Teaser A timeline of high-throughput omics data for neurodegeneration research shows that systems biology approaches have promising ability for the study of cellular and metabolic changes in Alzheimers and Parkinsons diseases, challenging the way researchers should define these diseases.

A systems biology approach for studying neurodegenerative diseases

Simon Lam[1], Abdulahad Bayraktar[1], Cheng Zhang[2], Hasan Turkez[3], Jens Nielsen[4], Jan Boren[5], Saeed Shoaie[1,2], Mathias Uhlen[2] and Adil Mardinoglu[1,2]

[1] Centre for Host-Microbiome Interactions, Faculty of Dentistry, Oral & Craniofacial Sciences, King's College London, London, SE1 9RT, UK
[2] Science for Life Laboratory, KTH – Royal Institute of Technology, Stockholm, SE-17121, Sweden
[3] Department of Medical Biology, Faculty of Medicine, Atatürk University, Erzurum, 25240, Turkey
[4] Department of Biology and Biological Engineering, Chalmers University of Technology, SE-41296, Gothenburg, Sweden
[5] Department of Molecular and Clinical Medicine, University of Gothenburg, The Wallenberg Laboratory, Sahlgrenska University Hospital, Gothenburg, SE-413 45, Sweden

Neurodegenerative diseases (NDDs), such as Alzheimer's (AD) and Parkinson's (PD), are among the leading causes of lost years of healthy life and exert a great strain on public healthcare systems. Despite being first described more than a century ago, no effective cure exists for AD or PD. Although extensively characterised at the molecular level, traditional neurodegeneration research remains marred by narrow-sense approaches surrounding amyloid β (Aβ), tau, and α-synuclein (α-syn). A systems biology approach enables the integration of multi-omics data and informs discovery of biomarkers, drug targets, and treatment strategies. Here, we present a comprehensive timeline of high-throughput data collection, and associated biotechnological advancements and computational analysis related to AD and PD. We hereby propose that a philosophical change in the definitions of AD and PD is now needed.

Introduction

NDDs, including AD and PD, are the leading cause of **disability-adjusted life years** (DALYs, see Glossary), or lost years of healthy life, accounting for 250.7 million DALYs, or 10.2% of all DALYs, worldwide in 2015. They are also the second-leading cause group of death, with 9.4 million, or 16.8% of global deaths in the same year [1]. Global population growth and increased life expectancy mean that ageing-related brain disorders pose an ever-increasing threat in developed countries, placing an unprecedented and growing strain on public healthcare structures. Although the epidemiology of AD and PD are well characterised at the molecular level,

Corresponding author: Mardinoglu, A. (adilm@scilifelab.se)



Adil Mardinoglu is an expert in the field of systems medicine, systems biology, computational biology, and bioinformatics. He was recruited as a professor of systems biology at the Centre for Host-Microbiome Interactions, King's College London, UK, where he leads a computational group. He also works as group leader at the Science for Life Laboratory (SciLifeLab) at KTH-Royal Institute of Technology in Sweden and leads a team of 25 researchers working in the area of computational biology, experimental biology, and drug development to develop new treatment strategies for metabolic diseases, neurodegenerative diseases, and certain type of cancers.



Simon Lam is a PhD student in the Mardinoglu lab at King's College London. He is interested in the multi-omics modelling of neurodegenerative diseases, with a particular focus on Parkinson's disease. He is constructing a high-resolution expression map of the human brain by generating brain region-specific and context-specific genome-scale metabolic models (GEMs) and analysing proteomics and single-cell transcriptomics data.



Abdulahad Bayraktar is also a PhD student in the Mardinoglu lab at King's College London. He is applying machine learning techniques and GEMs analysis to analyse multi-omics data sets to study neurodegenerative diseases, with an emphasis on Alzheimer's disease.

1359-6446/© 2020 Elsevier Ltd. All rights reserved.
https://doi.org/10.1016/j.drudis.2020.05.010

GLOSSARY

cDNA microarray array of DNA probes fixed to a solid surface; used for the measurement of the expression of large numbers of genes simultaneously.
Cerebrospinal fluid (CSF) body fluid in the brain and spinal cord, obtained by lumbar puncture (also known as spinal tap).
Deep brain stimulation (DBS) second-line therapy involving applying electrical pulses to stimulate nerve cells using electrodes surgically implanted deep in the brain.
Disability-adjusted life year (DALY) measure of disease burden; equal to number of years of healthy life lost because of ill health, disability, or early death.
Genome-scaleme tabolic model (GEM) computational model containing the full set of cellular reactions, substrates, products, stoichiometries, and directionalities, describing gene–protein–reaction relationships for entire metabolic pathways.
Immunohistochemistry selective staining of cells in a tissue section by exploiting the specificity of antibodies binding to proteins.
***In situ* hybridisation** molecular biology technique to study the localisation of RNA using complementary nucleic acids.
Induced pluripotent stem cell (iPCS) embryonic-like pluripotent stem cell generated from adult cells.
Mass spectrometry (MS) technique used to measure the mass of molecules.
Mini-mental state exam test of cognitive function.
Pseudotime analysis mapping of high-dimensional molecular data onto a series of single-dimensional latent trajectories.
RNA sequencing (RNA-seq) technique involving NGS to measure the quantity of RNA in a given sample.
Single-cell RNA sequencing (scRNA-seq) RNA sequencing applied to one cell.
Substantia nigra (SN) midbrain structure implicated in reward and movement function.
Transcranial magnetic stimulation (TMS) second-line non-invasive therapy involving applying magnetic fields to stimulate nerve cells in the brain.

current therapies targeting single genes and thinking have been met with limited clinical success [2,3]. In particular, the wholesale classification of NDD cases into labels such as AD and PD is not helpful and neglects the heterogeneous nature of these diseases. We attribute this problem to the relative lack of understanding of NDDs on a systems biology scale. Therefore, we propose that whole- and multi-omics strategies are the most attractive for the development of effective personalised therapy of NDDs. We highlight that these strategies are highly accessible given the recent advances in biotechnology and should be used in earnest.

In this review, we first describe current treatment strategies and their rationale given known AD and PD molecular pathology. We appraise the fundamental limitations of the current philosophy regarding AD and PD and offer an alternative paradigm for NDD research. Second, we present a comprehensive timeline of biotechnological and quality control advancements that have improved researchers' ability to study whole transcriptomes, proteomes, and metabolomes, and point to relevant successes that NDD research has been improved by as a result. Finally, we review the current state of integrated multi-omics systems biology work for NDDs that has enabled a reinterpretation of the way in which we define NDDs.

Current philosophy of Alzheimer's and Parkinson's diseases

AD and PD were first described in 1906 and 1817, respectively, and the underlying molecular mechanisms of these diseases are well characterised. AD is characterised by extracellular Aβ plaques and intracellular tau protein neurofibrillary tangles, whereas PD is characterised by α-syn inclusions in dopaminergic neurons of the **substantia nigra** (SN). As endogenous proteins, simple inhibition of any of these proteins is largely ineffective [4–6]. More recently, the 'central roles' of Aβ, tau, and α-syn have become the subject of controversy [7,8], leading to the beginning of a shift of focus away from just these species.

Rather than targeting single proteins specifically, front-line treatments aim to mitigate damage caused by neurodegeneration and ease symptoms. For patients with dementia, this involves cholinesterase inhibitors (ChEIs), such as donepezil, rivastigmine, and galantamine. The rationale for using ChEIs is to mitigate the decreasing acetylcholine levels due to loss of cholinergic neurons by neurodegeneration, and they have shown value in the short-term and sustained cognitive benefits [9]; however, they do not reverse any damage caused by neurodegeneration, and patients' individual responses to these drugs are highly variable [10].

For patients with PD, levodopa has been used clinically for over 50 years and remains the gold standard in PD pharmacotherapy [11]. Levodopa metabolises into dopamine in the brain, thus replenishing brain dopamine supplies, which are depleted through the loss of dopaminergic neurons. However, owing to the short plasma elimination half-life and poor oral bioavailability of levodopa, currently the best strategies involve continuous drug delivery via infusion [12]. In addition, similarly to AD therapies, it is ineffective in reversing damage caused by neurodegeneration and patients display variable levels in their response to it [13].

Alternatively, **transcranial magnetic stimulation** (TMS) has been used as a non-invasive nonpharmaceutical intervention for AD and PD, with positive effects reported [14,15]; however, controversy remains with regards to the safety of some TMS modes [16]. For the most severe PD cases, invasive **deep brain stimulatio**n (DBS) therapy is an option. DBS affords therapeutic benefits and mitigates levodopa-associated motor complications [17], but the risk of serious adverse events [17] prevents it from being a viable option for most patients with PD. Furthermore, DBS has been trialled for the treatment of AD, and has been shown to have positive effects on AD neurodegeneration-related brain atrophy [18].

Thus, the current therapeutic options for the treatment of AD and PD are limited to simply mitigating neurodegeneration and relieving symptoms rather than reversing damage done. The range of therapeutics available also makes it clear that AD and PD are treated as distinct diseases, but it is promising to see the TMS and DBS nonpharmaceutical techniques being applied widely to NDDs, despite their relative lack of understanding. Given the known clinical heterogeneity in patients' responses to frontline drugs for AD [10] and PD [13], we can hypothesise that such heterogeneity also exists at the genomic, transcriptomic,

TABLE 1

Summary of interrupted clinical trials for AD and PD[a]

Reason for interruption	Number of trials interrupted	
	AD	PD
Administrative reasons	37	50
No reason given	55	24
Poor recruitment	26	40
Endpoint unmet	24	8
Low efficacy	12	10
Superseded	10	5
Completed early	4	9
Deemed futile	5	2
Technical reasons	0	5
Adverse events	2	0
Agent nonspecificity	2	0
Benefit–risk balance	2	0
Not a clinical trial	0	2
Uptake inadequate	2	0
Low accrual	1	0

[a] Clinical interventions studying AD and PD conducted between 1999 and 2019 were retrieved from ClinicalTrials.gov.

proteomic, and metabolomic levels. In this case, it is unhelpful to use broad terms, such as AD and PD, without recognising that there is potentially huge variability from case to case.

