Exploring the Pannexin 1 interactome: *In silico* cross-analyses with postsynaptic proteins and neuropsychiatric disorder susceptibility genes

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The Pannexin 1 (Panx1) channel-forming protein is enriched in the central nervous system, and has been associated with several critical neurodevelopmental and plasticity functions; these include dendritic spine formation, neuronal network development, synaptic plasticity, and pathological brain states such as ischemia, epilepsy, and neurodegeneration. Despite major advances in understanding the properties and activation modes of Panx1, the Panx1 interactome remains largely uncharacterized. Considering that Panx1 has been implicated in critical neurodevelopmental and neurodegenerative processes and diseases, we investigated the Panx1 interactome (482 Panx1-interacting proteins) identified from mouse N2a cells. These proteins were cross-analyzed with the postsynaptic proteome of the adult mouse brain previously identified by mass spectrometry (LC-MS/MS), and neurodegenerative disease and neurodevelopmental disorder susceptibility genes previously identified by genome-wide association studies (GWAS); and then further investigated using various bioinformatics tools (PANTHER, GO, KEGG and STRING databases). A total of 104 of the Panx1-interacting proteins were located at the postsynapse, and 99 of these formed a 16-cluster protein-protein interaction (PPI) network (hub proteins: Eef2, Rab6A, Ddx39b, Mapk1, Fh1, Ndufv1 et cetera). The cross-analysis led to the discovery of proteins and candidate genes involved in synaptic function and homeostasis. Of particular note, our analyses also revealed that certain Panx1-interacting proteins are implicated in Parkinson disease, Alzheimer disease, Huntington disease, amyotrophic lateral sclerosis, schizophrenia, autism spectrum disorder and epilepsy. Altogether, our work revealed important clues to the role of Panx1 in neuronal function in health and disease by expanding our knowledge of the PPI network of Panx1, and unveiling previously unidentified Panx1-interacting proteins and networks involved in biological processes and disease.

Panx1 | PPIs | pathway analysis | neurodegenerative diseases | neurodevelopmental disorders | Parkinson disease | Alzheimer disease | chaperones | vesicle-mediated transport

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Introduction

Pannexin 1 (Panx1) is a channel-forming protein that is most commonly known for its role in mediating ATP release (Dahl, 2015; Lohman and Isakson, 2014) with context-specific (tissue and cell type expression, developmental stage and disease state) properties and regulation (reviewed by Chiu et al. (2018)). Although ubiquitously expressed throughout the body, Panx1 is enriched in the central nervous system (CNS; reviewed by Boyce et al. (2018)). Within the mouse cortex, it is highly expressed in neurons and oligodendrocytes (Ray et al., 2005; Vogt et al., 2005; Zhang et al., 2014; Zoidl et al., 2007)[see brainrnaseq.org]. Consistent with its reported role in regulating developmental cellular behaviours like proliferation and differentiation in a number of different cells and tissues from other groups, work in our lab has uncovered a role for Panx1 in regulating several aspects of neuronal development.

Our early work revealed that Panx1 promotes neural precursor cell maintenance *in vitro* (Wicki-Stordeur et al., 2012) and *in vivo* (Wicki-Stordeur et al., 2016). Conversely, we found that Panx1 inhibits neurite development in Neuro2a (N2a) and neural precursor cells (Wicki-Stordeur and Swayne, 2013). Similarly, cortical neurons from Panx1 knockout mice demonstrated an increased density of dendritic spines, as well as larger groups of co-activated neurons, also known as network ensembles (Sanchez-Arias et al., 2019). This regulation of neurites and dendritic spines connects nicely with our earlier discovery of Panx1 protein-protein interactions (PPIs) with dendritic spine regulating proteins, such as the actin-related protein (Arp) 2/3 complex components (Wicki-Stordeur and Swayne, 2013), and the microtubule-interacting protein collapsin-response mediator protein 2 (Crmp2) (Xu et al., 2018). A role for Panx1 in regulating neuronal development and connectivity is further supported by work revealing its role in hippocampal synaptic plasticity under naïve conditions (Ardiles et al., 2014; Gajardo et al., 2018; Prochnow et al., 2012), as well as recently reported connections to intellectual disability (Shao et al., 2016) and autism spectrum disorder (ASD) (Davis et al., 2012). Moreover, Panx1 has been implicated in synaptic dysfunction in ischemia (MacVicar and Thompson, 2010; Weilinger et al., 2016; Weilinger et al., 2013) and has been suggested to be involved in CNS dysfunction associated with Alzheimer disease (Orellana et al., 2011b) and Parkinson disease (Diaz et al., 2019).

Given the newly-discovered role in regulating development of dendritic spines and neuronal networks, our goal in this study was to further expand our analysis of the Panx1 interactome, that we previously reported on – partially in Wicki-Stordeur and Swayne (2013) and Wicki-Stordeur (2015), using *in silico* tools (Fig. 1). Firstly, we performed over-

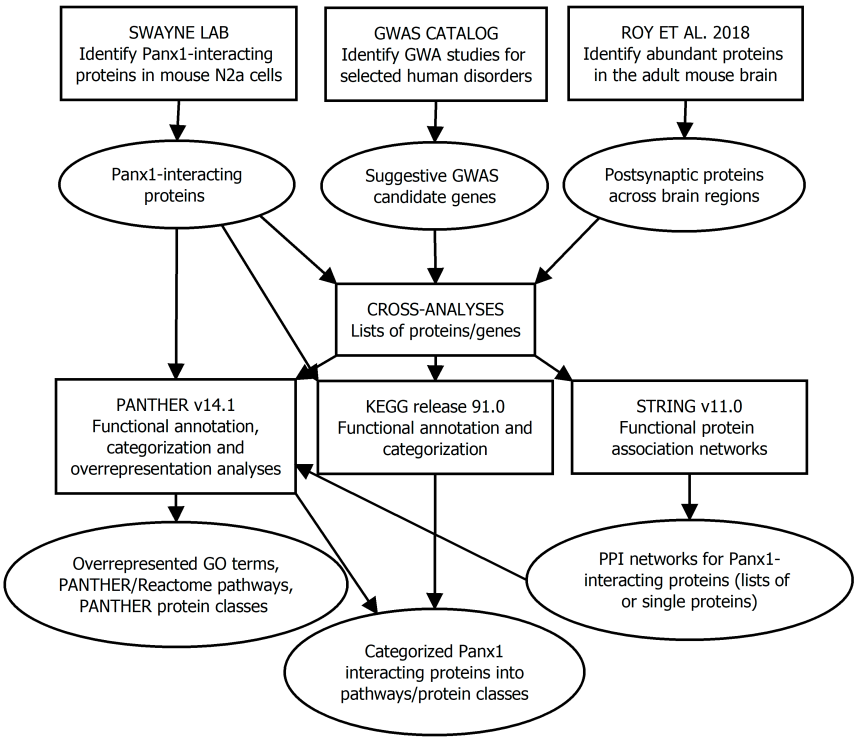

Fig. 1. Work flow for the current study from materials and methods to results. In this study, five subanalyses were conducted (further described in Fig. S1), which explored the: (1) Panx1 interactome in mouse N2a cells, (2) postsynaptic proteome in the adult mouse brain associated with the Panx1 interactome, and (3) neurodegenerative disease and neurodevelopmental disorder susceptibility genes associated with the Panx1 interactome. The data was obtained from three sources: Swayne lab [our lab], the genome-wide association study (GWAS) catalog (Buniello et al., 2019) and the original research article, Roy et al. (2018). The data were analyzed using the statistical computing environment R and the following databases: PANTHER [Protein ANalysis THrough Evolutionary Relationships] (Mi et al., 2019a; Mi et al., 2019b), KEGG [Kyoto Encyclopedia of Genes and Genomes] (Kanehisa et al., 2017; Kanehisa and Goto, 2000) and STRING [Search Tool for the Retrieval of Interacting Genes/Proteins] (Szklarczyk et al., 2019). In addition to the subanalyses, the findings across analyses were combined to provide a comprehensive overview for each neurodegenerative diseases and neurodevelopment disorders in relation to the Panx1 interactome.

representation analyses of the entire Panx1 interactome to get a better sense of its putative cellular and pathophysiological roles. Overrepresentation analyses determine if the Panx1-interacting proteins are present more than would be expected within the protein classes, cellular components and biological pathways under investigation. Next, to gain further insight into how Panx1 regulates dendritic spines, we performed a cross-analysis with the mouse brain postsynaptic proteome, and identified links to existing PPI networks. To further examine the link between Panx1 and neurodegenerative diseases and neurodevelopmental disorders associated with abnormalities of dendritic spines (recipients of excitatory inputs) (Forrest et al., 2018; Maiti et al., 2015; Penzes et al., 2011), we also cross-analyzed the Panx1 interactome with suggestive candidate genes identified by genome-wide association studies (GWAS). Here, we focused specifically on Parkinson disease, Alzheimer disease, amyotrophic lateral sclerosis (ALS), Huntington disease, schizophrenia, ASD and epilepsy. The outcomes of this work provide potential new insights into the role of Panx1 in the CNS and suggest links between Panx1 and neuropsychiatric disorders.

Materials and Methods

An overview of the study workflow can be found in Fig. 1 (for a more comprehensive work flow, see Fig. S1). For the comparisons, the R statistical computing environment v3.6.0 was applied.

Identification of the Panx1 interactome in mouse N2a cells. We previously identified the putative Panx1 interactome from mouse N2a neuroblastoma-derived cells (Wicki-Stordeur and Swayne, 2013), using methods that were comprehensively described in that work as well as in Wicki-Stordeur (2015). Briefly, proteins co-precipitating with Panx1-EGFP [enhanced green fluorescent protein] or EGFP from N2a cells, were identified by the UVIC-Genome BC Proteomics Centre using high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) followed by analysis with Proteome Discoverer v1.3.0.339 (Thermo Scientific) and Mascot v2.2 (Perkins et al., 1999) [percolator settings: Max delta Cn 0.05, Target false discovery rate (FDR) strict 0.01, Target FDR relaxed 0.05 with validation based on q-value]. The q-value refers to the minimal FDR at which a peptide spectrum match was accepted, as described by Brosch et al. (2009). To identify proteins selectively interacting with Panx1, all proteins co-precipitating with anti-GFP antibody from EGFP-expressing cells were removed from the list of Panx1 interactors. This paradigm was repeated 3 times and the results were pooled. The complete list of Panx1-interacting proteins identified from those experiments (not previously published in its entirety) was used as

input for the cross-analyses in the current study.

Functional annotation, categorization and overrepresentation analyses of the Panx1 interactome using the PANTHER database. Bioinformatics analysis of all proteins in our Panx1-interacting protein list (using UniProt accession numbers) was carried out using the PANTHER database v14.1 (Mi et al., 2013; Thomas et al., 2003), which is a curated database that includes several bioinformatics tools. The proteins (or their corresponding genes, depending on the nature of the analysis) were annotated to (i) PANTHER protein classes from the PANTHER protein class ontology, (ii) gene ontology (GO) terms within the biological process, molecular function and cellular component domains from the GO knowledge base and (iii) PANTHER pathways created using the CellDesigner tool, a modelling tool for biochemical networks (Funahashi et al., 2008; Mi et al., 2019a; Mi et al., 2013). Note that PANTHER pathways are similar to the GO biological process domain categories. First, the Panx1-interacting proteins were divided into PANTHER-defined categories termed 'protein classes'. Only protein classes that included at least 4 Panx1-interacting proteins were selected. Second, right-tailed Fisher's exact tests were used to identify overrepresented (present in greater abundance than would be expected) PANTHER protein classes, GO terms and PANTHER pathways. The nominal significance level α ($\alpha = 0.05$) was adjusted for multiple testing using the Bonferroni correction and thus, α divided by the number of independent tests was considered statistically significant. In addition, we selected the least specialized PANTHER protein class (to identify broad protein classes) and the most specialized GO term (to identify specialized cellular components, molecular functions and biological processes) within each category. Subsequently, only PANTHER protein classes, GO terms and PANTHER pathways with at least 10 annotated proteins/genes were presented (to reduce the likelihood of false positives). Parkinson disease (P00049) was the only PANTHER pathway that was significantly overrepresented in the Panx1 interactome. To get more information regarding links between the Panx1 interactome and other neurodegenerative disease pathways, we also categorized the Panx1 interactome to the PANTHER pathway Huntington disease (P00029) and at the time of the analysis there were no Panx1-interacting proteins present in the Alzheimer disease PANTHER pathways.

Cross-analysis between the Panx1 interactome and the adult mouse brain region-specific postsynaptic proteome from Roy et al. (2018). Roy et al. (2018) identified 1173 postsynaptic proteins in 8 weeks old male C57BL/6J mice (n = 6). This list (supplementary table 1) was used as an input in the current study aiming to identify Panx1-interacting proteins in the postsynaptic compartment, *in silico*. In brief, they dissected and homogenized brain tissues, isolated the postsynaptic densities (PSDs, which we refer to as postsynapse throughout this paper) separately for each brain region, and subjected these to LC-MS/MS and subsequently protein identification. To identify overlapping reviewed protein identifiers, the GenInfo Identifier (GI) numbers were converted to UniProt accession numbers to allow for comparison with the Panx1-interacting proteins identified in mouse N2a cells. As the data also contained unreviewed and archived protein identifiers, we mapped gene symbol aliases to current official gene symbols using the R package Limma v3.40.2 (Ritchie et al., 2015) and the organism R packages for mouse v3.8.2 (Carlson, 2019b). Subsequently, the lists were compared and combined.

PPI network for the Panx1 postsynaptic interactome in the adult mouse brain. The postsynaptic PPI network, based on interaction evidence [from STRING-defined categories: known interactions (curated databases, experimentally determined), predicted interactions (gene neighborhood, gene fusions, gene co-occurrence), and other (text mining, co-expression and protein homology)], was created for *Mus musculus* using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) v11.0 (Szklarczyk et al., 2019), and the identified Panx1-interacting postsynaptic proteins in the adult mouse brain were used as input. Edges (also termed PPIs) were formed if the interaction score was at least 0.4 (medium confidence, *"estimated likelihood that a given interaction is biologically meaningful, specific and reproducible, given the supporting evidence"* (Szklarczyk et al., 2017)), and the color of the edges in the PPI network represents the type of evidence that was used to identify the PPI. Clusters were identified using the unsupervised Markov Cluster (MCL) algorithm with inflation factor 1.8 (higher inflation factor leads to more clusters, but noting that *"MCL is remarkably robust to graph alterations"* (Brohee and van Helden, 2006; Enright et al., 2002; Van Dongen, 2000). Selected clusters were investigated further using the PANTHER database v14.1 (Mi et al., 2013; Thomas et al., 2003). Overrepresented Reactome pathways and GO terms were identified through right-tailed Fisher's exact tests and the statistical significance level ($p < 0.05$) was corrected for multiple testing using the Bonferroni correction. In order to glean the most detailed information, we selected only the most specialized overrepresented Reactome pathways and GO terms (Fabregat et al., 2018) for presentation here.

Categorization of the Panx1 interactome in N2a cells and postsynaptic Panx1-interacting proteins in the adult mouse brain to selected KEGG pathways. The Panx1-interacting proteins were categorized into selected Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways within the following categories: Organismal systems, human diseases and cellular processes. The selected KEGG pathways belonged to 9 subcategories, which were nervous system, development and regeneration, aging, environmental adaptation, neurodegenerative disease, substance dependence, transport and catabolism, cell growth and death, and cellular community – eukaryotes. To import information about *Mus musculus* from the KEGG database into the statistical computing environment R v3.6.0, the KEGG application programming interface was used (Kanehisa, 2019; Kanehisa et al., 2017; Kanehisa and Goto, 2000; Kanehisa et al., 2019).

For each KEGG pathway, the annotated genes were reported as Entrez gene IDs. Therefore, we converted the UniProt accession numbers for the Panx1 interactome and postsynaptic proteome to Entrez gene IDs using information stored in the publicly available database Ensembl (release 96) (Hunt et al., 2018) through the R package biomaRt v2.40.0 (Durinck et al., 2005; Durinck et al., 2009). Subsequently, semi-manual checking was used to ensure that all Entrez gene IDs were present. Entrez gene IDs were identified for the remaining proteins at gene level by (i) mapping gene symbol aliases to current official gene symbols using the R package Limma v3.40.2 (Ritchie et al., 2015) and the organism R package for mouse v3.8.2 (Carlson, 2019b), and (ii) converting the gene symbols as described above. The Panx1-interacting proteins identified in N2a cells and the adult mouse brain at the postsynapse were categorized into the selected KEGG pathways. Selected KEGG pathways were visualized using the KEGG Mapper (Kanehisa and Sato, 2019).

Cross-analysis between the Panx1 interactome and suggestive GWAS candidate genes involved in neurodegenerative diseases and neurodevelopmental disorders in humans. On 21/06/2019, we searched for 'Parkinson disease', 'Alzheimer disease', 'Huntington disease', 'Amyotrophic lateral sclerosis', 'Schizophrenia', 'Epilepsy' and 'Autism spectrum disorder' separately in the NHGRI-EBI GWAS catalog and downloaded the summary statistics (Buniello et al., 2019). For Parkinson disease, 31 studies and 276 associations were available; for Alzheimer disease, 84 studies and 1290 associations were available; for Huntington disease, 2 studies and 22 associations were available; for ALS, 28 studies and 309 associations were available; for schizophrenia, 95 studies and 2649 associations were available; for epilepsy, 30 studies and 129 associations were available; and for ASD, 20 studies and 490 associations were available.

Using the summary statistics from the GWAS catalog, we conducted cross-analyses between the Panx1-interacting proteins identified in N2a cells and the suggestive GWAS candidate genes potentially involved in the selected human neuropsychiatric disorders. For each dataset, single nucleotide polymorphism (SNP) associations without gene information were excluded and a list of mapped and reported genes was constructed. For each gene list, we mapped gene symbol aliases to current official gene symbols using the R package Limma v3.40.2 (Ritchie et al., 2015) and the organism R packages for mouse and human v3.8.2 (Carlson, 2019a, b). The human gene symbols were converted to mouse gene symbols using the R package biomaRt v2.40.0, including homology mapping (Durinck et al., 2005; Durinck et al., 2009), sending and retrieving queries from the publicly available database Ensembl (release 96) (Hunt et al., 2018). Then, we identified overlaps between the lists of genes.

Functional annotation and overrepresentation analyses: In cases where at least 15 genes from the GWAS intersected, functional annotation and overrepresentation analyses were conducted as described above to reveal potential biological functions. Here, UniProt accession numbers were extracted from the Panx1 interactome based on the information in the gene lists (current official gene symbols and mapped gene symbol aliases, e.g. a gene symbol alias for collapsin response mediator protein 1 (Crmp1) is dihydropyrimidinase-like 1 (Dpysl1)). Each protein list was analyzed separately and only PANTHER protein classes, GO terms and PANTHER pathways with at least 4 annotated genes/proteins were included (chosen to reduce the chance of false positives). If no overrepresented PANTHER protein classes, GO terms or PANTHER pathways were identified, we performed functional annotation and overrepresentation analyses of the full GWAS candidate gene list and compared it with the Panx1 interactome findings.

