Advanced Non-animal Models in Biomedical Research

Neurodegenerative Diseases

The collection of non-animal models described in this report is publicly available from the *JRC Data Catalogue.*

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Abstract

Neurodegenerative diseases cause progressive loss of cognitive and motor function and pose major challenges to societies with aging populations. Existing therapies for neurodegenerative diseases are limited, and typically only treat the symptoms rather than providing a cure. Although there has been considerable investment in research, based predominantly on animal models, progress in discovering and approving effective treatments has been poor.

The JRC's EU Reference Laboratory for alternatives to animal testing (EURL ECVAM) launched a study to collect current and emerging non-animal models used in the field of neurodegenerative diseases. The overall aim is to provide an inventory and scientific evaluation of innovative (human-based) non-animal models and approaches currently in use for basic and applied research in the field of neurodegenerative diseases, more specifically Alzheimer's and Parkinson's disease.

More than 13,000 abstracts were screened to review and catalogue different alternative methods developed or used between 2013 and 2018. The resulting inventory includes 568 models, ranging from biochemical and computational approaches to different types of cell cultures and procedures using *ex vivo* human material. The inventory supports increased adoption and acceptance of alternative methods in neurodegenerative disease research and provides insights into emerging trends and promising areas for further development.

568 models

simple cell cultures

3D cultures/organoids

brain-on-chip

Acknowledgements

1 Introduction

One of the major challenges facing Europe is its ageing population and the associated increase in diagnosed cases of neurodegenerative diseases.

Neurodegenerative diseases occur when cells of the nervous system (neurons) in the brain or spinal cord begin to deteriorate. Changes in these cells cause them to function abnormally and can eventually cause them to die. As neurons deteriorate, an individual may first experience relatively mild symptoms — problems with coordination or remembering names. In some cases, patients lose the ability to walk independently, think clearly, or generally function in the world. Ultimately, many of these diseases are fatal.

Amongst these disorders, dementia is responsible for the greatest burden of disease. Alzheimer's disease (AD) and related disorders affect over 8 million people in Europe, and this figure is expected to double in the next 30 years as the population ages (Alzheimer Europe, 2019).

Parkinson's disease (PD) is the second most common neurodegenerative disease. Population prevalence of PD increases from about 1% at age 60 to 4% by age 80 (Miller and O'Callaghan, 2015). The first sign of PD is usually subtle fatigue, discomfort, or shakiness. With advancing disease, memory lapses, depression and a "masked" or expressionless face become common. Additional symptoms include trembling, stiff/sore muscles, loss of spontaneous movement, difficulty swallowing and impaired coordination. PD is caused by a loss of brain cells (neurons) in a part of the brain called the substantia nigra. Normally, these neurons produce "dopamine," an essential chemical messenger in the brain. Once damaged, these neurons stop producing dopamine and compromise the brain's ability to control movement.

There is no way to prevent or cure PD. The most widely used drug — levodopa — allows neurons to make new dopamine. However, the drug may cause side effects and over time loses effectiveness.

Here below we summarise the animal models used in AD and PD research, as well as some of their main deficiencies.

1.1 Animal models of Alzheimer's disease

AD is a progressive dementia with classic pathologies comprising amyloid plaques in the brain, neurofibrillary tangles (NFTs) containing abnormal tau and neuronal degeneration. Symptoms include cognitive deficits, such as memory disruption and impaired judgment, disorientation, confusion, behavioural changes and difficulties moving, speaking and swallowing.

Some AD research is conducted on dogs, non-human primates, ageing rats and chemical- and lesion-induced rodents. Newer models include genetically modified zebrafish and the nematode worm *Caenorhabditis elegans*. However, the more dominant animal models used over the past 15 years have been transgenic (Tg) mice (Langley, 2014).

Most of the Tg mouse lines are based on one or several inserted human genes relevant to the amyloid hypothesis of AD causation (Howlett, 2011; Elder *et al.*, 2010). Some lines of Tg mice develop plaques and/or NFTs, a few show some neuronal loss and some have cognitive deficits. However, the disease dynamics differ and none of the models fully recapitulates human AD. Moreover, phenotypic similarities that are present are species- and strain-dependent.

