Brain-Site-Specific Proteome Changes Induced by Neuronal P60TRP Expression

Arulmani Manavalan[a, b]   Manisha Mishra[a, b]   Siu Kwan Sze[a]   Klaus Heese[c]

[a]School of Biological Sciences and [b]Institute of Advanced Studies, Nanyang Technological University, Singapore, Singapore; [c]Department of Biomedical Engineering, Hanyang University, Seoul, Korea

Abstract

p60 transcription regulator protein (p60TRP) facilitates the processing of the amyloid precursor protein towards the non-amyloidogenic pathway by inhibiting the β-secretase action. This protein was initially identified to be downregulated in the temporal lobe of brains from Alzheimer's disease patients. p60TRP is one of the G-protein-coupled receptor (GPCR)-associated proteins which directly influences the signalling capacity of GPCRs. In the present study, we investigated the brain-region-specific proteome profile of transgenic p60TRP mice to gain an insight into the molecular events mediated by the long-term effect of neuronal p60TRP overexpression on brain proteome changes and its potential implication for neuronal functions in the central nervous system. Using a proteomics research approach based on isobaric tags for relative and absolute quantitation, we identified 2,025 proteins, whereby 1,735 proteins were quantified, out of which 56 were found to be significantly altered in the cortex and/or hippocampus of neuronal transgenic neuronal p60TRP mice. Our data suggests that in vivo overexpression of neuronal p60TRP significantly affects cognitive and neuroprotective capacities.

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Introduction

G-protein-coupled receptors (GPCRs) represent one of the most abundant protein families that are involved in the modulation of numerous physiological functions thus represent major drug targets. The activity of GPCRs is tightly controlled by various interacting partners that modulate their membrane targeting, intracellular trafficking and signalling properties. One of the ways in which their activity is regulated is by the process of desensitisation and endocytosis, whereby agonist-activated receptors are rapidly and often reversibly silenced through removal from the cell surface. Following endocytosis, individual receptors can be sorted differentially between recycling endosomes and lysosomes, which control the reversibility of silencing. Thus, endocytosis can either serve as a mechanism for receptor resensitisation by delivering receptors back to the plasma membrane or facilitate receptor downregulation by serving as the first step towards targeting the receptors to lysosomal degradation. The sorting of receptors to the lysosomal pathway can be facilitated by interaction with an array of accessory proteins. Among them, the GPCR-associated sorting proteins (GPRASPs) have been shown to display a

A.M. and M.M. contributed equally to this study.

broad spectrum of interactions with GPCRs. In recent years, extensive research has identified and categorized numerous interacting partners of GPCRs and revealed the GPRASP family [1, 2]. In addition to their postulated role in the modulation of the postendocytic sorting of these receptors, recent data indicate that some of the GPRASP family members may modulate the transcriptional activity of the cell. The p60 transcription regulator protein (p60TRP), also known as GASP3 or BHLHB9, is one novel member of this recently discovered GPRASP family [3, 4]. Amongst the many distinguishing features of p60TRP it is noteworthy that it contains a myc-type bHLH-like domain at its C terminus which is a protein structural motif that characterizes transcription factors. Our studies have also shown that p60TRP regulates endocytic recycling of the δ-opioid receptor and is a predominant nervous system-associated protein that localizes to both the cytoplasm and the nucleus of cells [3, 4].

Our newly established transgenic p60TRP mice consistently revealed a tendency to repair Alzheimer's disease (AD)-related deficits by facilitating the proteolytic cleavage of the amyloid precursor protein towards the non-amyloidogenic pathway, higher synaptic plasticity as well as cognitive functions [4]. We applied the 2-dimensional (2D) liquid chromatography coupled with tandem mass spectrometry-based isobaric tag for relative and absolute quantification (2D-LC-MS/MS iTRAQ) technique into our clinically relevant in vivo mouse model for quantitative profiling of p60TRP-regulated brain-region-specific genes. Subsequently, we generated the proteome comprising the regulated proteins from the transgenic p60TRP mouse-derived brain (fig. 1). Our results show for the first time the long-term in vivo effect of neuronal p60TRP overexpression on brain-region-specific proteome changes and its potential implication for brain functions.

Materials and Methods

Reagents
Unless indicated, all reagents used for biochemical methods were purchased from Sigma-Aldrich (St. Louis, Mo., USA). Materials and reagents for SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) were from Bio-Rad (Bio-Rad Laboratories, Hercules, Calif., USA). The iTRAQ reagent multiplex kit was bought commercially (Applied Biosystems, Foster City, Calif., USA).

Antibodies
Anti-Acadl (acyl-coenzyme A dehydrogenase, long-chain, 1:1,000, rabbit polyclonal; Abcam, Cambridge, UK), anti-Gapdh (glyceraldehyde-3-phosphate dehydrogenase, 1:3,000, mouse monoclonal; Santa Cruz Biotechnology Inc., Santa Cruz, Calif., USA), anti-Mbp (myelin basic protein, 1:200, rat monoclonal; Abcam), anti-Mtap2 (microtubule-associated protein 2, 1:1,000, rabbit polyclonal; Abcam), anti-Sept2 (septin 2, 1:200, goat polyclonal; Santa Cruz Biotechnology), anti-Snca [synuclein (α), 1:1,000, mouse monoclonal; Santa Cruz Biotechnology], anti-Sncb (synuclein (β), 1:1,000, mouse monoclonal; Santa Cruz Biotechnology), anti-Sod1 (superoxide dismutase 1, 1:1,500, rabbit polyclonal; Abcam), anti-Synj1 (synaptojanin 1, polyphosphoinositide phosphatase, 1:1,000, rabbit polyclonal, mouse monoclonal; BD Biosciences, Clontech, Palo Alto, Calif., USA) and anti-Tuba8 (tubulin, α_8, 1:2,000, mouse monoclonal; Abcam).

Animal Material
Experimental procedures, including the killing of animals, were in accordance with the International Guiding Principles for Animal Research (World Health Organization) and were approved by the local Institutional Animal Care and Use Committee (Nanyang Technological University Institutional Animal Care and Use Committee). Throughout all protocols, mice were provided with food and water ad libitum and maintained in controlled conditions (12-hour light-dark cycle, 25°C). All offspring were weaned onto standard chow at 21 days of age, and metabolic tissue parameters were assessed at 18 months of age. Mouse tissues, i.e. from p60TRP transgenic mice [4] and control wild-type litter mates (C57BL/6J), were isolated after humane killing of the animals using approved anaesthetic methods. To analyse the tissue-specific protein expression, all efforts were made to minimize animal suffering and to reduce the number of animals used [4, 5].

Brain-Tissue-Specific Protein Expression Analysis
Brain tissues (cortex (Cx) and hippocampus (Hp)) were isolated from adult (approx. 18 months) transgenic p60TRP mice and wild-type litter mates (each group consisting of 3 males and 3 females, i.e. 6 mice) from each mouse strain tg38 and tg31, for both batches B-I and B-II, respectively (fig. 1). Briefly, tissues were excised, snap-frozen in liquid nitrogen, and then powdered using

Fig. 1. Schematic representation of the experimental design showing biological and technical replicates. Brain tissues were isolated from adult (approx. 18 months) transgenic p60TRP mice (tg) and wild-type litter mates (wt), each group (B-I and B-II) consisting of 3 males and 3 females, separately for each brain area (Cx and Hp) and for each mouse strain (tg38 and tg31); for B-I: 6 tg31 Cx and 6 tg31 Hp, 6 tg38 Cx and 6 tg38 Hp, 6 wt Cx and 6 wt Hp; for B-II: 6 tg31 Cx and 6 tg31 Hp, 6 tg38 Cx and 6 tg38 Hp, 6 wt Cx and 6 wt Hp. Following brain-derived tissue lysis, protein extracts were acetone precipitated, quantified, run on SDS-PAGE and subsequently digested. The quantitative proteomics analyses of transgenic p60TRP mouse-derived brains were performed by labelling with multiplex iTRAQ reagents (113–118) followed by electrostatic repulsion-hydrophilic interaction chromatography (ERLIC)-based fractionation, and LC-MS/MS-based multidimensional protein identification technology. The obtained data was analysed using ProteinPilot software and validated by quantitative Western blots. Finally, proteins were functionally classified into various subgroups.
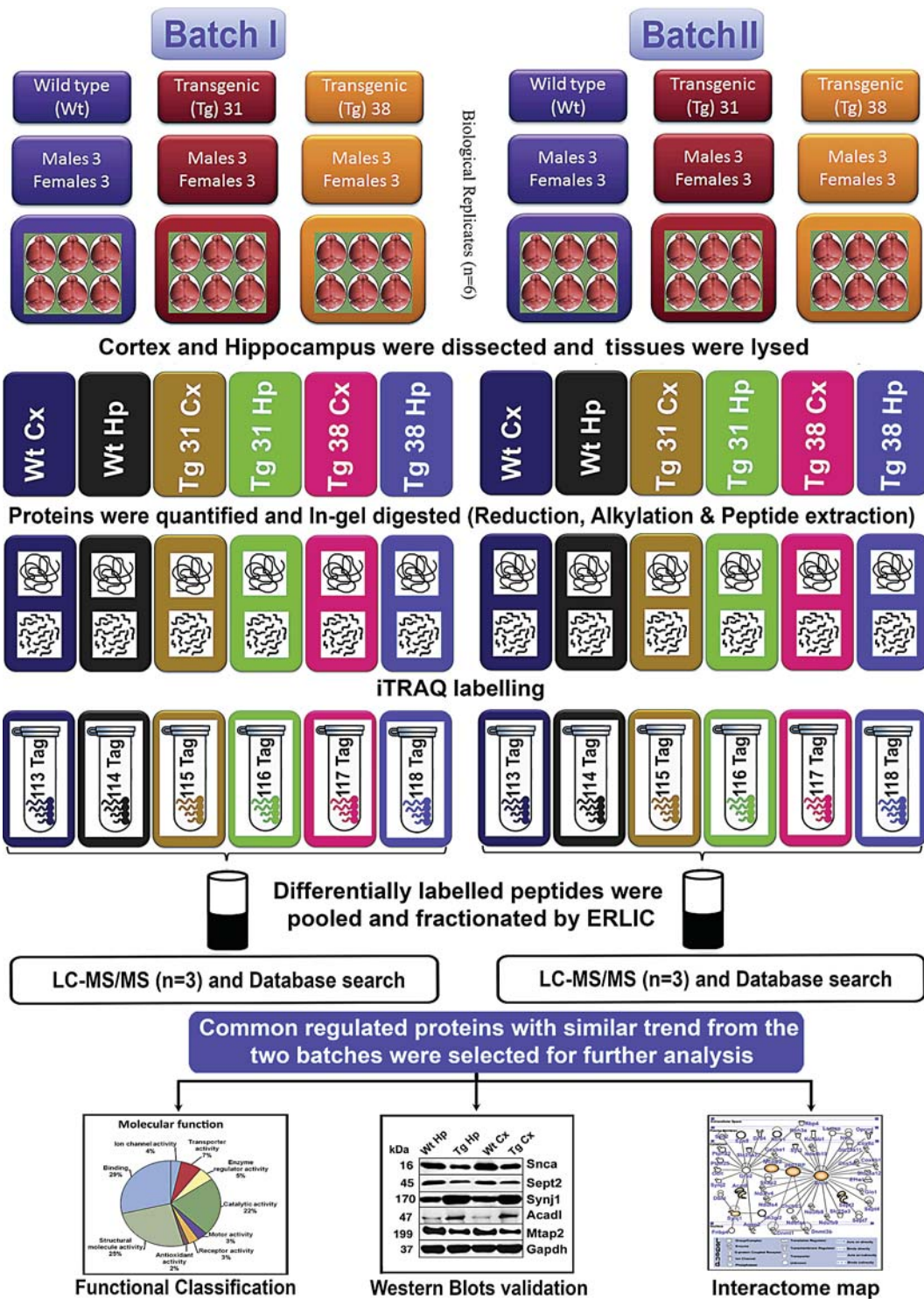
a mortar and pestle. Upon the addition of lysis buffer [2% SDS, 0.5 M triethyl ammonium bicarbonate buffer, 1 Complete™ protease inhibitor cocktail tablet (Roche, Mannheim, Germany) and 1 PhosSTOP phosphatase inhibitor cocktail tablet (Roche)], the samples were vortexed for 1 min and incubated on ice for an additional 45 min prior to homogenization (sonication parameters: amplitude, 23%; pulse: 5 s/5 s for 5 min) using a Vibra Cell high-intensity ultrasonic processor (Jencons Scientific Ltd., Leighton Buzzard, UK). After centrifugation (20,000 g/4°C/30 min), the supernatant was collected and stored at –80°C until further use. The protein concentration was quantified by a '2-D Quant' kit (Amersham, Piscataway, N.J., USA) according to the manufacturer's protocol.

