linear model was used to test for differential expression. Benjamini-Hochberg correction for multiple comparisons was used to control for type I error across all genes measured in this experiment.

Analysis of transcription factor binding and coregulated genes

We used GeneHancer[24] to gather information about predicted transcription factor binding sites (TFBS) within the TECPR2 locus [15]. GeneHancer was also used for identifying genes with a similar TFBS profile, so-called coregulated genes. Specifically, we focused on GeneHancer elements supported by at least two sources. We also restricted our analyses to factors predicted to bind at least two TECPR2-associated elements.

Mining of PPI data

Several PPI resources were mined to identify TECPR2 binding partners in human. These include BioGrid [25], ConsensusPathDB [26, 27], STRING [28], and GeneCards [15].

Phylogenetic profiling

We used Clustering by Inferred Models of Evolution (CLIME) [29] to identify evolutionarily conserved modules that include TECPR2. Genes with phylogenetic profiles resembling that of the human TECPR2 were also identified using PhyloGene [30], focusing on the top-ranked 50 co-evolved human genes.

Curated gene set analysis

The Molecular Signature Database (MSigDB), a collection of annotated gene sets, was used to explore curated gene sets from curated pathway databases, PubMed publications, and knowledge of domain experts [31, 32].

Pan-cancer TECPR2 expression and prognostic value analysis

Pan-cancer TECPR2 expression across The Genome Cancer Atlas (TGCA) samples was analyzed using Gene Expression Profiling Interactive Analysis (GEPIA) [33], which considers expression data of 9,736 tumor and 8,587 normal samples from TCGA and GTEx. Multiple testing correction was achieved using a Benjamini-Hochberg false discovery rate (FDR) threshold of 0.05.

Population variation analysis

Human population variation data was taken from gnomAD v2.1.1, aggregating whole exome sequence data from 141,456 individuals [34].

Functional enrichment analysis

Genes and proteins whose relation to TECPR2 was supported by at least two sources of information were subject to functional enrichment analysis using the Database for Annotation, Visualization and Integrated Discovery (DAVID) [35, 36], enabling the discovery of shared pathways and molecular mechanisms. Multiple testing correction was achieved using a Benjamini-Hochberg FDR threshold of 0.05.

Results

For a comprehensive understanding of TECPR2 function, we compiled and integrated independent layers of genome-scale functional data. Such combined information provides a clearer and broader interpretation of TECPR2 function than offered by each layer separately (Fig. 1). Information streams used to understand TECPR2 function included PPI, expression patterns, phylogenetic profiling, transcription factor binding, and population variation. The full list of genes and proteins and their relationships to TECPR2 is detailed in Table S1.

TECPR2 transcription factors and coregulated genes have developmental functions

Since genes that function together are often co-regulated at the transcriptional level, we first identified putative transcriptional regulators of TECPR2 and examined their other targets. Among transcription factors predicted to bind the TECPR2 promoter and nearby enhancer elements were the developmental factors KDM1A and CTBP1, both part of the notch signaling pathway [37]. The other targets of these factors include the neurological disease-associated CINP, DYNC1H1, and MIR431 genes [38]. Table S2 details the 335 factors predicted to bind multiple TECPR2-associated cis-regulatory elements (promoter/enhancers), and their 37 other gene targets. In light of its suggested developmental context, we next focused our analyses on neurodevelopmental expression.

TECPR2 expression resembles that of neurodevelopmental genes; it is strongly anticorrelated to ribosomal protein

expression and downregulated in AD and HD

We analyzed the expression patterns of 524 spatiotemporal samples representing typical human neurodevelopment. TECPR2 expression increased with prenatal age across all brain regions (Pearson's r = 0.48, P = 0.01, Fig. 2). During the postnatal period, no significant relationship between TECPR2 expression and age was detected (Pearson's r = 0.22, P = 0.26). No sex difference was detected during prenatal or postnatal development (P = 0.8 and P = 0.4, respectively).

Since genes that function together must be coexpressed, we next examined TECPR2-containing coexpression networks. In all, 578 genes showed a similar expression pattern in the developing human brain (Table S3). Of these, 184 were positively correlated with TECPR2, including many neurodevelopmental disease genes such as KIF1A [39], SHANK3 [40], TOM1L2 [41], ABCA2 [42], PLEKHA6 [43], ATXN1L [44], and CNTNAP1 [45]. Many of the negatively correlated genes (177 of 394 genes) encode ribosomal proteins (RPs), including RPL24, RPS3A, RPS6, RPS8, RPL15, RPL5, RPL11, RPL23, and RPS7, suggesting that TECPR2 function is negatively related to translation.

The release of these RPs, including RPL5, RPL11, RPL23, and RPS7, was previously shown to promote the accumulation of p53, which plays a pivotal role in neurodegenerative disorders via its upregulation [46]. Consistently, we found that TP53 expression is anticorrelated to TECPR2 expression across 31 human tissues in an age and sex adjusted model (Pearson's r = -0.405, $P = 2.33 \times 10^{-16}$). Consistently, we found that TECPR2 is downregulated in prefrontal cortex of individuals with AD and HD, two neurodegenerative diseases in in which p53 was shown to be upregulated [46] (AD adjusted $P = 2.83 \times 10^{-6}$, HD adjusted $P = 2.87 \times 10^{-9}$, Fig. 3).

We also examined TECPR2 co-expression partners across human tissues at both the RNA and protein levels. Table S4 details 19 genes whose expression pattern resembles that of TECPR2 across 69 human tissues, suggesting that their functions might be related. These include the Charcot Marie Tooth disease gene MTMR2, the coloboma of macula gene NRBP2, and other neurodevelopmental disease genes such as MACROD2 and NGFR.

Phylogenetic profiling reveals that many genes that have co-evolved with TECPR2 serve central neurodevelopmental functions

We also examined shared patterns of gene presence and absence across species, that can point to shared functions. For that purpose, we used two phylogenetic profiling approaches. The first, CLIME, identified seven genes predicted to have co-evolved with TECPR2. These include the neurodevelopmental disease genes BBS1, BBS9 [47], and TMOD2 [48], as depicted in Figure S1. The second approach PhyloGene, similarly revealed that many of the genes that have coevolved with TECPR2 serve

neurodevelopmental functions. As detailed in Table S5, these include ATXN1 [44], DLGAP4 [40], TRAK1 [49], and FILIP1 [50].

PPI highlight the role of TECPR2 in autophagy

Since interacting partners of known function can point to a shared function, we next mined several PPI databases. In all, 55 experimentally-validated TECPR2-binding partners were identified, as detailed in Table S6. For example, Figure S2 depicts an integrated 27-protein interaction network, showing that the majority of validated TECPR2 interactions are with proteins involved in autophagy. These include the autophagy-related ATG8 family members GABARAP, GABARAPL1, GABARAPL2, MAP1LC3B, and MAP1LC3C [9]. Moreover, a complementary approach implemented in The Search Tool for the Retrieval of Interacting Genes (STRING) identified three direct and ten indirect TECPR2 relationships, highlighting proteins whose dysfunction leads to TECPR2 deficiency-related phenotypes, including FBXL3 [51], ITCH [52], and ZFYVE26 [53]. These are depicted in Figure S3 and detailed in Table S6.