In translational science, predictive validity is crucial. Studies of Tg mice have certainly contributed to an understanding of some AD pathways. However, researchers have conducted more than 400 human trials of potential treatments for Alzheimer's disease but almost no new drugs have reached the market (King, 2018). Despite the blame placed on a variety of factors, one of the main sources of researchers' concern is the scientific relevance of the animal models that are used in the initial stages of drug development.

One of the main sources of researchers' concern is the scientific relevance of the animal models

1.2 Animal models of Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder and is characterised by loss of nigro-striatal dopaminergic neurons and aggregation of the α-synuclein-rich inclusions called Lewy bodies and Lewy neurites (Sharma *et al.*, 2013). Loss of striatal dopaminergic function in PD leads to resting tremor, bradykinesia, rigidity, and postural instability.

The cause of PD is unknown. Most cases are sporadic, however 15% of patients have a close relative (first degree) with the disease (Samii *et al.*, 2004) and up to 5% of cases have been linked to genes known to be associated with PD (Lesage and Brice, 2009). Treatment measures have been mostly focused on augmentation of dopaminergic signaling: pharmacologic treatments include levodopa

(a dopamine precursor), dopamine agonists, and MAO-B inhibitors (prevent dopamine metabolism). These drugs alleviate symptoms rather than treat the disease per se, and thus more effective research strategies are badly needed if therapies that slow progression, reverse or even prevent the disease are to be found (Savitt *et al.*, 2006).

Two main approaches are used to model PD in experimental animals: neurotoxins and genetics (Konnova *et al.*, 2018). Neurotoxin-based approaches include exposure of rodents or non-human primates to 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and agrochemicals such as the pesticide rotenone, the herbicide paraquat, and the fungicide maneb. Acute exposure to neurotoxins induces motor deficits and rapid nigro-striatal dopaminergic cell death by disrupting mitochondrial function and/or increasing oxidative stress, while chronic administration of neurotoxins induces progressive models which can include alpha-synuclein (α-synuclein) aggregates.

Genetic-based approaches to model Parkinson's disease include transgenic models and viral vector-mediated models based on genes linked to monogenic PD, including SNCA, LRRK2, UCH-L1, PRKN, PINK1, and DJ-1, as well as manipulation of dopaminergic transcription factors (Chang *et al.*, 2017). SNCA mutations, overexpression, and introduction of α-synuclein preformed fibrils induce toxic protein aggregates and variable nigro-striatal neurodegeneration and motor deficits, depending on the specific model.

Species, genetic background of a strain, and environment affect the display of symptoms and neurodegenerative hallmarks of animal models. Furthermore, there has been no translation from animal models of PD into a clinically proven neuroprotective or disease-modifying strategy. Many potentially neuroprotective compounds from a wide range of pharmacological classes have been identified in rodent and primate

models, and it is worrisome that so far none has proven effective in man.

1.3 Non-animal models for more human relevant research

Basic research strategies involving animal models of AD and PD have been unable to establish preventive measures and successful treatments. This is due primarily to the unreliability of the models used and poor translation of results to the human condition, leading to recommendations to shift the emphasis in research from animal models to the human patient (Cavanaugh, 2014).

This is in line with of the outcome of a workshop organised by the US National Academy of Sciences (Baine *et al.*, 2014): central nervous system (CNS) disorders have, compared with other disease areas, some of the highest failure rates for new compounds in advanced clinical trials. A frequently cited reason for CNS drugs failing because of lack of efficacy is the generally poor understanding of disease biology. There is a belief that advances in genetics and other new technologies can address this, by identifying new molecular targets and biomarkers.

Benam *et al.* (2015) highlight the need for a new paradigm for fundamental research into human diseases and for drug discovery research by maximising and combining the value of new engineering and molecular technologies with advanced human-based *in vitro* cell and tissue models. A more comprehensive framework merging together different read-outs and systems, e.g. novel human-specific *in vitro* models with induced pluripotent stem cells (iPSCs), organoid 3D models, or organ-on-chip with integrated microfluidic systems will drive progress.