iTRAQ Protocol

A detailed description of the 2D-LC-MS/MS iTRAQ procedures [6, 7], including postproteomic data verification by SDS-PAGE/Western blot analyses [8, 9], can be found in the online supplementary document (for all online suppl. material, see www.karger.com/doi/10.1159/000343672) as described previously.

Experimental Design

Six wild-type mice (3 males, 3 females) and 6 transgenic mice (3 males, 3 females, for each mouse strain) were used. For each set (B-I and B-II), we used 6 samples to perform iTRAQ (6 wild-type Cxs and Hps and 6 transgenic Cxs and Hps from both mouse strains, respectively, i.e. tg31-Cx, tg31-Hp, tg38-Cx, tg38-Hp) as 6 pooled biological replicates. All experiments were performed twice (B-I and B-II; fig. 1) with each set repeated 6 times (separately for each area, Cx and Hp, and for each mouse strain, tg38 and tg31, respectively). This was to ensure high confidence in the detection of brain-region-specific proteins regulated by neuronal p60TRP expression. The quality of the data set and instrumental reproducibility was then confirmed by comparing and combining 3 technical replicates [6] after the samples had been labelled with 113, 114, 115, 116, 117 and 118 isobaric tags and processed by LC-MS/MS.

In order to verify that the protein samples were indeed from the whole brain tissue proteome, the identified proteins were uploaded into JVirGel (http://www.jvirgel.de/), a database that creates a virtual 2D gel picture [10].

We used online databases, e.g. Panther (www.pantherdb.org), UniProt, NCBI, and 'softberry' (http://linux1.softberry.com/berry.phtml), to classify the functions of the iTRAQ-identified p60TRP-regulated proteins. STRING (Search Tool for the Retrieval of Interacting Genes, http://string-db.org/; *Mus musculus*, default mode) analysis was carried out to retrieve information about functional partnerships among proteins that constitute the core of complex cellular phenotypes in the transgenic p60TRP brain. Further biocomputational network analysis of the iTRAQ-identified p60TRP-regulated proteins using the Ingenuity Pathways Analysis (IPA, http://www.ingenuity.com) offered us additional valuable clues about the complex interactive link of these proteins in the brain.

Statistical Analysis

The data obtained in the Western blot analyses in this investigation are illustrated as means ± SD, and Student's t test was applied. IBM-SPSS (IBM Statistical Products and Service Solutions) for Windows version 19 was used to perform ANOVA followed by Fisher's protected least significant difference post hoc tests, when warranted. For the iTRAQ analysis, ProteinPilot Software 3.0 was used. To be considered statistically significant, we required a probability value to be at least <0.05 (95% confidence limit, $p < 0.05$) [5, 8, 9].

Results

Identification of p60TRP-Regulated Proteins in Mouse Brain Cx and Hp

Through iTRAQ we identified a total of 2,025 proteins (Cx and Hp), whereby 1,735 proteins were quantified [with a strict cut-off of unused ProtScore ≥2 as the qualification criteria, which corresponds to a peptide confidence level of 99% and an applied false discovery rate of 0.33% (<1.0%)]; out of 1,735 proteins 56 (Cx and Hp) showed altered protein expression levels that exhibited common trends for both transgenic mouse lines (fig. 1, tables 1–3, online suppl. tables; the cut-off for up- and downregulation was predefined at 1.2 and 0.83, respectively).

In particular, 24 proteins were significantly modulated in the Cx (table 1) and 37 proteins were significantly regulated in the Hp (table 2) suggesting a critical role of p60TRP in Hp compared to Cx functions. Comparing the altered proteins in these two groups, we could identify 5 proteins that were specifically modulated in the Hp and the Cx (table 3: Snca, Sept2, Mtap2, Acadl and Synj1), 32 proteins (e.g. Ndufv2, Dlg4, Cdh2, Sept9, Camk2a and Syn2) were specifically regulated only in the Hp but not in the Cx [table 2: 7 proteins upregulated and 25 proteins downregulated (22%:78%)], and 19 other proteins (e.g. Sncb, Arf3, Gria2, Sept6, Rin1, Dmxl2, Ranbp1 and Slc12a5) were specifically regulated in the Cx but not in the Hp [table 1: 8 proteins upregulated and 11 proteins downregulated (42%:58%)]. Remarkably, proteins modulated in both sites (Hp and Cx) showed the same trend in either site: if up- or downregulated in the Hp, then also up- or downregulated in the Cx (table 3). While those proteins modulated specifically in the Hp were mainly downregulated (22%:78%; table 2), proteins modulated specifically in the Cx were regulated almost in equal measure (42%:58%; table 1).

Biocomputational Classification of p60TRP-Regulated Proteins

During the classification process, our objective was also to identify the proteins' subcellular localization and action (fig. 2–4; tables 1–3). While the 56 regulated

Table 1. Functional classification of iTRAQ-quantified proteins differentially expressed in the Cx of p60TRP transgenic (tg Cx) mouse brains with reference to the wild-type Cx

Protein IDs	Gene symbols	Protein names	Biological process	Peptides (>95%) n	tg Cx/ wt Cx	p value	Subcellular location
Parkinson's disease							
IPI00131614.1	Sncb	β-Synuclein	Neurotransmitter secretion	5	0.57	0.03	Cytoplasmic
Huntington's disease							
IPI00221614.3	Arf3	ADP-ribosylation factor 3	Intracellular protein transport	21	0.76	0.03	Golgi
Receptor activity							
IPI00608015.1	Gria2	Glutamate receptor 2	Neurological system process	7	1.25	0.04	Plasma membrane
Chaperonic response							
IPI00230707.6	Ywhag	14-3-3 protein γ	Signal transduction	39	0.76	0.04	Cytoplasmic
GTPase activity/G protein							
IPI00473707.3	Sept6	Septin 6	GTP binding	9	1.49	0.02	Cytoplasmic
IPI00853932.1	Dmxl2	DmX-like protein 2	Intracellular protein transport	6	1.25	0.03	Cytoplasmic
IPI00321978.3	Ranbp1	Ran-specific GTPase-activating protein	Intracellular protein transport	4	0.82	0.04	Cytoplasmic
IPI00122131.1	Rin1	Ras and Rab interactor 1	Intracellular protein transport	2	0.7	0.05	Cytoplasmic
Phosphatases							
IPI00120374.1	Ppp2ca	Serine/threonine protein phosphatase 2A catalytic subunit α isoform	Immune system process	9	1.56	0.05	Cytoplasmic
Microtubule-associated protein/cytoskeletal protein							
IPI00311175.5	Tuba8	Tubulin α8 chain	Intracellular protein transport	114	1.29	0.01	Cytoplasmic
IPI00230751.2	Ctnna2	Catenin α2	Cellular component morphogenesis	11	1.37	0.01	Cytoplasmic
IPI00649104.1	Mapt	Microtubule-associated protein τ	Apoptosis	12	0.71	0.01	Nuclear
IPI00128973.1	Gap43	Neuromodulin	Axon choice point recognition	20	0.69	0.04	Membrane
IPI00115833.2	Mtap6	Microtubule-associated protein 6	Microtubule binding	25	0.49	0.01	Cytoplasmic
IPI00129519.3	Basp1	Brain acid soluble protein 1	Neurological system process	22	0.56	0.01	Cytoplasmic
IPI00153990.1	Actr1b	β-Centractin	Intracellular protein transport	9	0.68	0.01	Cytoplasmic
Transport protein							
IPI00877254.1	Slc12a5	Solute carrier family 12 member 5	Synaptic vesicle/cation transport	10	1.23	0.01	Plasma membrane
Other proteins							
IPI00874565.1	Nckap1	NCK-associated protein 1	Protein stabilization	8	1.24	0.05	Plasma membrane
IPI00471441.1	Ptms	Parathymosin	Nucleotide and nucleic acid metabolic process	6	0.47	0.02	Nuclear