Molecular signatures prognostic of various cancers include TECPR2

We next examined expert-curated expression-based gene sets that include TECPR2, identifying nine sets (Table S7). Of these, seven are informative of various cancers, including gene sets whose expression in optimally debulked ovarian tumors is associated with survival and those up-regulated in nasopharyngeal carcinoma. Genes shared across multiple curated sets include the neurodevelopmental genes BBS9, ATXN1, BACE1, FA2H, FILIP1, NBR1, TRAK1, and ZFYVE26, and the autophagy genes MAP1LC3B and AMBRA1.

TECPR2 expression is associated with survival in renal and liver cancers

In light of the identified TECPR2-containing curated sets, we examined TECPR2 expression in TCGA. TECPR2 was found to be expressed in all cancer tissues at ≥ 1 transcript per million (TPM). When comparing tumor to normal gene expression, TECPR2 was found to be significantly dysregulated in urothelial carcinoma, cholangiocarcinoma, diffuse large B cell lymphoma, acute myeloid leukemia, testicular germ cell tumors, and endometrioid carcinoma (Adjusted $P < 1 \times 10^{-3}$, Fig. 4A). Kaplan-Meier survival analyses showed a favorable effect for high TECPR2 expression in renal cancers, including clear cell renal cell carcinoma (CCRCC), papillary renal cell carcinoma (PRCC), and chromophobe renal cell carcinoma ($P = 6.5 \times 10^{-9}$, cut-off of 3.25 FPKM, Fig. 4B), and an unfavorable effect in hepatocellular carcinoma (HCC, $P = 5.7 \times 10^{-5}$, cut-off of 1.67 FPKM, Fig. 4C). Taken together, these findings suggest that TECPR2 dysregulation might have a high prognostic value for several cancer types.

Integrative functional analyses converge on a neurodevelopmental function in protein synthesis, autophagy, and splicing

To gain an integrative view of TECPR2 function and minimize experiment-specific bias, we focused our analyses on genes and proteins linked to TECPR2 via multiple independent lines of evidence (detailed above). In doing so, we examined the shared functions of 332 genes and proteins linked to TECPR2 function via multiple multimodal datasets (Table S8 and Fig. 1). Their functional enrichment analyses revealed a strong convergence on four molecular themes, namely protein synthesis, autophagy, splicing, and belonging to a neurodevelopmental gene expression program. Among pathways and mechanisms related to protein synthesis were the Reactome pathways “Formation of a pool of free 40S subunits” (Adjusted P = 8.01×10^{-26}), “Peptide chain elongation” (Adjusted P = 2.58×10^{-24}), and “SRP-dependent cotranslational protein targeting to membrane” (Adjusted P = 1.64×10^{-9}). Macroautophagy (Adjusted P = 0.01) and autophagosome (Adjusted P = 0.017) were some of the enriched concepts related to autophagy. The Reactome “mRNA Splicing - Major Pathway” (Adjusted P = 0.017) was most enriched among several splicing-related concepts. Finally, The Reactome pathways “Activation of anterior HOX genes in hindbrain development during early embryogenesis” (Adjusted P = 0.037) and “Downregulation of SMAD2/3:SMAD4 transcriptional activity” (Adjusted P = 0.038) are examples of enriched neurodevelopmental gene expression programs. These and other enriched functions are detailed in Table S8.

TECPR2 population variation demonstrates that its function is nonredundant

After gaining insight into the cellular functions of TECPR2, we further examined their centrality using human population variation data. Based on 141,456 exomes aggregated by gnomAD [34], TECPR2 was found to be extremely intolerant of loss-of-function (LoF) protein truncating variants (probability of LoF intolerance, pLI = 0.95 in ExAC[54]). The strong intolerance of TECPR2 to LoF variation is also supported by a low LoF observed/expected (oe) ratio of 0.22, 90% confidence interval (CI) = [0.14,0.35]. Conversely, its closely related paralog TECPR1, is highly tolerant to LoF variation (pLI = 0.000, oe = 0.36, 90% CI = [0.26, 0.5]). These results suggest that TECPR2 is a nonredundant core gene of the human cellular machinery.

Discussion

By integrating multimodal genome-scale functional data, we identified several likely functions of the currently under-characterized neurodegenerative disease gene TECPR2. Our analyses suggest that TECPR2 is a neurodevelopmental gene with diverse roles, including late autophagy and protein synthesis.

These roles are nonredundant, as demonstrated not only by the phenotypic implications of its dysfunction, but also by large scale population variation data.

Our work provides several lines of evidence that support the neurodevelopmental function of TECPR2. First, its neurodevelopmental expression is tightly correlated to that of many neurodevelopmental disease genes, including KIF1A [39], SHANK3 [40], TOM1L2 [41], ABCA2 [42], ATXN1L [44], FAM102A [55], IQSEC1-3 [56], and CNTNAP1 [45]. Consistently, transcription factors confidently predicted to regulate the TECPR2 locus are central regulators of development. These include KDM1A, SAP130, and CTBP1 [37]. Furthermore, many genes that have co-evolved with TECPR2 have a well-documented neurodevelopmental function, including DLGAP2-4 [40], MYT1 [57], TRAK1 [49], MYT1L [49], BBS1 and BBS9 [47]. In light of these results, the bona fide role of many of the aforementioned genes in synaptic function [39, 45, 56, 58], and the consistent expression trajectory of other synaptic genes [59], we speculate that TECPR2 might play an important role during synaptogenesis.

Moreover, our analyses confirm and expand the previously-reported role of TECPR2 in autophagy (Fig. 5). We provide multimodal evidence supporting the direct interactions of TECPR2 with autophagy receptors p62, NBR1, Stbd1, and AMBRA1, as well as indirect relationships with TOLLIP, TAX1BP, and FAM134B. Further evidence is provided to support the interactions of TECPR2 with the autophagy elongation regulators ATG3, ATG4, ATG5, and ATG7. We further provide supporting evidence for the role of TECPR2 in the homotypic fusion and protein sorting (HOPS) membrane tethering complex, which is required for the fusion phase [10, 60]. Aside from a direct interaction of TECPR2 with HOPS complex components VPS16, VPS18, VPS33A and VPS41, which were previously reported to be associated with TECPR2 [10, 60], another HOPS component, VPS11, was found to be co-expressed with TECPR2. Our results support the current view of TECPR2 as a positive regulator of autophagy [4, 61]. We suggest that the scope of TECPR2 involvement in autophagy is far greater than was previously acknowledged, and includes direct and indirect associations, especially to the later autophagy phases of elongation and regulation of autophagy selectivity. A summary of autophagy stages and the association of TECPR2 with each stage is depicted in Fig. 5.