A new framework will also enable a better understanding of disease causation and pathophysiology through deeper mechanistic analysis of disease related pathways – the normal cellular processes involving genes, proteins, and small molecules that lead to adverse human health effects when significantly perturbed by endogenous or exogenous factors (e.g. chemical toxicants).

Following the concepts underpinning Adverse Outcome Pathways (AOPs) in toxicology, 'biomedical AOPs' describe a chain of causally linked key events causing downstream effects at several biological levels and provide clear mechanistic rationale for diagnostic, preventative, and therapeutic interventions.

There are significant commonalities between the fields of human safety and biomedicine. They include, a) human biological pathways whose response encompasses efficacy, adaptation, and adversity; b) shared research tools and technologies (e.g. *in vitro* models, analytical approaches, computational modelling); and c) the benefits of better-structured and transparent weight-of-evidence decision-making frameworks, whether for chemical safety or drug efficacy, that can integrate all the data inputs.

To support the shift to a more human-relevant research paradigm, the JRC's EU Reference Laboratory for alternatives to animal testing (EURL ECVAM) launched a study to compile an inventory and carry out a scientific evaluation of innovative human-based non-animal models and approaches used for basic and applied research in neurodegenerative diseases, as published between 2013 and 2018.

The inventory covers *in vitro* models and *in silico* approaches, and in particular: human-specific (sub)cell and tissue-based models including two or three-dimensional cultures; human-induced pluripotent stem cells (iPSC); organ-on-a-chip devices incorporating microfluidics; *ex-vivo* approaches; and computational modelling.

2 Methodology

2.1 Selection and exclusion criteria

The agreed criteria for inclusion or exclusion of non-animal models and methods relevant for neurodegenerative diseases in this review are listed in Annex — Table 1 and Table 2.

The inclusion of non-animal models/methods was based on a combination of the agreed selection criteria.

2.2 Information sources

For this project, it was decided to use the open-access tool Web of Science (WoS) because of its high coverage of scientific resources and flexible tools for advanced searches. Moreover, WoS was the preferred tool compared to PubMed because of the higher likelihood to detect publications containing technologically advanced model systems, such as e.g. organ-on-chip approaches.

Within the Web of Science Core Collection, the Science Citation Index Expanded (available 1972–present), and the Emerging Sources Citation Index (available 2015–present) were selected to run the searches.

2.3 Search phrases used

The search phrases used to extract PD and AD papers and the results are given in Annex — Table 3 and Table 4, respectively. The search resulted in 4,740 abstracts for PD and 8,411 abstracts for AD.

The search phrase set #1 (related to a specific disease) and set #2 (related to disease specific features and endpoints) were different among PD, and AD while other phrases on the model systems (set #3) and combinations (set #4) were similar.

If endpoints described in the papes were relevant for both PD and AD, and/or in general for neurodegenerative diseases, the models were categories under the category NDD.

A list of tags for different groups of paper descriptors (e.g. type of paper, neurodegenerative disease, biological endpoint) were compiled and is available in Annex — Table 5.

2.4 Abstract retrieval

A total of 4,740 abstracts were retrieved for PD and 8,411 for AD. Each abstract was reviewed for relevance and, if accepted, tagged with reason for acceptance or rejection.

2.5 Model summary

It was agreed that the selected models be described and presented using a specially designed template based on the use of drop-down lists and fixed terminology, which allowed that the listed methods and models could be easily sorted, searched and filtered. (see Annex — Table 6).

The resulting dataset of non-animal models described is publicly available from the *JRC Data Catalogue*[1].

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1   *https://europa.eu/!bM83pv*

3 Results

3.1 Overview of main model categories for each areas

Figure 3.1 and Table 3.1 provide an overview of the numbers of categorised models/methods within each of the disease areas defined, respectively AD, PD or NDD. The table contains 588 entries (rows), representing a total of 568 models grouped in six different model categories derived from 519 publications.

In some cases, more than one relevant model is presented in the same publication, leading to more than one entry (row in table). For certain model systems (mainly cell models) authors have studied many different endpoints either or not in relation to disease hallmarks within the same model system. In that case, multiple entries were given for the model system.