The list contains quantitative information of the proteins from transgenic p60TRP mouse Cx compared with wild-type mice. These proteins have met the criteria [i.e. unused ProtScore >2.0, false discovery rate of 0.33% (<1.0%), change in expression levels of at least 1.2-fold (upregulation) or at least <0.833-fold (downregulation)] as defined in the experimental procedures. Peptides = The total number of peptides identified with >95% confidence; wt = wild-type mice; tg = transgenic p60TRP mice; NCK = non-catalytic region of tyrosine kinase.

proteins had a reasonable distribution in terms of biological process, molecular function and protein class (fig. 2), it is of interest to note that a substantial portion of the identified proteins that were specifically affected in the Hp were oxidoreductases (approx. 24%, fig. 3c), while none of the oxidoreductase subgroup proteins were altered in the Cx and, instead, the number of cytoskeletal proteins were enlarged in the Cx – hinting at enhanced synaptic plasticity (approx. 26%, fig. 2c). Interestingly, only in the Hp were p60TRP-regulated pro-

teins linked with the plasma membrane. Our proteomic data from AD brain-region-specific studies of human samples also revealed significant alterations in extracellular and plasma membrane-associated proteins in the Hp [unpubl. data]. This implicates that a tight regulation of plasma membrane-associated proteins is a prerequisite for normal functioning of the Hp.

We also realized that some of the p60TRP-regulated proteins could be partially linked with various neurodegenerative diseases such as Parkinson's (PD) or Hunting-

Table 2. Functional classification of iTRAQ-quantified proteins differentially expressed in the Hp of p60TRP transgenic (tg Hp) mouse brains with reference to the wild-type Hp

Protein IDs	Gene symbols	Protein names	Biological process	Peptides (>95%) n	tg Hp/ wt Hp	p value	Subcellular location
Parkinson's disease							
IPI00169925.2	Ndufv2	NADH dehydrogenase (ubiquinone) flavoprotein 2, mitochondrial	Apoptosis	10	4.02	0.02	Mitochondrial
Huntington's disease							
IPI00122094.2	Dlg4	Discs large homolog 4	Neurological system process	16	0.59	0.03	Plasma membrane
Receptor activity							
IPI00323134.1	Cdh2	Cadherin 2	Cell surface receptor-linked signal transduction	3	1.39	0.05	Plasma membrane
GTPase activity/G protein							
IPI00515330.1	Sept9	Septin 9	GTP binding	3	0.71	0.05	Cytoplasmic
IPI00405625.9	Cyfip2	Cytoplasmic FMR1-interacting protein 2	Signal transduction	20	0.58	0.04	Cytoplasmic
Kinases/phosphatases							
IPI00621806.2	Camk2a	Calcium/calmodulin-dependent protein kinase type II α-chain	Neural activity and plasticity	59	0.54	0.01	Cytoplasmic
IPI00136703.1	Ckb	Creatine kinase B type	Muscle contraction	84	0.75	0.02	Mitochondrial
Ubiquitin proteasome							
IPI00608097.1	Npepps	Puromycin-sensitive aminopeptidase	Proteolysis	17	0.62	0.04	Cytoplasmic
Microtubule-associated protein/cytoskeletal protein							
IPI00223377.1	Mbp	Myelin basic protein	Cellular component morphogenesis	23	1.91	0.03	Nuclear
IPI00124954.1	Kif5b	Kinesin 1 heavy chain	Intracellular protein transport	10	2.53	0.04	Cytoplasmic
IPI00454081.1	Kif21a	Kinesin-like protein KIF21A	Intracellular protein transport	3	2.07	0.01	Cytoplasmic
IPI00676243.2	Mtap1a	Microtubule-associated protein 1A	Protein binding	78	0.71	0.04	Cytoplasmic
IPI00319830.7	Spnb2	Isoform 1 of spectrin β-chain, brain 1	Actin filament capping	134	0.6	0.03	Cytoplasmic
IPI00134344.6	Spnb3	Spectrin β$_3$	Synaptic vesicle exocytosis	61	0.77	0.05	Cytoplasmic
Oxidative stress							
IPI00130589.8	Sod1	Superoxide dismutase (Cu-Zn)	Superoxide metabolic process	11	1.47	0.01	Cytoplasmic
IPI00876084.1	Gpd2	Glycerol-3-phosphate dehydrogenase, mitochondrial	Respiratory electron transport chain	23	0.69	0.01	Mitochondrial
IPI00459725.2	Idh3a	Isocitrate dehydrogenase (NAD) subunit α, mitochondrial	Tricarboxylic acid cycle	12	0.52	0.05	Mitochondrial
IPI00323592.2	Mdh2	Malate dehydrogenase, mitochondrial	Tricarboxylic acid cycle	52	0.54	0.02	Mitochondrial
Transport protein							
IPI00323571.1	Apoe	Apolipoprotein E	Lipid transport	8	1.28	0.02	Extracellular (secreted)
IPI00121576.2	Apoo	Apolipoprotein O		3	3.4	0.02	Plasma membrane
IPI00230165.1	Snap91	Synaptosomal-associated protein 91	Intracellular protein transport	23	0.55	0.03	Nuclear
IPI00469548.2	Syn2	Synapsin 2	Neurological system process	51	0.58	0.03	Cytoplasmic
IPI00648173.1	Cltc	Clathrin, heavy polypeptide	Intracellular protein transport	109	0.6	0.01	Nuclear
IPI00131695.3	Alb	Serum albumin	Transport	17	0.58	0.04	Extracellular (secreted)
IPI00649135.2	Gstm1	Glutathione S-transferase, mu 1	Glutathione transferase activity	26	0.7	0.05	Cytoplasmic
IPI00831055.2	Hbb-b1	β-Globin	Oxygen transport	15	0.72	0.03	Membrane-bound endoplasmic reticulum

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Table 2 (continued)

Protein IDs	Gene symbols	Protein names	Biological process	Peptides (>95%) n	tg Hp/ wt Hp	p value	Subcellular location
Mitochondrial proteins							
IPI00308882.4	Ndufs1	NADH ubiquinone oxidoreductase 75-kDa subunit, mitochondrial	Respiratory electron transport chain	28	0.63	0.01	Mitochondrial
IPI00122547.1	Vdac2	Voltage-dependent anion-selective channel protein 2	Anion transport	16	0.73	0.05	Mitochondrial
IPI00230754.5	Slc25a11	Mitochondrial 2-oxoglutarate/malate carrier protein	Cation transport	14	0.64	0.01	Mitochondrial
IPI00308162.3	Slc25a12	Calcium-binding mitochondrial carrier protein Aralar1	Cation transport	42	0.76	0.01	Membrane-bound mitochondrial
Other proteins							
IPI00462072.3	Eno1	α-Enolase	Glycolysis	118	0.61	0.02	Cytoplasmic
IPI00877205.1	Got1	Glutamate oxaloacetate transaminase 1, soluble	Aspartate biosynthetic process	39	0.5	0.01	Cytoplasmic

The list contains quantitative information of the proteins from transgenic p60TRP mouse Hp compared with wild-type mice. These proteins have met the criteria [i.e. unused ProtScore >2.0, false discovery rate of 0.33% (<1.0%), change in expression levels of at least 1.2-fold (upregulation) or at least <0.833-fold (downregulation)] as defined in the experimental procedures. Peptides = The total number of peptides identified with >95% confidence; wt = wild-type mice; tg = transgenic p60TRP mice.
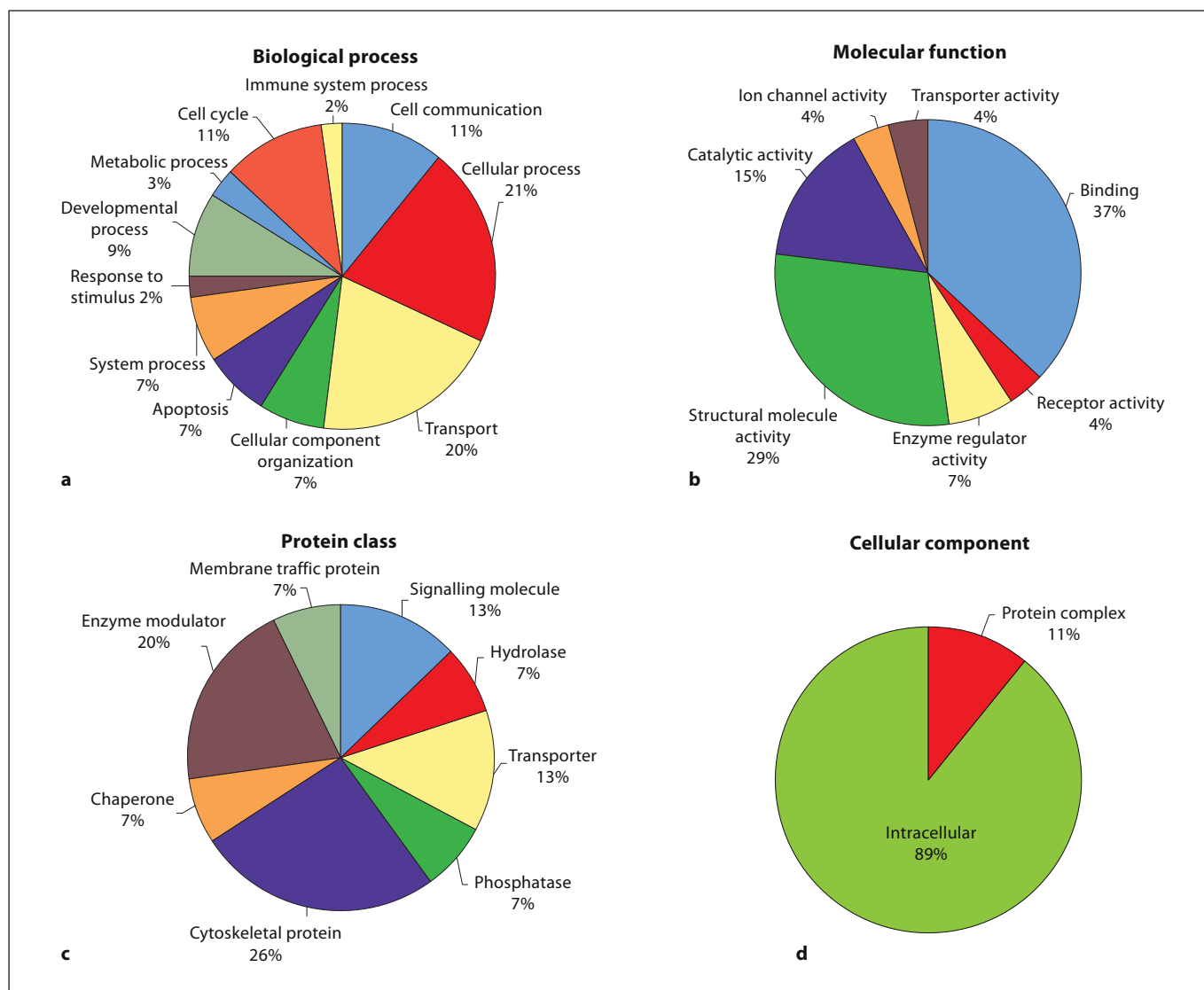
Table 3. Functional classification of iTRAQ-quantified proteins differentially expressed in the Cx and Hp of p60TRP transgenic mice with reference to the wild-type Cx and Hp