Therefore, we attribute the limited clinical success of AD and PD drugs (Tables 1 and 2) [2,3], including some high-profile drugs, such as bapineuzumab and parampanel, to the failure to recognise the complex, heterogeneous, multicomponent nature of NDDs. Thus, we also propose a philosophical shift away from the misleadingly precise labels of AD and PD and toward unified assessment of heterogeneous NDD, to guide pharmaceutical interventions of NDD as already seen in nonpharmaceutical interventions. In fact, there is evidence of this shift in thinking already taking place: there are direct associations between both AD and PD with comorbidities such as diabetes [19,20] and cardiovascular disease [21,22], and inverse associations with numerous types of cancer [23,24].

A good starting point for such an overarching assessment of NDD is by analysis of big biological data rather than focusing on single species in isolation. This approach has been successfully adopted in the study of liver diseases, such as hepatocellular carcinoma [25] and nonalcoholic fatty liver disease [26–28]. Given the wealth of data currently available in the NDD field, we posit that data-driven approaches can be readily applied to the neurodegeneration field. However, applying these approaches to the brain requires consideration of the functional heterogeneity across different regions of the brain, in contrast to the relatively homogeneous liver and, therefore, care must be taken to ensure quality sampling.

Timeline towards transcriptomic data-driven NDD research

To realise the goals of unifying heterogeneous NDD research, abolishing misleading labels, and moving away from single-target investigations in favour of systems biology approaches to studying NDDs, we must rely on big data. Data collection and quality control are vital for any high-quality downstream analysis. Here, we recount a short history of advancements in biotechnology and their subsequent adoption into brain and NDD research (Fig. 1 and Table 3).

Pioneering efforts: cDNA microarray

Low-throughput experimentation is expensive, time-consuming and often of limited use: focussing on single genes allows that gene to be exquisitely well characterised, but can rarely explain an entire disease alone. High-throughput technologies, such as microarrays, allow thousands of genes and quantitative trait loci to be analysed simultaneously, thus greatly increasing the volume of data while reducing cost and difficulty of data collection.

Although molecular hybridisation of nucleic acid molecules had been first applied experimentally as early as 1975, it was not until 1997 that the technology was miniaturised, automated, and applied to genome-wide analysis, and not until the new millennium that microarray hybridisation technology was applied to human brain samples.

TABLE 2

Summary of unsuccessful agents in AD and PD clinical trials[a,b]

Agent	Class	NCT IDs	Reasons for termination	Outlook
Azeliragon	RAGE antagonist	NCT02916056; NCT02080364	Low efficacy	Promising despite setbacks
Bapineuzumab	Monoclonal antibody against Aβ N-terminal region	NCT00916617; NCT00998764; NCT00996918; NCT00676143; NCT00667810	Endpoint unmet	Discontinued
Dimebon (Latrepirdine)	NMDAR antagonist	NCT00939783; NCT00704782; NCT00912288; NCT01066546	Low efficacy	Discontinued
Lanabecestat	BACE inhibitor	NCT02972658; NCT02245737; NCT02783573	Endpoint unmet	Discontinued
Pioglitazone	PPARγ agonist	NCT01931566; NCT02284906	Low efficacy	Discontinued
Solanezumab	Monoclonal antibody against Aβ mid-domain	NCT02760602; NCT01900665; NCT01127633	Endpoint unmet	Discontinued
Perampanel	AMPAR antagonist	NCT00427011; NCT00360412; NCT00505622; NCT00566462; NCT00360308	Low efficacy	Discontinued
Tavapadon	Dopamine D1/D5 receptor agonist	NCT02847650; NCT02687542	Low efficacy	Phase I favourable

[a] Clinical interventions were retrieved as in Table 1 in the main text. Shown are details of therapeutic agents used in at least two clinical trials which were then terminated or withdrawn.
[b] Abbreviations: AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; Aβ, amyloid β; BACE, β-secretase 1-cleaving enzyme; NMDAR, *N*-methyl-D-aspartate receptor; PPARγ, peroxisome-proliferator activator receptor γ; RAGE, receptor for advanced glycation endproducts.

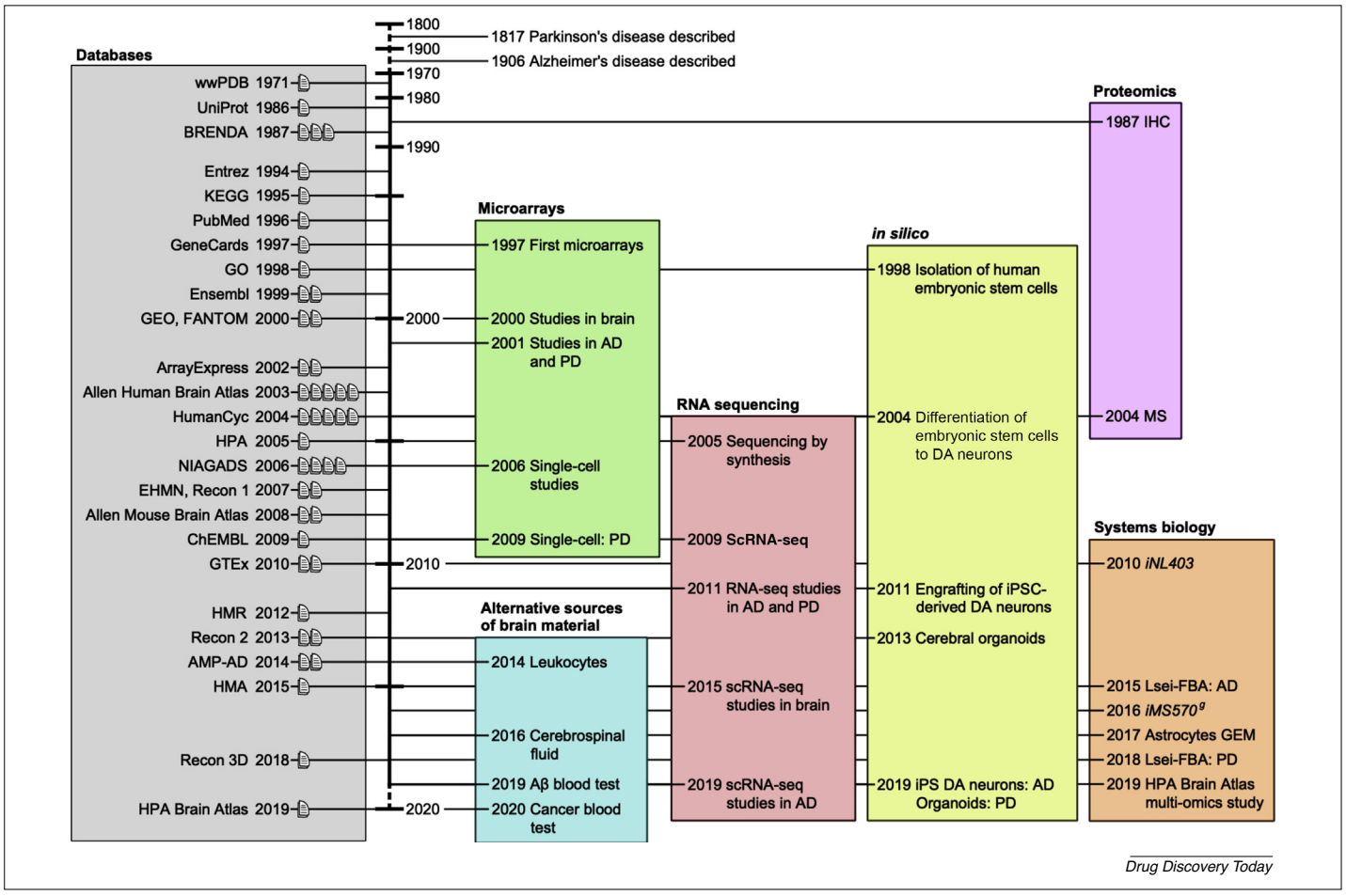

FIGURE 1

Timeline of biotechnological advancements and Alzheimer's (AD) and Parkinson's disease (PD) systems biology research. Chronology of major events in AD and PD research and technological advancements enabling genome-wide and multi-omics study. Years indicate first use or first description. Abbreviations: Aβ, amyloid β; DA, dopamine; GEM, genome-scale metabolic model; IHC, immunohistochemistry; iPSC, induced pluripotent stem cell; MS, mass spectrometry; RNA-seq, RNA sequencing; sc, single-cell. Abbreviations for databases are given in Table 3 in the main text.

In 2001 and 2002, **cDNA microarray** technology began to be used in AD and PD research. Microarray libraries of >6500 genes have been used to study gene expression in postmortem brain samples of patients with AD. These studies revealed wide-ranging trends across the genome, such as the upregulation of *PPP3CB* in the hippocampus [29], and the downregulation of synaptic vesicle protein synapsin II in the entorhinal cortex [30] in AD. For PD, microarray studies in PD model mice revealed differential expression of genes associated with functions and pathways, such as oxidative stress, inflammation, and glutamate toxicity, in the SN [31,32].

The large-scale findings of these studies were possible because of the whole-genome nature of microarray analysis. This was a significant step towards identifying factors and pathways that might otherwise have been missed in low-throughput studies, not to mention providing valuable data that can be reanalysed in the future. However, microarray technology is not without its limitations. First, microarrays rely on hybridisation, meaning that probes might have nonideal or arbitrarily different hybridisation kinetics, or that input RNA might cross-hybridise with an unintended probe, resulting in noise. Second, microarrays require a reference genome from which probes can be designed and, therefore, cannot be used for exploratory analysis of uncharacterised genes. Third, microarray technologies do not afford a wide dynamic range, owing to the background noise, as detailed earlier, resulting in decreased sensitivity to low-abundance transcripts and signal saturation at very high expression.