PPI network analyses in STRING: PPI network analyses were carried out, as described above (except here the PPI networks were created for *Homo sapiens*, and either low or medium confidence interaction scores were applied), for the Panx1-interacting proteins overlapping with the suggestive candidate genes identified by the GWAS. For each human disorder, if at least 3 Panx1-interacting proteins were identified, all proteins were used as input in STRING to construct the PPI networks. Otherwise, one or two Panx1-interacting proteins were used as input to create expanded PPI networks (only done for selected proteins).

Results

Comprehensive *in silico* classification of the characteristics of Panx1-interacting proteins in mouse N2a cells. We identified 482 Panx1-interacting proteins in mouse N2a cells and categorized them with PANTHER (protein analysis through evolutionary relationships) in order to designate the Panx1-interacting proteins into protein classes according to unifying functional characteristics (Mi et al., 2005). The 482 Panx1-interacting proteins fell into 34 PANTHER protein classes (Fig. 2). A total of 5 overrepresented (more abundant than expected) PANTHER protein classes (49.4% of the Panx1-interacting proteins were classified) were identified: Nucleic acid binding (n = 61, p = 2.84E-03), cytoskeletal proteins (n = 39, p = 1.51E-09), RNA binding proteins (n = 36, p = 7.10E-05), G-proteins (n = 13, p = 2.07E-02) and chaperones (n = 10, p = 1.16E-02) (Fig. 3A). The highest fold enrichment (actual number of Panx1-interacting proteins over the expected number of proteins) was identified for the protein class chaperone (PC00072) followed by the protein classes cytoskeletal proteins (PC00085) and G-proteins (PC00020, falls within the enzyme modulator PANTHER protein class (PC00095) identified through the categorization (Fig. 2)). The 10 Panx1-interacting proteins within the chaperone PANTHER protein class were: Calnexin (Canx), CAP-Gly domain-containing linker protein 2 (Clip2), peptidyl-prolyl cis-trans isomerase KBP1A (Fkbp1a), peptidyl-prolyl cis-trans isomerase FKBP4 (Fkbp4), 10 kDa heat shock protein, mitochondrial (Hspe1), prefoldin subunit 2 (Pfdn2), prostaglandin E synthase 3 (Ptges3), 14-3-3 protein sigma

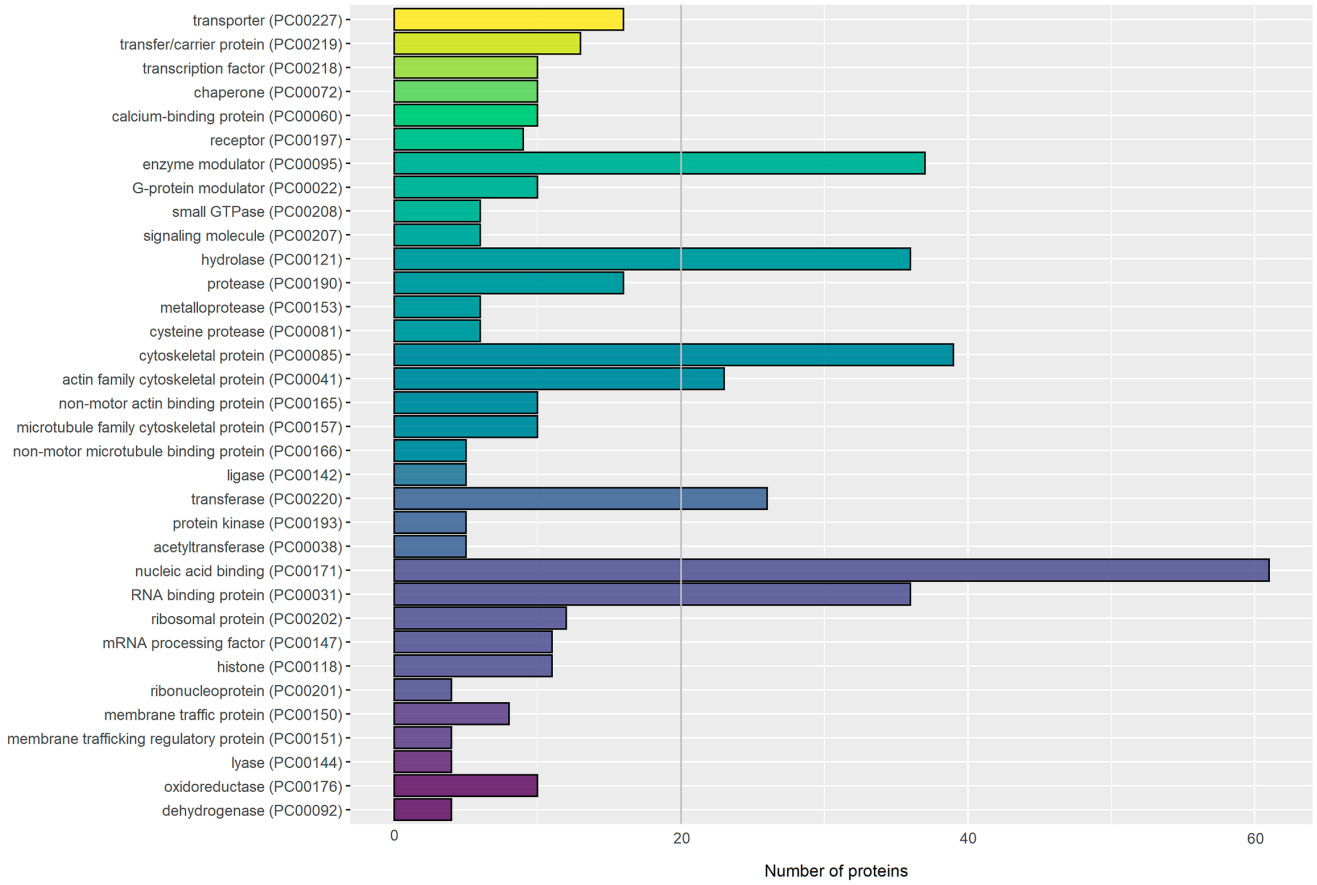

Fig. 2. Categorization of the Panx1 interactome, composed of 482 Panx1-interacting proteins, into PANTHER protein classes (cutoff: at least 4 proteins per PANTHER protein class). The proteins that make up the Panx1 interactome belong to 34 different PANTHER protein classes when applying the predefined cutoff (sorted by lineage and color coded; note that some of these are nested such that there can be overlap; for example, RNA binding proteins are also included within nucleic acid binding, see also Fig. 3B for additional visualization). The following 7 PANTHER protein classes held more than 20 annotated Panx1-interacting proteins: Enzyme modulator (PC00095), hydrolase (PC00121), cytoskeletal protein (PC00085), actin family cytoskeletal protein (PC00041), transferase (PC00220), nucleic acid binding (PC00171) and RNA binding protein (PC00031). Nevertheless, not all of these PANTHER protein classes were overrepresented in the Panx1 interactome (Fig. 3).

(Sfn), prefoldin subunit 3 (Vbp1) and 14-3-3 protein beta/alpha (Ywhab).

To increase our understanding of the function of the Panx1-interacting proteins at the gene level, we searched for overrepresented GO terms. Note that the database contained 63.3% of Panx1-interacting proteins. This analysis identified 7, 5, and 3 overrepresented GO terms for biological process, molecular function and cell component domain, respectively. The most overrepresented GO term within each domain, were intracellular protein transport (GO:0006886, p = 8.57E-13; Table S1 for full protein list), GTPase activity (GO:0003924, p = 2.78E-11) and actin cytoskeleton (GO:0015629, p = 5.72E-07). Four PANTHER pathways were overrepresented (23% of the Panx1-interacting proteins were classified) in which the most overrepresented pathway was Parkinson disease (P00049, p = 5.33E-04). All overrepresentation findings can be found in Fig. 3A and the Panx1-interacting proteins associated with the PANTHER pathway Parkinson disease are listed in Table 1. Twelve Panx1-interacting proteins were involved in that pathway. Overlaps exist between PANTHER protein classes, GO terms and PANTHER pathways (Fig. 3B-D). Regarding the PANTHER protein classes, RNA binding protein (PC00031) showed complete overlap with nucleic acid binding (PC00171) (Fig. 3B). Moreover, two and three Panx1-interacting proteins within the Parkinson disease PANTHER pathway belonged to the PANTHER protein class chaperone and were implicated in the PANTHER pathway cholecystokinin receptor (CCKR) signaling map, respectively (Fig. 3C). Considerable overlap was also observed between the GO terms, Ras protein signal transduction (GO:0007265), intracellular protein transport (GO:0006886, Table S1), vesicle-mediated transport (GO:0016192, Table S2 for full protein list) and GTPase activity (GO:0003924, Table S3 for full protein list), within the biological process and molecular function domains. Five Panx1-interacting proteins were annotated to each GO term, namely the Ras-related proteins Rab4A, Rab6A, Rab6B, Rab8A and Rab12 (Fig. 3D).

Cross-analysis of Panx1-interacting proteins with adult mouse brain postsynaptic proteins and *in silico* determination of PPI networks. As our initial proteomic identification of the Panx1-interactome was performed in mouse N2a cells (Wicki-Stordeur and Swayne, 2013), our findings will be affected by the characteristics of N2a cells,

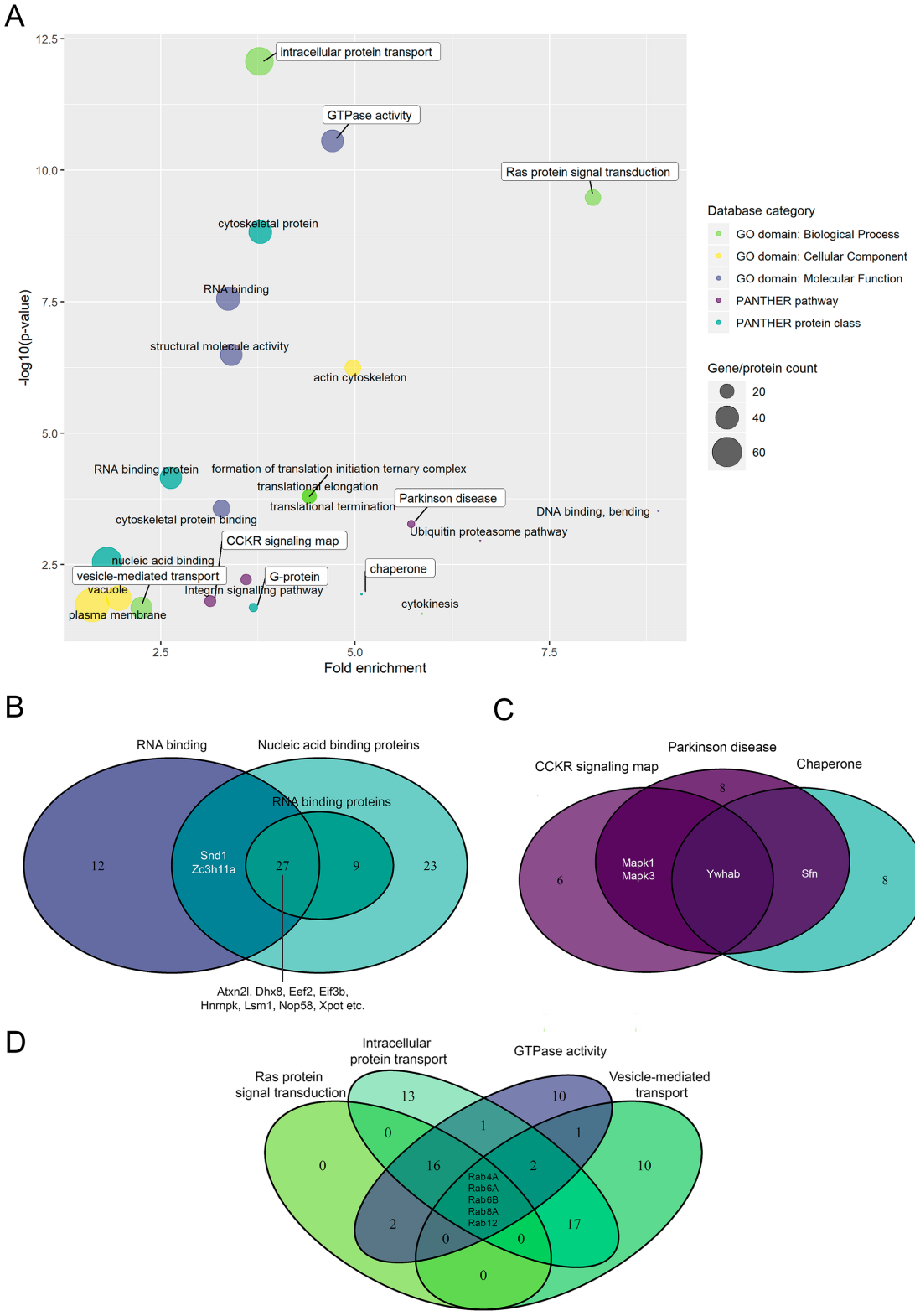

Fig. 3. Overrepresented PANTHER protein classes, Gene Ontology (GO) terms and PANTHER pathways in the Panx1 interactome identified in mouse N2a cells. **(A)** White-box labels are presented for PANTHER protein classes, GO terms and PANTHER pathways that are further discussed within this study. The connection between Panx1 and the cytoskeleton have already been reported and discussed in previous studies (Wicki-Stordeur and Swayne, 2013, 2014). **(B)** Venn diagram illustrating Panx1-interacting protein overlaps between the PANTHER protein classes, nucleic acid binding (PC00171) and RNA binding proteins (PC00031), and the GO term, RNA binding (GO:0003723). Twenty-seven Panx1-interacting proteins were annotated to each category. *(C)* Venn diagram illustrating Panx1-interacting protein overlaps between the PANTHER pathways, Parkinson disease (P00049) and cholecystokinin receptor (CCKR) signaling map (P06959), and the PANTHER protein class, chaperone (PC00072). Four Panx1-interacting proteins were annotated to more than one PANTHER protein class/pathway: Mitogen-activated protein kinase 1 (Mapk1), Mitogen-activated protein kinase 3 (Mapk3), 14-3-3 protein sigma (Sfn) and 14-3-3 protein beta/alpha (Ywhab). **(D)** Venn diagram illustrating Panx1-interacting protein overlaps between the GO terms, intracellular protein transport (GO:0006886), Ras protein signal transduction (GO:0007265) and vesicle-mediated transport (GO:0016192) and GTPase activity (GO:0003924), within the biological process and molecular function domains. Five Panx1-interacting proteins were annotated to each GO term: The Ras-related proteins Rab4A, Rab6A, Rab6B, Rab8A and Rab12. *Abbreviations: Atxn2l, ataxin-2-like protein; Dhx8, ATP-dependent RNA helicase DHX8; Eef2, elongation factor 2; Eif3b, eukaryotic translation initiation factor 3 subunit B; Hnrnpk, heterogeneous nuclear ribonucleoprotein K; Lsm1, U6 snRNA-associated Sm-like protein LSm1; Mapk1, mitogen-activated protein kinase 1; Mapk3, mitogen-activated protein kinase 3; Nop58, nucleolar protein 58; Rab4A, Ras-related protein Rab4A; Rab6A, Ras-related protein Rab6A; Rab6B, Ras-related protein Rab6B; Rab8A, Ras-related protein Rab8A; Rab12, Ras-related protein Rab12; Sfn, 14-3-3 protein sigma; Xpot, exportin-T; Ywhab, 14-3-3 protein beta/alpha.*

Table 1. Potential involvement of Panx1-interacting proteins in Parkinson disease and the cholecystokinin receptor (CCKR) signaling map. The PANTHER pathway Parkinson disease (P00049; p = 5.33E-04) was overrepresented in the Panx1-interacting protein list. Twelve Panx1-interacting proteins were implicated in this pathway. Additionally, the PANTHER pathway, CCKR signaling map (P06959), was overrepresented in the Panx1-interacting protein list (p = 1.58E-02) and in the list of reported and mapped genome-wide association study (GWAS) candidate genes involved in Alzheimer disease (p = 1.18E-03). Nine Panx1-interacting proteins were implicated in this pathway. There was, however, no overlap between the two lists but the proteins Map2k5, Mapk7 and Pik3r1 were represented at gene level in the list of suggestive GWAS candidate genes associated with Alzheimer disease.

Gene symbol	UniProt ID	Protein description
Parkinson disease (P00049)		
Hspa14	Q99M31	Heat shock 70 kDa protein 14
Hspa1b	P17879	Heat shock 70 kDa protein 1B
Hspa1l	P16627	Heat shock 70 kDa protein 1-like
Hspa2	P17156	Heat shock-related 70 kDa protein 2
Mapk1	P63085	Mitogen-activated protein kinase 1
Mapk3	Q63844	Mitogen-activated protein kinase 3
Mcm5	P49718	DNA replication licensing factor MCM5
Psma3	O70435	Proteasome subunit alpha type-3
Sept1	P42209	Septin-1
Sept2	P42208	Septin-2
Sfn	O70456	14-3-3 protein sigma
Ywhab	Q9CQV8	14-3-3 protein beta/alpha
CCKR signaling map (P06959)		
Acat1	Q8QZT1	Acetyl-CoA acetyltransferase, mitochondrial
Cckbr	P56481	Gastrin/cholecystokinin type B receptor
Elavl1	P70372	ELAV-like protein 1
Mapk1	P63085	Mitogen-activated protein kinase 1
Mapk3	Q63844	Mitogen-activated protein kinase 3
Pik3cb	Q8BTI9	Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit beta isoform
Rps6ka3	P18654	Ribosomal protein S6 kinase alpha-3
Slc18a2	Q8BRU6	Synaptic vesicular amine transporter
Ywhab	Q9CQV8	14-3-3 protein beta/alpha

nevertheless, this cell line was selected for its neural proteome and the requirement of abundance for LC-MS/MS identification (Hornburg et al., 2014). To begin to understand how this interactome could relate to the newly discovered role of Panx1 in dendritic spine development, we performed a cross-analysis with a postsynaptic proteome (Roy et al., 2018). A total of 104 out of the 482 Panx1-interacting proteins intersected with the set of proteins in the postsynaptic proteome of the adult mouse brain. To investigate if the Panx1-interacting proteins at the postsynapse were also overrepresented for Parkinson disease, we conducted another PANTHER pathway overrepresentation analysis. The PANTHER pathway Parkinson disease (P00049, p = 1.54E-02) was overrepresented with the following postsynaptic Panx1-interacting proteins: Heat shock 70 kDa protein 1-like (Hspa1l), heat shock-related 70 kDa protein 2 (Hspa2), mitogen-activated protein kinase 1 (Mapk1) and septin-2 (Sept2) and Ywhab (5 of out the 12 proteins presented in Table 1).