Protein IDs	Gene symbols	Protein names	Biological process	Peptides (>95%) n	tg Cx/ wt Cx	p value	tg Hp/ wt Hp	p value	Subcellular location
Parkinson's disease									
IPI00845803.1	Snca	α-Synuclein	Fatty acid metabolic process	4	0.79	0.05	0.78	0.03	Cytoplasmic
GTPase activity/G protein									
IPI00114945.1	Sept2	Septin 2	GTP binding	5	0.8	0.05	0.71	0.04	Cytoplasmic
Phosphatases									
IPI00850983.1	Synj1	Synaptojanin 1	Inositol phosphate dephosphorylation	5	2.75	0.05	2.88	0.05	Cytoplasmic
Mitochondrial proteins									
IPI00894588.1	Acadl	Long-chain acyl-CoA dehydrogenase	Fatty acid metabolism	3	1.31	0.05	1.41	0.05	Membrane-bound mitochondrial
Microtubule-associated protein/cytoskeletal protein									
IPI00895965.1	Mtap2	Microtubule-associated protein 2	Dendrite development	58	0.74	0.001	0.7	0.001	Cytoplasmic

The list contains quantitative information of the proteins from transgenic p60TRP mouse Hp and Cx compared with-wild type mice. These proteins have met the criteria [i.e. unused ProtScore >2.0, false discovery rate of 0.33% (<1.0%), change in expression levels of at least 1.2-fold (upregulation) or at least <0.833-fold (downregulation)] as defined in the experimental procedures. Peptides = The total number of peptides identified with >95% confidence; wt = wild-type mice; tg = transgenic p60TRP mice.

Fig. 2. Pie chart depicting the classification of p60TRP-regulated proteins in the Cx characterized by iTRAQ within the molecular function gene ontology category. Subcellular and functional categories were based on the annotations of gene ontology using the mouse genome informatics (MGI) GO_Slim Chart Tool. Analyses were done based on the following categories: biological process (**a**); molecular function (**b**); protein class (**c**), and cellular component (subcellular compartment, **d**).

ton's diseases (e.g. Snca/b, Ndufv2, Dlg4 and Arf3; tables 1–3), familial amyotrophic lateral sclerosis, schizophrenia and depressive disorder, suggesting a convergent nature of neurodegenerative mechanisms, hence linking different types of neurodegenerative disorders.

Data Validation by Western Blot

Following the database search and classification of proteins, Western blots were performed on randomly selected proteins to verify the iTRAQ values. Ten proteins, (Snca, Sept2, Synj1, Acadl, Mtap2, Mbp, Sod1, Sncb, Tuba8 and Gapdh) were used for validation (fig. 5). Gapdh was used as an internal control to ensure equal loading of the samples as its level was also unchanged in the iTRAQ analyses.

Remarkably, the Western blot images correlated very well and thus confirmed the iTRAQ values obtained.

Biocomputational Network Analyses

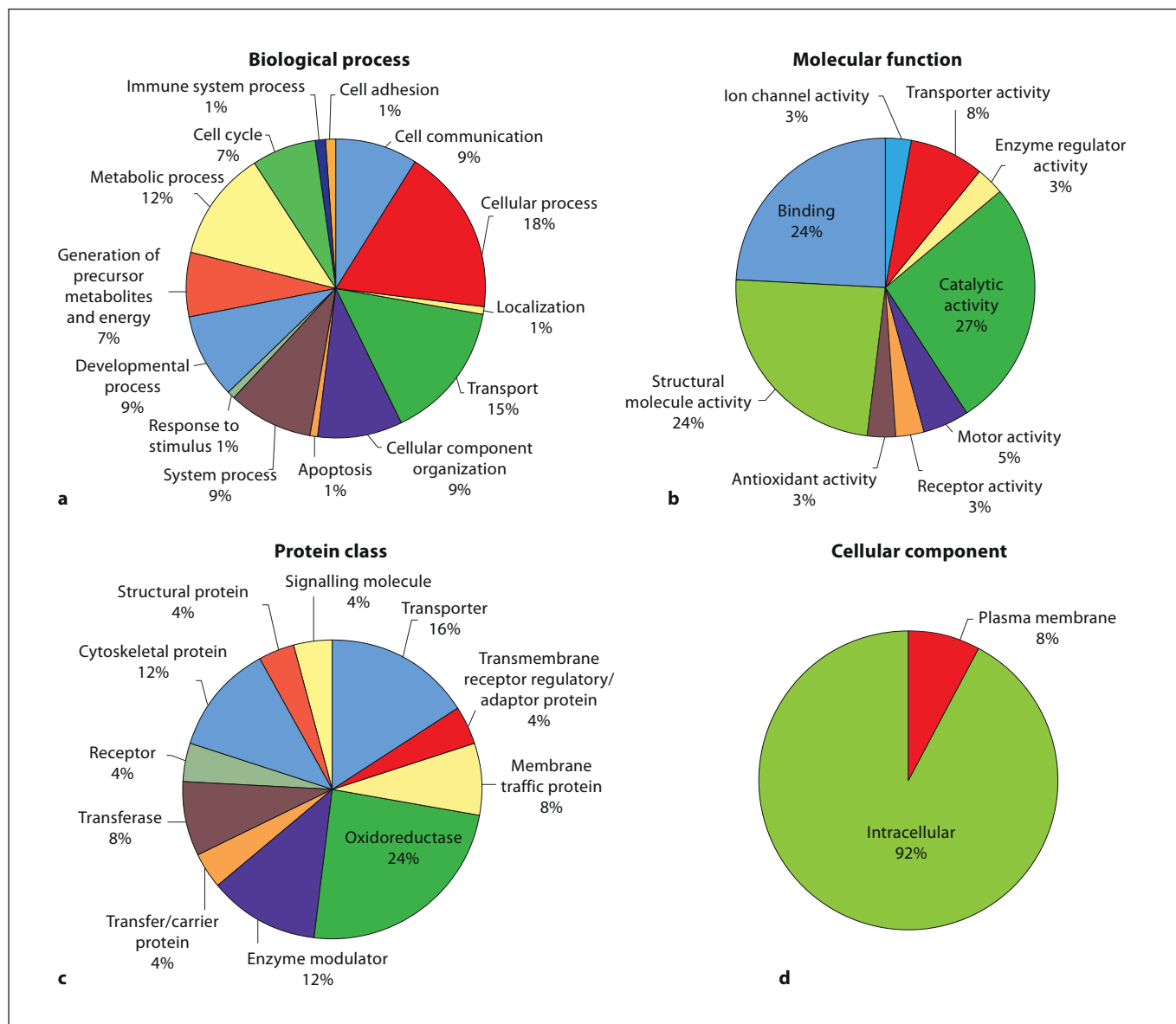The networks formed by the interacting proteins provided us crucial scaffolds for modelling the brain

Biological process

a

Molecular function

b

Protein class

c

Cellular component

d

Fig. 3. Pie chart depicting the classification of p60TRP-regulated proteins in the Hp characterized by iTRAQ within the molecular function gene ontology category. Subcellular and functional categories were based on the annotations of gene ontology using the mouse genome informatics (MGI) GO_Slim Chart Tool. Analyses were done based on the following categories: biological process (**a**); molecular function (**b**); protein class (**c**), and cellular component (subcellular compartment, **d**).