The former two studies [29,30] used postmortem human brain tissue as a sample source, whereas the latter two studies used mouse models [31,32]. It is worth discussing the choice of sample source in terms of sample quality and precision. Postmortem brain tissue allows the dissection and precise sampling of internal structures, which is desirable given that different brain regions are associated with diverse functions and might be differentially affected by disease. However, there are also disadvantages. First, postmortem brains are not readily sourced, so sample sizes for individual studies are invariably small. Second, the brain deteriorates rapidly after death, thereby impacting the quality of the data if sampling is not done in a timely manner. Third, postmortem brains can only be sampled once per donor, making longitudinal studies impossible. Fourth, cause of death and final physiological state can drastically affect the RNA content of the postmortem brain at the time of decease. Finally, antemortem events and clinical phenotypes affect postmortem brain RNA quality, but this

TABLE 3

Comparison of public databases and materials suitable for integrating into multi-omics systems biology or downstream analysis[a]

Name	Year	Omics layer(s)	Data collection method(s)
Worldwide Protein Data Bank (wwPDB)	1971	Ligome, proteome	Molecular structure determination; user submission; manual curation
UniProt	1986	Proteome	Manual curation
Braunschweig Enzyme Database (BRENDA)	1987	Chemome, ligome	Manual curation from literature
Online Mendelian Inheritance in Man (OMIM)	1987	Phenome	Manual curation from literature
DNA Data Bank of Japan (DDBJ)	1987	Genome, transcriptome	NGS; TLS; TSA; WGS; user submission
Entrez Gene	1994	Genome	Imported from DDBJ and GenBank; manually curated
Kyoto Encyclopedia of Genes and Genomes (KEGG)	1995	Chemome, genome, metabolome, pathways, proteome	Manual curation, Imported from GenBank, RefSeq
PubMed	1996	Literature	Imported from MEDLINE and peer-reviewed journals
GeneCards	1997	Genome, proteome	Imported from various databases
Gene Ontology (GO)	1998	Pathways	Manual curation
Ensembl	1999	Genome	Annotation
LocusLink	1999	Genome	Retired: now exists as Entrez Gene
Gene Expression Omnibus (GEO)	2000	Genome	Microarray, NGS
Functional Annotation of the Mammalian Genome (FANTOM)	2000	Transcriptome	CAGE, RNA-seq
ArrayExpress	2002	Transcriptome	RNA-seq
miRBase	2002	Transcriptome (miRNA)	RNA-seq
Allen Human Brain Atlas	2003	Transcriptome	Microarray, RNA-seq, scRNA-seq
Human Protein Reference Database (HPRD)	2003	Proteome	Annotation, Imported from OMIM, PubMed, RefSeq
Reactome	2003	Pathways	Imported from ChEBI, Ensembl, miRBase, UniProt
Lipid Maps® Lipidomics Gateway Structure Database (LMSD)	2003	Chemome (lipidome)	Manual curation
Reference Sequence (RefSeq)	2003	Genome, proteome, transcriptome	Manual curation
Encyclopedia of Human Genes and Metabolism (HumanCyc)	2004	Genome, metabolome	Imported from Ensembl, GenBank, LocusLink
PubChem	2004	Literature	User submission
H-Invitational Database	2004	Genome	Manual curation
Integrated Relational Enzyme Database (IntEnz)	2004	Proteome (enzyme)	Imported from BRENDA; annotation to standardise nomenclature from Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB) and Swiss Institute of Bioinformatics (SIB) Enzyme Nomenclature Database (ENZYME)
Chemical Components in the Protein Data Bank in Europe (PDBeChem)	2004	Chemome	Imported from wwPDB
Human Protein Atlas (HPA)	2005	Proteome	Antibody profiling
Human Metabolome Database (HMDB)	2006	Metabolome, pharmacometabolome	MS, NMR
Human Proteome Organization (HUPO) Brain Proteome Project (BPP)	2006	Proteome	User submission
National Institute on Aging Genetics of Alzheimer's Disease Data Storage Site (NIAGADS)	2006	Genome	GWAS, WES, WGS
ProteomeXchange	2006	Proteome	User submission
Edinburgh Human Metabolic Network (EHMN)	2007	Metabolome	Reconstruction from Ensembl, Entrez Gene, GeneCards, KEGG, UniProt
Recon 1	2007	Metabolome	Reconstruction from BRENDA, Entrez Gene, GeneCards, RefSeq, and literature
Allen Mouse Brain Atlas	2008	Transcriptome	ISH
Chemical Entities of Biological Interest (ChEBI)	2008	Chemome	Imported from ChEMBL, IntEnz, KEGG, PDBeChem
ChEMBL	2009	Chemome	Manual curation
Genotype-Tissue Expression (GTEx)	2010	Transcriptome	Microarray, SNP array, RNA-seq, WES, WGS
HepatoNet	2010	Metabolome	Manual curation, reconstruction from BRENDA, GEO, KEGG, Reactome, UniProt
Human Metabolic Reaction (HMR)	2012	Metabolome	Reconstruction from GO, HPA, KEGG, UniProt
PD Map	2013	Interactome	Manual curation

TABLE 3 (*Continued*)

Name	Year	Omics layer(s)	Data collection method(s)
Recon 2	2013	Metabolome	Reconstruction from Recon 1; annotation from ChEBI, EHMN, GO, HMDB, KEGG, PubChem; manual curation of reactions from PubMed
Accelerating Medicines Partnership — Alzheimer's Disease (AMP-AD)	2014	Epigenome, genome, proteome, transcriptome	User submission
Literome	2014	Interactome, phenome	Imported from PubMed; natural language processing
Human Metabolic Atlas (HMA)	2015	Various	Imported from BRENDA, ChEBI, EHMN, Ensembl, HepatoNet, HMDB, HumanCyc, KEGG, LMSD, PubChem, Recon 1, Recon 2, UniProt
Recon3D	2018	Metabolome	Reconstruction from HMR 2.00; manual curation of reactions
HPA Brain Atlas	2020	Transcriptome, proteome	Imported from Allen Mouse Brain Atlas, GTEx, FANTOM5; RNA-seq, immunohistochemistry

[a] Abbreviations: CAGE, cap analysis of gene expression; GWAS, genome-wide association study; ISH, in situ hybridisation; sc, single-cell; TLS, targeted locus study; TSA, transcriptome shotgun assembly; WES, whole-exome sequencing; WGS, whole-genome sequencing. Abbreviations for resource names are shown in brackets, where applicable.

is likely to be unavoidable regardless of the sample method. Mouse models of disease do not suffer from such small sample sizes, and death–sampling interval and antemortem events are more easily controllable, but require translation of medicine from mice to humans. Despite the advantages of mouse models, modern NDD research studies mostly depend on human and human-derived samples, such as postmortem brain tissue, because of the functional complexity of the brain and difficulty in cross-species translation of medicine.

Next generation: RNA sequencing

DNA sequencing was first described in 1977 by the chain termination inhibition, or sequencing-after-synthesis, method. The more efficient next-generation sequencing (NGS), or sequencing-by-synthesis, method was first described by 454 Life Sciences Corporation in 2005, and further developed by companies such as Illumina and Applied Biosystems. **RNA sequencing** (RNA-seq) begins with reverse transcription to cDNA, so any RNA that can be extracted to an acceptable purity can be sequenced. RNA-seq quickly grew in popularity because of the advantages it confers over microarrays. First, RNA-seq is not dependent on a reference genome, meaning it can sequence any arbitrary RNA sequence without probes, making it more suitable for exploratory and hypothesis-free experiments. Second, RNA-seq is based on annealing and synthesis rather than on hybridisation; therefore, it does not suffer from cross-hybridisation, offering lower background noise, higher sensitivity, and wider dynamic range. Finally, RNA sequencing depth can be increased to detect lowly expressed transcripts, which are often hidden in the background noise on microarrays and, therefore, are difficult to detect.

RNA-seq began to be used in NDD research in earnest shortly after the technology became available. In a 2011 study, AD researchers utilised the Illumina system to investigate expression in total brain and transcriptomes in the frontal and temporal lobes [33], revealing downregulation of *TFR1*, *SLIT1*, and *PPP3CB* in AD compared with controls. *TFR1* and *SLIT1* had been implicated in brain development and neuropathy previously [34,35]. In a 2013 study of expression in the less-studied parietal cortex [36], again using the Illumina system, investigators found upregulation of lipid metabolism genes, in line with associations between neurodegeneration and steroidogenesis and fatty acid biosynthesis [37], but downregulation of RNA-processing genes in AD brains compared with controls. In 2014, researchers sequenced long noncoding (lnc)RNA from blood leukocytes of patients with PD and controls undergoing DBS [38]. They detected 3495 out of >7000 known brain-expressed lncRNAs and identified differentially expressed lncRNAs, differential promoter usage, and alternative splicing of genes. In a 2016 study, researchers used RNA-seq to identify differentially expressed sequencing tags (DESTs) in the **cerebrospinal fluid** (CSF) of patients with PD and controls [39]. They identified a total of 201 DESTs, including potential biomarkers *BMP7*, *SCN9A*, and *Dnmt1*, which had been associated with PD previously, as well as potential lncRNA biomarkers.

RNA-seq has allowed parallels to be drawn between novel findings and previously known interactions in the neuropathy field. This is testament to the sensitive, high-throughput nature of the technology. However, with such volumes of relatively easy-to-collect data, there are bound to be inconsistencies from study to study. This is illustrated in the example of *PPP3CB*, which was identified as upregulated in the 2001 microarray study [29] but downregulated in the 2011 RNA-seq study [33]. This could be because of technical limitations of microarrays, as explained earlier; it could be a spurious result because of the low sample size of one AD temporal lobe versus four in the microarray study; or it could represent important true biology as a source of heterogeneity, and, therefore, would have been missed without high-throughput methods. Importantly, reanalysis of these results is possible for any researcher because the raw reads of all of the above studies are available for download online via the NCBI Short Read Archive or Gene Expression Omnibus (GEO).

Notably, the 2014 and 2016 RNA-seq studies used blood cells and CSF as the source of RNA as opposed to the traditionally used postmortem brain tissue. CSF and blood cells are valuable alternative sources of brain RNA because they allow multiple timepoint sampling and are less intrusive to obtain. However, sampling from CSF is still prone to misinterpretation. First, it is less precise. CSF represents circulating factors from whole brain, thus making it difficult to study a single region of interest exclusively. In blood samples, this problem is exacerbated because organs other than the brain contribute RNA to the RNA pool. This means that

transcripts of interest that are expressed in multiple tissues (or organs) cannot easily be traced back to the tissues (or organs) of interest, thereby limiting the interpretability of any results. Second, the scope of discovery is limited. This was demonstrated in the 2014 leukocyte study, which could only identify less than half of lncRNAs known to be expressed in the brain. This means that intracellular, or otherwise noncirculating, transcripts will be under-represented or not represented at all. This is further accentuated by the presence of the blood–brain barrier (BBB), which might confer little or no permeability to transcripts between the brain and the circulatory system. In CSF samples, this does not represent a problem because CSF is obtained via lumbar puncture (also known as a spinal tap), which traverses the blood–CSF barrier [40]. Third, alternative sampling does not discount external or environmental variables. Particularly for blood samples, sample content can change rapidly in response to, for example, personal lifestyle, fasting state, or time of day. However, environmental variables can be controlled for more easily than for postmortem brain samples because multiple blood or CSF samples can be taken from the same patient.