In terms of PPI networks, PPIs existed for 99 Panx1-interacting postsynaptic proteins using the predefined interaction score threshold (overview can be found in Table 2 and visualization in Fig. S2). Dynein cytoplasmic 1 heavy chain 1 (Dync1h1), vesicle-fusing ATPase (Nsf), and Crmp1 had the highest confidence scores (most likely protein matches); and eukaryotic translation elongation factor 2 (Eef2), guanine nucleotide-binding protein subunit beta-2-like 1 (Gnb2l1), and eukaryotic translation elongation factor 1 gamma (Eef1g) had the most 'edges' (i.e. STRING-defined interactions to other Panx1-interacting proteins). Cluster analysis revealed 16 clusters of varying size within the PPI network. The size of each cluster ranged from as small as 2 to as many as 28 postsynaptic Panx1-interacting proteins (Table 2). Based on the composition of the PPI network, we were particularly interested in further exploring cluster 1. The top 22 postsynaptic Panx1-interacting proteins, ranked by the highest number of edges, all belonged to cluster 1. Also, cluster 1 was directly connected to all of the other clusters within the PPI network constructed by at least 4 postsynaptic Panx1-interacting proteins. Therefore, we next sought to identify the most specialized overrepresented GO terms and Reactome pathways for the postsynaptic Panx1-interacting proteins within cluster 1 (Fig. 4). For cluster 1, the most significantly overrepresented GO terms within the biological process, cellular component and molecular function domains were translation (GO:0006412, n = 13), cytosolic small ribosomal subunit (GO:0022627, n = 5) and structural constituent of ribosome (GO:0003735, n = 7), respectively (Fig. 4).

Categorization of postsynaptic and non-postsynaptic Panx1-interacting proteins within selected KEGG cellular processes, human disease and organismal systems pathways. To further categorize the Panx1 interactome and gain insight into their biological function and in-

Table 2. The adult mouse brain and postsynaptic Panx1-interacting protein clusters within the PPI network. The results presented in this table include findings from Roy et al. (2018) (postsynaptic proteins and confidence scores) and from the current study (Panx1-interacting proteins, clusters and number of edges). Roy et al. (2018) investigated 7 different brain regions, namely the cerebellum, caudal cortex, medial cortex, frontal cortex, hippocampus, hypothalamus and striatum. A total of 99 out of the 104 postsynaptic Panx1-interacting proteins (confidence scores ranging from 43.45 to 3632.83) were connected. Within the PPI network, formed by physical and functional PPIs (Szklarczyk et al., 2019), sixteen clusters were detected containing 2 to 28 proteins per cluster. Clusters, protein complex or functional modules, have been described to *"demonstrate greater similarity among proteins in the same cluster than in different clusters"* (Wang et al., 2010). Within the PPI network, hubs (*"highly connected protein nodes"* (He and Zhang, 2006)) tend to have a higher essentiality, and thus biological importance, than non-hubs (Zotenko et al., 2008). The postsynaptic Panx1-interacting proteins with the most edges (also termed PPIs) were Eef2, Gnb2l1 and Eef1g (cluster 1). The remaining 5 Panx1-interacting postsynaptic proteins without associations (interaction scores threshold: $\geq$ 0.4) were: Cell cycle exit and neuronal differentiation protein 1 (Cend1), myosin light polypeptide 6 (Myl6), programmed cell death 6-interacting protein (Pdcd6ip), staphylococcal nuclease domain-containing protein 1 (Snd1) and ubiquitin-associated protein 2-like (Ubap2l).

Cluster no	Gene symbol	Protein description	Confidence score	Number of edges
Cluster 1	Eef1g	Elongation factor 1-gamma	404.30	25
	Eef2	Elongation factor 2	455.32	27
	Eif3b	Eukaryotic translation initiation factor 3 subunit B	174.07	19
	Eprs	Bifunctional glutamate/proline–tRNA ligase	209.66	24
	Gars	Glycine–tRNA ligase	43.45	5
	Gnb2l1	Receptor of activated protein C kinase 1	518.86	26
	Hspa1l	Heat shock 70 kDa protein 1-like	612.23	13
	Hspa2	Heat shock-related 70 kDa protein 2	1241.14	13
	Hspa4l	Heat shock 70 kDa protein 4L	430.55	12
	Hspd1	60 kDa heat shock protein, mitochondrial	2248.62	19
	Hspe1	10 kDa heat shock protein, mitochondrial	263.42	15
	Kpnb1	Importin subunit beta-1	107.69	7
	Phb2	Prohibitin-2	698.10	7
	Plec	Plectin	1719.89	17
	Ppib	Peptidyl-prolyl cis-trans isomerase B	76.41	4
	Rpl4	Ribosomal protein L4	348.59	19
	Rpl7a	Ribosomal protein L7A	109.64	15
	Rpl13	Ribosomal protein L13	127.67	17
	Rplp0	60S acidic ribosomal protein P0	219.11	17
	Rps3a1	40S ribosomal protein S3a	200.88	18
	Rps6	40S ribosomal protein S6	81.63	19
	Rps11	Ribosomal protein S11	65.34	17
	Rps18	40S ribosomal protein S18	198.96	15
	Rpsa	40S ribosomal protein SA	220.68	19
	Slc25a5	ADP/ATP translocase 2	1373.48	11
	St13	Hsc70-interacting protein	134.66	5
	Vars	Valine–tRNA ligase	202.73	17
	Vdac3	Voltage-dependent anion-selective channel protein 3	717.67	3
Cluster 2	Arl8b	ADP-ribosylation factor-like protein 8B	101.31	1
	Cd47	Leukocyte surface antigen CD47	488.73	2
	Gdi1	Rab GDP dissociation inhibitor alpha	947.56	5
	Map1lc3b	Microtubule-associated proteins 1A/1B light chain 3B	57.49	3
	Nsf	Vesicle-fusing ATPase	3503.42	5
	Rab3C	Ras-related protein Rab3C	458.36	4
	Rab6A	Ras-related protein Rab6A	174.70	10
	Rab11B	Ras-related protein Rab11B	159.64	5
	Rab14	Ras-related protein Rab14	546.40	7
	Rab35	Ras-related protein Rab35	236.23	5
	Vps51	Vacuolar protein sorting-associated protein 51 homolog	146.82	2
Cluster 3	Ddx5	Probable ATP-dependent RNA helicase DDX5	183.80	5
	Ddx39b	Spliceosome RNA helicase Ddx39b	144.50	10
	Hdlbp	Vigilin	190.56	2
	Hnrnpab	Heterogeneous nuclear ribonucleoprotein A/B	104.90	7
	Hnrnph2	Heterogeneous nuclear ribonucleoprotein H2	140.78	5
	Hnrnpk	Heterogeneous nuclear ribonucleoprotein K	598.91	9
	Hnrnpl	Heterogeneous nuclear ribonucleoprotein L	236.99	5
Cluster 4	Actr1a	Alpha-centractin	119.03	4
	Clasp2	CLIP-associating protein 2	339.83	1
	Cyb5r3	NADH-cytochrome b5 reductase 3	150.95	2
	Dync1h1	Cytoplasmic dynein 1 heavy chain 1	3632.83	8
	Fkbp4	Peptidyl-prolyl cis-trans isomerase FKBP4	68.33	5
	Mapk1	Mitogen-activated protein kinase 1	454.21	10
	Ywhab	14-3-3 protein beta/alpha	502.92	3

Table 2. Continued.

Cluster no	Gene symbol	Protein description	Confidence score	Number of edges
Cluster 5	Eno2	Gamma-enolase	1007.98	8
	Fh1	Fumarate hydratase, mitochondrial	513.91	9
	Gfap	Glial fibrillary acidic protein	594.72	1
	Lasp1	LIM and SH3 domain protein 1	154.28	1
	Pfkl	ATP-dependent 6-phosphofructokinase, liver type	381.50	3
	Taldo1	Transaldolase	63.78	7
Cluster 6	Atp5j2	ATP synthase subunit f, mitochondrial	84.13	4
	Etfb	Electron transfer flavoprotein subunit beta	380.58	5
	Ndufa8	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8	351.27	4
	Ndufv1	NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial	1069.63	8
	Ppa1	Pyrophosphatase (inorganic) 1	126.03	4
	Uqcrq	Cytochrome b-c1 complex subunit 8	246.81	3
Cluster 7	Actr3	Actin-related protein 3	276.51	6
	Actr3b	Actin-related protein 3B	49.44	3
	Coro2b	Coronin-2B	68.43	3
	Gsn	Gelsolin	114.49	6
	Tnik	Traf2 and NCK-interacting protein kinase	129.54	1
Cluster 8	Ddb1	DNA damage-binding protein 1	61.30	3
	Otub1	Ubiquitin thioesterase OTUB1	136.31	3
	Ube2n	Ubiquitin-conjugating enzyme E2 N	144.26	3
	Ubqln2	Ubiquilin-2	140.42	11
	Usp9x	Probable ubiquitin carboxyl-terminal hydrolase FAF-X	79.30	2
Cluster 9	Marcks	Myristoylated alanine-rich C-kinase substrate	306.98	1
	Phgdh	D-3-phosphoglycerate dehydrogenase	317.59	4
	Slc7a5	Large neutral amino acids transporter small subunit 1	204.00	2
	Slc16a1	Monocarboxylate transporter 1	66.00	2
Cluster 10	Acat1	Acetyl-CoA acetyltransferase, mitochondrial	1011.50	2
	Hadhb	Trifunctional enzyme subunit beta, mitochondrial	420.91	4
	Oxct1	Succinyl-CoA:3-ketoacid coenzyme A transferase 1, mitochondrial	572.19	4
	Tnpo2	Transportin-2	76.16	3
Cluster 11	Actn1	Alpha-actinin-1	2139.41	4
	Csrp1	Cysteine and glycine-rich protein 1	384.68	1
	Sccpdh	Saccharopine dehydrogenase-like oxidoreductase	128.16	1
Cluster 12	Gna11	Guanine nucleotide-binding protein subunit alpha-11	262.22	2
	Gna13	Guanine nucleotide-binding protein subunit alpha-13	212.89	2
	Gnb2	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2	947.17	4
Cluster 13	Canx	Calnexin	722.26	10
	Rtn3	Reticulon-3	640.72	2
	Tmx3	Protein disulfide-isomerase TMX3	109.74	1
Cluster 14	Ppp1r7	Protein phosphatase 1 regulatory subunit 7	83.18	1
	Sept2	Septin-2	83.10	4
	Sept9	Septin-9	161.42	1
Cluster 15	Ahcyl2	Putative adenosylhomocysteinase 3	784.35	4
	Anp32a	Acidic leucine-rich nuclear phosphoprotein 32 family member A	96.02	1
Cluster 16	Crmp1	Dihydropyrimidinase-related protein 1	3082.18	1
	Dpysl3*	Dihydropyrimidinase-related protein 3	765.49	1

*Also referred to as Crmp4

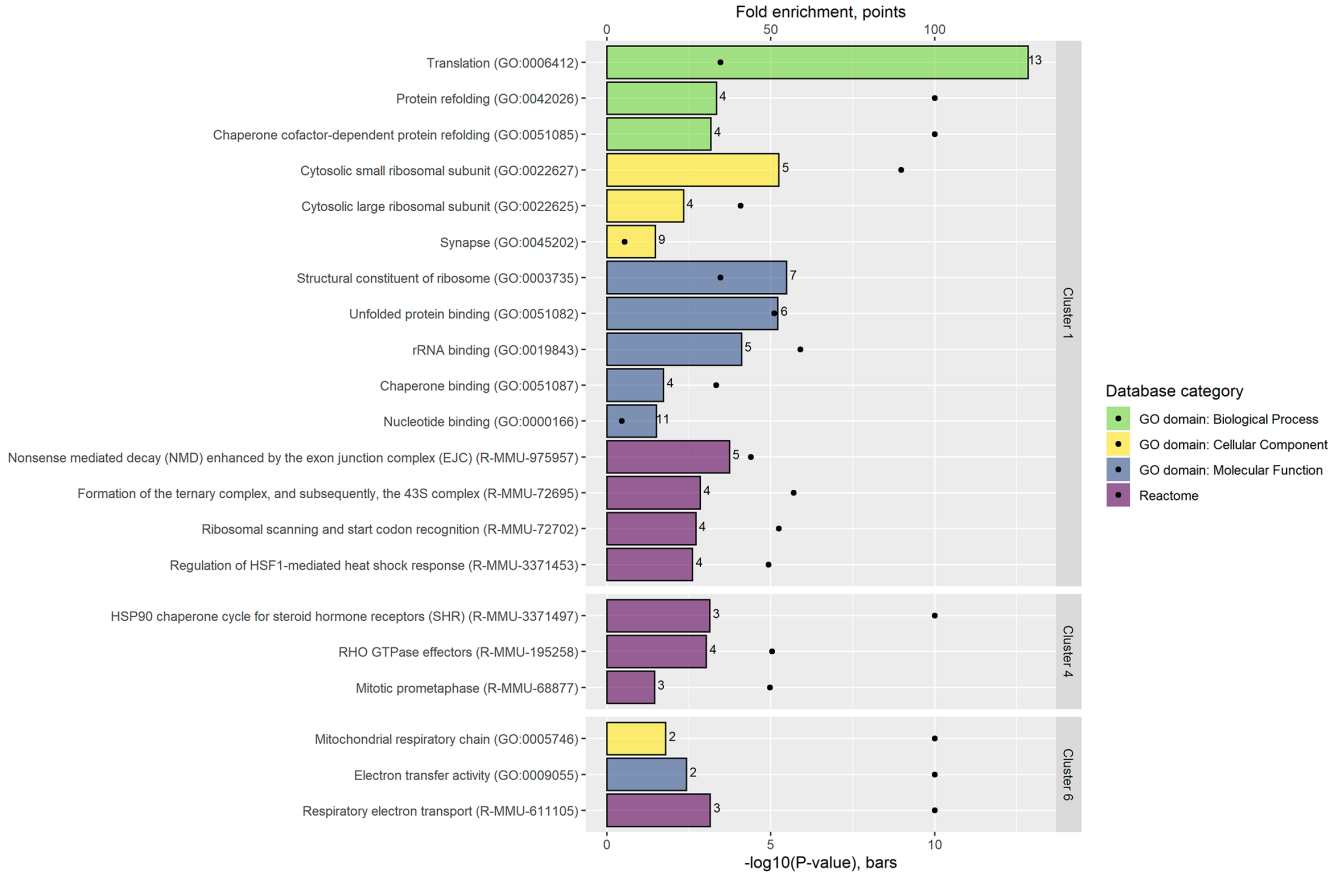

Fig. 4. Overrepresented Gene Ontology (GO) terms and Reactome pathways for cluster 1, 4 and 6 within the postsynaptic mouse STRING protein-protein interaction (PPI) network. The Panx1-interacting proteins that formed the postsynaptic mouse STRING PPI network are presented in Table 2. Clusters within PPI networks are thought to be associated with specific biological functions, and can be protein complex or functional modules (Wang et al., 2010). For cluster 1, eleven overrepresented GO terms and 4 Reactome pathways were identified. The most significant GO term was translation (GO:0006412) and those with the highest fold enrichment were protein refolding (GO:0042026) and chaperone cofactor-dependent protein refolding (GO:0051085). For cluster 4, three overrepresented Reactome pathways were identified. The Reactome pathway that was most significant and had the highest fold enrichment score was HSP90 chaperone cycle for steroid hormone receptors (SHR) (R-MMU-3371497). For cluster 6, two overrepresented GO terms and 1 Reactome pathway were identified with the Reactome pathway, respiratory electron transport (R-MMU-611105), being the most significant. The disorder-specific Panx1-interacting proteins were primarily represented within these clusters (Fig. 9, Fig. 12). The numbers beside the bars represent the protein/gene count for the GO term or Reactome pathway in question.

volvement in disease, we selected 43 KEGG pathways of interest in the KEGG database (68.3% of the Panx1 interactome in mouse N2a cells overlapped with the KEGG database). A total of 100 Panx1-interacting proteins were annotated to the selected KEGG pathways of which 26 of those were present at the postsynapse in the adult mouse brain (Fig. 5). Seven, 7, 8 and 1 Panx1-interacting proteins were annotated to the KEGG pathways Parkinson disease (mmu05012), Alzheimer disease (mmu05010), Huntington disease (mmu05016) and ALS (mmu05014), respectively. We observed some overlap between the neurodegenerative diseases. For example, NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8 (Ndufa8), NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial (Ndufv1) and cytochrome b-c1 complex subunit 8 (Uqcrq) were implicated in Parkinson disease, Alzheimer disease, and Huntington disease (Fig. 5). Visualization of the KEGG pathways, Parkinson disease and Alzheimer disease, can be found in Fig. 6 and Fig. 7.