Additionally, we detected a strong negative correlation between TECPR2 expression and the expression of genes related to protein synthesis in the developing human brain. Dysfunction of different types of RPs was previously associated with neurodevelopmental abnormalities [62, 63]. The proposed link between TECPR2 and ribosomal protein function should be directly examined in future studies, in physiological and pathological conditions.

An energy balance is maintained between protein synthesis and autophagy, which are both coregulated by mTORC1 [64]. An imbalance between these processes is a hallmark of several diseases, including AD [13], HD [65] and various types of cancer [64]. Our analysis strongly supports the association of TECPR2 with these conditions (Fig. 3 and Fig. 4). These results suggest that TECPR2 could serve as an informative biomarker for these disorders.

A tightly regulated balance between autophagy and protein synthesis is critical not only for maintaining a bioenergetic homeostasis, but also for controlling a proteomic one. Fine-tuned proteostasis is essential during synaptogenesis, where rapid local protein synthesis is required for synapse formation [66], and local autophagy is essential for removing various synaptic cargos, including synaptic vesicles and scaffold proteins [67]. Moreover, synaptic function crucially depends on protein homeostasis, the uninterrupted synthesis and degradation of synaptic proteins, both at the presynaptic and postsynaptic termini [66]. Autonomous synaptic control depends on the crosstalk between the local autophagy and protein synthesis machineries [68]. Accumulating evidence suggests that disruptions in synaptic proteostasis may play a central role in the pathogenesis of severe neurodegenerative disorders, including AD, HD and Parkinson's disease (PD) [69]. In light of the multimodal evidence supporting the role of TECPR2 as a bridge between autophagy and protein synthesis, its suggested involvement in synaptogenesis, and its well-supported interactions with synaptic autophagy proteins such as KIFA1 and ATG9, we hypothesize that TECPR2 might serve as a marker for synaptic homeostasis in health and disease.

Accordingly, our work identifies direct and indirect relationships between TECPR2 and other neurodegenerative disease genes. For example, KIF1A, in which pathogenic variants cause hereditary sensory and autonomic neuropathy type II [39], was found to directly interact with TECPR2 at the protein level, while being tightly coregulated at the mRNA level during human neurodevelopment. TOM1L2, a PD candidate also related to autophagy [41], and FA2H, in which pathogenic variants cause an autosomal recessive type of HSP [70], were both found to be robustly correlated with TECPR2 expression during human brain development. Other neurodegenerative disease genes such as MTMR2, in which pathogenic variants cause Charcot-Marie-Tooth disease type 4B, an autosomal recessive demyelinating neuropathy [71], and NGFR, previously suggested to be involved in AD [72], were found to be tissue co-expression partners of TECPR2. Furthermore, TECPR2 was found to have coevolved with genes implicated in neurodegenerative disorders such as ATXN1 [44] and KIAA1217 [73].

Conclusions

Taken together, our results suggest that TECPR2 might serve as an indicator for the homeostasis between autophagy and protein synthesis, and a biomarker for diseases characterized by their imbalance. Nonetheless, the exact mechanisms by which TECPR2 is related to the identified functions and their alterations in disease remain to be determined. The information compiled in our analyses points to a central role of TECPR2 in the developing human brain, which should be further characterized in future studies. This study represents a first comprehensive integrated analysis of TECPR2 function across multiple layers of molecular information. Such diverse pool of information allowed us to provide better insight into TECPR2 functions and profoundly expand our current understanding of this disease gene. We speculate that TECPR2 might play an important role during synaptogenesis by linking local protein synthesis and local autophagy in developing neurons.

In summary, this study suggests that TECPR2 might be a hub gene bridging between protein synthesis, autophagy, and brain function in health and disease. With no existing cure for TECPR2-related disorders, the need to improve our understanding of this gene is imperative. For this purpose, the knowledge acquired by this integrative study is intended to serve as a substrate for focused mechanistic investigations into TECPR2 function, which will ultimately lead to the identification of much-needed therapeutic options for TECPR2-related neurodegenerative disorders.

List Of Abbreviations

TECPR2, tectonin beta-propeller repeat containing 2; TECPR, Tectonin beta-propeller repeat-containing protein; WD, tryptophan-aspartic acid repeat; LoF, Loss of Function; ID, intellectual disability; HSP, hereditary spastic paraplegia; LC3, MAPLC3; ER, endoplasmic reticulum; PPI, protein-protein interactions; AD, Alzheimer's disease; HD, Huntington's disease; HIPED, Human Integrated Protein Expression Database; GTEx, Genotype Tissue Expression; TMM, trimmed mean of M-values; GEO, Gene Expression Omnibus; TFBS, transcription factor binding sites; CLIME, Clustering by Inferred Models of Evolution; MSigDB, The Molecular Signature Database; TGCA, The Genome Cancer Atlas; GEPIA, Gene Expression Profiling Interactive Analysis; FDR, false discovery rate; DAVID, Database for Annotation, Visualization and Integrated Discovery; RPs, ribosomal proteins; STRING, The Search Tool for the Retrieval of Interacting Genes; TPM, transcripts per million; CCRCC, clear cell renal cell carcinoma; PRCC, papillary renal cell carcinoma; HOPS, homotypic fusion and protein sorting; PD, Parkinson's disease.

Declarations

Ethics approval and consent to participate. Not applicable.

Consent for publication. Not applicable.

Availability of data and materials. All data analyzed in this study is included in this published article (and its supplementary information files). GTEx data used for the analyses described in this manuscript were obtained from dbGaP accession number phs000424.v6.p1.

Competing interests. The authors declare no competing interests.

Funding. The GTEx Project was supported by the Common Fund of the Office of the Director of the National Institutes of Health, and by NCI, NHGRI, NHLBI, NIDA, NIMH, and NINDS. AE was supported by NIH grant P50MH106933.

Authors' contributions. IS analyzed the data, and wrote the manuscript. JS helped interpreting the results. AE designed the current study, wrote the manuscript, and provided critical input on data analyses.

Acknowledgements. We thank Jerry Eichler and Shahar Mizrahi for fruitful discussions.