Postmortem brain, CSF, and blood samples each have their merits in terms of precision of data (postmortem brain), non-intrusiveness of sampling (blood), or the compromise (CSF). It cannot be ignored that non- or less intrusive modes of sampling for diagnosis are desirable to minimise patient discomfort as well as to maximise hospital accessibility to the procedure. Indeed, a diagnostic blood test was recently developed for Aβ [41] and multiple cancers [42]. In the pursuit of discovery, however, precise data and interpretable results are paramount and, therefore, postmortem brain samples continue to be in use to this day. Although we described earlier the advantages of region-specific brain samples over total brain samples, they too still have their limitations: most profoundly that samples from a specific brain region still contain a heterogeneous mix of distinct cell types of varying abundances. As such, the sequencing of RNA purified from single cells vital for cell population-independent basic biology and for potentiating the usefulness of bulk tissue and region-specific samples through the use of cell population mapping, allowing standards to be built for these samples.

Higher resolution: single-cell transcriptomics

In addition to the points laid out earlier, single-cell transcriptomics is attractive because of its potential to reveal the RNA profile of a single cell type rather than a population of potentially heterogeneous cells, thus revealing the underlying single cell archetypes comprising the population. However, any modern sequencing technique requires on the order of micrograms of input material: this is easily obtainable from bulk tissue samples containing hundreds of thousands of cells, but for a single cell, the material needs to be amplified.

An amplification strategy allowing single-cell transcriptomics analysis was established as early as 2006 when microarrays still dominated the transcriptomics scene. This was then adapted for use with RNA-seq [known as **single-cell RNA-seq** (scRNA-seq)] in 2009 and then first applied to the human brain in 2015. Today, the field of single-cell transcriptomics has matured, and there are now many specialised applications and configurations for carrying out scRNA-seq experiments [43].

In 2009, an early single-cell microarray-based approach was used to perform genome-wide expression profiling and single nucleotide polymorphism (SNP) association analysis using single SN neurons from patients with PD [44]. Researchers found significant upregulation of *PDXK* and downregulation of *TRAPPC4*, which have known roles in vitamin B6/dopamine metabolism and in vesicle transport, respectively, in patients with PD compared with controls. In addition, the researchers also identified a SNP within *PDXK* to be associated with increased risk of PD, confirmed across three independent cohorts. By focussing the study on single cells in the SN, signals in the gene expression data were guaranteed to originate from a single neuron, as opposed to a heterogeneous mix of cell types in the sample. However, problems with this study include not sorting neurons by cell type, so cell type-specific transcriptomics within the SN were not considered.

In 2019, a droplet-based scRNA-seq approach was used to identify whole-genome RNA expression differences in the prefrontal cortexes of postmortem brains of individuals with AD pathology and healthy controls from the Religious Order Study and Rush Memory and Aging Project (ROSMAP) [45]. Investigators found distinct transcriptional profiles of differentially expressed genes among six major cell types, and that differentially expressed genes in astrocytes, microglia, and oligodendrocytes were mostly upregulated, whereas oligodendrocyte precursor cells, excitatory neurons, and inhibitory neurons had mostly downregulated genes in individuals with high Aβ load compared with individuals with no or low Aβ load. The investigators observed clustering of individual transcriptomic profiles by Aβ load and by cell type, with several marker genes being identified for each subcluster. Examples of these include *BEX1* and *LINGO1*, which have known roles in axonal regeneration and myelination, respectively. This study methodologically expands on the 2009 microarray-based study because it assesses transcriptomic expression profiles in different cell types, allowing differential expression analysis to be carried out with much finer precision.

These studies demonstrate the potential for single-cell transcriptomic methods to reveal and address the underlying heterogeneity in NDDs at a level of precision that is not possible by bulk transcriptomics. It accentuates the need to stratify patients by their differing cellular expression profiles and thereby the need for per-stratum or personalised therapy. Crucially, it is able to identify potential biomarkers of disease on a cell type-specific basis and provide single-cell transcriptomic expression profiles, feasibly allowing the re-exploration of cell population changes in bulk RNA-seq data.

In vitro*: induced pluripotent stem cells and brain organoids*

Aside from postmortem brain tissue, CSF, and blood samples as sources of transcriptomic data, lab-grown sources such as **induced pluripotent stem cells** (iPSCs) have been used in NDD research in recent years. Since the first isolation of human embryonic stem cells (hESCs) from human blastocysts in 1998, the first differentiation of hESCs into dopamine neurons in 2004, and the first *in vivo* engrafting of human iPSC-derived dopamine neurons into animal models in 2011, iPSCs are now of intense interest in central nervous system research as well as more widely in biology.

To illustrate this point, iPSCs were used in a 2019 study to analyse gene expression differences in patients with PD with the

GBA N370S point mutation [46]. Researchers found downregulation of genes involved in neuronal development, neuronal differentiation, and synaptic signalling, and upregulation of genes involved in cell cycle progression, mitosis, and zinc ion transport in *GBA* mutant cell lines compared with controls. This demonstrates that highly informative data can be obtained from non-deceased individuals as an alternative to CSF or blood samples.

However, even within the iPSC lines, cellular heterogeneity still exists because cells are unlikely to progress through the same developmental stages at precisely the same time. To address this, the researchers then used single-cell analysis, revealing a pseudotime continuum of disease development, with transcriptomes ranging from more control-like to less control-like. Gene expression was seen to vary along the pseudotime axis: genes involved in neuronal function, tau (*MAPT*) splicing, and microtubule function were downregulated early, whereas genes involved in the endoplasmic reticulum (ER) stress response were upregulated late. Therefore, pseudotime could be deduced from gene expression acting not only as simple disease biomarkers, but also as a proxy of disease progression stage.

Described in 2013, cerebral organoids have been used to model human brain development and microcephaly [47]. Cerebral organoids are miniature brain-like structures grown in 3D culture that display cortical organisation, discrete region identity, neuronal function, and coordinated motor stimulation. Organoids can be cultured from patient-derived stem cells and closely recapitulate *in vivo* development. Protocols for culturing organoids for the brain, liver, pancreas, colon, and lung, among others, have been described.

In 2019, midbrain floor plate neural progenitor cells derived from patients with PD were used to culture patient-specific midbrain organoids [48]. The researchers verified that the organoids could recapitulate human brain developmental behaviours, such as formation of midbrain dopaminergic neurons, expression of markers *TH*, *FOXA2*, *EN1*, and *LMX1A*, and electrophysiological function. They then modelled PD pathology with organoids by culturing stem cells with the *LRRK2* G2019S mutation, and found decreased *TH*/*FOXA2* double-positive signatures in PD organoids compared with controls. PD neurons also exhibited reduced dendrite bifurcation and branching.

These studies are examples of the powerful insights that can be gained through the use of iPSCs and cerebral organoids. As *in vitro* methods, these techniques eliminate a great deal of noise and variability generated from *in vivo* systems and are more easily controlled. iPSC lines and cerebral organoids can be produced en masse and can be derived from patients, thereby allowing longitudinal studies and personalised therapy. Finally, combining *in* vitro techniques with scRNA-seq allows **pseudotime analysis** of disease progression, a powerful method to exploit cellular heterogeneity. However, potential problems still exist in terms of translatability: the artificial environment of *in vitro* methods might not accurately simulate native conditions *in vivo*, and the absence of external signals means that care must be taken when interpreting the results from *in vitro* studies.

Timeline towards proteomic data-driven NDD research

In the case of proteomics, the study of all protein content of interest, the story becomes complicated. The instruments and techniques that have been used extensively for the determination and quantitation of peptides and proteins began to be developed >50 years ago. In addition to improvements in the instruments, advances in reagents, labelling, and imaging devices, and the creation of software and protein databases, such as wwPDB, SWISS-PROT, UniProt, led to the beginning of proteomics during the mid-1990s [49]. Despite what 'proteomics' implies, studying all protein content is not possible and undesirable with the current technology. By contrast, the use of **mass spectrometry** (MS) and 2D gel electrophoresis, for instance, allows for the determination of thousands of proteins and their abundances. The determination of post-translational modifications (PTMs), and protein–protein and drug–protein interactions is also an important part of proteomics studies. Each method serves better on separate tasks; therefore, more and more researchers implement them together in their studies, including in AD and PD research [50–53]. Proteomics-based strategies have also been used for biomarker discovery, validation, and screening for several diseases, such as cancer [54].

Under the microscope: immunohistochemistry

The study of proteins has a long history spanning over 100 years. In 1890, Von Behring discovered serum antibodies, and used them to treat diphtheria and tetanus. Seven years later, Kraus determined the reaction between antibodies and antigens: protein–protein interactions. In 1932, Heidelberger attached dyes to antigens. In 1941, Coons designed the first fluorescent antibody labels visible under light microscopy. These developments led to **immunohistochemistry**, the technique for the detection and localisation of proteins and antibodies within tissues and cells using specifically binding antigens. The interaction can be observed under different types of microscope using dyes excited enzymatically (chromogenic dyes) or by light (fluorescent dyes), attached to the specific antigen or another antigen to improve precision.

We see an early use of immunohistochemistry in neurodegenerative diseases around 1990. In 1987, researchers demonstrated that amyloid deposits of postmortem aged brain comprised Aβ protein, similar to those found in senile plaques of AD and PD, evidencing the link between these diseases and ageing [55]. Several studies showed the associations of lysosomal proteases (cathepsins) [56] and nerve growth factor receptors [57] with protein aggregation in AD brain but not PD brain, in the following years. Other proteins, such as tau protein, ubiquitin, and tyrosine hydroxylase, were observed in the SN of patients with PD and in the spinal cord of patients with amyotrophic lateral sclerosis in studies using immunohistochemical procedures [58].