Some Panx1-interacting proteins (Fig. 5) involved in the neurodegenerative diseases (Parkinson disease, Alzheimer disease, and Huntington disease), based on the KEGG categorization, were annotated to the KEGG pathways alcoholism (mmu05034) and thermogenesis (mmu04714). For Parkinson disease, the Panx1-interacting proteins were also implicated in KEGG pathways such as synaptic vesicle cycle (mmu04721), cellular senescence (mmu04218), necroptosis (mmu04217) and ferroptosis (mmu04216). Despite overrepresentation of heat shock 70 kDa protein 1B (Hspa1b), Hspa1l, Hspa2, Mapk1, mitogen-activated protein kinase 3 (Mapk3), DNA replication licensing factor MCM5 (Mcm5), Sfn, and Ywhab in the PANTHER Parkinson disease (P00049) pathway (Table 1, Fig. 5), these same proteins were not found in the KEGG Parkinson disease pathway (even though they were in the database). Although not found in the Parkinson disease KEGG pathway, Mcm5, Sfn, and Ywhab were found in the cell cycle KEGG pathway (mmu04110), as well as Hspa1b, Hspa1l, and Hspa2 in the KEGG pathways endocytosis (mmu04144) and longevity regulating pathway (mmu04213). For Alzheimer disease, the Panx1-interacting proteins were also implicated in the KEGG pathways circadian entrainment (mmu04713) and long-term potentiation (mmu04720); and for Huntington dis-

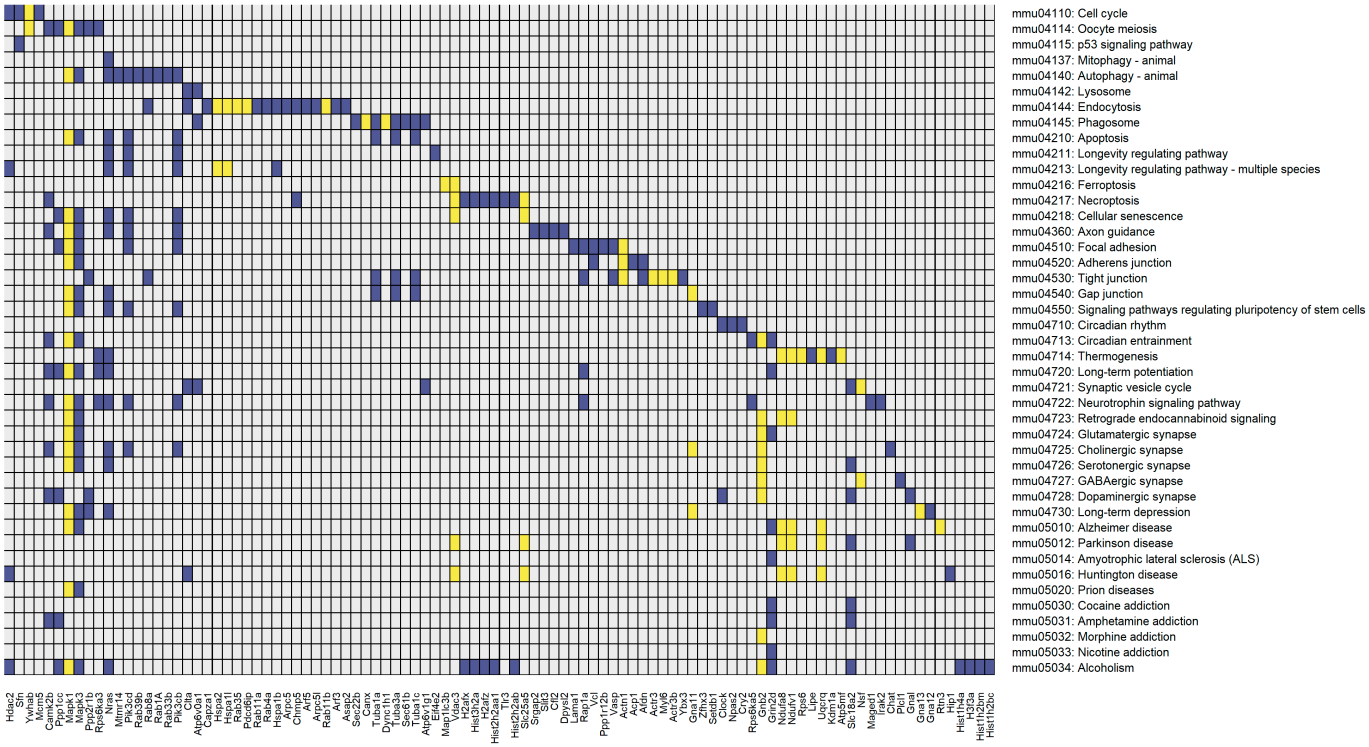

Fig. 5. Categorization of Panx1-interacting proteins into KEGG pathways of interest (Panx1 interactome in N2a cells (blue and yellow); Panx1-interacting proteins at the postsynapse in the adult mouse brain (yellow)). A total of 100 Panx1-interacting proteins were categorized into the 43 KEGG pathways. Twenty-six of the categorized Panx1-interacting proteins were also identified at the postsynapse in the adult mouse brain. The categorization led to identification of 7 additional Panx1-interacting proteins involved in Parkinson disease (mmu05012), including NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8 (Ndufa8), NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial (Ndufv1) and cytochrome b-c1 complex subunit 8 (Uqcrq). These Panx1-interacting proteins were also associated with Alzheimer disease (mmu05010) and Huntington disease (mmu05016), and depending on the Panx1-interacting protein, retrograde endocannabinoid signaling (mmu04723) and thermogenesis (mmu04714). Other Panx1-interacting proteins identified for Parkinson disease can be found in Table 1 and Table 3. *Abbreviations: Acp1, low molecular weight phosphotyrosine protein phosphatase; Actn1, alpha-actinin-1; Actr3, actin-related protein 3; Actr3b, actin-related protein 3B; Afdn, afadin ; Arf3, ADP-ribosylation factor 3; Arf5, ADP-ribosylation factor 5; Arpc5, actin-related protein 2/3 complex subunit 5; Arpc5l, actin-related protein 2/3 complex subunit 5-like protein; Asap2, Arf-GAP with SH3 domain, ANK repeat and PH domain-containing protein 2; Atp5mf, ATP synthase subunit f, mitochondrial; Atp6v0a1, V-type proton ATPase 116 kDa subunit a isoform 1; Atp6v1g1, V-type proton ATPase subunit G 1; Camk2b, calcium/calmodulin-dependent protein kinase type II subunit beta; Canx, calnexin; Capza1, F-actin-capping protein subunit alpha-1; Cfl2, cofilin-2; Chat, choline O-acetyltransferase; Chmp5, charged multivesicular body protein 5; Clock, circadian locomoter output cycles protein kaput; Clta, clathrin light chain A; Cry2, cryptochrome-2; Dpysl2, dihydropyrimidinase-related protein 2 (also referred to as Crmp2); Dync1h1, cytoplasmic dynein 1 heavy chain 1; Eif4e2, eukaryotic translation initiation factor 4E type 2; Gna11, guanine nucleotide-binding protein subunit alpha-11; Gna12, guanine nucleotide-binding protein subunit alpha-12; Gna13, guanine nucleotide-binding protein subunit alpha-13; Gnal, guanine nucleotide-binding protein G(olf) subunit alpha; Gnb2, guanine nucleotide-binding protein G(l)/G(S)/G(T) subunit beta-2; Grin2d, glutamate ionotropic receptor NMDA type subunit 2D; H2afx, histone H2AX; H2afz, histone H2A.Z; H3f3a, histone H3.3; Hdac2, histone deacetylase 2; Hip1, huntingtin-interacting protein 1; Hist1h2bc, histone H2B type 1-C/E/G; Hist1h2bm, histone H2B type 1-M; Hist1h4a, histone H4; Hist2h2ab, histone H2A type 2-B; Hist2h2aa1, histone H2A type 2-A; Hist3h2a, histone H2A type 3; Hspa1b, heat shock 70 kDa protein 1B; Hspa1l, heat shock 70 kDa protein 1-like; Hspa2, heat shock-related 70 kDa protein 2; Irak2, interleukin-1 receptor-associated kinase-like 2; Kdm1a, lysine-specific histone demethylase 1A; Lama1, laminin subunit alpha-1; Lipe, hormone-sensitive lipase; Maged1, melanoma-associated antigen D1; Map1lc3b, microtubule-associated proteins 1A/1B light chain 3B; Mapk1, mitogen-activated protein kinase 1; Mapk3, Mitogen-activated protein kinase 3; Mcm5, DNA replication licensing factor MCM5; Mtmr14, myotubularin-related protein 14; Myl6, myosin light polypeptide 6; Ndufa8, NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8; Ndufv1, NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial; Npas2, neuronal PAS domain-containing protein 2; Nras, GTPase NRas; Nsf, vesicle-fusing ATPase; Pdcd6ip, programmed cell death 6-interacting protein; Pik3cb, phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit beta isoform; Pik3cd, phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit delta isoform; Plcl1, inactive phospholipase C-like protein 1; Ppp1cc, serine/threonine-protein phosphatase PP1-gamma catalytic subunit; Ppp1r12b, protein phosphatase 1 regulatory subunit 12B; Ppp2r1b, serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A beta isoform; Rab1A, Ras-related protein Rab1A; Rab4A, Ras-related protein Rab4A; Rab8A, Ras-related protein Rab8A; Rab11A, Ras-related protein Rab11A; Rab11B, Ras-related protein Rab11B; Rab33B, Ras-related protein Rab33B; Rab35, Ras-related protein Rab35; Rab39B, Ras-related protein Rab39B; Rap1A, Ras-related protein Rap1A; Rps6, 40S ribosomal protein S6; Rps6ka3, ribosomal protein S6 kinase alpha-3; Rps6ka5, ribosomal protein S6 kinase alpha-5; Rtn3, reticulon-3; Sec22b, vesicle-trafficking protein SEC22b; Sec61b, protein transport protein Sec61 subunit beta; Setdb1, histone-lysine N-methyltransferase SETDB1; Sfn, 14-3-3 protein sigma; Slc18a2, synaptic vesicular amine transporter; Slc25a5, ADP/ATP translocase 2; Slit3, slit homolog 3 protein; Srgap2, SLIT-ROBO Rho GTPase-activating protein 2; Tlr3, toll-like receptor 3; Tuba1a, tubulin alpha-1A chain; Tuba1c, tubulin alpha-1C chain; Tuba3a, tubulin alpha-3 chain; Uqcrq, cytochrome b-c1 complex subunit 8; Vasp, vasodilator-stimulated phosphoprotein; Vcl, vinculin; Vdac3, voltage-dependent anion-selective channel protein 3; Ybx3, Y-box-binding protein 3; Ywhab, 14-3-3 protein beta/alpha; Zfhx3, zinc finger homeobox protein 3.*

ease, it was for the KEGG pathways synaptic vesicle cycle (mmu04721), cellular senescence (mmu04218), necroptosis (mmu04217), ferroptosis (mmu04216), endocytosis (mmu04144), apoptosis (mmu04210), and longevity regulating pathway (mmu04213). The ALS-associated Panx1-interacting protein, glutamate ionotropic receptor NMDA type subunit 2D (Grin2d), was also annotated to other KEGG pathways; for example, circadian entrainment (mmu04713), long-term potentiation (mmu04720), and different pathways related to substance abuse.

Panx1-interacting protein representation within GWAS for neurodegenerative diseases and neurodevelopmental disorders in humans.

GWAS and neurodegenerative diseases. Additional Panx1-interacting proteins potentially involved in the neurodegenerative diseases, Parkinson disease (3 Panx1-interacting proteins, Fig. 8A-C, including the expanded PPI networks for each of these proteins), Alzheimer disease (18 Panx1-interacting proteins), Huntington disease (1 Panx1-interacting protein), and ALS (1 Panx1-interacting protein) were identified by cross-analyzing the Panx1 interactome in mouse N2a cells with GWAS hits, and are presented in Table 3. The Panx1-interacting proteins, mitogen-activated protein kinase kinase kinase kinase 4 (Map4k4) and MAM domain-containing glycosylphosphatidylinositol anchor protein 2 (Mdga2), were identified for both Parkinson disease and Alzheimer disease in the GWAS. We did not identify other overlapping Panx1-interacting proteins when comparing the findings from the cross-analyses for the neurodegenerative diseases, Parkinson disease, Alzheimer disease, Huntington disease, and ALS (Fig. 9A). Nevertheless, these neurodegenerative diseases had 6 Panx1-interacting proteins in common with the neurodevelopmental disorders investigated

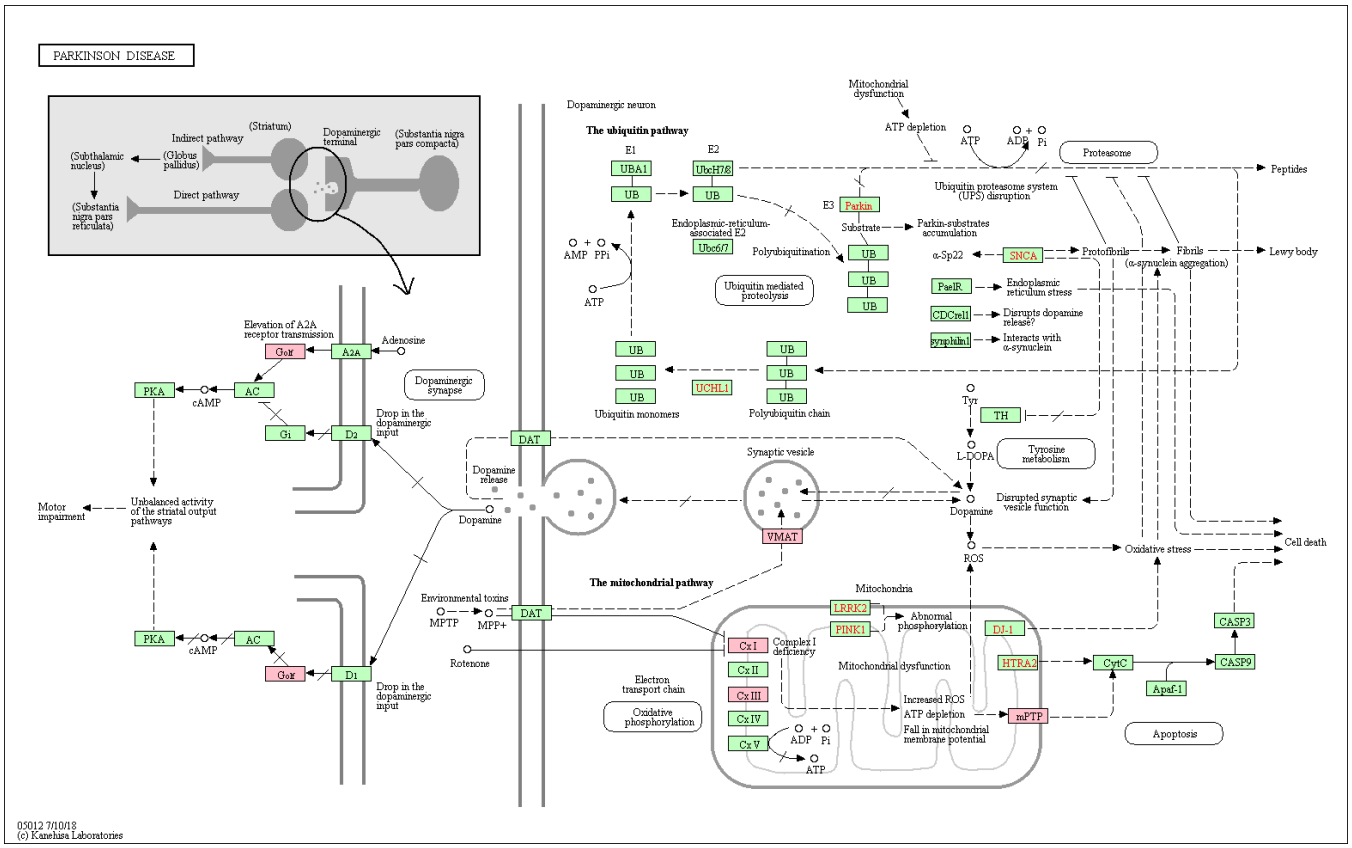

Fig. 6. The Parkinson disease KEGG pathway (mmu05012) highlighting components related to the Panx1 interactome (red boxes). The Panx1-interacting proteins involved in and highlighted for this KEGG pathway were guanine nucleotide-binding protein G(olf) subunit alpha (Gnal, highlight Golf), NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8 (Ndufa8, highlight CxI), NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial (Ndufv1, highlight CxI), synaptic vesicular amine transporter (Slc18a2/Vmat2, highlight VMAT), ADP/ATP translocase 2 (Slc25a5, highlight mPTP), cytochrome b-c1 complex subunit 8 (Uqcrq, highlight CxIII) and voltage-dependent anion-selective channel protein 3 (Vdac3, highlight mPTP). Of these, 71.4% were located at the postsynapse in the adult mouse brain (Fig. 5). Image credit: Kanehisa and Sato (2019). *Other abbreviations: CxI/III, complex I/III; mPTP, mitochondrial permeability transition pore.*

within the current study, namely collagen alpha-1(XII) chain (Col12a1), dystonin (Dst), eukaryotic peptide chain release factor subunit 1 (Etf1), inactive phospholipase C-like protein 1 (Plcl1), DNA polymerase nu (Poln), and Map4k4 (Table 3).

For Alzheimer disease, eighteen Panx1-interacting proteins overlapped with the reported and mapped GWAS candidate genes (Table 3). Therefore, we performed functional annotation and overrepresentation analysis for this protein list. We did not identify any overrepresented PANTHER protein classes, GO terms, or PANTHER pathways for these Panx1-interacting proteins. Nevertheless, by lowering the predefined protein count threshold (threshold used to reduce the risk of type I errors), the circadian clock system (P00015, p = 1.37E-04) PANTHER pathway was overrepresented. For the full GWAS candidate gene list for Alzheimer disease, the following 5 GO terms and PANTHER pathways were overrepresented: Cell junction (GO:0030054, p = 1.79E-02), cell adhesion molecule binding (GO:0050839, p = 2.23E-02), cadherin signaling pathway (P00012, p = 3.28E-02), CCKR signaling map (P06959, p = 1.18E-03), and Alzheimer disease-presenilin pathway (P00004, p = 2.45E-02). The PANTHER pathway CCKR signaling map was also overrepresented in the list of Panx1-interacting proteins in mouse N2a cells (p = 1.58E-02) (Fig. 3A,C, Table 1).

GWAS and neurodevelopmental disorders. Our cross-analyses with GWAS led to the discovery of Panx1-interacting proteins potentially involved in the neurodevelopmental disorders, including schizophrenia (38 Panx1-interacting proteins), ASD (24 Panx1-interacting proteins), and epilepsy (3 Panx1-interacting proteins) (Table 3, Fig. 10A-B, including the PPI networks for the schizophrenia and ASD-associated proteins). There was a considerable overlap between Panx1-interacting proteins associated with schizophrenia and those associated with ASD based on the GWAS hits in which 23 Panx1-interacting proteins overlapped (95.8% of the ASD-associated Panx1-interacting proteins). Additionally, the Panx1-interacting protein, protein tyrosine phosphatase receptor type K (Ptprk), was associated with both schizophrenia and epilepsy whereas no overlap was observed between ASD and epilepsy (Fig. 9B, Table 3).