system level mechanisms of cellular events involved in p60TRP-mediated brain functions. It revealed the functional link among metabolically modulated proteins specifically regulated in the Cx and Hp, respectively (fig. 6, 7). In particular, it shows the link between p60TRP and Cdk proteins – a possible pathway through which p60TRP can modulate synaptic plasticity [4, 11], eventually via influencing the anaphase-promoting complex as shown previously by others [5, 12] –, and the link between p60TRP and Ipo5, thus implicating a potential role of p60TRP within the nucleus. Besides, p60TRP's influence on oxidoreductases (Nduf, Sod1) and heat shock proteins (Hspd1 and Hspe1) may make the vulnerable Hp more stable against oxidative stress (fig. 7). Moreover, the physical interaction of p60TRP with Pp2ca connects it with several essential and life-
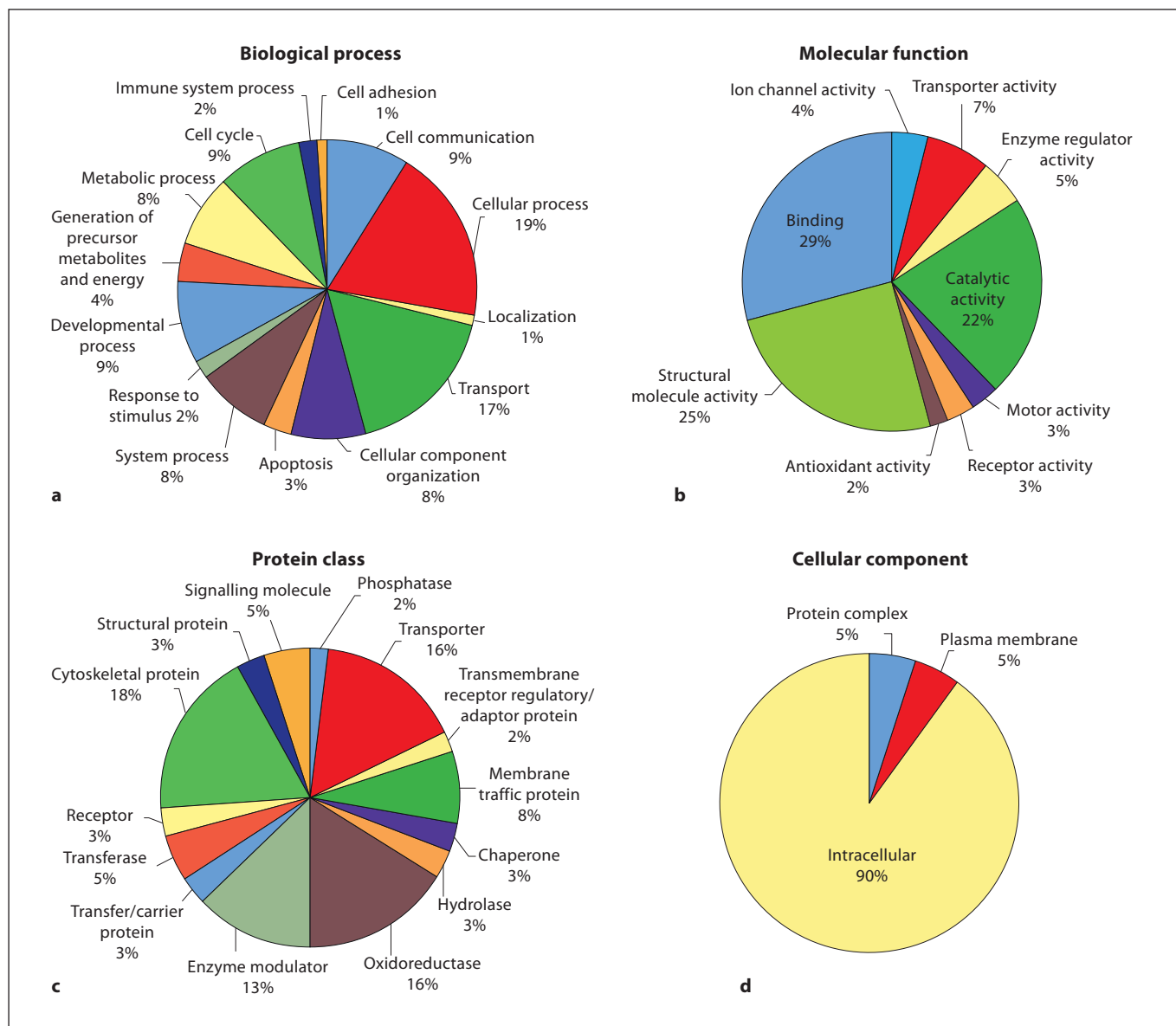
Fig. 4. Pie chart depicting the classification of p60TRP-regulated proteins in the Hp and Cx characterized by iTRAQ within the molecular function gene ontology category. Subcellular and functional categories were based on the annotations of gene ontology using the mouse genome informatics (MGI) GO_Slim Chart Tool. Analyses were done based on the following categories: biological process (**a**); molecular function (**b**); protein class (**c**), and cellular component (subcellular compartment, **d**).

sustainable metabolic processes such as survival, synaptogenesis and long term potentiation.

Considering p60TRP's influence on AD-related signalling [4], IPA could demonstrate p60TRP's impact on cortical and hippocampal proteins and the affected network with synaptic plasticity-relevant proteins such as Ntm, Ntrk1, Lsamp, Mtap2, Snca, Synj1/2, Syn2 and others (fig. 8).

Discussion

GPCRs are considered as the largest superfamily of membrane proteins that translate extracellular signals into intracellular messages and serve as one of the best targets for diverse therapeutic applications. p60TRP has recently been found as novel member of the GPRASP family [3] and the in vivo relevance of p60TRP-dependent
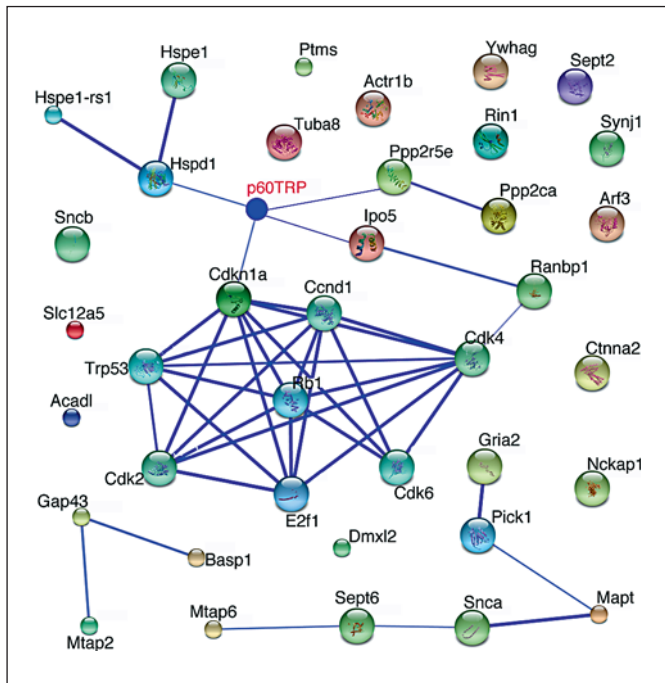
Fig. 5. Western blot validation of iTRAQ results by randomly selected proteins significantly regulated in transgenic p60TRP mouse-derived brains (common trends in both transgenic p60TRP mouse strains tg38 and tg31) compared with controls. **a** Western blots using proteins that showed similar trends in the Hp and Cx. Synj1 and Acadl levels increased while Snca, Sept2 and Mtap2 levels were reduced in p60TRP transgenic mice. **b** Western blots using proteins that showed significant changes either in the Hp (upper panel: Mbp and Sod1 with increased protein expression levels) or Cx (lower panel: Sncb decreased while Tuba8 increased). In **a** and **b**, Gapdh was used as internal control and it was unchanged in the iTRAQ analysis as well. **c** Quantitative analyses of the Western blots shown in **a** and **b**. Western blot experiments were performed at least 3 times for statistical quantification and analyses (n = 3). Values (= relative protein expression) represent the ratio of densitometric scores for the respective Western blot data, and statistical error was indicated as mean ± SD (* p < 0.05) using the Gapdh bands as reference. The Western blots correlated with the iTRAQ values obtained. **d** The histogram indicates similar ratios of the iTRAQ expression levels (tg p60TRP Hp vs. wt Hp and tg p60TRP Cx vs. wt Cx if compared with the Western blot ratios for selected proteins (**c**).
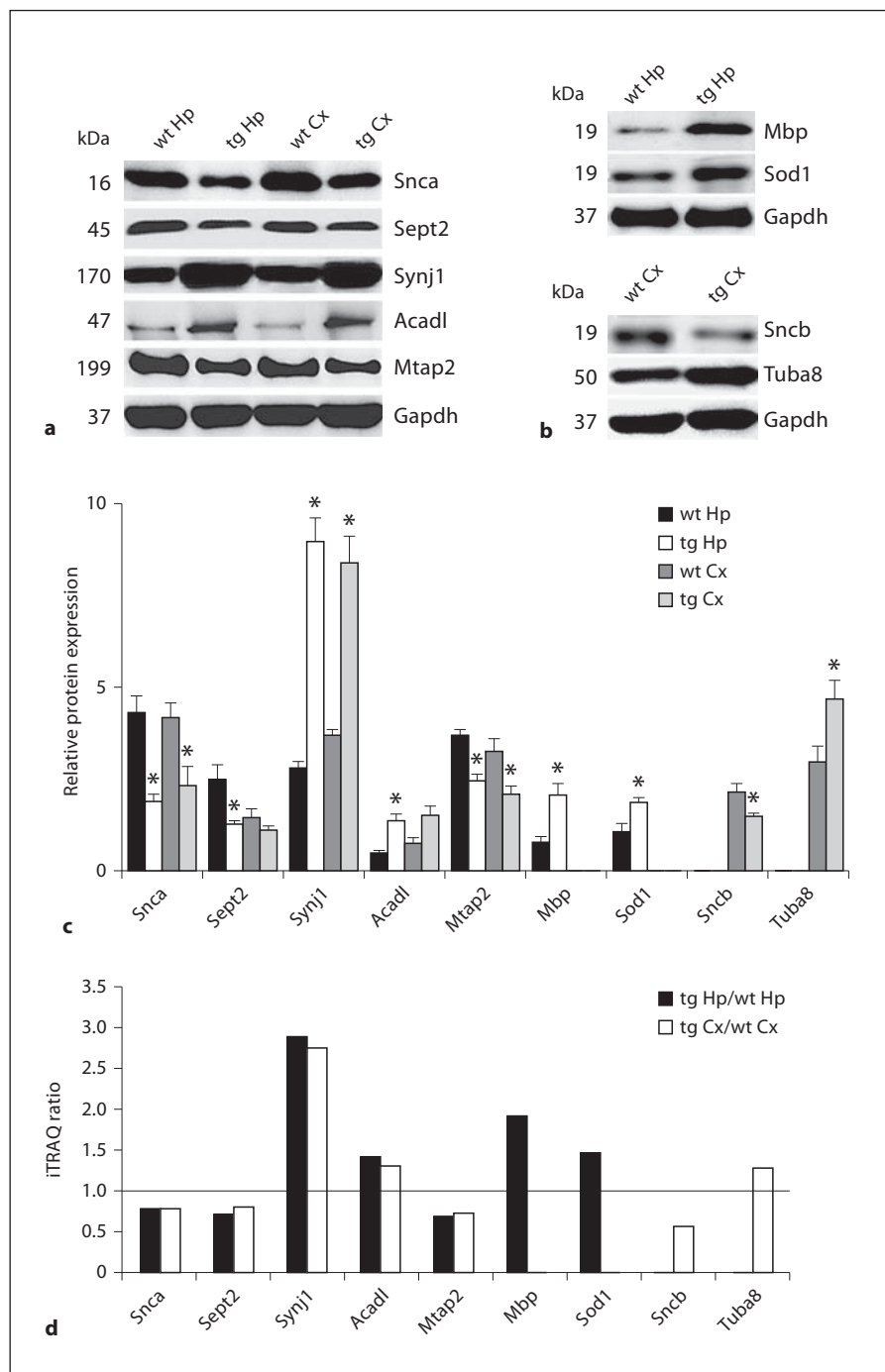
signalling has also begun to be verified in a mouse model overexpressing neuronal p60TRP [4]. Further considering p60TRP's neuroprotective effects in AD, one could predict its feasibility in the treatment of AD as well as other neurodegenerative disorders. Hence, here we investigated in vivo the long-term effect of neuronal p60TRP overexpression on the brain-region-specific proteome metabolism. Successively, 56 proteins with altered expression were identified in the Hp and Cx of transgenic p60TRP mice.