In the next paragraphs, each of the model categories is evaluated using the methodological properties in relation to the relevance to study disease features or the scientific aim for each of the three disease areas. The format of the dataset allows for a quantitative analysis for several parameters (e.g. disease feature, application/aim, biological endpoint, status of method) by retrieving information through filtering on the fixed fields, as pre-defined in the drop-down lists of the compiled models table.

As a general overview, we present the analysis of the applications and/or aims, the specifics of the model system, the disease features and biological endpoints they focus on, as well as the throughput of the listed methods/models per category and per disease focus (or merged where deemed appropriate). A general summary offers some qualitative reflections on the categorisation efforts and relevance of models in the context of NDD area.
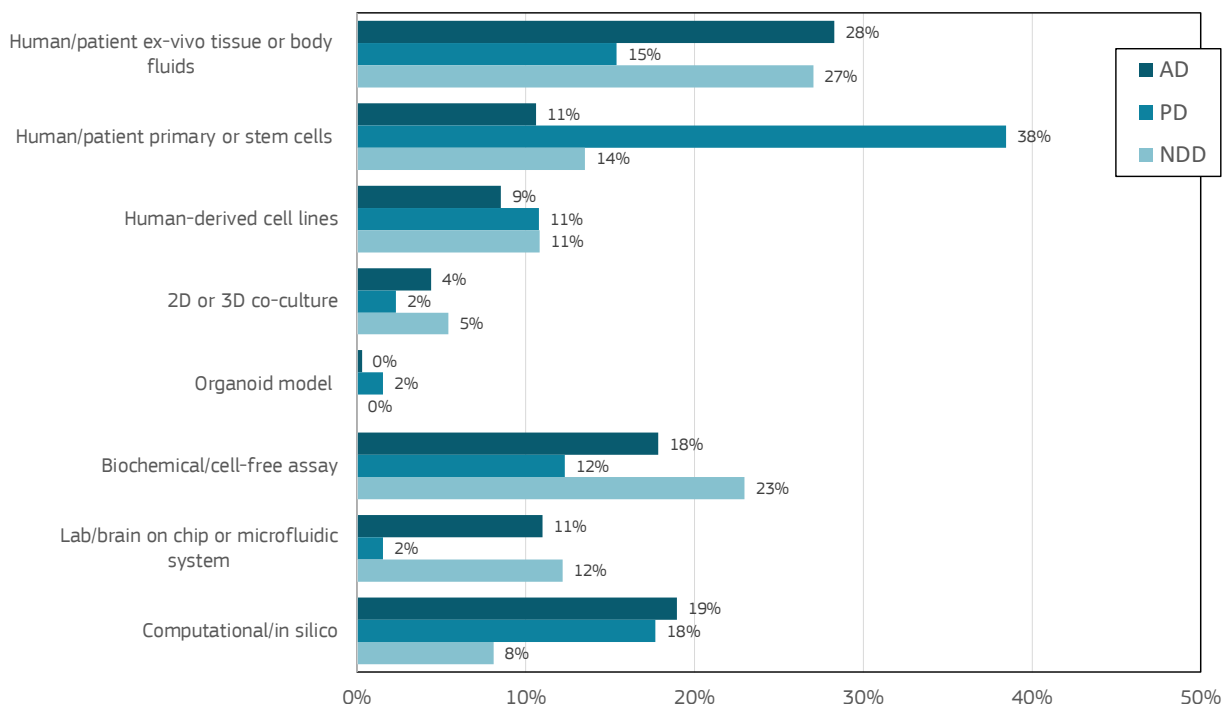
Figure 3.1:   Distribution of model categories among three disease areas.

Table 3.1: Summary table of different model systems.