More than 15 Panx1-interacting protein encoding genes, identified through GWAS cross-analysis, were associated with increased susceptibility to develop *both* schizophrenia and ASD. The Panx1-interacting proteins identified for both neurodevelopmental disorders (Table 3) were overrepresented in the following PANTHER protein classes, GO terms, and PANTHER pathways: Histone (PC00118, n = 4), chaperone-mediated protein folding (GO:0061077, n = 4),

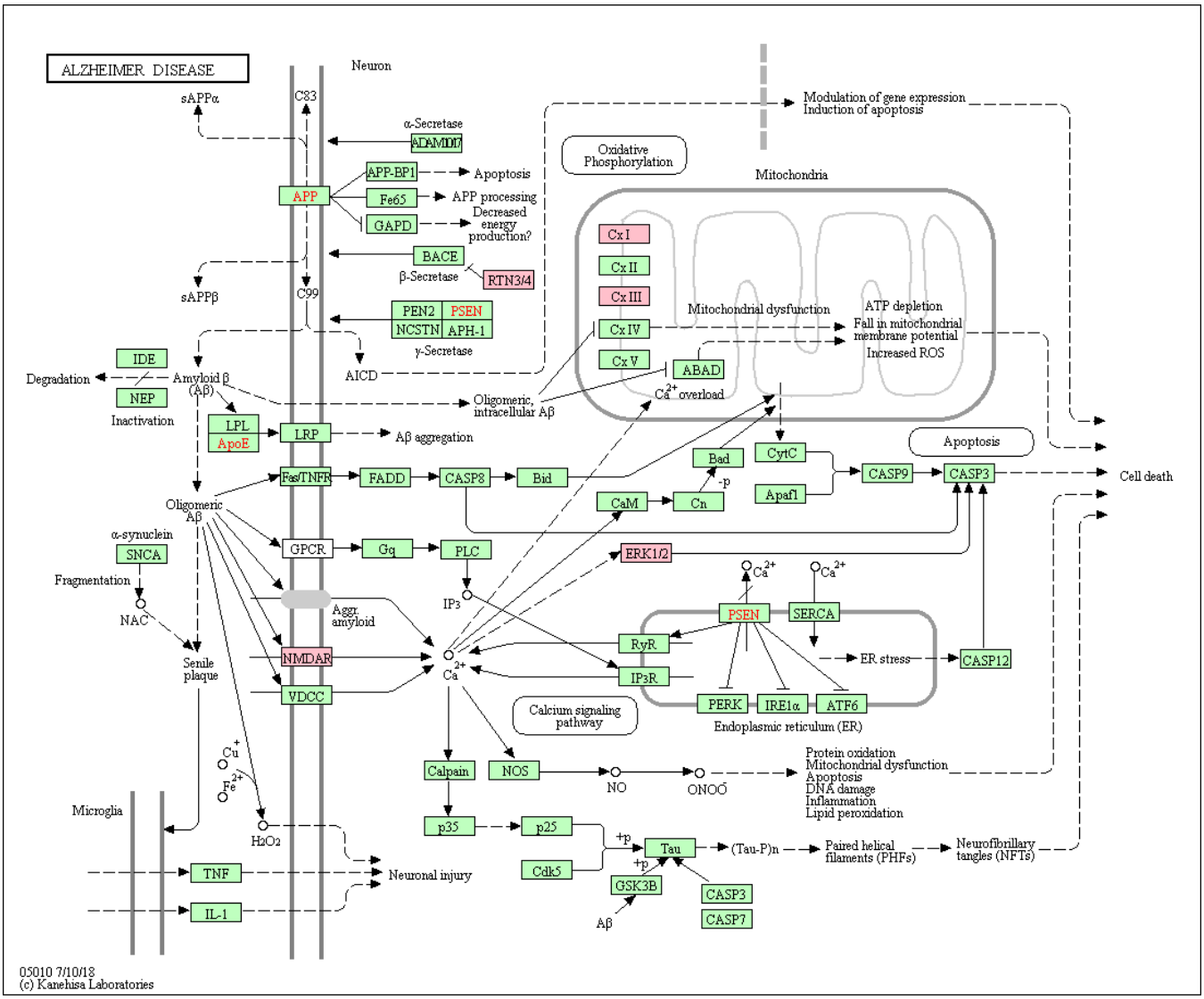

Fig. 7. The Alzheimer disease KEGG pathway (mmu05010) highlighting components related to the Panx1 interactome (red boxes). The Panx1-interacting proteins involved in and highlighted for this KEGG pathway were glutamate ionotropic receptor NMDA type subunit 2D (Grin2d, highlight NMDAR), mitogen-activated protein kinase 1 (Mapk1, highlight ERK1/2), mitogen-activated protein kinase 3 (Mapk3, highlight ERK1/2), NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8 (Ndufa8, highlight CxI), NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial (Ndufv1, highlight CxI), reticulon-3 (Rtn3, highlight RTN3/4) and cytochrome b-c1 complex subunit 8 (Uqcrq, highlight CxIII). Of these, 71.4% were located at the postsynapse in the adult mouse brain (Fig. 5). Image credit: Kanehisa and Sato (2019). *Other abbreviations: CxI/III, complex I/III.*

cellular component assembly (GO:0022607, n = 4-5), DNA binding, bending (GO:0008301, n = 4), and apoptosis signaling pathway (P00006, n = 4). An overview of the findings, including the corresponding Panx1-interacting proteins, fold enrichment and Bonferroni-corrected p-values, are available in Table 4.

Comparison of cross-analyses of Panx1-interacting proteins/encoding genes implicated in neurodegenerative diseases and neurodevelopmental disorders in humans, and brain region specificity.

Panx1-interacting proteins and Parkinson disease. For Parkinson disease, a total of 22 Panx1-interacting proteins were revealed (Fig. 9A,C) of which 21 out of the 22 Panx1-interacting proteins formed a 3-cluster PPI network (Fig. 11A, low confidence interaction score applied). Twelve, 7, and 3 Panx1-interacting proteins were identified for Parkinson disease through the PANTHER overrepresentation analysis, KEGG categorization and GWAS cross-analysis, respectively. The Panx1-interacting proteins potentially associated with Parkinson disease based on the GWAS hits, Map4k4, Mdga2 and Nsf, did not overlap with those identified through the overrepresented PANTHER pathway, Parkinson disease (P00049, p = 5.33E-04) (Fig. 8A-C, Table 3). The 5 postsynaptic Panx1-interacting proteins contained within this PANTHER pathway were least abundant in the striatum (Hspa1l, Hspa2 and Sept2) and cerebellum (Mapk1 and Ywhab) (Table 1, Table 2, Fig. 12A). Focusing on the GWAS, one of the Panx1-interacting proteins potentially associated with increased susceptibility to develop Parkinson disease, namely Nsf, was also identified at the postsynapse in the adult mouse brain (Table 2, Table 3). Across the brain regions under investigation, the highest protein abundance of Nsf was identified in the hippocampus and the lowest in the cerebel-

Table 3. Putative Panx1-interacting proteins associated with multiple neurodegenerative diseases and neurodevelopmental disorders in humans based on genome-wide association study (GWAS) hits. Panx1-interacting proteins at gene level overlapped with the reported and mapped suggestive GWAS candidate genes involved in Alzheimer disease, Huntington disease, amyotrophic lateral sclerosis (ALS), schizophrenia, epilepsy and Autism Spectrum Disorder (ASD). Intersecting genes indicate the number of genes present in both the Panx1 interactome and GWAS datasets, and the percentage provides the proportion of intersecting genes in relation to the total number of suggested and mapped GWAS candidate genes.

Disease/disorder	Intersecting genes (%)	List of intersecting genes/proteins*	References
Parkinson disease▼	3 (1.3)	Map4k4, Mdga2, Nsf	[1]
Alzheimer disease▼	18 (2.6)	Col12a1, Cry2, Ddx18, Dst, Etf1, G3bp1, Lama1, Map4k4, Mdga2, Plcl1, Poln, Psmc3, Rab3D, Senp7, Tmem94, Txn1, Usp6nl, Vasp	[2]
Huntington disease▼	1 (2.6)	Mfsd10	[3]
ALS▼	1 (0.4)	Rab3gap2	[4]
Schizophrenia▲	38 (2.6)	Acads, Actr1a, Agpat1, Atp5mpl, Bdh2, Car12, Cnot1, Col12a1, Ddx39b, Dph2, Dst, Efl1/Eftud1, Etf1, Gmip, Golga4, Hist1h1c, Hist1h1t, Hist1h2bc, Hist1h2bm, Hspa1b, Hspa1l, Hspe1, Ik, Lsm1, Map4k4, Mapk3, Pcbp3, Plcl1, Poln, Psmd6, Ptprk, Rab39, Rps18, St13, Tnik, Vars, Zdbf2, Zfhx3	[5]
Epilepsy▲	3 (2.4)	Camsap2, Olfml3, Ptprk	[6]
ASD▲	24 (3.7)	Actr1a, Agpat1, Atp5mpl, Cnot1, Ddx39b,Dph2, Etf1, Golga4, Hist1h1c, Hist1h1t, Hist1h2bc, Hist1h2bm, Hspa1b, Hspa1l, Hspe1,Lsm1, Map4k4, Mapk3, Plcl1, Rab39, Rps18, St13, Vars, Xrn2	[7]

▼ neurodegenerative disease; ▲ neurodevelopmental disorder
[1] (Chang et al., 2017; Hamza et al., 2010; Hu et al., 2016; Liu et al., 2011; Simon-Sanchez et al., 2009)
[2] (Chung et al., 2018; Cummings et al., 2012; Furney et al., 2011; Herold et al., 2016; Jun et al., 2016; Jun et al., 2017; Kunkle et al., 2019; Lee et al., 2017; Logue et al., 2011; Marioni et al., 2018; Mez et al., 2017; Nazarian et al., 2019; Zhu et al., 2019)
[3] (Moss et al., 2017)
[4] (Xie et al., 2014)
[5] (Aberg et al., 2013; Amare et al., 2018; Athanasiu et al., 2015; Autism Spectrum Disorders Working Group of The Psychiatric Genomics Consortium, 2017; Bergen et al., 2012; Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013; Drago and Kure Fischer, 2018; Goes et al., 2015; Ikeda et al., 2019; Lencz et al., 2013; Li et al., 2017a; Li et al., 2017b; Pardinas et al., 2018; Pardinas et al., 2019; Potkin et al., 2009; Purcell et al., 2009; Ripke et al., 2013; Ruderfer et al., 2014; Saito et al., 2016; Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium, 2011; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Shi et al., 2011; Wang et al., 2013; Wolthusen et al., 2015; Yu et al., 2018)
[6] (Berghuis et al., 2019; Guo et al., 2012; International League Against Epilepsy Consortium on Complex Epilepsies, 2018)
[7] (Autism Spectrum Disorders Working Group of The Psychiatric Genomics Consortium, 2017; Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013; Grove et al., 2019)
* *Abbreviations:* See Fig. 7.

lum (Fig. 12A). Through the KEGG categorization, five additional postsynaptic Panx1-interacting proteins were revealed (Ndufa8, Ndufv1, ADP/ATP translocase 2 (Slc25a5), Uqcrq and voltage-dependent anion-selective channel protein 3 (Vdac3)) (Fig. 5). For Parkinson disease, the Panx1-interacting proteins at the postsynapse were mainly contained within cluster 1 and 6 in the postsynaptic PPI network (Table 2, Fig. 12B).

Panx1-interacting proteins and Alzheimer disease. For Alzheimer disease, a total of 25 Panx1-interacting proteins were revealed (Fig. 9A,C) of which 20 out of the 25 Panx1-interacting proteins formed a 3-cluster PPI network (Fig. 11B, low confidence interaction score applied). Seven and 18 Panx1-interacting proteins were identified for Alzheimer disease through KEGG categorization and GWAS cross-analysis, respectively. Through KEGG categorization, five postsynaptic Panx1-interacting proteins associated with Alzheimer disease were revealed, namely Mapk1, Ndufa8, Ndufv1, Reticulon-3 (Rtn3), and Uqcrq (Fig. 5). Three of those were contained within cluster 6 in the postsynaptic PPI network (Table 2, Fig. 12B).

Panx1-interacting proteins and Huntington disease. For Huntington disease, a total of 17 Panx1-interacting proteins were revealed (Fig. 9A,C). Eight, 1, and 10 Panx1-interacting proteins were identified for Parkinson disease through KEGG categorization, GWAS cross-analysis and PANTHER categorization, respectively. All, except one of these Panx1-interacting proteins, formed a 3-cluster PPI network when a low confidence interaction score was applied (Fig. 11D). Clathrin light chain A (Clta) and Hip1 were annotated by both the KEGG and PANTHER database. Huntingtin-interacting protein 1 (Hip1) was represented in the list of Panx1-interacting proteins associated to the actin cytoskeleton and involved in cytoskeletal protein binding and vesicle-mediated transport (Fig. 3A). For the KEGG categorization, the postsynaptic Panx1-interacting proteins associated with Huntington disease (Ndufa8, Ndufv1, Slc25a5, Uqcrq and Vdac3) were identical to those identified for Parkinson disease (Fig. 5). These postsynaptic Panx1-interacting proteins were contained within cluster 1 and 6 in the PPI network (Table 2, Fig. 12B). In addition, the postsynaptic Panx1-interacting protein cytoplasmic dynein 1 heavy chain 1 (Dync1h1) was revealed with PANTHER Huntington disease categorization.

Panx1-interacting proteins and ALS. For ALS, a total of 2 Panx1-interacting proteins were revealed (Fig. 9A,C) for which KEGG categorization and GWAS cross-analysis

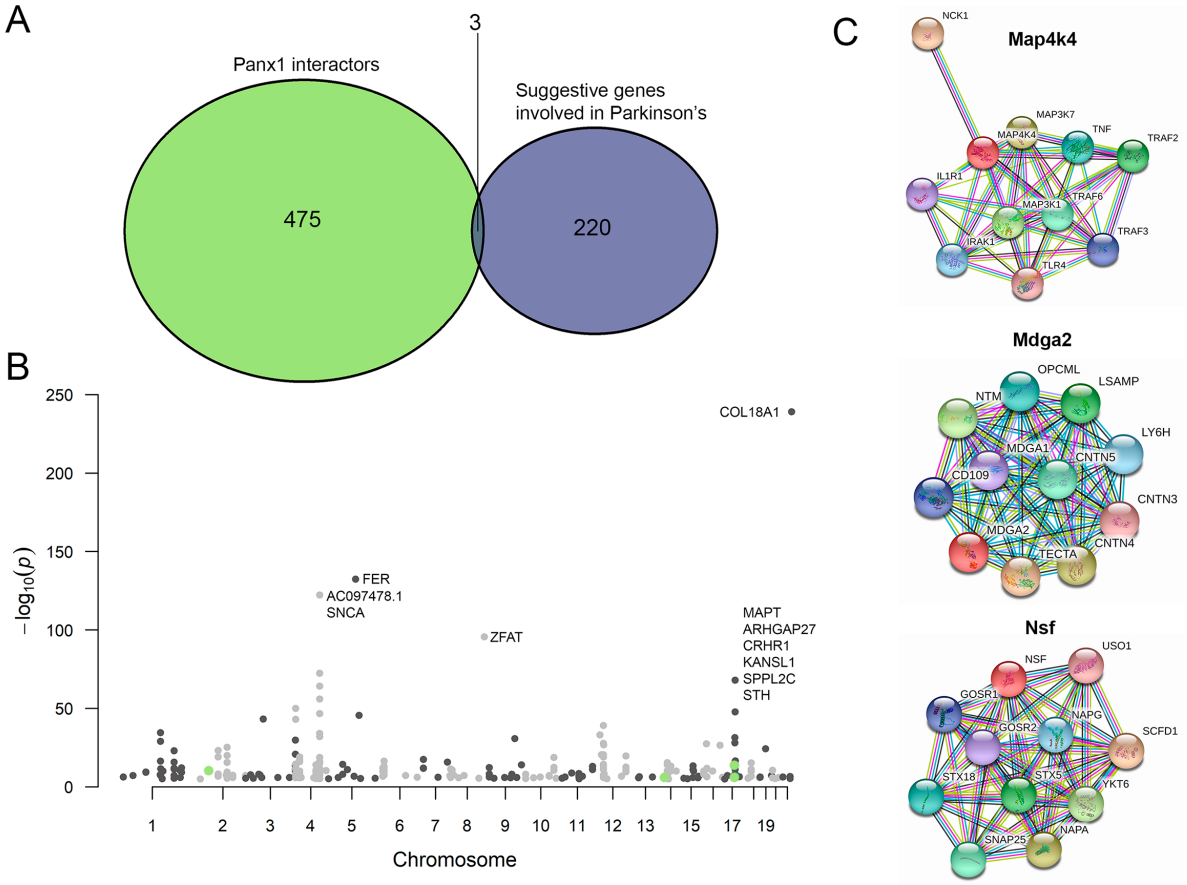

Fig. 8. The Panx1 interactome, Parkinson disease and human STRING protein-protein interaction (PPI) networks. (A) The Venn diagram displays the gene overlap between the reported and mapped genome-wide association study (GWAS) candidate genes involved in Parkinson disease and the Panx1-interacting proteins. The Venn diagram shows that the two gene lists have 3 genes in common: Map4k4, Mdga2 and Nsf. **(B)** The Manhattan plot illustrates significant single nucleotide polymorphism (SNP) associations of Parkinson disease reported in the NHGRI-EBI GWAS catalog. The SNP associations that led to the discovery of these suggestive candidate genes are highlighted (green) in the Manhattan plot. Based on the GWAS catalog, these SNPs are located in the following chromosomal regions: 2q11.2 (Chang et al., 2017), 14q21.3 (Hu et al., 2016) and 17q21.31 (Hamza et al., 2010; Liu et al., 2011; Simon-Sanchez et al., 2009). **(C)** Human STRING PPIs for Map4k4, Mdga2 and Nsf (1 cluster per PPI network). Based on the STRING analyses (top 3 Gene Ontology (GO) terms within the biological process domain), the PPI networks for Map4k4, Mdga2 and Nsf were involved in (i) positive regulation of immune response (GO:0050778), regulation of response to stress (GO:0080134) and positive regulation of mitogen-activated protein (MAP) kinase activity (GO:0043406), (ii) cell adhesion (GO:0007155), neuron recognition (GO:0008038) and nervous system development (GO:0007399); and (iii) membrane fusion (GO:0061025), Golgi vesicle transport (GO:0048193) and membrane organization (GO:0061024), respectively. *Abbreviations: CD109, CD109 antigen; CNTN3, contactin-3; CNTN4, contactin-4; CNTN5, contactin-5; GOSR1, Golgi SNAP receptor complex member 1; GOSR2, Golgi SNAP receptor complex member 2; IL1R1, Interleukin-1 receptor type 1; IRAK1, interleukin-1 receptor-associated kinase 1; LSAMP, Limbic system-associated membrane protein; LY6H, Lymphocyte antigen 6H; MAP3K1, Mitogen-activated protein kinase kinase kinase 1; MAP3K7, Mitogen-activated protein kinase kinase kinase 7; MAP4K4, mitogen-activated protein kinase kinase kinase kinase 4; MDGA1, MAM domain-containing glycosylphosphatidylinositol anchor protein 1; MDGA2, MAM domain-containing glycosylphosphatidylinositol anchor protein 2; NAPA, alpha-soluble NSF attachment protein; NAPG, gamma-soluble NSF attachment protein; NCK1, cytoplasmic protein NCK1; NSF, vesicle-fusing ATPase; NTM, neurotrimin; OPCML, opioid-binding protein/cell adhesion molecule; SCFD1, Sec1 family domain-containing protein 1; SNAP25, synaptosomal-associated protein 25; STX5, syntaxin-5; STX18, syntaxin-18; TECTA, alpha-tectorin; TLR4, toll-like receptor 4; TNF, tumor necrosis factor; TRAF2, TNF receptor-associated factor 2; TRAF3, TNF receptor-associated factor 3; TRAF6, TNF receptor-associated factor 6; USO1, general vesicular transport factor p115; YKT6, synaptobrevin homolog YKT6.*

each identified one Panx1-interacting protein, respectively. A 2-cluster PPI network was formed using Rab3 GTPase-activating protein non-catalytic subunit (Rab3gap2) and Grin2d as input (Fig. 11C). The proteins within the PPI network were involved in multiple PANTHER pathways, namely the ionotropic glutamate receptor pathway (P00037), metabotropic glutamate receptor group I/III pathway (P00041/ P00039), and muscarinic acetylcholine receptor 1 and 3 signaling pathway (P00042).