References

1. Oz-Levi D, Gelman A, Elazar Z, Lancet D. TECPR2. Autophagy. 2013;9(5):801-2.
2. Bento CF, Renna M, Ghislat G, Puri C, Ashkenazi A, Vicinanza M, et al. Mammalian autophagy: how does it work? Annual Review of Biochemistry. 2016;85:685-713.
3. Heimer G, Oz-Levi D, Eyal E, Edvardson S, Nissenkorn A, Ruzzo EK, et al. TECPR2 mutations cause a new subtype of familial dysautonomia like hereditary sensory autonomic neuropathy with intellectual disability. European Journal of Paediatric Neurology. 2016;20(1):69-79.
4. Oz-Levi D, Ben-Zeev B, Ruzzo Elizabeth K, Hitomi Y, Gelman A, Pelak K, et al. Mutation in TECPR2 Reveals a Role for Autophagy in Hereditary Spastic Paraparesis. The American Journal of Human Genetics. 2012;91(6):1065-72.
5. Covone AE, Fiorillo C, Acquaviva M, Trucco F, Morana G, Ravazzolo R, et al. WES in a family trio suggests involvement of TECPR2 in a complex form of progressive motor neuron disease. Clinical genetics. 2016;90(2):182-5.
6. Gatto E, Turjanski A, Nemirovsky S, Bustamante J, Cesarini M, Espay A. TECPR2 mutations and HSP combined phenotype. Neurology. 2017;88(16 Supplement):P5. 029.
7. Hahn K, Rohdin C, Jagannathan V, Wohlsein P, Baumgärtner W, Seehusen F, et al. TECPR2 associated neuroaxonal dystrophy in Spanish water dogs. PloS one. 2015;10(11):e0141824.
8. Mizushima N, Komatsu M. Autophagy: Renovation of Cells and Tissues. Cell. 2011;147(4):728-41.
9. Behrends C, Sowa ME, Gygi SP, Harper JW. Network organization of the human autophagy system. Nature. 2010;466(7302):68-76.
10. Stadel D, Millarte V, Tillmann Kerstin D, Huber J, Tamin-Yecheskel B-C, Akutsu M, et al. TECPR2 Cooperates with LC3C to Regulate COPII-Dependent ER Export. Molecular Cell. 2015;60(1):89-104.
11. Le Grand JN, Chakrama FZ, Seguin-Py S, Fraichard A, Delage-Mourroux R, Jouvenot M, et al. GABARAPL1 (GEC1): Original or copycat? Autophagy. 2011;7(10):1098-107.
12. Alexander J, Stroebel T, Georgitsi M, Schuster M, Penz T, Bock C, et al. TECPR2 a positive regulator of autophagy is implicated in healthy brain ageing. bioRxiv. 2017:157693.
13. Shafei MA, Harris M, Conway ME. Divergent Metabolic Regulation of Autophagy and mTORC1—Early Events in Alzheimer's Disease? Frontiers in Aging Neuroscience. 2017;9(173).
14. BrainSpan Atlas of the Developing Human Brain. www.brainspan.org. Accessed 10 January 2020.
15. Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, et al. The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses. Current protocols in bioinformatics. 2016;54(1):1.30.1-1..3.
16. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics (Oxford, England). 2014;30(15):2114-20.
17. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR: ultrafast universal RNA-seq aligner. Bioinformatics. 2012;29(1):15-21.

18. STAR based ENCODE Long RNA-Seq processing pipeline www.github.com/ENCODE-DCC/long-rna-seq-pipeline. Accessed 10 January 2020.
19. Anders S, Pyl PT, Huber W. HTSeq—a Python framework to work with high-throughput sequencing data. Bioinformatics. 2014;31(2):166-9.
20. Frankish A, Diekhans M, Ferreira A-M, Johnson R, Jungreis I, Loveland J, et al. GENCODE reference annotation for the human and mouse genomes. Nucleic Acids Research. 2018;47(D1):D766-D73.
21. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 2009;26(1):139-40.
22. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Research. 2015;43(7):e47-e.
23. Narayanan M, Huynh JL, Wang K, Yang X, Yoo S, McElwee J, et al. Common dysregulation network in the human prefrontal cortex underlies two neurodegenerative diseases. Molecular Systems Biology. 2014;10(7):743.
24. Fishilevich S, Nudel R, Rappaport N, Hadar R, Plaschkes I, Iny Stein T, et al. GeneHancer: genome-wide integration of enhancers and target genes in GeneCards. Database (Oxford). 2017;2017:1-17.
25. Stark C, Breitkreutz B-J, Reguly T, Boucher L, Breitkreutz A, Tyers M. BioGRID: a general repository for interaction datasets. Nucleic Acids Research. 2006;34(Database issue):D535-D9.
26. Kamburov A, Pentchev K, Galicka H, Wierling C, Lehrach H, Herwig R. ConsensusPathDB: toward a more complete picture of cell biology. Nucleic Acids Research. 2010;39(Database issue):D712-D7.
27. Kamburov A, Wierling C, Lehrach H, Herwig R. ConsensusPathDB—a database for integrating human functional interaction networks. Nucleic Acids Research. 2008;37(Database issue):D623-D8.
28. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, et al. STRING v10: protein–protein interaction networks, integrated over the tree of life. Nucleic Acids Research. 2015;43(D1):D447-D52.
29. Li Y, Ning S, Calvo SE, Mootha VK, Liu JS. Bayesian Hidden Markov Tree Models for Clustering Genes with Shared Evolutionary History. Ann Appl Stat. 2019;13(1):606-37.
30. Sadreyev IR, Ji F, Cohen E, Ruvkun G, Tabach Y. PhyloGene server for identification and visualization of co-evolving proteins using normalized phylogenetic profiles. Nucleic Acids Research. 2015;43(W1):W154-W9.
31. Liberzon A, Subramanian A, Pinchback R, Thorvaldsdóttir H, Tamayo P, Mesirov JP. Molecular signatures database (MSigDB) 3.0. Bioinformatics. 2011;27(12):1739-40.
32. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. Proceedings of the National Academy of Sciences. 2005;102(43):15545-50.
33. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Research. 2017;45(W1):W98-W102.

34. Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Wang Q, et al. Variation across 141,456 human exomes and genomes reveals the spectrum of loss-of-function intolerance across human protein-coding genes. bioRxiv. 2019:531210.
35. Huang DW, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Research. 2008;37(1):1-13.
36. Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nature protocols. 2008;4(1):44-57.
37. Zhang R, Engler A, Taylor V. Notch: an interactive player in neurogenesis and disease. Cell Tissue Res. 2018;371(1):73-89.
38. Tu W-Y, Simpson JE, Highley JR, Heath PR. Spinal muscular atrophy: Factors that modulate motor neurone vulnerability. Neurobiology of Disease. 2017;102:11-20.
39. Rivière J-B, Ramalingam S, Lavastre V, Shekarabi M, Holbert S, Lafontaine J, et al. KIF1A, an Axonal Transporter of Synaptic Vesicles, Is Mutated in Hereditary Sensory and Autonomic Neuropathy Type 2. The American Journal of Human Genetics. 2011;89(2):219-30.
40. Rasmussen AH, Rasmussen HB, Silahtaroglu A. The DLGAP family: neuronal expression, function and role in brain disorders. Molecular Brain. 2017;10(1):43.
41. Ferrari R, Kia DA, Tomkins JE, Hardy J, Wood NW, Lovering RC, et al. Stratification of candidate genes for Parkinson's disease using weighted protein-protein interaction network analysis. BMC genomics. 2018;19(1):452.
42. Wang Q, Li M, Yang Z, Hu X, Wu H-M, Ni P, et al. Increased co-expression of genes harboring the damaging de novo mutations in Chinese schizophrenic patients during prenatal development. Scientific Reports. 2015;5:18209.
43. Spellmann I, Rujescu D, Musil R, Giegling I, Genius J, Zill P, et al. Pleckstrin homology domain containing 6 protein (PLEKHA6) polymorphisms are associated with psychopathology and response to treatment in schizophrenic patients. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2014;51:190-5.
44. Zhang C, Browne A, Child D, Divito JR, Stevenson JA, Tanzi RE. Loss of function of ATXN1 increases amyloid beta-protein levels by potentiating beta-secretase processing of beta-amyloid precursor protein. The Journal of biological chemistry. 2010;285(12):8515-26.
45. Zou Y, Zhang W-f, Liu H-y, Li X, Zhang X, Ma X-f, et al. Structure and function of the contactin-associated protein family in myelinated axons and their relationship with nerve diseases. Neural Regeneration Research. 2017;12(9):1551-8.
46. Chang JR, Ghafouri M, Mukerjee R, Bagashev A, Chabrashvili T, Sawaya BE. Role of p53 in neurodegenerative diseases. Neurodegenerative Diseases. 2012;9(2):68-80.
47. Bennouna-Greene V, Kremer S, Stoetzel C, Christmann D, Schuster C, Durand M, et al. Hippocampal dysgenesis and variable neuropsychiatric phenotypes in patients with Bardet–Biedl syndrome underline complex CNS impact of primary cilia. Clinical Genetics. 2011;80(6):523-31.

48. McMillan EL, Kamps AL, Lake SS, Svendsen CN, Bhattacharyya A. Gene expression changes in the MAPK pathway in both Fragile X and Down syndrome human neural progenitor cells. Am J Stem Cells. 2012;1(2):154-62.
49. Gilissen C, Hehir-Kwa JY, Thung DT, van de Vorst M, van Bon BW, Willemsen MH, et al. Genome sequencing identifies major causes of severe intellectual disability. Nature. 2014;511(7509):344-7.
50. Yagi H, Oka Y, Komada M, Xie M-J, Noguchi K, Sato M. Filamin A interacting protein plays a role in proper positioning of callosal projection neurons in the cortex. Neuroscience Letters. 2016;612:18-24.
51. Ansar M, Paracha SA, Serretti A, Sarwar MT, Khan J, Ranza E, et al. Biallelic variants in FBXL3 cause intellectual disability, delayed motor development and short stature. Human Molecular Genetics. 2018;28(6):972-9.
52. Lohr NJ, Molleston JP, Strauss KA, Torres-Martinez W, Sherman EA, Squires RH, et al. Human ITCH E3 Ubiquitin Ligase Deficiency Causes Syndromic Multisystem Autoimmune Disease. The American Journal of Human Genetics. 2010;86(3):447-53.
53. Chang J, Lee S, Blackstone C. Spastic paraplegia proteins spastizin and spatacsin mediate autophagic lysosome reformation. The Journal of Clinical Investigation. 2014;124(12):5249-62.
54. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature. 2016;536:285-91.
55. Mao F, Wang L, Zhao X, Xiao L, Li X, Liu Q, et al. Mutations involved in post-transcriptional dysregulation contribute to six neuropsychiatric disorders. bioRxiv. 2018:175844.
56. Um JW. Synaptic functions of the IQSEC family of ADP-ribosylation factor guanine nucleotide exchange factors. Neuroscience Research. 2017;116:54-9.
57. Vasconcelos FF, Castro DS. Coordinating neuronal differentiation with repression of the progenitor program: Role of the transcription factor MyT1. Neurogenesis. 2017;4(1):e1329683.
58. Verpelli C, Dvoretskova E, Vicidomini C, Rossi F, Chiappalone M, Schoen M, et al. Importance of Shank3 in regulating metabotropic glutamate receptor 5 (mGluR5) expression and signaling at synapses. J Biol Chem. 2011;286(40):34839–50.
59. Kang HJ, Kawasawa YI, Cheng F, Zhu Y, Xu X, Li M, et al. Spatio-temporal transcriptome of the human brain. Nature. 2011;478:483–9.
60. Jiang P, Nishimura T, Sakamaki Y, Itakura E, Hatta T, Natsume T, et al. The HOPS complex mediates autophagosome–lysosome fusion through interaction with syntaxin 17. Molecular Biology of the Cell. 2014;25(8):1327-37.
61. Shpilka T, Weidberg H, Pietrokovski S, Elazar Z. Atg8: an autophagy-related ubiquitin-like protein family. Genome Biology. 2011;12(7):226.
62. Klauck SM, Felder B, Kolb-Kokocinski A, Schuster C, Chiocchetti A, Schupp I, et al. Mutations in the ribosomal protein gene RPL10 suggest a novel modulating disease mechanism for autism. Molecular Psychiatry. 2006;11:1073-84.

63. Uechi T, Nakajima Y, Nakao A, Torihara H, Chakraborty A, Inoue K, et al. Ribosomal Protein Gene Knockdown Causes Developmental Defects in Zebrafish. PLOS ONE. 2006;1(1):e37.
64. Lindqvist LM, Tandoc K, Topisirovic I, Furic L. Cross-talk between protein synthesis, energy metabolism and autophagy in cancer. Current Opinion in Genetics & Development. 2018;48:104-11.
65. Harding RJ, Tong YF. Proteostasis in Huntington's disease: disease mechanisms and therapeutic opportunities. Acta pharmacologica Sinica. 2018;39(5):754-69.
66. Cioni JM, Koppers M, Holt CE. Molecular control of local translation in axon development and maintenance. Curr Opin Neurobiol. 2018;51:86-94.
67. Liang Y. Emerging Concepts and Functions of Autophagy as a Regulator of Synaptic Components and Plasticity. Cells. 2019;8(1).
68. Birdsall V, Waites CL. Autophagy at the synapse. Neurosci Lett. 2019;697:24-8.
69. Taoufik E, Kouroupi G, Zygogianni O, Matsas R. Synaptic dysfunction in neurodegenerative and neurodevelopmental diseases: an overview of induced pluripotent stem-cell-based disease models. Open Biol. 2018;8(9):180138.
70. González-González MC, Gutierrez M, de la Mota CC, Jareño NM, Gonzalez S, Cava F. Next generation sequencing for rapid diagnosis of a rare early onset spastic paraplegia: A novel pathological variant in FA2H gene. Revista del Laboratorio Clínico. 2018;11:222-6.
71. Vaccari I, Dina G, Tronchère H, Kaufman E, Chicanne G, Cerri F, et al. Genetic Interaction between MTMR2 and FIG4 Phospholipid Phosphatases Involved in Charcot-Marie-Tooth Neuropathies. PLOS Genetics. 2011;7(10):e1002319.
72. Cozza A, Melissari E, Iacopetti P, Mariotti V, Tedde A, Nacmias B, et al. SNPs in neurotrophin system genes and Alzheimer's disease in an Italian population. Journal of Alzheimer's Disease. 2008;15(1):61-70.
73. Zhang C, Lu J, Liu B, Cui Q, Wang Y. Primate-specific miR-603 is implicated in the risk and pathogenesis of Alzheimer's disease. Aging (Albany NY). 2016;8(2):272-90.