	AD			PD			NDD			Total # model systems/ category across diseases areas
	#Models	#Papers	#Entries	#Models	#Papers	#Entries	#Models	#Papers	#Entries	
Human/patient ex-vivo tissue or body fluids (brain biopsy, CSF, post-mortem)	103	93	103	20	18	20	20	17	20	143
Human/patient primary or stem cells	39	38	39	50	50	63	10	10	10	99
Human-derived cell lines	31	29	31	14	14	19	8	8	8	53
2D or 3D co-culture	16	16	16	3	2	3	4	4	6	23
Organoid model	1	1	1	2	2	2	0	0	0	3
Biochemical/cell-free assays	65	64	65	16	16	16	17	17	17	98
Lab/brain on chip or microfluidic systems	40	40	40	2	2	2	9	9	9	51
Computational (*in silico*) model	69	69	69	23	23	23	6	6	6	98
Sum of models/disease area	**364**			**130**			**74**			**568**

3.2 Human/patient *ex vivo* tissue or body fluids

A total of 128 publications were retained after full-text evaluations of papers and resulted into respectively 103 relevant models for AD, 20 models for PD and 20 models for other NDD.

3.2.1 Application/aim

In the AD area, almost two thirds of all models/methods (n=57) were focused on generating greater insight into the disease mechanism. About a third (n=34) were primarily aimed at improved diagnostics. A minority of models zoomed in on disease therapy development or experimental model development.

A similar trend is seen for PD, where two-thirds of all models/methods (n=12) were focused on generating greater insight into the disease mechanism while a third (n=8) were primarily aimed at improved diagnostics.

Likewise, for the broader NDD area, the majority of models/methods (n=10) were focused on generating greater insight into the disease mechanism. Eight methods were primarily aimed at diagnostics, while two methods focused on experimental model development. Similar patterns are clear from the distribution graph with more than 50% of the models/methods overall focusing to the disease mechanism for the three disease areas (Figure 3.2).

Table 3.2:   Applications/aims for Human/patient *ex vivo* tissue or body fluids model for three disease areas.

Application/aim	AD (n=103)		PD (n=20)		NDD (n=20)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
Diagnosis of disease	34	29	8	7	8	6
Disease mechanism (exp/theor)	57	55	12	11	10	9
Disease therapy developm	9	7	0	0	0	0
Model/method development – experim	2	1	0	0	2	2
Other	1	1	0	0	0	0
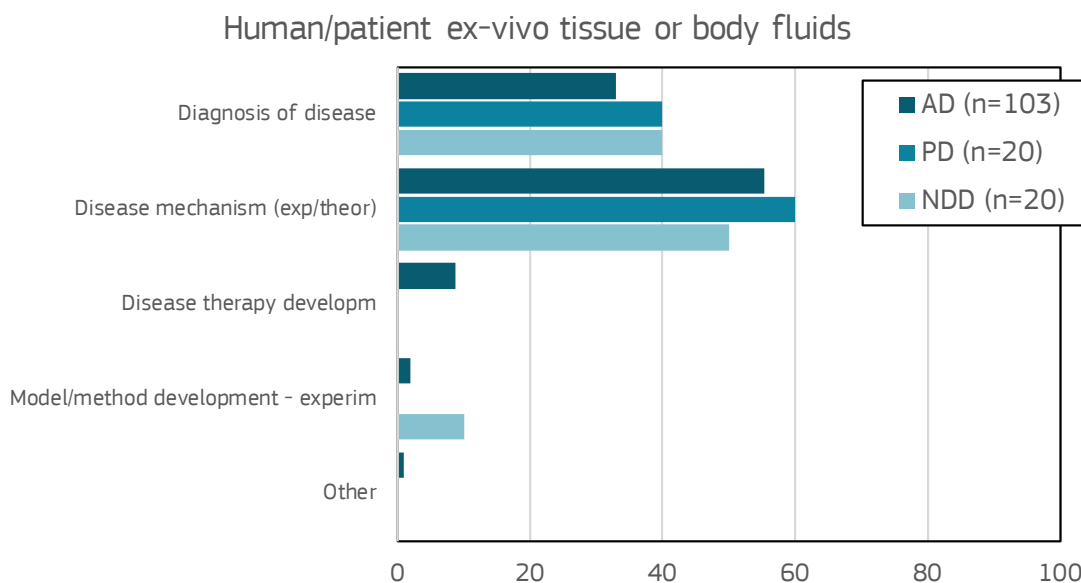
Figure 3.2:   Relative distribution of fields for model system within Human/patient *ex vivo* tissue or body fluids model (% of models).