Panx1-interacting proteins and neurodevelopmental disorders. Through the GWAS cross-analysis, we identified 38, 24, and 3 Panx1-interacting proteins with a potential link to schizophrenia, ASD, and epilepsy, respectively (Fig. 9B,C). The neurodevelopmental disorders investigated in the present study were not covered by the KEGG and PANTHER databases and therefore, no additional Panx1-interacting proteins were discovered for this group. Notably, in addition to the overlap identified by the GWAS cross-analysis, the full list of Panx1-interacting proteins associated with the neurodegenerative diseases revealed that three more proteins (Hspa1b, Hspa1l and Mapk3) overlapped with those identified for schizophrenia and ASD.

For the neurodevelopmental disorders, eight of the Panx1-interacting proteins were present at the postsynapse in the adult mouse brain, particularly those associated with schizophrenia and ASD (Table 3, Fig. 12A). These postsynaptic Panx1-interacting proteins were: actin related protein 1A (Actr1a), spliceosome RNA helicase Bat1 (Ddx39b), heat shock 70 kDa protein 1-like (Hspa1l), Hspe1, 40S ribosomal protein S18 (Rps18), hsc70-interacting protein (St13), traf2 and NCK-interacting protein kinase (Tnik), and valine–tRNA ligase (Vars). The highest protein abundances were mainly detected in the hypothalamus (Actr1a, Ddx39b and Hspe1) and striatum (Rps18, Tnik and Vars) (Table 2, Table 3, Fig. 12A). These postsynaptic Panx1-interacting proteins

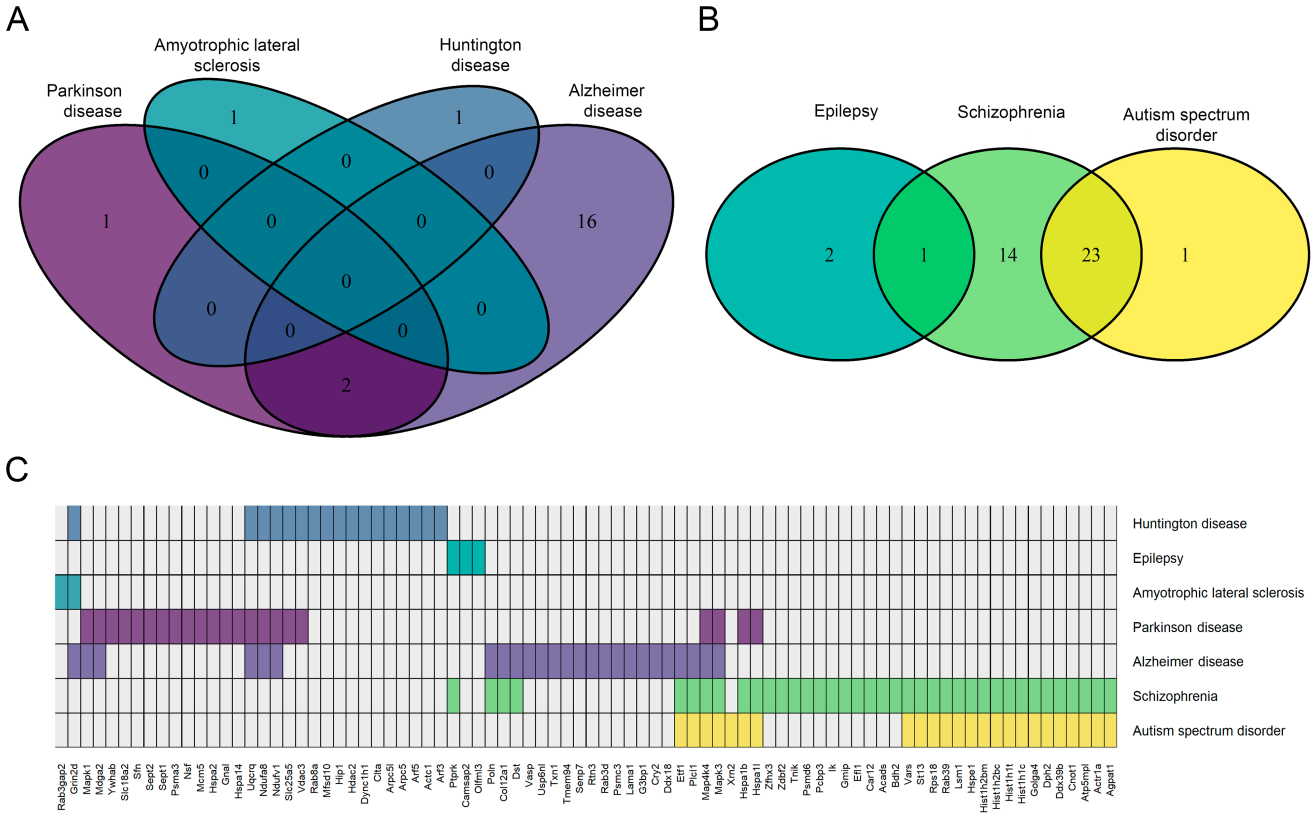

Fig. 9. Panx1-interacting proteins associated with neurodegenerative diseases and neurodevelopmental disorders in humans based on genome-wide association study (GWAS) findings and other cross-analyses. (A) Venn diagram displaying what Panx1-interacting proteins the neurodegenerative diseases, Parkinson disease, Alzheimer disease, Huntington disease and amyotrophic lateral sclerosis (ALS), have in common. The Panx1-interacting proteins, Mdga2 and Map4k4, were associated with both Parkinson disease and Alzheimer disease. **(B)** Venn diagram visualizing overlap between Panx1-interacting proteins associated with the neurodevelopmental disorders, schizophrenia, epilepsy and Autism Spectrum Disorder (ASD). The Panx1-interacting proteins, Actr1a, Agpat1, Atp5mpl, Cnot1, Ddx39b, Dph2, Etf1, Golga4, Hist1h1c, Hist1h1t, Hist1h2bc, Hist1h2bm, Hspa1b, Hspa1l, Hspe1, Lsm1, Mapk3, Map4k4, Plcl1, Rab39, Rps18, St13, Vars, were associated with both schizophrenia and ASD. Only one Panx1-interacting protein, namely Ptprk, was linked to both epilepsy and schizophrenia. **(C)** Overview of the Panx1-interacting proteins that we identified for Parkinson disease, Alzheimer disease, Huntington disease, ALS, schizophrenia, epilepsy and ASD across the analyses (colored boxes). Multiple Panx1-interacting proteins were associated with more than one neurodegenerative disease or neurodevelopmental disorder. *Abbreviations: Acads, short-chain specific acyl-CoA dehydrogenase, mitochondrial; Actc1, Actin, alpha cardiac muscle 1; Actr1a, alpha-centractin; Agpat1, 1-acyl-sn-glycerol-3-phosphate acyltransferase alpha; Arf3, ADP-ribosylation factor 3; Arf5, ADP-ribosylation factor 5; Arpc5, actin-related protein 2/3 complex subunit 5; Arpc5l, actin-related protein 2/3 complex subunit 5-like protein; Atp5mpl, ATP synthase subunit ATP5MPL, mitochondrial; Bdh2, 3-hydroxybutyrate dehydrogenase type 2; Camsap2, calmodulin-regulated spectrin-associated protein 2; Car12, carbonic anhydrase 12; Cltа, clathrin light chain A; Cnot1, CCR4-NOT transcription complex subunit 1; Col12a1, collagen alpha-1(XII) chain; Cry2, cryptochrome-2; Ddx18, ATP-dependent RNA helicase DDX18; Ddx39b, spliceosome RNA helicase DDX39B; Dph2, 2-(3-amino-3-carboxypropyl)histidine synthase subunit 2; Dst, dystonin; Dync1h1, cytoplasmic dynein 1 heavy chain 1; Eif1, elongation factor-like GTPase 1 (also referred to as Eftud1); Etf1, eukaryotic peptide chain release factor subunit 1; G3bp1, Ras GTPase-activating protein-binding protein 1; Gmip, GEM-interacting protein; Gnal, guanine nucleotide-binding protein G(olf) subunit alpha; Golga4, golgin subfamily A member 4; Grin2d, glutamate ionotropic receptor NMDA type subunit 2D; Hdac2, histone deacetylase 2; Hip1, huntingtin-interacting protein 1; Hist1h1c, histone H1.2; Hist1h1t, histone H1t; Hist1h2bc, histone H2B type 1-C/E/G; Hist1h2bm, histone H2B type 1-M; Hspa14, heat shock 70 kDa protein 14; Hspa1b, heat shock 70 kDa protein 1B; Hspa1l, heat shock 70 kDa protein 1-like; Hspa2, heat shock-related 70 kDa protein 2; Hspe1, 10 kDa heat shock protein, mitochondrial; Ik, protein red; Lama1, laminin subunit alpha-1; Lsm1, U6 snRNA-associated Sm-like protein LSm1; Map4k4, mitogen-activated protein kinase kinase kinase kinase 4; Mapk1, mitogen-activated protein kinase 1; Mapk3, Mitogen-activated protein kinase 3; Mcm5, DNA replication licensing factor MCM5; Mdga2, MAM domain-containing glycosylphosphatidylinositol anchor protein 2; Mfsd10, major facilitator superfamily domain-containing protein 10; Ndufa8, NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8; Ndufv1, NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial; Nsf, vesicle-fusing ATPase 1; Olfml3, olfactomedin-like protein 3; Pcbp3, poly(rC)-binding protein 3; Plcl1, inactive phospholipase C-like protein 1; Poln, DNA polymerase nu; Psma3, proteasome subunit alpha type-3; Psmc3, 26S proteasome regulatory subunit 6A; Psmd6, 26S proteasome non-ATPase regulatory subunit 6; Ptprk, receptor-type tyrosine-protein phosphatase kappa; Rab39, Ras-related protein Rab39; Rab3D, Ras-related protein Rab3D; Rab3gap2, Rab3 GTPase-activating protein non-catalytic subunit; Rab8A, Ras-related protein Rab8A; Rps18, 40S ribosomal protein S18; Rtn3, reticulon-3; Senp7, sentrin-specific protease 7; Sept1, septin-1; Sept2, septin-2; Sfn, 14-3-3 protein sigma; Slc18a2, Synaptic vesicular amine transporter; Slc25a5, ADP/ATP translocase 2; St13, Hsc70-interacting protein; Tmem94, transmembrane protein 94; Tnik, TRAF2 and NCK-interacting protein kinase; Txn1, thioredoxin; Uqcrq, cytochrome b-c1 complex subunit 8; Usp6nl, USP6 N-terminal-like protein; Vars, valine–tRNA ligase; Vasp, vasodilator-stimulated phosphoprotein; Vdac3, voltage-dependent anion-selective channel protein 3; Xrn2, 5'-3' exoribonuclease 2; Ywhab, 14-3-3 protein beta/alpha; Zdbf2, DBF4-type zinc finger-containing protein 2; Zfhx3, zinc finger homeobox protein 3.*

were mainly contained within cluster 1 in the PPI network (Table 2, Fig. 12B).

Biological functions of clusters within the postsynaptic PPI network in the context of the neurodegenerative diseases and neurodevelopmental disorders. The suggestive Panx1-interacting proteins involved in the neurodegenerative diseases and neurodevelopmental disorders are contained within cluster 1-4, 6-7 and 13-14 in the postsynaptic PPI network in the adult mouse brain, but primarily cluster 1, 4 and 6 (Table 2, Fig. 12B). Cluster 1 has been described previously with the most significant finding being overrepresentation of the GO term translation (GO:0006412) within the biological process domain. The postsynaptic Panx1-interacting proteins contained within cluster 4 and 6 were involved in a number of biological functions, including heat shock protein 90 (hsp90) chaperone cycle for steroid hormone receptors (SHR) (R-MMU-3371497, n = 3) and respiratory electron transport (R-MMU-611105, n = 3), respectively (Fig. 4).

Discussion

Here we used up-to-date protein classification paradigms (PANTHER, GO and KEGG analyses), a PPI networks database (Szklarczyk et al., 2019), and a newly characterized dataset describing enriched postsynaptic proteins across multiple regions in the adult mouse brain (Roy et al., 2018). Our findings complement previously known role(s) of Panx1 in the regulation of neuronal networks, synaptic plasticity, and pathological brain states, and support and/or reveal new potential avenues of research with regards to the role of Panx1 in CNS physiology and pathophysiology.

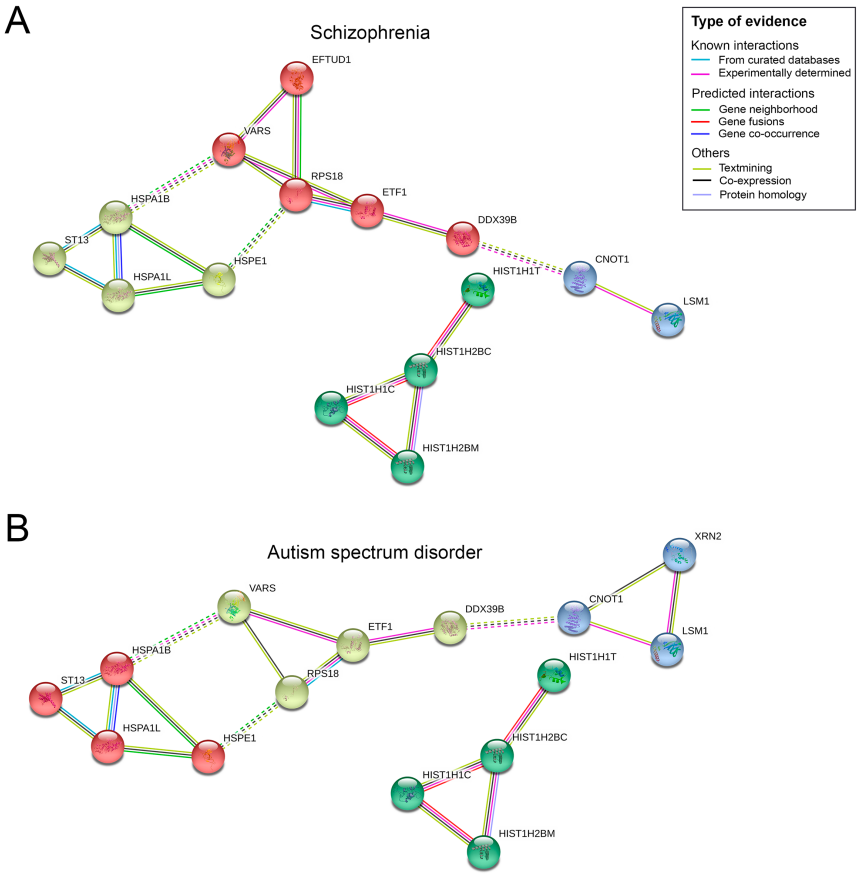

Fig. 10. Human STRING protein-protein interaction (PPI) networks for schizophrenia and Autism spectrum disorder (ASD) based on GWAS findings. (A) HHuman STRING PPI network for the 38 Panx1-interacting proteins identified to overlap with schizophrenia. A 4-cluster PPI network was formed by 15 Panx1-interacting proteins within this group. **(B)** Human STRING PPI network for the 24 Panx1-interacting proteins identified to overlap with ASD. PPIs were only observed for 15 Panx1-interacting proteins within this group which formed 4 clusters. *Abbreviations: CNOT1, CCR4-NOT transcription complex subunit 1; DDX39B, spliceosome RNA helicase DDX39B; ETF1, eukaryotic peptide chain release factor subunit 1; EFTUD1, elongation factor-like GTPase 1 (also referred to as Efl1); HIST1H1C, histone H1.2; HIST1H1T, histone H1t; HIST1H2BC, histone H2B type 1-C/E/G; HIST1H2BM, histone H2B type 1-M; HSPA1B, heat shock 70 kDa protein 1B, HSPA1L, heat shock 70 kDa protein 1-like; HSPE1, 10 kDa heat shock protein, mitochondrial; LSM1, U6 snRNA-associated Sm-like protein LSm1; RPS18, 40S ribosomal protein S18; ST13, Hsc70-interacting protein; VARS, valine–tRNA ligase; XRN2, 5'-3' exoribonuclease 2.*

Table 4. Panx1-interacting proteins potentially increasing the susceptibility to develop schizophrenia and autism spectrum disorder (ASD) and biological functions. Overrepresented PANTHER protein classes, Gene Ontology (GO) terms and PANTHER pathways for the Panx1-interacting proteins intersecting with the mapped and reported genome-wide association study (GWAS) candidate genes associated with schizophrenia and ASD. The fold enrichment score varies from 19.24 to >100, which is a measure of the actual number of Panx1-interacting proteins over the expected number of proteins within each category.

ID, Protein class/term/pathway	Gene/protein list	Fold enrichment (p-value)	
		Schizophrenia	ASD
PC00118, Histone	Hist1h1t, Hist1h2bm, Hist1h2bc, Hist1h1c	34.01 (1.46E-03)	53.86 (2.18E-04)
GO:0061077, Chaperone-mediated protein folding	Hspe1, Hspa1l, Hspa1b, St13	93.88 (2.38E-04)	>100 (3.47E-05)
GO:0022607, Cellular component assembly	Hist1h1t, Hist1h2bm, Hist1h2bc, Hist1h1c, (Dst*)	45.13 (1.76E-04)	57.17 (1.23E-03)
GO:0008301, DNA binding, bending	Hist1h1t, Hist1h2bm, Hist1h2bc, Hist1h1c	45.13 (1.12E-03)	71.46 (1.66E-04)
P00006, Apoptosis signaling pathway	Hspa1l, Hspa1b, Map4k4, Mapk3	19.24 (9.52E-03)	30.46 (1.46E-03)

Abbreviations: Dst, dystonin; Hist1h1t, histone H1t; Hist1h2bm, histone H2B type 1-M; Hist1h2bc, histone H2B type 1-C/E/G; Hist1h1c, histone H1.2; Hspa1b, heat shock 70 kDa protein 1B; Hspa1l, heat shock 70 kDa protein 1-like; Hspe1, 10 kDa heat shock protein, mitochondrial; Map4k4, mitogen-activated protein kinase kinase kinase kinase 4; Mapk3, mitogen-activated protein kinase 3; St13, Hsc70-interacting protein. *For Schizophrenia, Dst was also contained in the gene/protein list.