In the following sections, the specific functional roles of several of these iTRAQ-identified proteins, their rele-
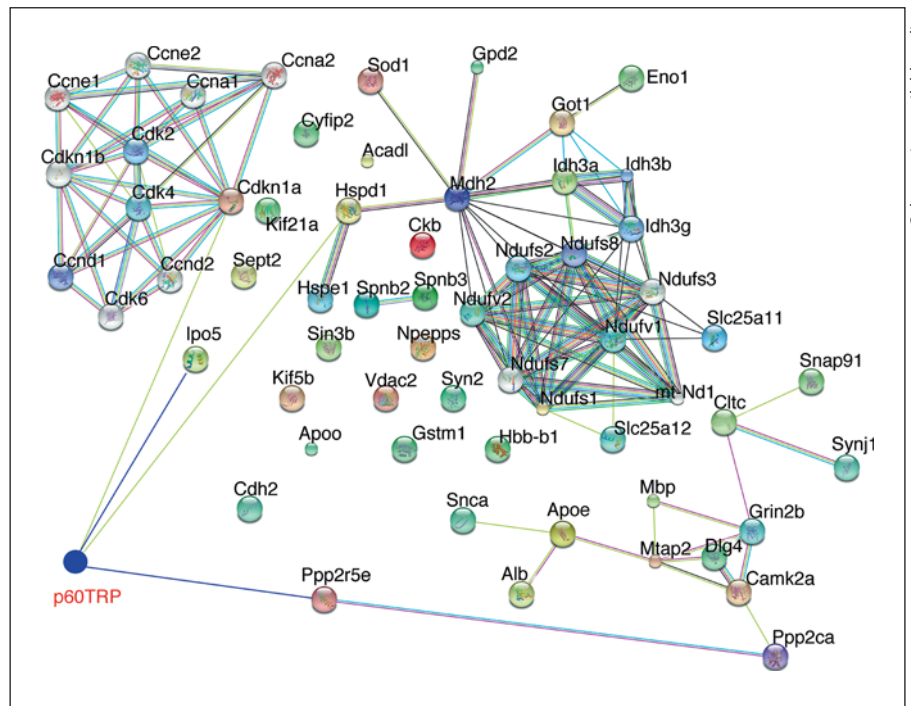
Fig. 6. STRING-9.0 analysis of transgenic p60TRP-modulated proteins in the brain cortex: Different coloured lines represent the types of evidence for the association. Network display: Nodes are either coloured (if they are directly linked to the input as in the tables 1 and 3) or white (nodes of a higher iteration/depth). Edges, i.e. predicted functional links, consist of up to eight lines: one colour for each type of evidence.

vance to brain diseases and the potential consequences of their p60TRP-mediated modified expressions are discussed.

Neuronal p60TRP Expression Specifically Mediates the Regulation of Cortical Proteins Crucially Involved in Brain Functions
Gria2 Protein

The Gria2 gene product (also known as GluA2, GluR-K2, GluR2 or GluR-B) belongs to a family of ionotropic glutamate receptors (GluRs) that are sensitive to α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA), and function as ligand-activated cation channels. Regulation of AMPA receptors (AMPARs) has emerged as a key mechanism of long-term potentiation and long-term depression during processes related to synaptic plasticity [13, 14]. Analysis from post-mortem brains revealed a particularly prominent reduction of AMPAR subunit Gria1 and Gria2 expression in the prefrontal Cx of patients with schizophrenia. This finding supports and expands the hypothesis of glutamatergic dysfunction in the Cx during pathophysiological processes of schizophrenia [15]. Taken together, p60TRP may enhance synaptic plasticity through its positive impact on Gria2. Thus, interference with the cross-talk between p60TRP-GPCR and AMPAR signalling pathways may eventually become of interest for the treatment of diseases such as schizophrenia.

Fig. 7. STRING-9.0 analysis of transgenic p60TRP-modulated proteins in the brain hippocampus: differently coloured lines (colours seen in the online version only) represent the types of evidence for the association. Network display: nodes are either coloured (if they are directly linked to the input as in tables 2 and 3) or white (nodes of a higher iteration/depth). Edges, i.e. predicted functional links, consist of up to 8 lines, with 1 colour for each type of evidence.
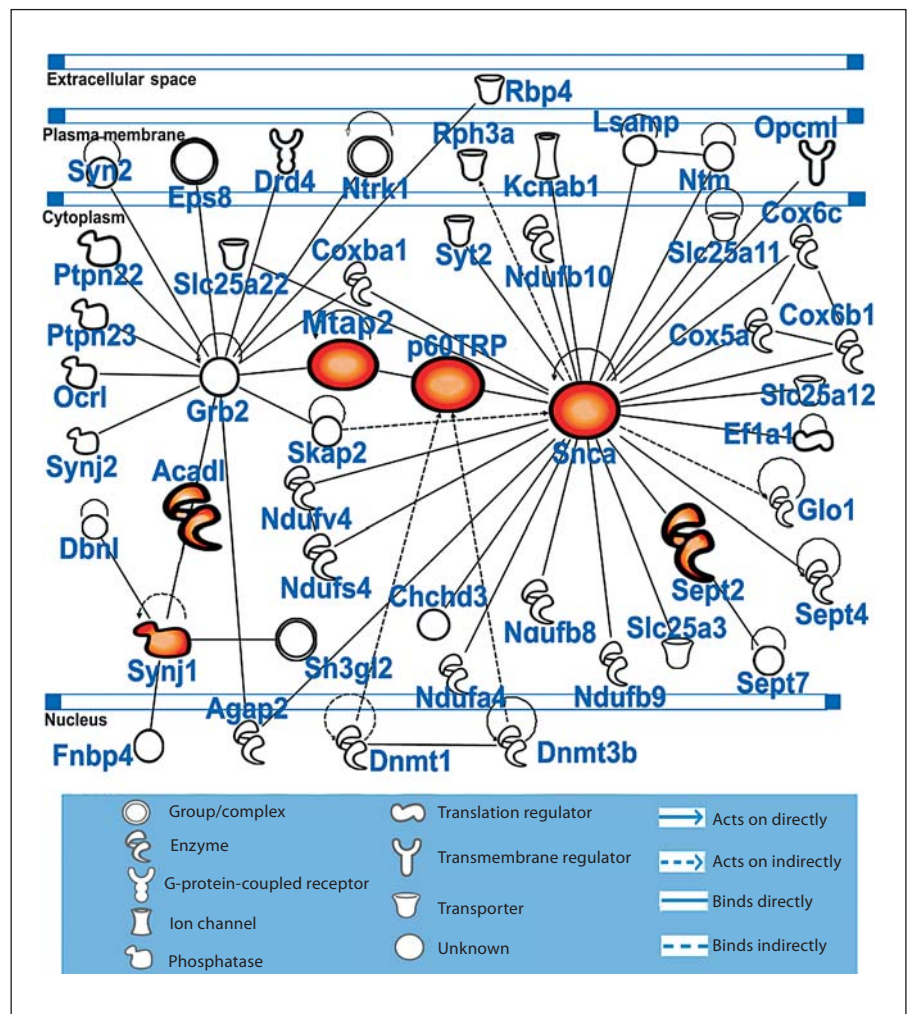
Rin1 Protein

Rin proteins serve as guanine nucleotide exchange factors for Rab5 GTPases. Rin1 is the prototype of this new family that has been shown to play an important role in the endocytosis of various receptors including the epidermal growth factor receptor [16–19]. Rin1 is preferentially expressed in postnatal forebrain neurons in which it is localized in dendrites and physically associated with Ras, suggesting a role in Raf-Mek-Erk-pathway-mediated postsynaptic neuronal plasticity. It has been shown that Rin1 modulates neuronal plasticity in forebrain neurons where it blocks fear conditioning and amygdala long-term potentiation to inhibit the acquisition of fear memories [20–22]. The results indicate that Rin1 serves as an inhibitory modulator of neuronal plasticity in aversive memory formation [23]. An underlying mechanism of Rin1 function in amygdala is to downregulate EphA4 signalling by promoting its endocytosis [24]. Accordingly, p60TRP may strengthen synaptic plasticity through the downregulation of Rin1. Moreover, p60TRP mice revealed higher tendency to explore their surroundings and reduced Rin1 activity would serve as a tool to provoke acquisition of fear conditioning; hence, p60TRP mice are more clever compared to their wild type littermates.

Dmxl2 Protein

Dmxl2 binds both GDP/GTP exchange protein and GTPase-activating protein for the Rab3 small G protein family that regulates Ca^{2+}-dependent exocytosis of neurotransmitters [25, 26], Dmxl2 is also a functional regulator of mammalian Notch signalling [27], and thereby modulating neurogenesis [28, 29]. Its upregulation by p60TRP may cause enhanced neuronal synaptic activities resulting in increased synaptic plasticity.