Interest in immunohistochemistry in AD and PD studies increased during the 1990s until peaking around 2012. The frequency of animal model studies and studies regarding the effect of drugs and other compounds from this period draws our attention. For instance, a 2012 study showed possible protective effects of curcumin on the degeneration of nigral dopaminergic neurons in a rat model of PD [59], whereas another showed similar effects for paroxetine [60]. Both indicated anti-inflammatory properties and the inhibition of oxidative stress for the relief of symptoms. Supporting this, another study highlighted presynaptic glutamate receptors in the SN as potential targets for symptomatic and neuroprotective relief in PD [61]. For the treatment of AD, ameliorative effects of BMP-9 in reducing the amyloid plaque burden

were shown in immunofluorescence images of BMP-9-treated hippocampus under confocal microscopy [62]. However, supporting evidence has only been provided by murine models thus far [63].

In addition, observing PTMs is also possible by immunohistochemical studies. Phosphorylation of UBQLN, a component of the protein ubiquitin–proteasome system, in the hippocampus of patients with AD [64], phosphorylation of tau protein in the hippocampus of insulin-resistant rats [65], and ubiquitination of tau in yeast two-hybrid screen [66] are among these findings.

Mass data: mass spectrometry

MS, originally used for the quantitative analysis of complex hydrocarbon mixtures, was first reconfigured for gas chromatography MS for the determination of the primary structure of a protein by Klaus Kiemann in 1963. For almost a decade, it could barely compete with the analytical success of the contemporary methods, but it established its place in peptide studies by the routinisation of high-performance liquid chromatography (HPLC). Many instruments derived later from MS during the following decades, including fast atom bombard MS, electrospray ionisation MS (ESI), matrix-assisted laser desorption-ionisation (MALDI), and tandem MS.

MS is based on the digestion or disruption of proteins (or any amino acid-based compound) into short amino acid fragments, the determination of these sequences based on their size, charge, and other chemical properties, and the evaluation of data to determine protein identities, quantify protein abundances, and determine PTMs if present. The technique allows for the analysis of both simple compounds comprising a single antibody and samples with thousands of proteins [67]. Nonetheless, the evaluation of data is the general bottleneck, requiring the use of appropriate analysis software, knowledgebases cataloguing signal values for sequences and peptides, and protein databases. Thus, it can be said that MS has been reborn with proteomics. The increasing number of articles in PubMed with the terms 'mass spectrometry' and MS applications such as HPLC and MALDI since the 1990s supports this point.

AD and PD studies inevitably benefit from this trend. Postmortem brain has been used to determine differentially expressed proteins in patients with disease [51,68]. In a quantitative proteomic analysis of 80 postmortem human brain tissues (i.e., frontal cortex and anterior cingulate gyrus) sampled from control, AD, PD and comorbid AD/PD cases, tandem mass tag and synchronous precursor selection-based MS3 MS were utilised. Mass spectra were searched against UniProt, SWISS-PROT, and TrEMBL, and deposited into the ProteomeXchange Consortium. In total, 11 840 proteins were identified, 8955 of which were common to both tissues and 828 overlapped with Human Brain Atlas RNA-seq data set, or 65% of highly expressed transcripts when considering RNA-seq transcripts per million values >1 [69]. According to its authors, this study was one of the most in-depth human protein proteomes and stands as a valuable resource for further studies. Indeed, we see that many proteomics studies in NDDs make use of data and procedures from this study [70–73].

CSF [74–76], iPS cells [77], blood [78–80], and many other sample types [81] are also being analysed for the discovery of disease biomarkers. As explained earlier, CSF has the advantage of being in direct continuity with the brain. Proteomic profiling of CSF from individuals with familial AD (FAD; i.e., carriers of mutations on proteins such as PSEN1 and amyloid precursor protein) by LC-ESI-MS on a high-resolution time-of-flight showed overlapping protein changes between individuals with presymptomatic and symptomatic FAD and those with late-onset AD [75]. A recent study showed that the use of immunoprecipitation MS together with immunoassay gives less-biased, reproducible results between different assays and laboratories for the detection of α-syn forms in CSF [82].

Despite the aforementioned advantages, CSF might not be preferable because of its invasiveness, making it non-ideal for long-term screening of present and possible patients even if this were possible [83]. Blood is a common sample of human studies. However, the BBB severely limits the determination of possible protein biomarkers. A trial for this task on PD on nearly 300 subjects displayed weak outputs. Selected reaction monitoring (SRM), a method used in tandem MS, was performed to detect blood glycoproteins. Twelve out of 50 candidate glycoproteins were reliable and consistently identified in blood, whereas only three were significantly expressed or present among groups. There was no good specificity or correlation between possible biomarkers and the disease severity [80]. In another study, the decrease in serum apolipoprotein E (ApoE) levels of patients with AD linked to the lower **mini-mental state exam** scores by use of SRM [79]. By contrast, the need for MS to detect blood ApoE does not seem effective, considering that it is commonly detected by biochemical assays [84].

Multi-omics data integration

Through recent advances in high-throughput transcriptomic, proteomic, and metabolic technologies, researchers now have unprecedented access to high-throughput data from countless studies. Reanalysis and re-interpretation of these data allow investigators to create integrated multi-omic models of NDD, which are desirable because they allow more factors to be considered than would be possible when considering only a single omic.

Repositories of multi-omics data in NDD

Researchers wishing to reanalyse previously generated data face myriad options in terms of independent data repositories (Table 3). These often offer raw data files from disconnected studies differing in sampling precision, data collection technology, and sample source. Here, we discuss repositories offering reorganised, preprocessed, quality-checked data that are more helpful to researchers. The Human Protein Atlas Brain Atlas [85] is a repository describing regional expression of >17 000 protein-coding genes across ten anatomical regions for three species: human, mouse, and pig. Investigators integrated expression data from the GTEx and FANTOM5 databases, ***in situ*** **hybridisation** data from the Allen Mouse Brain Atlas, and in-house-generated RNA-seq and antibody-based immunofluorescence data to determine normalised expression (NX) and subcellular localisation. Processed NX data are available for download at the convenience of the researcher. At the time of writing, only data from healthy individuals are available for download.

The Accelerating Medicines Partnership – Alzheimer's Disease (AMP-AD) [86] initiative is a centralised repository of data from

studies in humans, mice, fruit flies, and rats, which includes high-profile studies such as the aforementioned ROSMAP study, studies from the Mayo Clinic, and the Adult Changes in Thought study. AMP-AD studies, data, and publications are searchable by data type, disease, species, and brain region, and offer raw and processed data downloads after approval by application.

The Parkinson's Disease Map [87] is comprehensive pool of molecular information based on 1485 publications and presented as an interaction map. It organises and summarises pathways, genes, and molecules implicated in PD, allowing researchers to rapidly identify gaps in our understanding, and to reconcile novel findings in the context of known interactions already on the map. The map is available for download in systems biology mark-up language and, therefore, is compliant with common *in silico* simulation pipelines (discussed in the next section).

Taken together, the pooling of raw data into unified repositories of processed data is an important step to maximise the immediate availability of big data in NDD research. This breaking of silos also improves ease of data reanalysis.

Genome-scale metabolic models of human brain

Systems biology has benefitted from vast amounts of processed data from curated repositories as discussed above. Equipped with big multi-omic data, it has become possible to generate simulation-ready **genome-scale metabolic models** (GEMs) of the human brain. GEMs encompass the entire set of cellular reactions and their associated substrates, products, stoichiometries, and directionalities, enabling the holistic study of relationships between metabolic pathways and perturbations in a quantitative manner. These models have already been successful in providing deeper mechanistic insight into disease and therapy as well as drawing new hypotheses.

Systems biology has provided simulation-ready GEMs of disease in other human tissues before [26]. Given a sufficiently detailed model, GEMs can be perturbed to simulate systemic and targeted administration of therapeutics and, therefore, suggest any adverse effects that could be exerted on the wider system (Fig. 2). GEMs have also been integrated with regulatory, protein–protein interactions, and signalling networks to study the alterations in biological functions other than metabolism [88,89]. Human brain tissue-specific GEMs have been generated and used to compare the metabolism in brain and other human tissues (Fig. 3).

In 2010, one early such metabolic model, *iNL403* [90], was generated by integrating expression data from the Human Protein Reference Database, H-Invitational Database, and the Human Proteome Organisation into an existing manually curated generic human metabolic network, Recon 1. This GEM was analysed to model energy metabolism in glutamatergic, γ-aminobutarate (GABA)-ergic, and cholinergic neurons. By applying microarray data from patients with AD to the model, the investigators could