Figures

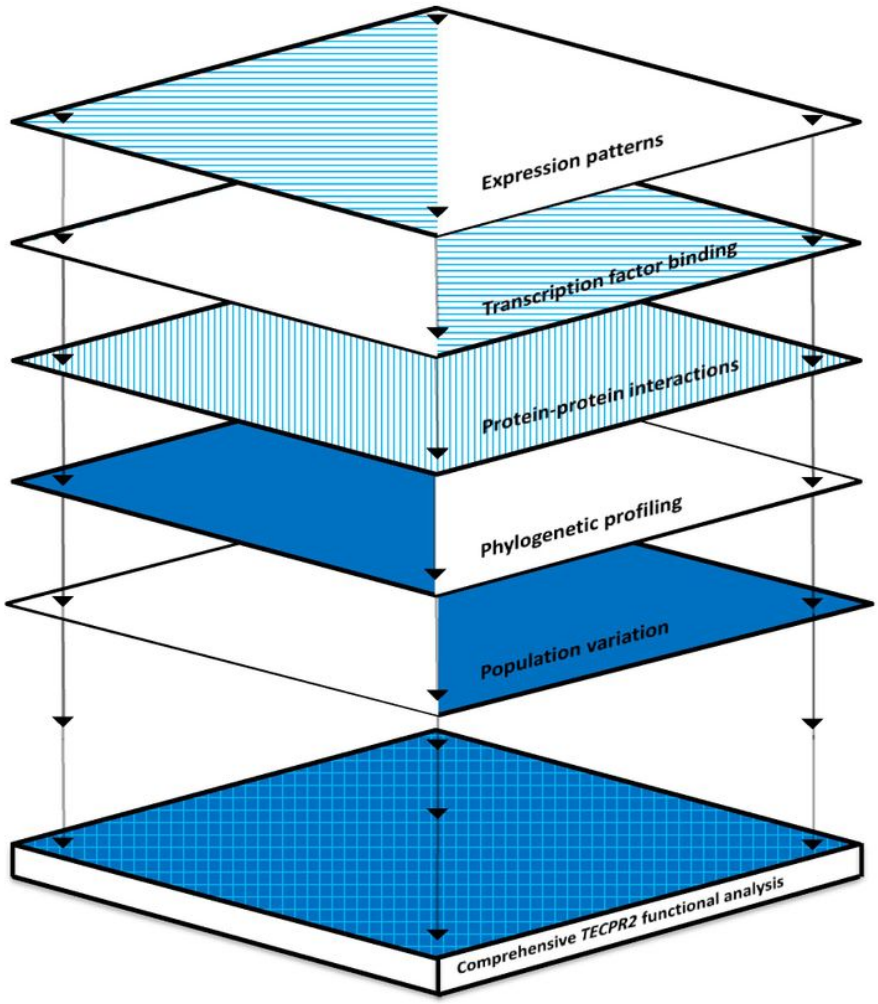

Figure 1

Multiple layers of information compiled for a comprehensive functional analysis of TECPR2.

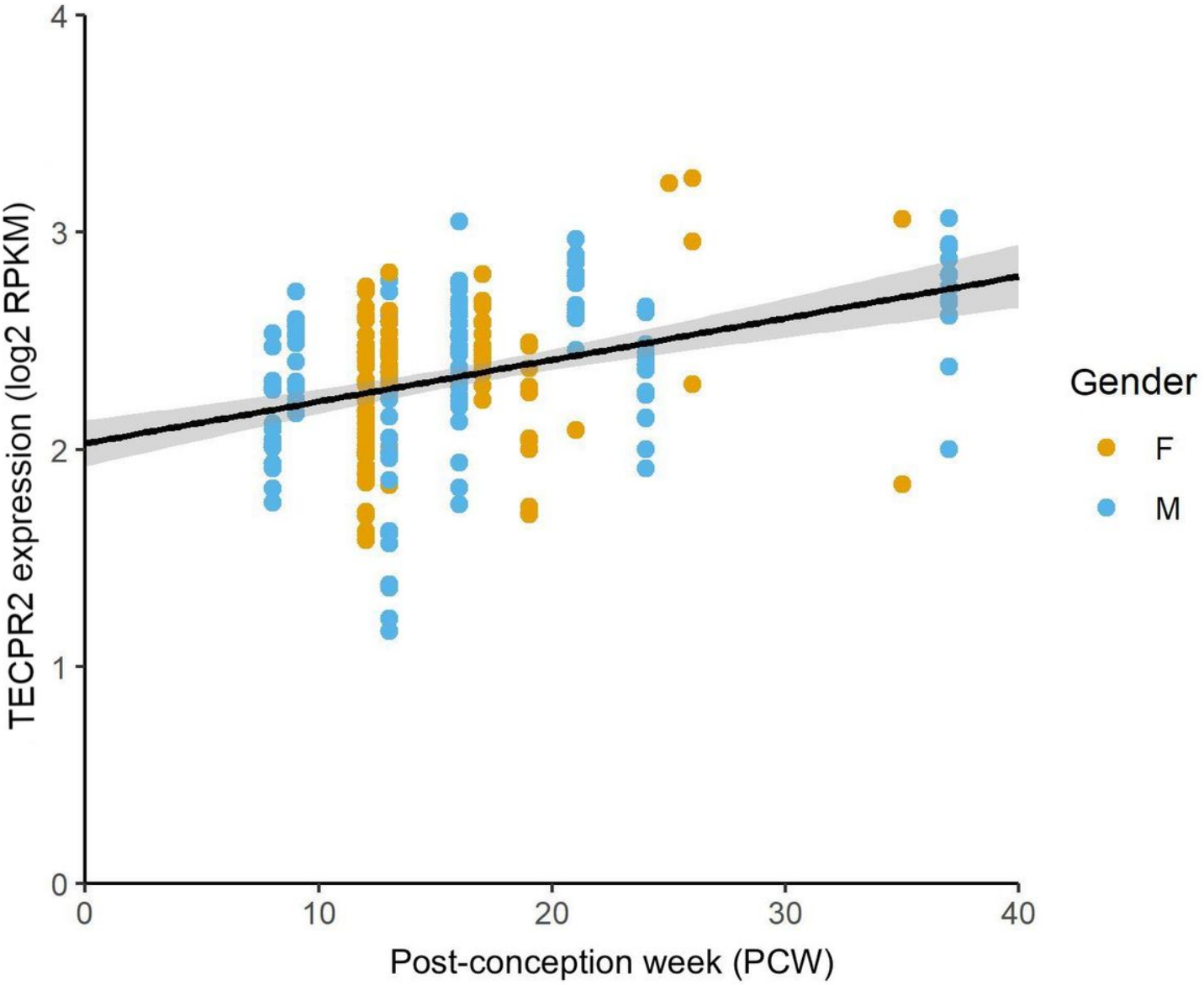

Figure 2

TECPR2 expression increases during prenatal brain development (Pearson's r=0.48, P=0.01). In 237 samples, aged 8 to 36 post conception weeks (PCW), TECPR2 expression increased with age, across all brain regions tested, with no significant differences between males and females. Female samples are shown in orange, male samples in blue. See www.brainspan.org for sample details.