Fig. 8. Interactome maps demonstrating direct and indirect relationships among the iTRAQ-identified proteins with altered expression in the Cx and Hp of p60TRP transgenic mice. IPA was done to reveal the network of p60TRP-regulated proteins. The interactome shows that the 5 proteins identified by our iTRAQ analysis (Acadl, Mtap2, Sept2, Snca and Synj1) regulate either directly or indirectly the activities of several biomarkers and proteins involved in neurological diseases.

Basp1 Protein

Similar to Gap43, the brain abundant membrane-attached signal protein 1 (Basp1) is intensively present in nerve endings [30], and increased expression has been associated with the neuritic and synaptic dysfunction and pathogenesis of schizophrenia [31]. p60TRP-mediated downregulation of Basp1 might be associated with normal synaptic functions mediating neuroprotection.

Sncb Protein

Among the proteins downregulated in p60TRP transgenic mouse brains was Sncb, a protein involved in neurotransmitter release from nerve terminals and in the integration of signal transduction and membrane vesicle trafficking at nerve terminals. The synucleins (α, β and γ) are highly homologous proteins thought to play a role in regulating neurotransmission and are found abundantly in presynaptic terminals [32]. Besides, the proteins have the functional ability to induce membrane curvature and to convert large vesicles into highly curved membrane tubules and are thus involved in vesicle trafficking and endocytosis [33]. The Sncb protein is highly homologous to Snca and has been shown to be highly expressed in the substantia nigra of the brain, a region of neuronal degeneration in patients with PD; however, no direct relation to PD has been established; in contrast, Sncb rather inhibits Snca protein aggregation [34–36].

Sept6 Protein

Septins are an evolutionarily conserved group of GTP-binding and filament-forming proteins that belong to the large superclass of P-loop GTPases. Recent studies reveal that septins associate with microtubules, microtubule-associated proteins and microtubule motors, and are also involved in dendritic spine formation [37, 38]. Additionally, they are involved in processes controlling neurotransmitter release [39]. Functions identified to date include also cell apoptosis, DNA damage response, vesicle trafficking, cytoskeletal reorganization and membrane dynamics, and alterations of these septin GTPase scaffolds, by mutation or expression changes, have also been associated with a variety of neurological disorders such as AD and PD [40–42]. For Sept6 it could be demonstrated that it has an increase in expression from embryonic day 15 to postnatal day 70, and is abundantly expressed in axons and growth cones of developing neurons, found in neuronal vesicles of presynaptic terminals [43]. In addition, it has been shown that the transmembrane myelin protein Mal interacts with the cytoskeleton protein Sept6 for membrane compartmentalization in myelin internodes [44]. The significance of its upregulation by p60TRP, however, needs further investigations.

Slc12a5 Protein

Slc12a5 is an integral membrane K^+-Cl^- cotransporter that can function in either a net efflux or influx pathway, depending on the chemical concentration gradients of K^+ and Cl^-. The encoded protein can act as a homomultimer or as a heteromultimer with other K^+-Cl^- cotransporters, to maintain Cl^- homeostasis in neurons. Increase expression in Slc12a5 correlates with the previous findings of an increased vesicular glutamate transporter Slc17a7 [4] and is pivotal for the maturation of the CNS GABAergic neurotransmission system [45, 46].

14-3-3 Protein γ

14-3-3 Protein γ (Ywhag) belongs to the 14-3-3 family of proteins which mediate signal transduction by binding to phosphoserine-containing proteins. It has been shown to interact with Raf1 and protein kinase C, proteins involved in various signal transduction pathways. Ywhag interacts with Lrrk2 (leucine-rich repeat protein kinase 2) and Syna to protect dopaminergic neurons from α-synuclein toxicity. Ywhag's downregulation is probably associated with the reduced expression of Snca/b [47, 48]. An upregulation might rather cause oncogenic Mapk and Pi3k signalling activation and cell transformation [49]. Similarly, the p60TRP-induced inhibition of Actr1b could be linked to the regulation of Snca/b [50].

Arf3 Protein

ADP ribosylation factors are highly conserved approximately 20-kDa guanine nucleotide-binding proteins that play crucial roles in neuronal intracellular vesicular transport and in regulations of phospholipid-modifying enzyme activities and cytoskeletons [51, 52]. Findings by Suzuki et al. [53] suggest that ADP ribosylation factors are differentially involved in some processes essential to nerve regeneration as well as neuronal differentiation and maturation. Thus, the regulatory influence of p60TRP on Arf3 could have a wider impact on neuronal differentiation (e.g. neuronal stem cells activated and recruited to affected areas upon nerve injury) and neuronal regeneration in the CNS.

Tuba8 Protein

It is conceivable that despite its low expression level in the CNS, Tuba8 serves some unique function that is es-

sential to the developing brain, and mutations in Tuba8 might be involved in polymicrogyria and optic nerve hypoplasia [54].

Ppp2ca Protein

Increased expression of Ppp2ca correlates with higher protein phosphatase 2A activity in transgenic p60TRP mice and is of interest in view of AD-related signalling [4] though other recent findings report that a genetic variation in the tau protein phosphatase-2A pathway is not associated with AD risk [55].

Gap43 Protein

GAP43 promotes axon growth by multiple synergistic mechanisms that potentiate the intrinsic motility of the elongating processes, while reducing their sensitivity to environmental inhibition [56, 57].

Ptms Protein

Ptms is a ubiquitous small acidic nuclear protein that is thought to be involved in cell cycle progression, proliferation and cell differentiation though is function in the brain is still unknown [58]. Data suggest a role of Ptms in the remodelling of higher-order chromatin structure through modulation of histone H_1 interaction with nucleosomes and point to its involvement in chromatin-dependent functions [59].

Neuronal P60TRP Expression Specifically Mediates the Regulation of Hippocampal Proteins Crucially Involved in Brain Functions

Ndufv2 Protein

The NADH-ubiquinone oxidoreductase complex (complex I) of the mitochondrial respiratory chain catalyses the transfer of electrons from NADH to ubiquinone, and consists of at least 43 subunits. The complex is located in the inner mitochondrial membrane. Ndufv2 encodes the 24-kDa subunit of complex I and is involved in electron transfer. Mutations in Ndufv2 are implicated in PD, bipolar disorder and schizophrenia [60–66].

Dlg4 Protein

Dlg4 (also known as PSD95) is a member of the membrane-associated guanylate kinase family and is recruited into N-methyl-D-aspartate receptor (NMDAR) and potassium channel clusters to regulate the strength of synaptic activity. Dlg4 interacts with several other scaffolding proteins to serve as anchorages also for ionotropic AMPA-selective GluRs such as Gria2 [67]. Modifications of Dgl proteins function in the glutamatergic synapse, such as

alterations of their interaction with NMDAR regulatory subunits, and are common events in several neurodegenerative disorders [68–71]. Decreased expression of NMDAR-associated proteins has been found in the frontal Cx of elderly patients with schizophrenia [72] while decreased levels of Dlg4 have been reported in the Hp from subjects with amnestic mild cognitive impairment [73] and in the inferior temporal Cx of AD subjects [74]; in contrast, a significant increase in Dlg4 protein in Brodmann's area 9 of AD patients suggests a brain-site-specific change in NMDAR trafficking and may represent a novel marker of functional significance for the disease [75]. TrkB receptor and PKMζ, two critical regulators of synaptic plasticity, facilitate Dlg4 targeting to synapses [76]. Thus, p60TRP can influence synaptic plasticity directly via modulation of ionotropic AMPARs (Gria2) and/or indirectly via its modulatory role on Dlg4/NMDAR levels.

Camk2a Protein

The Ca^{2+}/calmodulin-dependent serine/threonine protein kinase Camk2a is crucial for several aspects of plasticity at glutamatergic synapses such as hippocampal NMDAR-dependent long-term potentiation and spatial learning [77]. Postsynaptic secretion of brain-derived neurotrophic factor and neurotrophin-3 from hippocampal neurons also depends on Camk2, Ca^{2+} and protein kinase A signalling [78]. Camk2a deficiency causes an immature dentate gyrus, dysregulated behaviours and impaired neuronal development as observed in psychiatric disorders [79].

Another interesting point is that active proteolysis by the ubiquitin-proteasome pathway system has emerged as a new molecular mechanism that controls synaptic plasticity [80]. Besides, the neuronal ubiquitin-proteasome pathway system is itself rapidly and dynamically regulated by synaptic activity. Blockade of action potentials inhibits the activity of the proteasome, whereas the up-regulation of action potentials increases the activity of the proteasome. Camk2a stimulates proteasome activity and directly phosphorylates Rpt6, a subunit of the 19S (PA700) subcomplex of the 26S proteasome. This is a novel mechanism whereby p60TRP can influence Camk2a activity that may regulate the proteasome in neurons to facilitate remodelling of synaptic connections through protein degradation [81]. Since p60TRP mediates the upregulation of excitatory ionotropic GluR signalling mechanisms via Dlg4 and Gria2, the downregulation of Camk2a might be via a feedback loop to control synaptic plasticity in order to avoid excitatory neurotoxicity [82–85].

Sod1 Protein

Sod1 binds copper and zinc ions and is one of two isozymes responsible for scavenging free superoxide radicals/reactive oxygen species in the cell. Mutant Sod1 has been associated with familial amyotrophic lateral sclerosis [86, 87]. The upregulation of Sod1 might also be via a feedback loop to control synaptic plasticity in order to avoid excitatory neurotoxicity mediated by oxidative stress and reactive oxygen species due to the enhanced levels of Gria2 and Dlg4 [85, 88].

Sept9 Protein

Sept9 can substitute for septins of the Sept2 group and partially for Sept7 [42, 89]. Since Sept9 is a member of the cytoskeleton-related septin family, which is highly expressed in glia cells in neuronal tissues, neuronal p60TRP modulates also glia functions. Sept9 is thought to function through interaction with other septins and small GTPase rho-mediated signalling. Sequence alterations in Sept9 are known to cause hereditary neuralgic amyotrophy. Sequence variations in Sept9 causing hereditary neuralgic amyotrophy are thus likely to alter modes of interaction with partner molecules in cells, and consequently contribute to the pathogenesis of hereditary neuralgic amyotrophy [90–92]. While p60TRP mediated reduced Sept9 expression levels, its overexpression would probably cause malignant brain glia tumors [93].