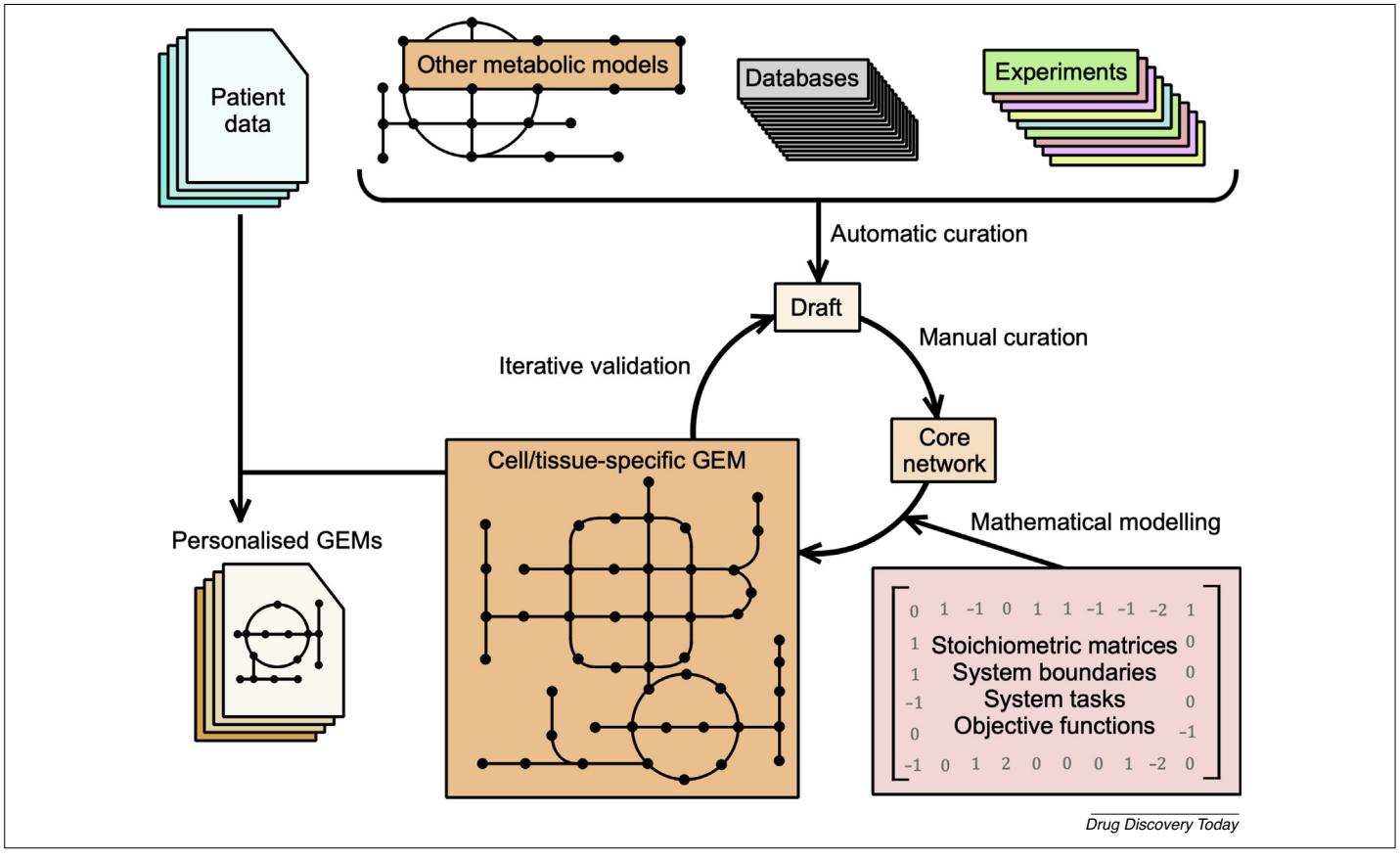

FIGURE 2

Reconstruction of genome-scale metabolic models. A draft network is constructed by automatic curation followed by manual curation of annotation data from public databases and experimental results. Generic metabolic models can also be used to aid generation of a core network. The core network is converted into a computational model by integrating metabolic and fluxomic data to yield a cell/tissue-specific genome-scale metabolic model (GEM). Personalised GEMs can be created by applying patient-specific transcriptomic and/or proteomic data to the cell/tissue-specific GEM.

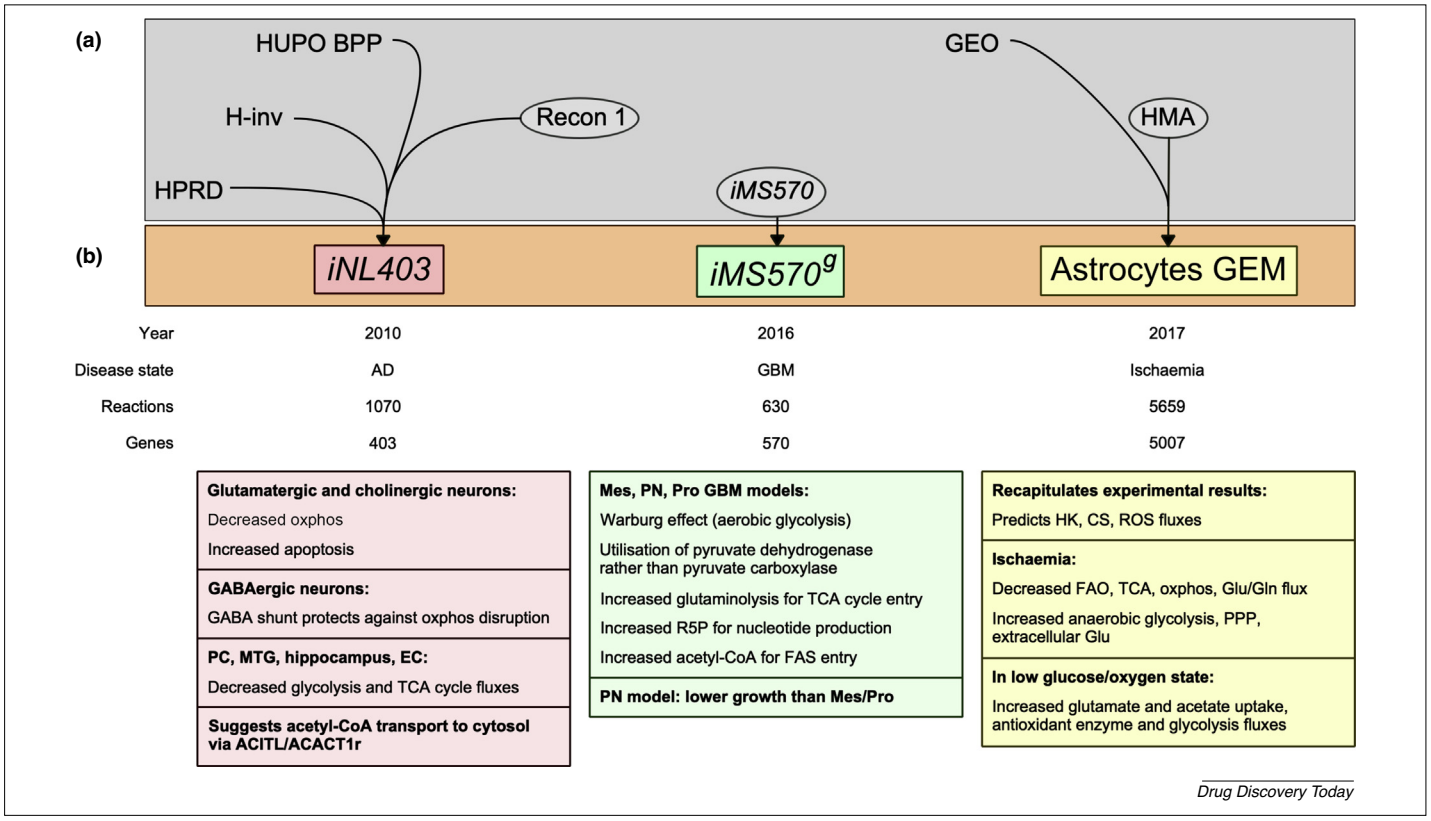

FIGURE 3

Summary of generation and results of genome-scale metabolic models for brain. **(a)** Reconstruction of genome-scale metabolic models (GEMs) using public resources. Resources in ellipses are GEMs or other repositories of metabolic reactions. Abbreviations for databases are as in Table 3 in the main text. **(b)** Properties of GEMs and results of GEM analysis. Abbreviations: AD, Alzheimer's disease; CS, citrate synthase; EC, entorhinal cortex; FAS, fatty acid synthesis; GABA, γ-aminobutyric acid; GBM, glioblastoma, HK, hexokinase; Mes, mesenchymal; MTG, middle temporal cortex; oxphos, oxidative phosphorylation; PC, posterior cingulate cortex; PN, proneural; PPP, pentose phosphate pathway; Pro, proliferative; R5P, ribose 5-phosphate; ROS, reactive oxygen species; TCA, tricarboxylic acid. After [90,91,94].

study the changes in energy metabolism in AD. The investigators perturbed the model by decreasing α-ketoglutarate dehydrogenase activity, which is known to be associated with AD, *in silico*, and predicted decreases in oxidative phosphorylation and increases in apoptosis in glutamatergic and cholinergic neurons but not GABAergic neurons. The model suggested the GABA shunt, which is active only in GABAergic neurons, to protect against disruption of the TCA cycle and oxidative phosphorylation fluxes. In addition, investigators also identified a pathway for transporting mitochondrial acetyl-CoA into the cytosol for acetylcholine synthesis, which they suggested could involve ACITL or ACACT1r enzymes.

Separately, GEMs have also been constructed for modelling glioblastoma (GBM). Published in 2016, the GBM-specific GEM *iMS570*^{g} [91] builds upon a previous GEM, *iMS570* [92], which itself is based on a comprehensive stoichiometric reaction model [93]. Each iteration of model reconstruction was conducted by literature search. The researchers mapped microarray data on three subtypes of GBM and peritumoral tissue onto *iMS570*^{g} to confer disease context specificity. *In silico* modelling of glycolytic TCA cycle fluxes yielded previously characterised descriptions of metabolic alterations in GBM, such as the well-established Warburg effect and the more recently described pyruvate utilisation by pyruvate dehydrogenase in GBM rather than pyruvate carboxylase. In addition, the three GBM models also suggested increased glutaminolysis for increased TCA entry at α-ketoglutarate via glutamate, increased ribose 5-phosphate for promotion of production of nucleotides, and increased acetyl-CoA for enhanced fatty acid synthesis entry.

In 2017, an astrocyte-specific GEM [94] was constructed by integrating transcriptomic data from the Gene Expression Omnibus and metabolic data from the Human Metabolic Atlas and similarly displayed accuracy to the literature; in particular, changes in the fluxes through hexokinase, citrate synthase, and reactive oxygen species from oxidative phosphorylation were correctly predicted by the model. By modelling ischaemia *in silico*, the GEM revealed decreased fluxes in fatty acid oxidation, TCA cycle, oxidative phosphorylation, and glutamate/glutamine interconversion; and increased fluxes in anaerobic glycolysis and the pentose phosphate pathway. Furthermore, in a low-glucose, low-oxygen environment, the GEM predicted increased glutamine and acetate uptake and heightened fluxes through antioxidant enzymes and glycolysis.

In 2018, the newly described least-squares with equalities and inequalities flux balance analysis (Lsei-FBA) algorithm was used to predict flux changes in a small-scale brain metabolic reaction network, using RNA expression data from patients with PD as the predictor [95]. The investigators predicted region-specific changes, including upregulation of electron transport chain genes in the SN and upregulation of glycolytic genes in the globus

pallidus. This contrasts with previous findings with (Lsei-FBA) from patients with AD, reported by the same group in 2015 [96], which predicted the concerted downregulation of TCA cycle genes and activation of the GABA shunt, in line with the findings of *iNL403*.

Taken together, these studies were able to recapitulate the results of many presystems experiments, reach similar conclusions with independent systems-level simulations, and suggest further predictions to be tested experimentally. This further demonstrates the suitability for systems biology approaches to be applied to the study of heterogeneous diseases, such as NDD.