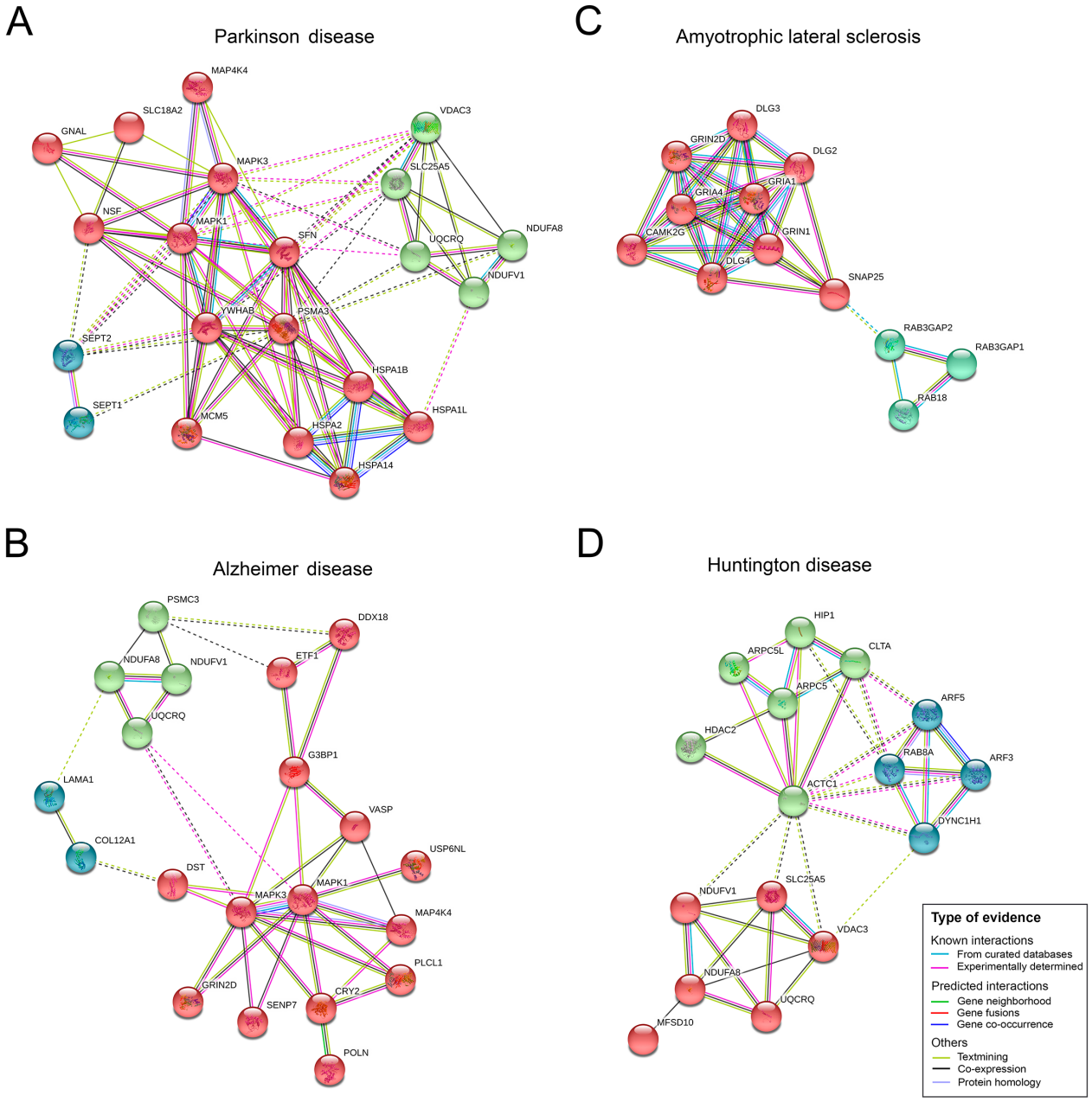

Fig. 11. Human STRING protein-protein interaction (PPI) networks for the neurodegenerative diseases, Parkinson disease, Alzheimer disease, amyotrophic lateral sclerosis (ALS) and Huntington disease based on all findings. (A) For Parkinson disease, a 3-cluster PPI network were formed by 21 Panx1-interacting proteins out of the 22 Panx1-interacting proteins that we identified across analyses (Fig. 9). To form the network, we had to lower the interaction score threshold ($\geq$ 0.150). Based on the STRING analysis, this PPI network was, in part, involved in protein refolding (GO:0042026), and regulation of establishment of protein localization (GO:0070201). **(B)** For Alzheimer disease, a 3-cluster PPI network was formed by 20 Panx1-interacting proteins out of the 25 Panx1-interacting proteins that we identified (Fig. 9). As for Parkinson disease, we had to lower the interaction score threshold ($\geq$ 0.150) to form this network. This PPI network was, in part, involved in regulation of Golgi organization and inheritance (GO:1903358, GO:0090170), caveolin-mediated endocytosis (GO:0072584) and small molecule binding (GO:0036094) based on the STRING analysis. **(C)** TThe rare disorder ALS affects approximately 1-8 per 100,000 people (Couratier et al., 2016). The expanded ALS 2-cluster PPI network was created based on the Panx1-interacting proteins, Rab3 GTPase-activating protein non-catalytic subunit (Rab3gap2) and glutamate ionotropic receptor NMDA type subunit 2D (Grin2d), that we identified for ALS (Fig. 9). The proteins in the PPI network were, in part, involved in the following PANTHER pathways: Ionotropic glutamate receptor pathway (P00037), metabotropic glutamate receptor group I/III pathway (P00041/ P00039) and muscarinic acetylcholine receptor 1 and 3 signaling pathway (P00042). **(D)** Huntington disease affects less than 1-6 per 100,000 people (Pringsheim et al., 2012), and can also be considered a rare disorder. The 3-cluster PPI network for Huntington disease was created using the full list of Huntington disease-associated Panx1-interacting proteins that we identified in this study (Fig. 9). The interaction score threshold was relaxed ($\geq$ 0.150) in order to form the network consisting of 16 Panx1-interacting proteins out of the 17 suggestive Panx1-interacting proteins we identified for Huntington disease. *Abbreviations: ACTC1, actin, alpha cardiac muscle 1; ARF3, ADP-ribosylation factor 3; ARF5, ADP-ribosylation factor 5; ARPC5, actin-related protein 2/3 complex subunit 5; ARPC5L, actin-related protein 2/3 complex subunit 5-like protein; CAMK2G, calcium/calmodulin-dependent protein kinase type II subunit gamma; CLTA, clathrin light chain A; COL12A1, collagen alpha-1(XII) chain; CRY2, cryptochrome-2; DDX18, ATP-dependent RNA helicase DDX18; DLG2, disks large homolog 2; DLG3, disks large homolog 3; DLG4, disks large homolog 4; DST, dystonin; DYNC1H1, cytoplasmic dynein 1 heavy chain 1; ETF1, eukaryotic peptide chain release factor subunit 1; G3BP1, Ras GTPase-activating protein-binding protein 1; GNAL, guanine nucleotide-binding protein G(olf) subunit alpha; GRIA1, glutamate receptor 1; GRIA4, Glutamate receptor 4; GRIN1, glutamate ionotropic receptor NMDA type subunit 1; GRIN2D, glutamate ionotropic receptor NMDA type subunit 2D; HDAC2, histone deacetylase 2; HIP1, huntingtin-interacting protein 1; HSPA1B, heat shock 70 kDa protein 1B; HSPA1L, heat shock 70 kDa protein 1-like; HSPA2, heat shock-related 70 kDa protein 2; HSPA14, heat shock 70 kDa protein 14; LAMA1, laminin subunit alpha-1; MAP4K4, mitogen-activated protein kinase kinase kinase kinase 4; MAPK1, mitogen-activated protein kinase 1; MAPK3, mitogen-activated protein kinase 3; MCM5, DNA replication licensing factor MCM5; MFSD10, major facilitator superfamily domain-containing protein 10; NDUFA8, NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8; NDUFV1, NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial; NSF, vesicle-fusing ATPase 1; PLCL1, inactive phospholipase C-like protein 1; POLN, DNA polymerase nu; PSMA3, proteasome subunit alpha type-3; PSMC3, 26S proteasome regulatory subunit 6A; RAB3GAP1, Rab3 GTPase-activating protein catalytic subunit; RAB3GAP2, Rab3 GTPase-activating protein non-catalytic subunit; RAB8A, Ras-related protein Rab8A; RAB18, Ras-related protein Rab18; SENP7, sentrin-specific protease 7; SEPT1, septin-1; SEPT2, septin-2; SFN, 14-3-3 protein sigma; SLC18A2, synaptic vesicular amine transporter; SLC25A5, ADP/ATP translocase 2; SNAP25, synaptosomal-associated protein 25; UQCRQ, cytochrome b-c1 complex subunit 8; USP6NL, USP6 N-terminal-like protein; VASP, vasodilator-stimulated phosphoprotein; VDAC3, voltage-dependent anion-selective channel protein 3; YWHAB, 14-3-3 protein beta/alpha.*

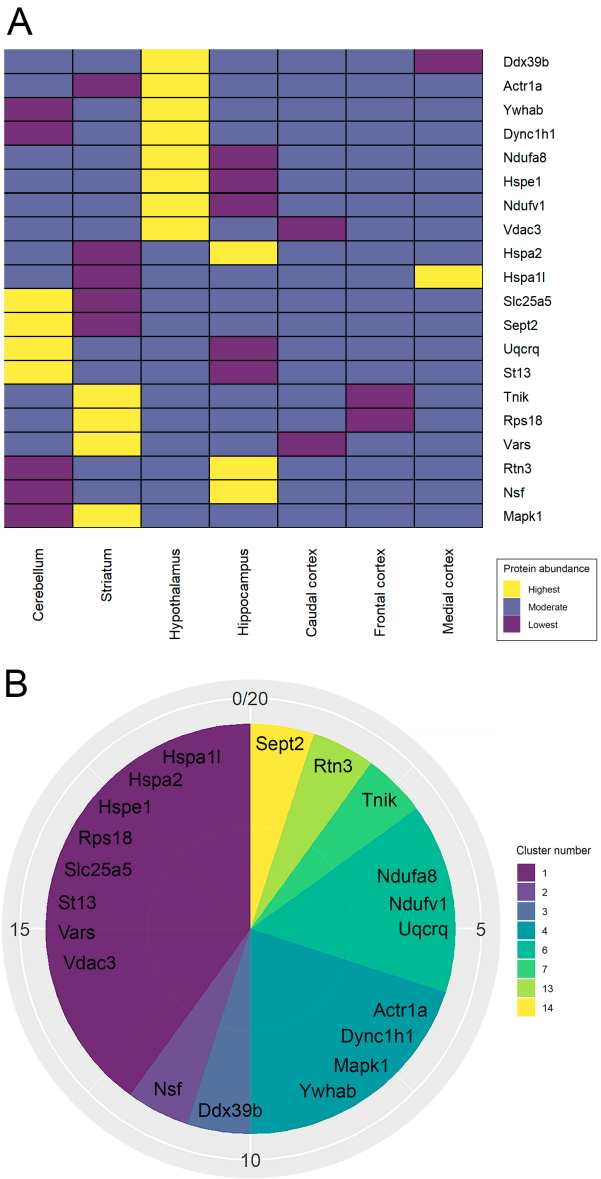

Fig. 12. Protein abundance across brain regions in adult mice from Roy et al. (2018) for the suggestive postsynaptic Panx1-interacting proteins associated with neurodegenerative diseases and neurodevelopmental disorders in humans and links to the postsynaptic mouse STRING protein-protein interaction (PPI) network. (A) Protein abundance of the disorder-associated Panx1-interacting proteins (Fig. 9) that were located at the postsynapse in the adult mouse brain. Highest, moderate and lowest protein abundance are represented as yellow, blue and purple colors, respectively. None of the disorder-associated Panx1-interacting proteins were highly abundant in the caudal and frontal cortex. Three or more disorder-associated Panx1-interacting proteins were highly abundant in the cerebellum, striatum, hypothalamus and hippocampus, and least abundant in the same brain regions, except hypothalamus. **(B)** These 20 postsynaptic Panx1-interacting proteins that potentially are associated with Parkinson disease (11 Panx1-interacting proteins), Alzheimer disease (5 Panx1-interacting proteins), Huntington disease (6 Panx1-interacting proteins), schizophrenia (8 Panx1-interacting proteins) and autism spectrum disorder (7 Panx1-interacting proteins) were contained within cluster 1-4, 6-7 and 13-14 in the postsynaptic mouse STRING PPI network. The top 3 clusters were cluster 1, 4 and 6 meaning those with the highest proportion of proteins related to disease. Suggestive biological functions of these clusters are presented in Fig. 4. Abbreviations: Actr1a, alpha-centractin; Ddx39b, spliceosome RNA helicase DDX39B; Dync1h1, cytoplasmic dynein 1 heavy chain 1; Hspa1l, heat shock 70 kDa protein 1-like; Hspa2, heat shock-related 70 kDa protein 2; Hspe1, 10 kDa heat shock protein, mitochondrial; Mapk1, mitogen-activated protein kinase 1; Ndufa8, NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8; Ndufv1, NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial; Nsf, vesicle-fusing ATPase 1; Rps18, 40S ribosomal protein S18; Rtn3, reticulon-3; Sept2, septin-2; Slc25a5, ADP/ATP translocase 2; St13, Hsc70-interacting protein; Tnik, TRAF2 and NCK-interacting protein kinase; Uqcrq, cytochrome b-c1 complex subunit 8; Vars, valine–tRNA ligase; Vdac3, voltage-dependent anion-selective channel protein 3; Ywhab, 14-3-3 protein beta/alpha.

Panx1 interactome in the postsynapse, PPIs and neurological diseases. Our initial goal in this study was to get in-depth insight into the putative cellular roles of Panx1-interacting proteins by performing basic classification analysis using PANTHER, and next to identify those proteins enriched at the postsynapse and their connections within the PPI network. The initial PANTHER pathway analysis of the full Panx1 interactome (Fig. 3) revealed an overrepresentation of Panx1-interacting proteins within the PANTHER pathway Parkinson disease. Because this disease first affects specific brain regions regulating motor function (Davie, 2008), we selected a proteomic study that accounted for differential enrichment of postsynaptic proteins within various brain regions (Roy et al., 2018). The unanticipated discovery of the connection of Panx1 with Parkinson disease, together with the role of Panx1 we recently discovered in dendritic spine development, which is aberrant in both neurodegenerative diseases and neurodevelopmental disorders (Forrest et al., 2018), led us to further downstream focus on neurological diseases (Fig. 9A-C). We highlight selected findings below.

Postsynaptic Panx1 interacting proteins in intracellular protein transport. The top GO term in the Panx1 interactome within the biological process domain was intracellular protein transport, which includes membrane trafficking proteins like the Rab GTPases (Ras-related protein in brain (Touchot et al., 1987); belong to the Ras family of small monomeric GTPases), as well as cytoskeleton-regulating proteins. Note that cytoskeleton-regulating proteins have been discussed previously elsewhere (Wicki-Stordeur, 2015; Wicki-Stordeur and Swayne, 2013; Xu et al., 2018). In fact, 20 different Rab proteins were identified as Panx1 interactors (Table S1) and several of these were also found in the mouse postsynaptic PPI network (Table 2, additional information about the hub proteins in the mouse postsynaptic PPI network can be found in Table 5, e.g. Rab6A within cluster 2). Rab GTPases are membrane-associated molecular switches (GTP-"on"/active, GDP-"off"/inactive), that define the identity of specific subcellular compartments where they are localized, and recruit various effector proteins to direct trafficking of membranes and protein cargo between these subcellular compartments (reviewed by Hutagalung and Novick (2011), Zerial and McBride (2001), Pylypenko et al. (2018) and Grosshans et al. (2006)). Of the 5 Panx1-interacting Rabs found in the mouse postsynaptic PPI network (Table 2), Rab11 is specifically known to regulate different aspects of AMPA receptor trafficking within the postsynaptic compartment (reviewed in (Mignogna and D'Adamo, 2018). Another critical membrane trafficking protein, Nsf was present in the cross-analyses for both postsynaptic proteins and Parkinson disease risk genes from the GWAS cross-analysis, and Alzheimer disease (Kundra et al., 2017). In addition to the canonical role it plays working with soluble Nsf attachment protein (SNAP) in the disassembly of protein complexes, Nsf also regulates the trafficking of AMPA receptors (Kiral et al., 2018; Osten et al., 1998; Zhao et al., 2007). We now require additional experimental validation to determine whether these interactions are direct, and whether Panx1 is a

client protein (i.e. a cargo protein or target of the trafficking protein) or a functional partner (i.e. effector that mediates or regulates the function of the trafficking protein).

Panx1 interacting proteins in neurodegenerative diseases: a putative chaperone/scaffold connection. Together, our analyses revealed links between the Panx1 interactome and Parkinson disease, Alzheimer disease, Huntington disease and ALS. Initially, several Panx1-interacting chaperone proteins were identified amongst the proteins annotated to the PANTHER pathway Parkinson disease as well as enriched in the postsynapse (Table 1, Table 2), including members of the heat shock protein 70 kDa (Hsp70) and 14-3-3 protein families. Molecular chaperones are highly conserved proteins that maintain protein homeostasis by regulating proper folding, oligomerization, and degradation of proteins. Via interactions with co-chaperones that regulate their localization and function, members of the Hsp70 (also known as Hspa) family of chaperones use the energy derived from ATP hydrolysis to maintain proper folding and/or turnover of synaptic proteins, and thereby, synaptic integrity (reviewed by Gorenberg and Chandra (2017)). As such, dysfunction of Hsp70 and other chaperone protein families has been implicated in several neurodegenerative diseases (for reviews see Kampinga and Bergink (2016), Ebrahimi-Fakhari et al. (2011), Bobori et al. (2017); Roodveldt et al. (2017) and Lackie et al. (2017)). Members of the Hsp70 family identified in the PANTHER pathway Parkinson disease were also identified in our cross-analysis with susceptibility genes associated with schizophrenia and ASD (Fig. 9). Although not detected in the Parkinson disease PANTHER analysis, two Hsp70 co-chaperones (DnaJ/Hsp40 family members) Dnaja1 and Dnaja4 were identified as putative Panx1-interacting proteins. Like their co-chaperones (Gorenberg and Chandra, 2017), DnaJ/Hsp40 family members have also been implicated in Parkinson disease (Hasegawa et al., 2018). Additionally, genetic variants of Dnaja1 have been linked to intellectual disability and seizures (Alsahli et al., 2019), and Dnaja1 also reportedly opposes Hsp70-mediated stabilization of tau, a key microtubule-binding protein involved in both Parkinson and Alzheimer disease pathogenesis (Abisambra et al., 2012). From the list of Panx1-interacting proteins, Hspa2 was also present in postsynaptic proteome of the adult mouse brain and found most abundant in the hippocampus (Fig. 12A, note that chaperone expression decreases with increasing age (Gorenberg and Chandra, 2017)). Overall these findings underscore the importance of chaperones in neuronal and synaptic proteostasis and highlight a potential implication of Panx1 in their regulation.