Neuronal p60TRP Expression Specifically Mediates the Regulation of Cortical and Hippocampal Proteins Crucially Involved in Brain Functions

Synj1 Protein

Endocytosis of the synaptic vesicle is a complicated process, in which many proteins and lipids participate. Phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$] plays important roles in the process, and the dynamic regulation of this lipid is one of the key events. Synj1 is the major constitutively active phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase in rodent brain that regulates the levels of membrane $PI(4,5)P_2$ [94]. Cyclin-dependent kinase 5 phosphorylates Synj1 and inhibits the inositol 5-phosphatase activity of Synj1, whereas dephosphorylation by calcineurin stimulates such activity [95].

Besides, SYNJ1 maps to human chromosome 21 and is thus a candidate for an involvement in the AD-related disease Down's syndrome, a complex disorder resulting from the overexpression of trisomic genes. $PI(4,5)P_2$ metabolism is altered in the brain of Ts65Dn mice, the most commonly used model of Down's syndrome. This defect is rescued by restoring Synj1 to disomy in these Ts65Dn mice and is recapitulated in transgenic mice overexpressing Synj1. These transgenic mice also exhibit cognitive deficits, suggesting that $PI(4,5)P_2$ dyshomeostasis caused by a gene dosage imbalance for Synj1 may contribute to brain dysfunction and cognitive disabilities in Down's syndrome [96]. In addition, mutation analysis of SYNJ1 revealed it as a possible candidate gene for chromosome 21q22-linked bipolar disorder [97].

Acadl Protein

Long-chain acyl-CoA dehydrogenase (Acadl or LCAD) belongs to the acyl-CoA dehydrogenase family, which is a family of mitochondrial flavoenzymes involved in the first step of long-chain fatty acid β-oxidation and branched chain amino-acid metabolism. This protein is one of the 4 enzymes that catalyse the initial step of mitochondrial β-oxidation of straight-chain fatty acid. Defects in this gene are the cause of LCAD deficiency, leading to non-ketotic hypoglycaemia [98, 99]. Long-chain fatty acids are an important source of energy; however, the role of long-chain fatty acid oxidation in the brain (that does not rely on fat for energy) is poorly understood. Compartmentalization of acyl-CoA dehydrogenases in the human CNS suggests that β-oxidation in the brain participates in different functions other than generating energy, for example the synthesis and/or degradation of unique cellular lipids and catabolism of aromatic amino acids, compounds that are vital to neuronal function; e.g. it may play a role in the turnover of lipid membrane unsaturated fatty acids that are essential for membrane integrity and structure [100, 101].

Sept2 Protein

As other ρ-GTPases [102, 103], Sept2 is crucially involved in modelling neurite outgrowth during neuronal differentiation and a tight regulation of it expression is necessary [104]. Similarly to a number of other proteins that are related to G-protein-signalling pathways, such as Ranbp1, Rin1 and Sept9, p60TRP downregulates Sept2 in the Cx and Hp during a receptor (e.g. Gria2) resensitisation process. The observation that Sept6 is upregulated in the Cx might be another spatial brain-region-specific event that needs to be investigated further.

Mtap2 Protein

Mtap2 regulates and organizes microtubules and may serve as a scaffolding protein for neuromodulatory activities. Neuronal activity-dependent dynamic changes
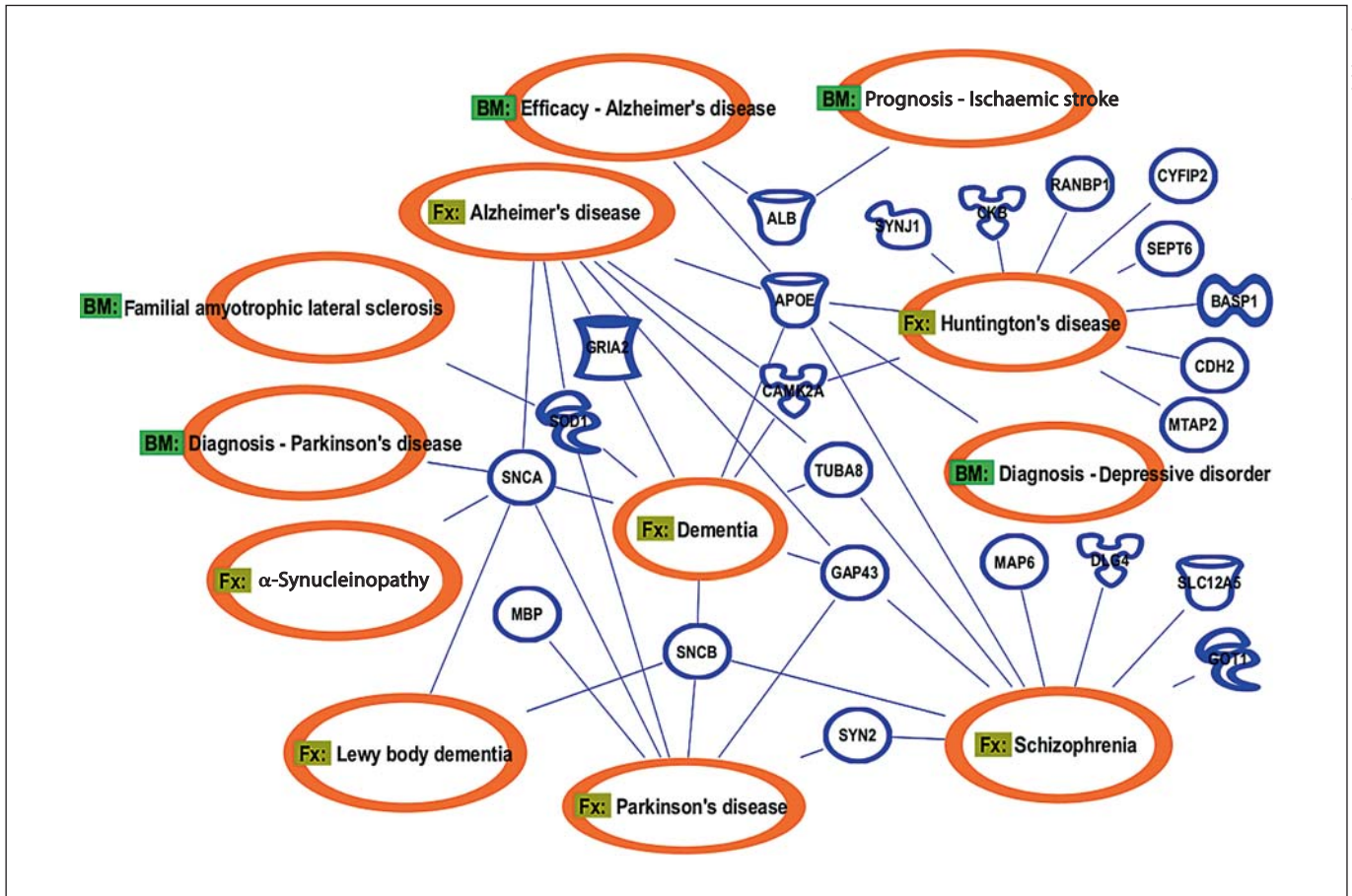
Fig. 9. p60TRP-associated neurological disorders. IPA-based network analysis of iTRAQ-identified proteins of mouse brain Cx and Hp exhibits p60TRP-regulated biomarkers (BM) and proteins linked with disease function (Fx). p60TRP directly regulates diagnostic, prognostic and efficacy biomarkers of PD, depressive disorder, ischaemic stroke, familial amyotrophic lateral sclerosis and AD, respectively.

of microtubules are important to maintain neuronal morphology and function and are spatial and temporal-dependent processes during synaptic plasticity [105]. A spatially and temporally dependent downregulation of indices of dendrites and spines, such as Mtap2, during neurosynaptic plasticity processes has been reported previously [106]. Additionally, a high Mtap2-associated microtubule turnover that accompanies synaptic plasticity and memory formation might be another reason for a temporal/spatial change in Mtap2 levels in the Cx and Hp [107]. Further experiments have also to identify the specific Mtap2 isoforms involved in this mechanism.

Snca Protein

α-Synuclein is a member of the synuclein family, which also includes β- and γ-synuclein. Synucleins are abundantly expressed in the brain and α- and β-synucleins inhibit phospholipase D_2 selectively. Snca may serve to integrate presynaptic signalling and membrane trafficking. Snca is a natively unfolded protein which plays a central role in the control of dopaminergic neuronal functions and which is thought to be critically implicated in PD pathophysiology. Defects in Snca have been implicated in the pathogenesis of PD [108]. PD is characterized by a progressive loss of dopaminergic neurons of the nigrostriatal system and by the presence of Lewy bodies, proteinaceous inclusions mainly composed of filamentous Snca aggregates. Indeed, besides the fact that Snca is the main protein component of Lewy bodies, genetic studies showed that mutations and multiplications of the Snca gene are responsible for the onset of familial forms of PD. A large body of evidence indicates that Snca pa-

thology at dopaminergic synapses may underlie the onset of neuronal cell dysfunction and degeneration in the PD brain, particularly neurodegeneration caused by presynaptic Snca aggregation [109, 110]. In addition, abnormalities of Snca and NMDARs are implicated in the pathogenesis of PD through mechanisms involving oxidative stress and excitotoxicity as Snca may promote clathrin-mediated NMDAR endocytosis [111, 112] and large Snca oligomers enhance both pre- and post-synaptic AMPAR-mediated synaptic transmission, thereby aggravating intracellular calcium dyshomeostasis and contributing to excitotoxic nerve cell death in synucleinopathies [113].

In conclusion, neuronal p60TRP overexpression may contribute to improved cognitive functions by promoting synaptic proteins such as Gria2 or Dmxl2 [4]. p60TRP's potential to modulate temporal and spatial brain-site-specific signalling pathways related to synaptic plasticity and neuroregenerative processes (fig. 9) may thus open new therapeutic avenues for the treatment of various CNS diseases such as AD, PD, amyotrophic lateral sclerosis, bipolar disorder or schizophrenia.

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