Concluding remarks

In conclusion, research into AD and PD has come a long way since their first description. This research has seen marked progress from the latter part of the 20th century, in concert with increasing accessibility to new genome-wide technologies. It is no coincidence that biological achievements fell hand-in-hand with biotechnological advancements. However, despite decades-long research efforts, no cure exists for either disease.

With the advent of high-throughput data generation and integration of data from multiple 'omics, we now see evidence to treat AD and PD not as discrete diseases, but as individual, heterogeneous forms of NDD. Advancements in transcriptomic and proteomic analysis have allowed multiple genes and pathways to be identified as characteristics of neurodegeneration, shifting focus away from Aβ, tau, and α-syn, whereas GEMs have allowed system-wide personalised metabolic modelling, biomarker identification, treatment guidance, and hypothesis generation.

In the pursuit of precise data, the pioneering work on cerebrospinal fluid, blood leukocytes, iPSCs, and cerebral organoids as alternatives to postmortem brain tissue has revolutionised the accessibility of brain material. Although postmortem brain tissue remains the most widely used source, more data-rich and less intrusive approaches are key if personalised medicine in the NDD field are to work, and this work has already paved the way for new experimental design and a blood test for Aβ.

Traditional clinical progress for AD and PD treatment has been blighted by failures and setbacks and, when compounded with lengthy drug development pipelines, it is unclear whether or when current approaches will eventually develop an effective treatment. From the late 20th century to today, our understanding of NDDs at the transcriptomic, proteomic, and metabolomic levels has improved thanks to biotechnological advancements allowing easy high-throughput data generation and patient-specific modelling of systems *in silico*.

Thus, we challenge the traditional definitions of AD and PD and the notion that these diseases are fundamentally governed by Aβ, tau, and α-syn. We recommend disease patients be appraised not as patients with AD or PD, but as patients with NDD with specific, and now measurable, molecular characteristics. It is unreasonable to envision a single cure for such a heterogeneous disease; to address the heterogeneity, we should use high-throughput multi-omics data collection, *in silico* system-wide modelling, biomarker identification, stratification of disease, and per-stratum therapy.

Author contributions

S.L. and A.B. wrote the manuscript; all authors were involved in the editing and reviewing of the manuscript.

Acknowledgment

This work was supported by the Knut and Alice Wallenberg Foundation.

References

1 GBD 2015 Neurological Disorders Collaborator Group (2017) Global, regional, and national burden of neurological disorders during 1990–2015, a systematic analysis for the Global Burden of Disease Study 2015. *Lancet Neurol.* 16, 877–897

2 Cummings, J.L. *et al.* (2014) Alzheimer's disease drug-development pipeline: few candidates, frequent failures. *Alzheimer's Res. Ther.* 6, 37

3 Oertel, W. and Schulz, J.B. (2016) Current and experimental treatments of Parkinson disease: a guide for neuroscientists. *J. Neurochem.* 139, 325–337

4 Laird, F.M. *et al.* (2005) BACE1, a major determinant of selective vulnerability of the brain to amyloid-β amyloidogenesis, is essential for cognitive, emotional, and synaptic functions. *J. Neurosci.* 25, 11693–11709

5 Cohen, P. and Goedert, M. (2004) GSK3 inhibitors: development and therapeutic potential. *Nat. Rev. Drug Discov.* 3, 479–487

6 Brundin, P. *et al.* (2017) Therapeutic approaches to target alpha-synuclein pathology. *Exp. Neurol.* 298, 225–235

7 Kametani, F. and Hasegawa, M. (2018) Reconsideration of amyloid hypothesis and tau hypothesis in Alzheimer's disease. *Front. Neurosci.* 12, 25

8 Collier, T.J. *et al.* (2016) Is alpha-synuclein loss-of-function a contributor to parkinsonian pathology? Evidence from non-human primates. *Front. Neurosci.* 10, 12

9 Farlow, M.R. and Cummings, J.L. (2007) Effective pharmacologic management of Alzheimer's disease. *Am. J. Med.* 120, 388–397

10 Liberini, P. *et al.* (1996) Lewy-body dementia and responsiveness to cholinesterase inhibitors: a paradigm for heterogeneity of Alzheimer's disease? *Trends Pharmacol. Sci.* 17, 155–160

11 Poewe, W. *et al.* (2010) Levodopa in the treatment of Parkinson's disease: an old drug still going strong. *Clin. Interv. Aging* 5, 229–238

12 Nyholm, D. *et al.* (2005) Duodenal levodopa infusion monotherapy vs oral polypharmacy in advanced Parkinson disease. *Neurology* 64, 216–223

13 Wijemanne, S. and Jankovic, J. (2015) Dopa-responsive dystonia – clinical and genetic heterogeneity. *Nat. Rev. Neurol.* 11, 414–424

14 Dong, X. *et al.* (2018) Repetitive transcranial magnetic stimulation for the treatment of Alzheimer's disease: a systematic review and meta-analysis of randomized controlled trials. *PLoS One* 13, e0205704

15 Moisello, C. *et al.* (2015) TMS enhances retention of a motor skill in Parkinson's disease. *Brain Stimul.* 8, 224–230

16 Wassermann, E.M. (1998) Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5-7, 1996. *Electroencephalogr. Clin. Neurophysiol. Evoked Potentials* 108, 1–16

17 Deuschl, G. *et al.* (2006) A randomized trial of deep-brain stimulation for Parkinson's disease. *N. Engl. J. Med.* 355, 896–908

18 Sankar, T. *et al.* (2015) Deep brain stimulation influences brain structure in Alzheimer's disease. *Brain Stimul.* 8, 645–654

19 De La Monte, S.M. and Wands, J.R. (2008) Alzheimer's disease is type 3 diabetes-evidence reviewed. *J. Diabetes Sci. Technol.* 2, 1101–1113

20 Sandyk, R. (1993) The relationship between diabetes mellitus and Parkinson's disease. *Int. J. Neurosci.* 69, 125–130

21 Stampfer, M.J. (2006) Cardiovascular disease and Alzheimer's disease: common links. *J. Intern. Med.* 260, 211–223

22 Kotagal, V. *et al.* (2014) Modifiable cardiovascular risk factors and axial motor impairments in Parkinson disease. *Neurology* 82, 1514–1520

23 Driver, J.A. *et al.* (2012) Inverse association between cancer and Alzheimer's disease: results from the Framingham Heart Study. *BMJ* 344, 19

24 Bajaj, A. *et al.* (2010) Parkinson's disease and cancer risk: a systematic review and meta-analysis. *Cancer Causes Control* 21, 697–707

25 Ozcan, M. *et al.* (2020) Improvement in the current therapies for hepatocellular carcinoma using a systems medicine approach. *Adv. Biosyst.* . http://dx.doi.org/10.1002/adbi.202000030 Published online April 8. 2020

26 Lam, S. *et al.* (2020) Addressing the heterogeneity in liver diseases using biological networks. *Brief. Bioinform.* . http://dx.doi.org/10.1093/bib/bbaa002 Published online March 23, 2020

27 Bosley, J. *et al.* (2017) Improving the economics of NASH/NAFLD treatment through the use of systems biology. *Drug Discov. Today* 22, 1532–1538

28 Mardinoglu, A. *et al.* (2018) Systems biology in hepatology: approaches and applications. *Nat. Rev. Gastroenterol. Hepatol.* 15, 365–377

29 Hata, R. *et al.* (2001) Up-regulation of calcineurin aβ mRNA in the Alzheimer's disease brain: assessment by cDNA microarray. *Biochem. Biophys. Res. Commun.* 284, 310–316

30 Ho, L. *et al.* (2001) Altered expression of a-type but not b-type synapsin isoform in the brain of patients at high risk for Alzheimer's disease assessed by DNA microarray technique. *Neurosci. Lett.* 298, 191–194

31 Grünblatt, E. *et al.* (2001) Gene expression analysis in N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mice model of Parkinson's disease using cDNA microarray: effect of R-apomorphine. *J. Neurochem.* 78, 1–12

32 Mandel, S. *et al.* (2002) Early and late gene changes in MPTP mice model of Parkinson's disease employing cDNA microarray. *Neurochem. Res.* 27, 1231–1243

33 Twine, N.A. *et al.* (2011) Whole transcriptome sequencing reveals gene expression and splicing differences in brain regions affected by Alzheimer's disease. *PLoS One* 6, e16266

34 Levy, J.E. *et al.* (1999) Transferrin receptor is necessary for development of erythrocytes and the nervous system. *Nat. Genet* 21, 396–399

35 Kaneko, N. *et al.* (2010) New neurons clear the path of astrocytic processes for their rapid migration in the adult brain. *Neuron* 67, 213–223

36 Mills, J.D. *et al.* (2013) RNA-Seq analysis of the parietal cortex in Alzheimer's disease reveals alternatively spliced isoforms related to lipid metabolism. *Neurosci. Lett.* 536, 90–95

37 Wollmer, M.A. (2010) Cholesterol-related genes in Alzheimer's disease. *Biochim. Biophys. Acta* 1801, 762–773

38 Soreq, L. *et al.* (2014) Long non-coding RNA and alternative splicing modulations in Parkinson's leukocytes identified by RNA sequencing. *PLoS Comput. Biol.* 10, e1003517

39 Hossein-Nezhad, A. *et al.* (2016) Transcriptomic profiling of extracellular RNAs present in cerebrospinal fluid identifies differentially expressed transcripts in Parkinson's disease. *J. Parkinsons Dis.* 6, 109–117

40 Engelhardt, B. and Sorokin, L. (2009) The blood-brain and the blood-cerebrospinal fluid barriers: function and dysfunction. *Semin. Immunopathol.* 31, 497–511

41 Schindler, S.E. *et al.* (2019) High-precision plasma β-amyloid 42/40 predicts current and future brain amyloidosis. *Neurology* . http://dx.doi.org/10.1212/wnl.0000000000008081 Published online August 1, 2019

42 Liu, M.C. *et al.* (2020) Sensitive and specific multi-cancer detection and localization using methylation signatures in cell-free DNA. *Ann. Oncol.* . http://dx.doi.org/10.1016/j.annonc.2020.02.011 Published online March 30, 2020

43 Ziegenhain, C. *et al.* (2017) Comparative analysis of single-cell RNA sequencing methods. *Mol. Cell* 65, 631–643

44 Elstner, M. *et al.* (2009) Single-cell expression profiling of dopaminergic neurons combined with association analysis identifies pyridoxal kinase as Parkinson's disease gene. *Ann. Neurol.* 66, 792–798