■ 3.2.2 Model system

Overall, for AD, the primary *ex-vivo* material used was CNS (brain) tissue. Approximately a quarter of the models/methods were based on the use of cerebrospinal fluid (CSF) or blood, respectively. Two studies used abdominal adipose arterioles and urine as source material (categorised as other).

Papers relevant for PD also indicated that the main *ex-vivo* material used was CNS (brain) tissue. The remainder of the methods was based on using human/patient CSF, and to a lesser extent blood, two types of body fluids which markedly differ in terms of accessibility. One method relied on pancreatic tissue to study α-synuclein deposits.

In the NDD disease category of models/ methods, focusing not specifically on one neurodegenerative disorder, the prime *ex-vivo* material used was CNS (brain) tissue as well. Only two methods were based on the use of CSF or blood, while one study used exosomes (categorised as other).

Table 3.3: Model systems for Human/patient *ex vivo* tissue or body fluids model for three disease areas.

Type	AD (n=103)		PD (n=20)		NDD (n=20)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
Brain (or CNS) tissue	54	54	11	11	15	14
CSF	25	24	5	5	2	2
Blood	21	21	3	3	2	2
Other	2	2	1	1	1	1
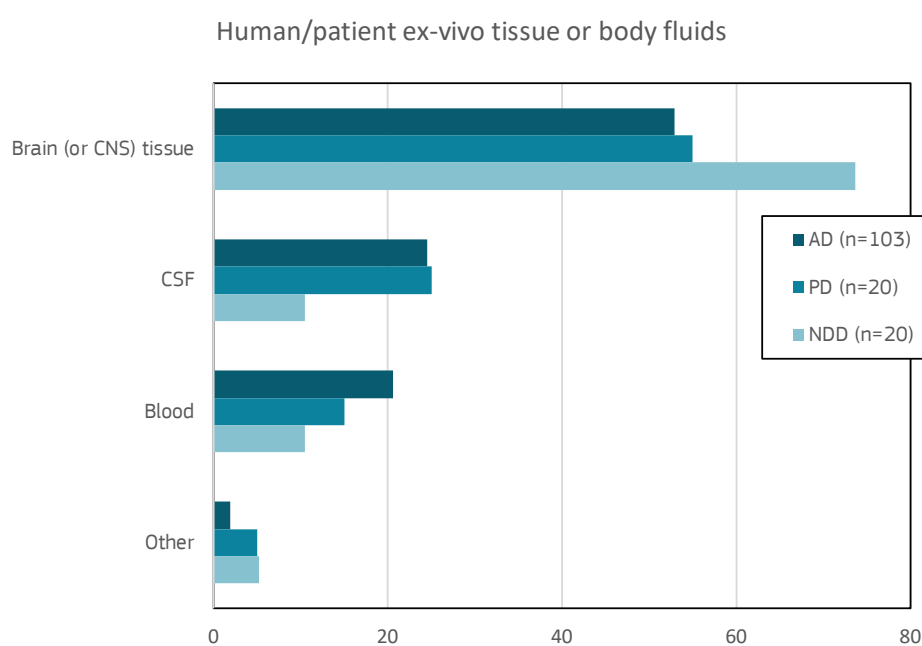
Figure 3.3: Relative distribution of fields for model system within Human/patient *ex vivo* tissue or body fluids model (% of models).

3.2.3 Disease feature

The majority of AD models/methods based on *ex-vivo* patient material focused around protein aggregation, in this case tau or Aβ. About a third were applied for exploratory purposes or not focused on any disease feature at all (na). A handful of methods were applied to investigate cognitive problems, energy metabolism deregulation, neuronal loss and neuro-inflammation disturbances associated with AD.

Similar to AD, >50% of the PD models/methods based on *ex-vivo* patient material focused around protein aggregation (Figure 3.4). In the PD studies, the α-synuclein protein is the subject. A few models/methods were used for exploratory purposes or not focused on any disease feature at all. Two methods were applied to investigate energy metabolism disturbances in PD.

As expected, for methods that were not aimed at one specific neurodegenerative disorder, the focus to a specific disease feature in the NDD group was less clear with relatively more methods scoring 'not applicable' (na).

Overall, considering the three disease areas, about half of all models/methods did focus on protein aggregation mechanisms, as a common feature of the different neurodegenerative diseases.