Additionally, we found members of the 14-3-3 scaffold/chaperone protein family in the PANTHER pathway Parkinson disease and postsynapse (Table 1, Table 2). This class of proteins are known to regulate tau (Papanikolopoulou et al., 2018), and have been implicated in a number of neurological diseases (reviewed by Kaplan et al. (2017)). Notably, one of the 14-3-3 proteins was also identified through the enriched CCKR signaling map PANTHER pathway (Table 1, Fig. 3A,C). The involvement of CCKRs and related signaling pathways have been described in schizophrenia, anxiety/mood disorders, and addiction (reviewed by Lee and Soltesz (2011); Ballaz (2017)); as well as in neurodegenerative diseases (in this study, specifically Alzheimer disease).

Further exploration and validation of the interaction between Panx1 and chaperone/scaffold proteins is now warranted. Similar to trafficking proteins, it will be important to determine if these interactions are direct, and whether Panx1 is a client protein or a functional partner with respect to the interactor.

Parkinson disease. Panx1 has only very recently been implicated in Parkinson disease in an *in vitro* model system (Diaz et al., 2019). The pathophysiologies of sporadic and genetic forms of Parkinson disease are thought to involve a combination of environmental factors and genetic susceptibility (for review see (Kalia and Lang, 2015)). Dysfunctional protein aggregation and oxidative stress are two primary proposed mechanisms (Betarbet et al., 2006; Moore et al., 2005). Some of the chaperone proteins involved in mitigating protein aggregation that have been implicated in Parkinson disease and identified by PANTHER analysis are described above. KEGG Parkinson disease analysis identified 5 Panx1-interacting proteins that associate with the mitochondrial membrane (Fig. 5, Fig. 6, Table 5): Ndufa8 and Ndufv1, subunits of complex I of the electron transport chain; Uqcrq, a subunit of complex III of the electron transport chain; and Slc25a5 and Vdac3, subunits of the mitochondrial permeability transition pore (mPTP) complex. An increase in mitochondrial permeability through the mPTPs can induce cell death (Kroemer et al., 2007); but how Panx1, not known to be associated with mitochondria, is able to interact with these mitochondrial proteins, has yet to be determined. Additional Panx1-interacting proteins of note identified in the Parkinson disease KEGG pathway were the synaptic vesicular amine transporter (Slc8a2/Vmat2) and the guanine nucleotide-binding protein G(olf) subunit alpha (Gnal). VMAT/Vmat2 is the H^+-ATPase antiporter which packages dopamine (and other monoamines) into synaptic vesicles and also sequesters potentially harmful excessive cytosolic dopamine in dopaminergic neurons of the substantia nigra (German et al., 2015; Lohr and Miller, 2014). On the other hand, Gnal differentially mediates cAMP signalling in adenosine-2 receptor (A2AR)-enriched nigrostriatal neurons and dopamine 1/2 receptor (D1/2R)-enriched neurons (Fuxe et al., 2007; Goto, 2017; Morigaki et al., 2017). Taken together, Panx1-interacting proteins identified in Parkinson disease through the KEGG categorization revealed multiple molecular players already implicated in key putative mechanisms of disease.

Alzheimer disease. Panx1 has previously been implicated in Alzheimer disease-associated neuronal death in *in vitro* models (Giaume et al., 2019; Orellana et al., 2011a; Orellana

Table 5. Panx1-interacting proteins within the postsynaptic protein-protein interaction (PPI) network in the adult mouse brain in health and disease based on existing literature. This table covers the Panx1-interacting proteins with the most edges (i.e., associations to other Panx1-interacting proteins) within each cluster, and clusters formed by at least 4 Panx1-interacting proteins. This resulted in a total of 12 Panx1-interacting proteins of which several are associated with the development of inherited disorders and diseases such as amyotrophic lateral sclerosis (ALS) and spinocerebellar ataxia (Online Mendelian Inheritance in Man (MIM) database) (Amberger and Hamosh, 2017), and are implicated in mitochondrial dynamics (reviewed by Nsiah-Sefaa and McKenzie (2016)).

Cluster	Protein*	Function and disease	Reference
1	Eef2	- Registered MIM phenotype associated with Eef2: Spinocerebellar ataxia 26 (609306, autosomal dominant inheritance). - Potentially involved in Alzheimer disease-related synaptic dysfunction through Eef2 kinase activation and AMP-activated protein kinase hyperactivation.	(Ma et al., 2014)
2	Rab6A	- The expression of the trafficking Ras-related protein Rab6A was upregulated in Alzheimer disease brains, especially the temporal cortex. - The RAB6A gene was identified as a hub gene of the protein homeostasis system associated with the Alzheimer disease metastable subproteome.	(Kundra et al., 2017; Scheper et al., 2007)
3	Ddx39b	- In addition to the potential involvement of Ddx39b in schizophrenia and Autism spectrum disorder (ASD), a genetic variant in the 5' UTR of Ddx39b has been associated with an increased risk of multiple sclerosis. - Polymorphisms in the promoter of DDX39B alter the risk of developing Alzheimer disease	(Galarza-Munoz et al., 2017; Gnjec et al., 2008)
4	Mapk1	- Mapk1 variants have been linked to bipolar disorder and the status of major depressive disorder. - In striatal cells derived from a Huntington disease knock-in mice, activation of Mapk1 seems to affect mitochondrial function and fission through dynamin-related protein 1. - Mitochondrial dynamics are important during neurodevelopment, but also adult neurogenesis. It is believed that mitochondrial mutation and damage affect cognitive processing in neurodevelopmental and neurodegenerative diseases.	(Calabro et al., 2019; Calabro et al., 2016; Khacho et al., 2019; Roe and Qi, 2018)
5	Fh1	- Registered MIM phenotype associated with Fh1: Fumarase deficiency (606812, autosomal recessive inheritance). - A central feature of Parkinson disease is mitochondrial dysfunction. The expression of VEGF-B in rat midbrain cultures, with its neuroprotective properties, correlated with Fh1 and other nuclear-encoded mitochondrial genes such as Etfb, Ndufv and Hadhb. In coherence, cluster 5, 6 and 10 are closely connected in the postsynaptic protein-protein interaction network (Table 2).	(Yue et al., 2014)
6	Ndufv1	- Registered MIM phenotype associated with Ndufv1: Mitochondrial complex I deficiency, nuclear type 4 (618225, autosomal recessive inheritance). - Ndufv1 (mitochondrial complex I subunit) was expressed at mRNA and protein level in the striatum, cerebellum, prefrontal cortex and parieto-occipital cortex from subjects with schizophrenia, bipolar depression and major depression postmortem (varying expressional patterns).	(Ben-Shachar and Karry, 2008)
7	Actr3	- Fine mapping of single nucleotide polymorphisms (SNPs) in a Latino cohort led to the discovery of ACTR3 as a suggestive candidate gene for bipolar disorder.	(Gonzalez et al., 2019)
7	Gsn	- Registered MIM phenotype associated with Gsn: Amyloidosis, Finnish type (105120, autosomal dominant inheritance). In chicken embryos, Gsn (actin-binding protein with anti-apoptotic properties) was highly expressed in structures such as brain vesicles, midbrain and neural tube. Later in development, a high Gsn expression was observed in cranial and dorsal root ganglia. - The expression of Gsn was downregulated in the postmortem dorsolateral prefrontal cortex from people with chronic schizophrenia as compared with controls.	(Hakak et al., 2001; Mazur et al., 2016)
8	Ubqln2	- Registered MIM phenotype associated with Ubqln2: ALS 15, with or without frontotemporal dementia (300857, X-linked dominant inheritance). - Ubqln2 (ubiquilins interact with chaperones) has been linked to progressive degeneration of motor neurons, aberrant accumulation of proteins and skeletal muscle denervation atrophy, features of ALS. - Another study indicated that neuronal cell death in Ubqln2 transgenic and knockout rats primarily was due to gain of functions.	(Chen et al., 2018; Wu et al., 2015)

Table 5. Continued.

Cluster	Protein*	Function and disease	Reference
9	Phgdh	- Registered MIM phenotype associated with Phgdh: Phosphoglycerate dehydrogenase deficiency (601815, autosomal recessive inheritance). - Phgdh (enzyme of the novo L-Ser synthetic pathway) is involved in maintenance of nucleotide metabolism and carbon metabolism homeostasis. In addition, L-Ser deficiency has been linked to severe malformation of CNS structures.	(Hamano et al., 2016; Reid et al., 2018)
10	Hadhb	- Registered MIM phenotype associated with Hadhb: Trifunctional protein deficiency (609015, autosomal recessive inheritance). - Mitochondrial trifunctional protein deficiency has been described by Diebold et al. (2019) as "a rare autosomal recessive metabolic disorder characterized by phenotypic heterogeneity ranging from severe, early-onset, cardiac disease to milder, later-onset, myopathy and neuropathy". - Hadhb (mitochondrial protein) affects beta-oxidation of fatty acids in mitochondria, crucial for maintaining the energy homeostasis.	(Diebold et al., 2019; Zhou et al., 2012)
10	Oxct1	- Registered MIM phenotype associated with Oxct1: Succinyl CoA:3-oxoacid CoA transferase deficiency (245050). - The expression of Oxct1 (affects response to oxidative stress) was downregulated in peripheral leukocytes from pre-symptomatic Huntington disease carriers and Huntington disease patients.	(Chang et al., 2012)

**Abbreviations:* Actr3, actin-related protein 3; Ddx39b, spliceosome RNA helicase DDX39B; Eef2, elongation factor 2; Fh1, fumarate hydratase, mitochondrial; Gsn, gelsolin; Hadhb, trifunctional enzyme subunit beta, mitochondrial; Mapk1, mitogen-activated protein kinase 1; Ndufv1, NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial; Oxct1, succinyl-CoA:3-ketoacid coenzyme A transferase 1, mitochondrial; Phgdh, D-3-phosphoglycerate dehydrogenase; Rab6A, Ras-related protein Rab6A; Ubqln2, ubiquilin-2.

et al., 2011b). The Panx1-interacting proteins identified through the KEGG categorization, reticulon-3 (Rtn3) and Mapk1/3 (ERK1/2 signaling pathway), have been implicated in Alzheimer disease pathology (Fig. 5, Fig. 7). Rtn3, a protein regulating early secretory pathway membrane trafficking (Wakana et al., 2005) interacts with and negatively regulates beta-secretase 1 (BACE1) (He et al., 2004; Murayama et al., 2006). Rtn3 deletion leads to increased BACE1 activity facilitating beta-amyloid deposition in mouse models of Alzheimer disease, while overexpression leads to reduced beta-amyloid deposition at the expense of preformed dystrophic neurites and decreased cognitive function (Hu et al., 2007; Shi et al., 2009). Mapk3 is a kinase implicated in the phosphorylation of the microtubule-associated protein tau as well as beta-amyloid processing (Kirouac et al., 2017; Zhao et al., 2002), and is up-regulated Alzheimer disease patient brains (Chen and Mobley, 2019; Pei et al., 2002). Although Noel et al. (2015) reported that that Mapk3/ERK1 is not involved in tau phosphorylation, another report targeting Mapk3/ERK1 prevented tau hyperphosphorylation and aggregation (Siano et al., 2019). Moreover, Indrigo et al. (2018) reported that modulation of Mapk3/ERK1 promoted neuroprotective effects in mouse models of Alzheimer disease, Parkinson disease, and Huntington disease, and facilitated hippocampal synaptic plasticity.

The pathophysiological changes (e.g. synaptic loss, hyperphosphorylated tau) observed in people with Alzheimer disease have been linked to activation of extrasynaptic NMDA receptors. Activation of extrasynaptic NMDA receptors induces cell death whereas synaptic NMDA receptors are known to possess neuroprotective properties (reviewed by Hardingham and Bading (2010)). In this context, another Panx1-interactor in the Alzheimer disease KEGG pathway was Grin2d (also known as GluN2D; Fig. 5, Fig. 7)), one of the seven identified subunits of NMDA receptors: GluN1, GluN2A, GluN2B, GluN2C, GluN2D, GluN3A, and GluN3B (reviewed by Paoletti (2011)). More specifically, synaptic dysfunction in Alzheimer disease is thought to be secondary to altered NMDA receptor function, leading to aberrant handling of intracellular Ca^{2+}, excitotoxicity, loss of synapses, and ultimately, neuronal death (reviewed by Mota et al. (2014)). Moreover, modulation of NMDA receptor function with the low-affinity NMDA blocker memantine is one of the main therapeutic options for moderate to severe Alzheimer disease (reviewed by Danysz and Parsons (2003); Wang and Reddy (2017)). Although the specific relationship between Panx1 and GluN2D remains to be explored, work by others have implicated Panx1 in synaptic dysfunction during anoxia/ischemia and excitotoxicity through a mechanism involving a noncanonical metabotropic NMDA signalling pathway coupling Src kinases to Panx1 pore opening (Weilinger et al., 2016; Weilinger et al., 2012).

As with Parkinson disease, multiple Panx1-interacting proteins involved in mitochondrial function were identified in our Alzheimer disease KEGG categorization, namely Ndufa8 and Ndufv1 (subunits of complex I of the electron transport chain) and Uqcrq (subunit of complex III of the electron transport chain). Altered mitochondria function has been noted as one of the pathophysiological mechanisms underlying Alzheimer disease (Moreira et al., 2010). For example, Lunnon et al. (2017) reported significant differences in gene expression of Ndufa8 and Uqcrq in blood samples from patients with Alzheimer disease and mild cognitive impairments. Another study applied quantitative proteomics to brain samples from Alzheimer disease patients and found decreased levels of multiple proteins involved in mitochondrial

metabolism, mainly those of complex I and III of electron transport chain (Adav et al., 2019). However, as noted above how Panx1, not known to be present in mitochondria, is able to interact with these proteins, has yet to be determined.

Study limitations. To determine overlap between the Panx1 interactome and genetic risk factors for neurodevelopmental disorders and neurodegenerative diseases, we used selected GWAS. GWAS are based on SNP-phenotype associations so the link to the candidate genes is inferred and not necessarily direct. Moreover, GWAS are limited by the use of restricted populations (predominantly European), additive genetic models, and easy-to-measure phenotypes (Tam et al., 2019). Crmp2 (also referred to as Dpysl2), a microtubule-regulating protein present in our Panx1 interactome and experimentally validated as a direct interaction that also occurs in brain tissue by our group (Wicki-Stordeur, 2015; Xu et al., 2018) has been independently linked to schizophrenia (Braunschweig et al., 2013) and Alzheimer disease (Cole et al., 2007) through post-translational mechanisms, but was not identified in our genetic risk cross-analysis with GWAS as being associated with these neuropsychiatric disorders. This highlights the need for additional studies implementing gene-to-gene and gene-to-environment analyses, more diverse populations, wider range of phenotypes (deep phenotypes and composite traits), and application of alternative inheritance models in this kind of studies. Similarly, other Panx1-interacting proteins associated with disease might similarly be implicated in post translational disease mechanisms that would not be detected by genetic risk cross-analysis. It is also not surprising that some postsynaptic proteins were not identified by Roy et al. (2018) given that LC-MS/MS is abundance biased and thus proteins expressed at relatively lower levels are less likely to be detected. Additionally, it is possible that proteins enriched at postsynaptic compartments of inhibitory interneurons, like Mdga2, may not be as likely to be enriched as those present primarily at excitatory postsynaptic compartments with the methods used. Future studies comparing developmental synaptic proteomes using proximity proteomics will provide additional insights into the role of Panx1 in dendritic spines and nascent synapses.

In terms of PPI databases, some limitations of traditional PPI networks have been previously described by Halakou et al. (2017) and Szklarczyk et al. (2017), for example splice isoforms, conformational changes, surface area and binding sites of the proteins were not considered in this analysis. We found that several known postsynaptic proteins that we identified as Panx1-interacting proteins were not identified by the cross-analysis with the adult mouse brain postsynaptic proteome, such as calcium/calmodulin-dependent protein kinase type II subunit beta (Camk2b) (Hell, 2014), Arp2/3 components (Kim et al., 2013; Spence et al., 2016), alpha-actinin-1 (Matt et al., 2018), neurofibromin (NF1) (Hsueh et al., 2001; Oliveira and Yasuda, 2014), and Mdga2 (Pettem et al., 2013). Moreover, some notable interactions between these proteins and other proteins of interest in the lab were missed by our bioinformatics approach. For example, NF1, a NMDA receptor-interacting protein (Husi et al., 2000) and Ras GTPase that regulates dendritic spines (Oliveira and Yasuda, 2014), interacts with and regulates the microtubule-regulating protein Crmp2 (Moutal et al., 2018), another Panx1-interacting identified by us, that itself regulates dendritic spines and NMDA receptor trafficking (Brustovetsky et al., 2014). Crmp2 also interacts with Crmp1, which was identified as a postsynaptic protein. Using less stringent settings within the STRING database, all of these connections were revealed. Taking these limitations into account, it is reasonable to speculate that our results presented here highlighting important connections between Panx1-interacting proteins (Table 2) actually underestimate the complexity of already established links in the Panx1 interactome. Ultimately, experimental validation is now required to determine whether these interactions are important in the pathways and neurological diseases identified by our bioinformatic analyses.

Conclusions

Overall, this work highlights important new insights on the role of Panx1 in the CNS, both in health and disease. Our expanded protein classification revealed putative new aspects of Panx1 function in cells. Our cross-analyses revealed intriguing overlap between Panx1-interacting proteins, postsynaptic proteins, and suggestive GWAS candidate genes associated with neurodegenerative diseases (Parkinson disease, Alzheimer disease, Huntington disease and ALS) and neurodevelopmental disorders (schizophrenia, epilepsy and ASD) that now warrant further validation and mechanistic investigation.

ACKNOWLEDGEMENTS

This project was supported by operating grants from the Canadian Institutes of Health Research (MOP142215), the Natural Sciences and Engineering Research Council of Canada (NSERC; 402270-2011), and the University of Victoria Division of Medical Sciences to LAS. LAS was also supported by a Michael Smith Foundation for Health Research and British Columbia Schizophrenia Society Foundation Scholar Award (5900). LEWS was supported by a Vanier Canada Graduate Scholarship (NSERC). LAS is also grateful for infrastructure support from the Canada Foundation for Innovation (29462) and the BC Knowledge Development Fund (804754) for the Leica SP8 confocal microscope system.

Author contributions: LEWS identified the Panx1 interactome in mouse N2a cells, SDF performed the bioinformatics analyses, and SDF, JCSA and LAS wrote the first draft of the manuscript. All authors contributed to the writing process and approved the final manuscript.

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Supplementary Note 1:

Fig. S1: Comprehensive overview of the workflow within the current study from research questions, material and methods to results..

Fig. S2: Mouse STRING protein-protein interaction (PPI) network of 99 Panx1-interacting proteins located at the postsynapse. Eight out of the 16 clusters within the PPI network were composed of at least 5 Panx1-interacting proteins..

Table S1: Fifty-four Panx1-interacting proteins within the overrepresented intercellular protein transport (GO: 0006886) GO term.

Table S2: Thirty-five Panx1-interacting proteins within the overrepresented vesicle-mediated transport (GO:0016192) GO term.

Table S3: Thirty-seven Panx1-interacting proteins within the overrepresented GTPase activity (GO:0003924) GO term.