45 Mathys, H. *et al.* (2019) Single-cell transcriptomic analysis of Alzheimer's disease. *Nature* 570 332–227

46 Lang, C. *et al.* (2019) Single-cell sequencing of iPSC-dopamine neurons reconstructs disease progression and identifies HDAC4 as a regulator of Parkinson cell phenotypes. *Cell Stem Cell* 24, 93–106

47 Lancaster, M.A. *et al.* (2013) Cerebral organoids model human brain development and microcephaly. *Nature* 501, 373–379

48 Smits, L.M. *et al.* (2019) Modeling Parkinson's disease in midbrain-like organoids. *NPJ Park. Dis* 5, 1–8

49 Kraus, A. and Dunn, M. (2016) Proteomics: the first 15 years. *Proteomics* 16, 1–3

50 Bandopadhyay, R. *et al.* (2004) The expression of DJ-1 (PARK7) in normal human CNS and idiopathic Parkinson's disease. *Brain* 127, 420–430

51 Owen, J.B. *et al.* (2009) Proteomics-determined differences in the concanavalin-A-fractionated proteome of hippocampus and inferior parietal lobule in subjects with Alzheimer's disease and mild cognitive impairment: implications for progression of AD. *J. Proteome Res.* 8, 471–482

52 Choi, J. *et al.* (2004) Oxidative modifications and down-regulation of ubiquitin carboxyl-terminal hydrolase L1 associated with idiopathic Parkinson's and Alzheimer's diseases. *J. Biol. Chem.* 279, 13256–13264

53 Pemberton, S. *et al.* (2011) Hsc70 protein interaction with soluble and fibrillar α-synuclein. *J. Biol. Chem.* 286, 34690–34699

54 Xing, X. *et al.* (2016) The application of proteomics in different aspects of hepatocellular carcinoma research. *J. Proteomics* 145, 70–80

55 Coria, F. *et al.* (1987) Brain amyloid in normal aging and cerebral amyloid angiopathy is antigenically related to Alzheimer's disease β-protein. *Am. J. Pathol.* 129, 422–428

56 Cataldo, A.M. and Nixon, R.A. (1990) Enzymatically active lysomal proteases are associated with amyloid deposits in Alzheimer brain. *Proc. Natl. Acad. Sci. U. S. A.* 87, 3861–3865

57 Mufson, E.J. and Kordower, J.H. (1992) Cortical neurons express nerve growth factor receptors in advanced age and Alzheimer disease. *Proc. Natl. Acad. Sci. U. S. A.* 89, 569–573

58 Hirano, A. (1992) Amyotrophic lateral sclerosis and parkinsonism-dementia complex on Guam: immunohistochemical studies. *Keio J. Med.* 41, 6–9

59 Du, X.X. *et al.* (2012) Curcumin protects nigral dopaminergic neurons by iron-chelation in the 6-hydroxydopamine rat model of Parkinson's disease. *Neurosci. Bull.* 28, 253–258

60 Chung, Y.C. *et al.* (2010) Paroxetine prevents loss of nigrostriatal dopaminergic neurons by inhibiting brain inflammation and oxidative stress in an experimental model of Parkinson's disease. *J. Immunol* 185, 1230–1237

61 Austin, P.J. *et al.* (2010) Symptomatic and neuroprotective effects following activation of nigral group III metabotropic glutamate receptors in rodent models of Parkinson's disease. *Br. J. Pharmacol.* 160, 1741–1753

62 Burke, R.M. *et al.* (2013) BMP9 ameliorates amyloidosis and the cholinergic defect in a mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. U. S. A.* 110, 19567–19572

63 Lauzon, M.A. *et al.* (2015) Growth factor treatment to overcome Alzheimer's dysfunctional signaling. *Cell Signal.* 27, 1025–1038

64 Mizukami, K. *et al.* (2014) Immunohistochemical analysis of ubiquilin-1 in the human hippocampus: association with neurofibrillary tangle pathology. *Neuropathology* 34, 11–18

65 Peng, D. *et al.* (2013) Hyperphosphorylation of tau protein in hippocampus of central insulin-resistant rats is associated with cognitive impairment. *Cell. Physiol. Biochem.* 32, 1417–1425

66 Flach, K. *et al.* (2014) Axotrophin/MARCH7 acts as an E3 ubiquitin ligase and ubiquitinates tau protein *in vitro* impairing microtubule binding. *Biochim. Biophys. Acta Mol. Basis Dis.* 1842, 1527–1538

67 Vogel, C. and Marcotte, E.M. (2008) Calculating absolute and relative protein abundance from mass spectrometry-based protein expression data. *Nat. Protoc.* 3, 1444–1451

68 Lachén-Montes, M. *et al.* (2019) Unveiling the olfactory proteostatic disarrangement in Parkinson's disease by proteome-wide profiling. *Neurobiol. Aging* 73, 123–134

69 Ping, L. *et al.* (2018) Global quantitative analysis of the human brain proteome in Alzheimer's and Parkinson's Disease. *Sci. Data* 5, 180036

70 Swarup, V. *et al.* (2019) Identification of conserved proteomic networks in neurodegenerative dementia. *BioRxiv* 2019, 825802

71 Rangaraju, S. *et al.* (2018) Quantitative proteomics of acutely-isolated mouse microglia identifies novel immune Alzheimer's disease-related proteins. *Mol. Neurodegener.* 13, XXX–YYY

72 Johnson, E.C.B. *et al.* (2018) Deep proteomic network analysis of Alzheimer's disease brain reveals alterations in RNA binding proteins and RNA splicing associated with disease. *Mol. Neurodegener.* 13, XXX–YYY

73 Fernandez, C.G. *et al.* (2019) The role of apoE4 in disrupting the homeostatic functions of astrocytes and microglia in aging and Alzheimer's disease. *Front. Aging Neurosci.* 10, XXX–YYY

74 Choe, L. *et al.* (2007) 8-Plex quantitation of changes in cerebrospinal fluid protein expression in subjects undergoing intravenous immunoglobulin treatment for Alzheimer's disease. *Proteomics* 7, 3651–3660

75 Ringman, J.M. *et al.* (2012) Proteomic changes in cerebrospinal fluid of presymptomatic and affected persons carrying familial Alzheimer disease mutations. *Arch. Neurol.* 69, 96–104

76 Núñez Galindo, A. *et al.* (2019) Analysing cerebrospinal fluid proteomes to characterize central nervous system disorders: a highly automated mass spectrometry-based pipeline for biomarker discovery. *Methods Mol. Biol.* 1959, 89–112

77 Jiang, H. *et al.* (2019) Proteomic study of a Parkinson's disease model of undifferentiated SH-SY5Y cells induced by a proteasome inhibitor. *Int. J. Med. Sci.* 16, 84–92

78 Jiang, R. *et al.* (2019) Differential proteomic analysis of serum exosomes reveals alterations in progression of Parkinson disease. *Medicine* 98, e17478

79 Han, S.H. *et al.* (2014) Both targeted mass spectrometry and flow sorting analysis methods detected the decreased serum apolipoprotein e level in Alzheimer's disease patients. *Mol. Cell Proteomics* 13, 407–419

80 Pan, C. *et al.* (2014) Targeted discovery and validation of plasma biomarkers of Parkinsons disease. *J. Proteome Res.* 13, 4535–4545
81 Wang, S. *et al.* (2019) Proteomic analysis of urinary extracellular vesicles reveal biomarkers for neurologic disease. *EBioMedicine* 45, 351–361
82 Mollenhauer, B. *et al.* (2019) Antibody-based methods for the measurement of α-synuclein concentration in human cerebrospinal fluid – method comparison and round robin study. *J. Neurochem* 149, 126–138
83 Bateman, R.J. *et al.* (2012) Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *N. Engl. J. Med.* 367 795–780
84 Wang, C. *et al.* (2014) Meta-analysis of peripheral blood apolipoprotein E levels in Alzheimer's disease. *PLoS One* 9, e89041
85 Sjöstedt, E. *et al.* (2020) An atlas of the protein-coding genes in the human, pig, and mouse brain. *Science* 367, eaay5947
86 Hodes, R.J. and Buckholtz, N. (2016) Accelerating Medicines Partnership: Alzheimers Disease (AMP-AD) Knowledge portal aids Alzheimers drug discovery through open data sharing. *Expert Opin. Ther. Targets* 20, 389–391
87 Fujita, K.A. *et al.* (2014) Integrating pathways of Parkinson's disease in a molecular interaction map. *Mol. Neurobiol.* 49, 88–102
88 Lee, S. *et al.* (2016) Dysregulated signaling hubs of liver lipid metabolism reveal hepatocellular carcinoma pathogenesis. *Nucleic Acids Res.* 44, 5529–5539
89 Lee, S. *et al.* (2016) Integrated network analysis reveals an association between plasma mannose levels and insulin resistance. *Cell Metab.* 24, 172–184
90 Lewis, N.E. *et al.* (2010) Large-scale in silico modeling of metabolic interactions between cell types in the human brain. *Nat. Biotechnol* 28, 1279–1285
91 Özcan, E. and Çakir, T. (2016) Reconstructed metabolic network models predict flux-level metabolic reprogramming in glioblastoma. *Front. Neurosci.* 10, 156
92 Sertbaş, M. *et al.* (2014) Systematic analysis of transcription-level effects of neurodegenerative diseases on human brain metabolism by a newly reconstructed brain-specific metabolic network. *FEBS Open Bio.* 4, 542–553
93 Çakir, T. *et al.* (2007) Reconstruction and flux analysis of coupling between metabolic pathways of astrocytes and neurons: application to cerebral hypoxia. *Theor. Biol. Med. Model.* 4, 48
94 Martín-Jiménez, C.A. *et al.* (2017) Genome-scale reconstruction of the human astrocyte metabolic network. *Front. Aging Neurosci.* 9, 23
95 Supandi, F. and van Beek, J.H.G.M. (2018) Computational prediction of changes in brain metabolic fluxes during Parkinson's disease from mRNA expression. *PLoS One* 13, e0203687
96 Gavai, A.K. *et al.* (2015) Using bioconductor package BiGGR for metabolic flux estimation based on gene expression changes in brain. *PLoS One* 10, e0119016