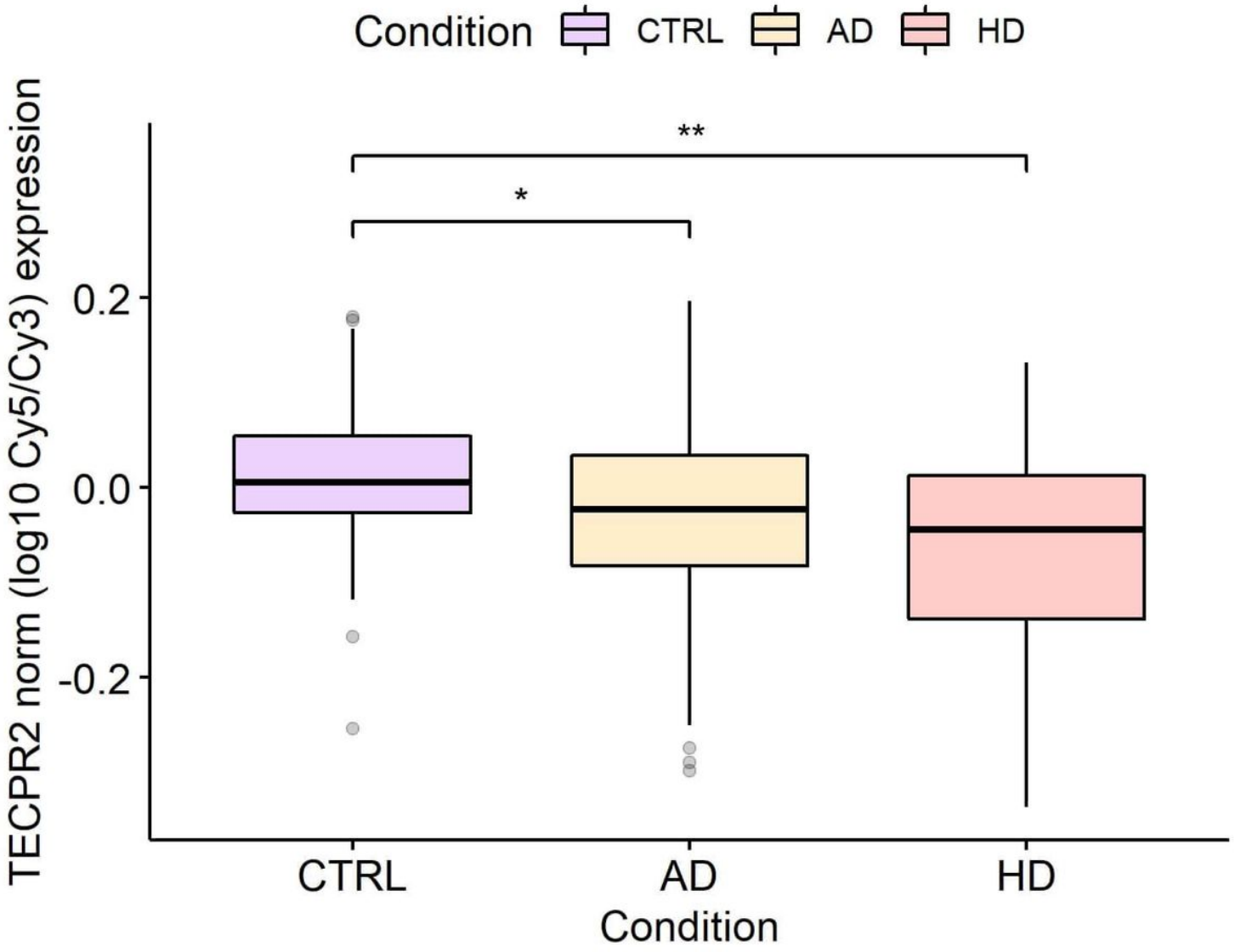

Figure 3

TECPR2 downregulation in postmortem prefrontal cortex from individuals with AD and HD. Shown are boxplots of normalized TECPR2 expression in postmortem prefrontal cortex from 310 individuals with AD (orange), 157 individuals with HD (red) and 157 neurotypical controls (purple). *Adjusted P = 2.83x10-6; **Adjusted P = 2.87x10-9.

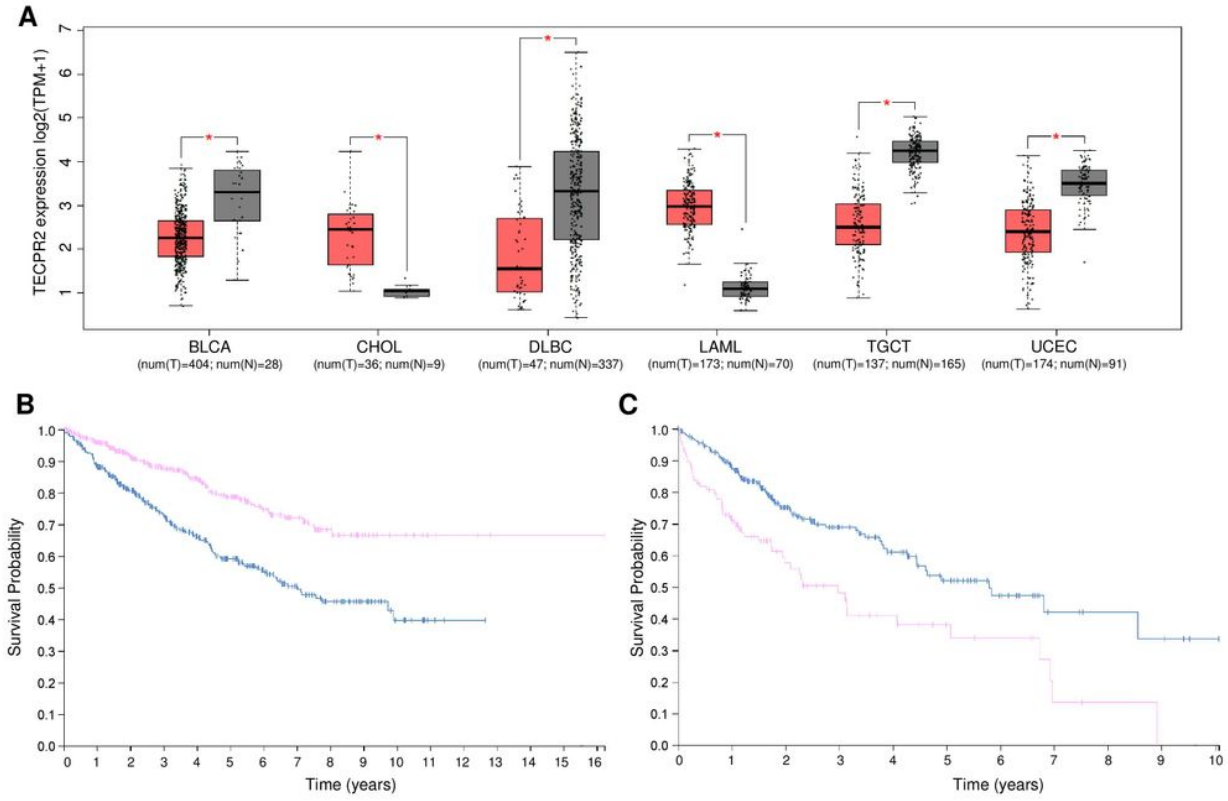

Figure 4

TECPR2 dysregulation and prognostic value in cancer. (A) Among 31 TCGA cancers, TECPR2 was found to be significantly dysregulated in bladder urothelial carcinoma (BLCA), cholangiocarcinoma (CHOL), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), acute myeloid leukemia (LAML), testicular germ cell tumors (TGCT), and uterine corpus endometrial carcinoma (UCEC). Normalized tumor expression is shown in red, matched normal tissue and relevant GTEx sample expression is in gray. The number of samples in each comparison is noted. Adjusted P < 0.001. (B) Kaplan-Meier survival plot based on TECPR2 expression in renal cancers, including clear cell renal cell carcinoma (CCRCC), papillary renal cell carcinoma (PRCC), and chromophobe renal cell carcinoma (P = 6.5x10-9). The pink line depicts the survival of individuals whose tumor samples had a normalized TECPR2 expression ≥ 3.25 FPKM; The blue line depicts the survival of individuals whose tumor samples had a normalized TECPR2 expression < 3.25 FPKM. (C) Kaplan-Meier survival analysis based on TECPR2 expression in hepatocellular carcinoma (HCC, P = 5.7x10-5, cut-off of 1.67 FPKM, similar representation as in B).

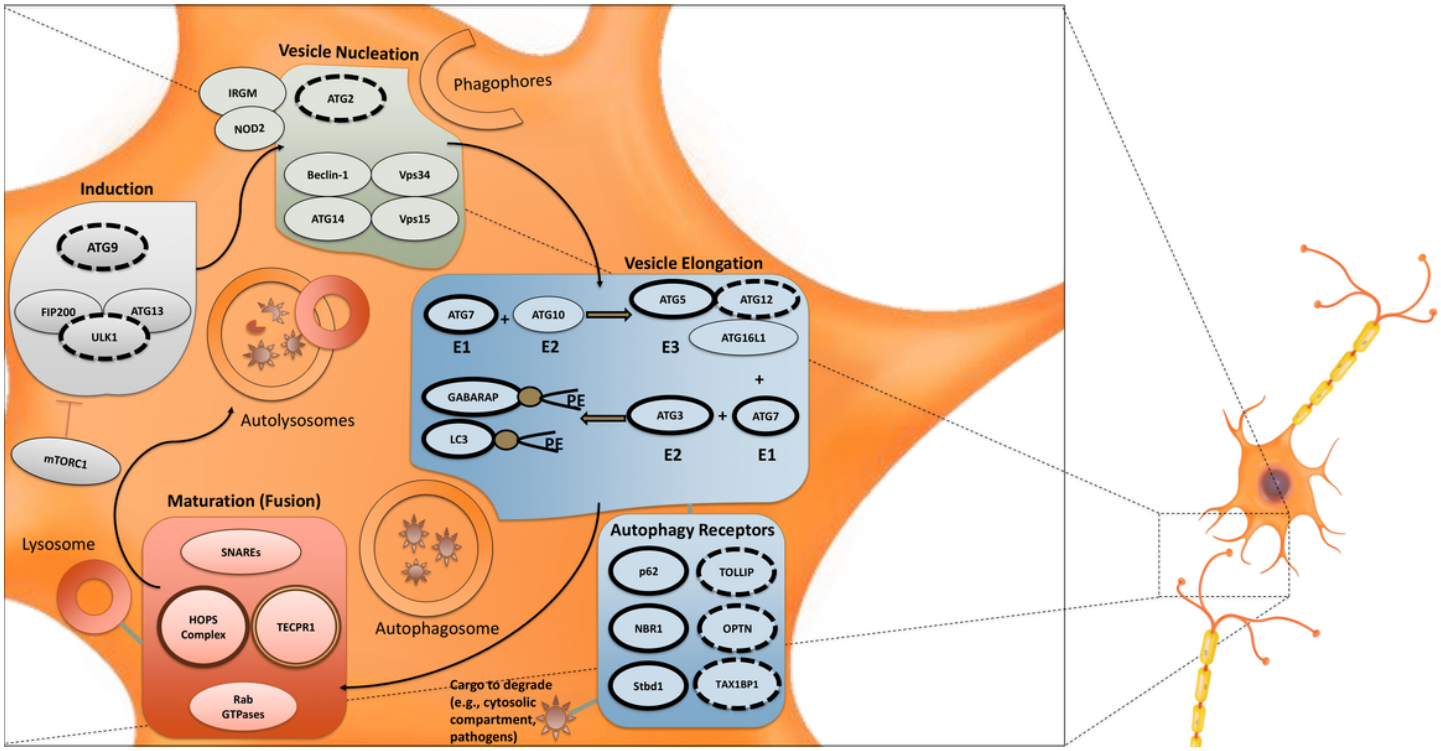

Figure 5

A scheme of autophagy stages and the main proteins involved in each stage. Proteins that are directly associated with TECPR2 are highlighted in thick lines. Proteins that are indirectly associated with TECPR2 are shown in dashed lines. The evolutionary relation between TECPR2 and TECPR1 is highlighted by a double line.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigureS3.jpg
- TableS2Transcriptionalregulatorscoregulatedtargets.xlsx
- TableS1allTECPR2relatedgenesandproteins.xlsx
- TableS5Phylogeneticprofiling.xlsx
- TableS6Proteinproteininteractions.xlsx
- TableS7Curatedgenesets.xlsx
- TableS8Integratedfunctionalenrichment.xlsx
- FigureS2.jpg
- SupplementaryFigureTablelegends.docx
- TableS4Tissuecoexpressionpartners.xlsx
- FigureS1.JPG

- TableS3Neurodevelopmentalcoexpression.xlsx