Table 3.4:   Disease features for Human/patient *ex vivo* tissue or body fluids model for three disease areas.

Disease feature	AD (n=103)		PD (n=20)		NDD (n=20)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
Cognitive functional problems	1	1	0	0	0	0
Energy metabolism	4	4	2	1	2	2
Exploratory/ no specific feature	28	26	4	3	1	1
Neuronal loss	6	5	0	0	0	0
Neuro-inflammation	8	7	0	0	2	2
Protein aggregation	54	47	11	11	8	6
Not applicable	2	2	3	3	7	6
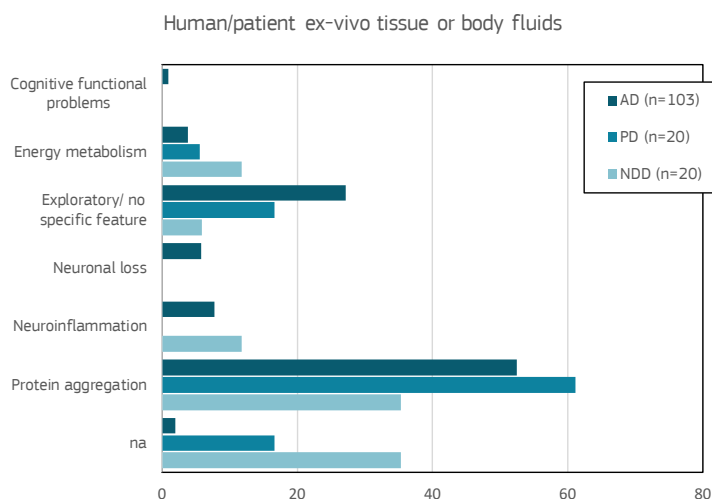
Figure 3.4:   Relative distribution of disease features for Human/patient *ex vivo* tissue or body fluids model (% of models).

3.2.4 Biological endpoints

Reflecting the large focus on protein aggregation in the previous section, the attention in terms of biological endpoints in the AD disease area is also on the amyloid peptide, and to a lesser extent on tau. One in five methods generally aimed at identifying gene or protein expression differences, as well as epigenetic changes.

In the field of models for PD research, the studies also reflect again the large focus on protein aggregation. The attention in terms of biological endpoints is on α-synuclein dysfunction and Lewy bodies. A few generally aimed at identifying gene or protein expression differences, as well as epigenetic changes. In the NDD group, there is a wide variety in biological endpoints for the methods and models without one specific disease focus.

Table 3.5: Biological endpoints for Human/patient *ex vivo* tissue or body fluids model for three disease areas.

Biological endpoints	AD (n=103)		PD (n=20)		NDD (n=20)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
Activation of microglia	2	2	0	0	0	0
Changed gene expression	4	4	2	2	1	1
Changed protein expression	17	15	2	2	3	3
DNA damage	1	1	0	0	0	0
Endoplasmic reticulum stress	0	0	0	0	1	1
Epigenetic changes	2	2	2	1	1	1
Impaired synaptic/vesicular function, transport or morphology	2	2	0	0	2	1
Inflammation	5	4	0	0	1	1
Lysosomal dysfunction	1	1	0	0	0	0
MicroRNA changes	0	0	0	0	2	2
Mitochondrial dysfunction	0	0	2	1	1	1
Multiple biological endpoints	8	7	0	0	3	1
Oxidative/nitrosative stress	6	6	1	1	0	0
Protein dysfunction: amyloid peptide (any version)	30	25	0	0	1	1
Protein dysfunction: ApoE	1	1	0	0	0	0
Protein dysfunction: α-synuclein	0	0	7	7	0	0
Protein dysfunction: BACE1	1	1	0	0	0	0
Protein dysfunction: gamma secretase (or comp.) –PSEN	1	1	0	0	0	0
Protein dysfunction: Lewy bodies	0	0	4	4	0	0
Protein dysfunction: multiple	4	4	0	0	1	1
Protein dysfunction: Tau (phosphorylation)	8	7	0	0	1	1
Not applicable	0	0	0	0	1	1
Other	10	9	0	0	0	0
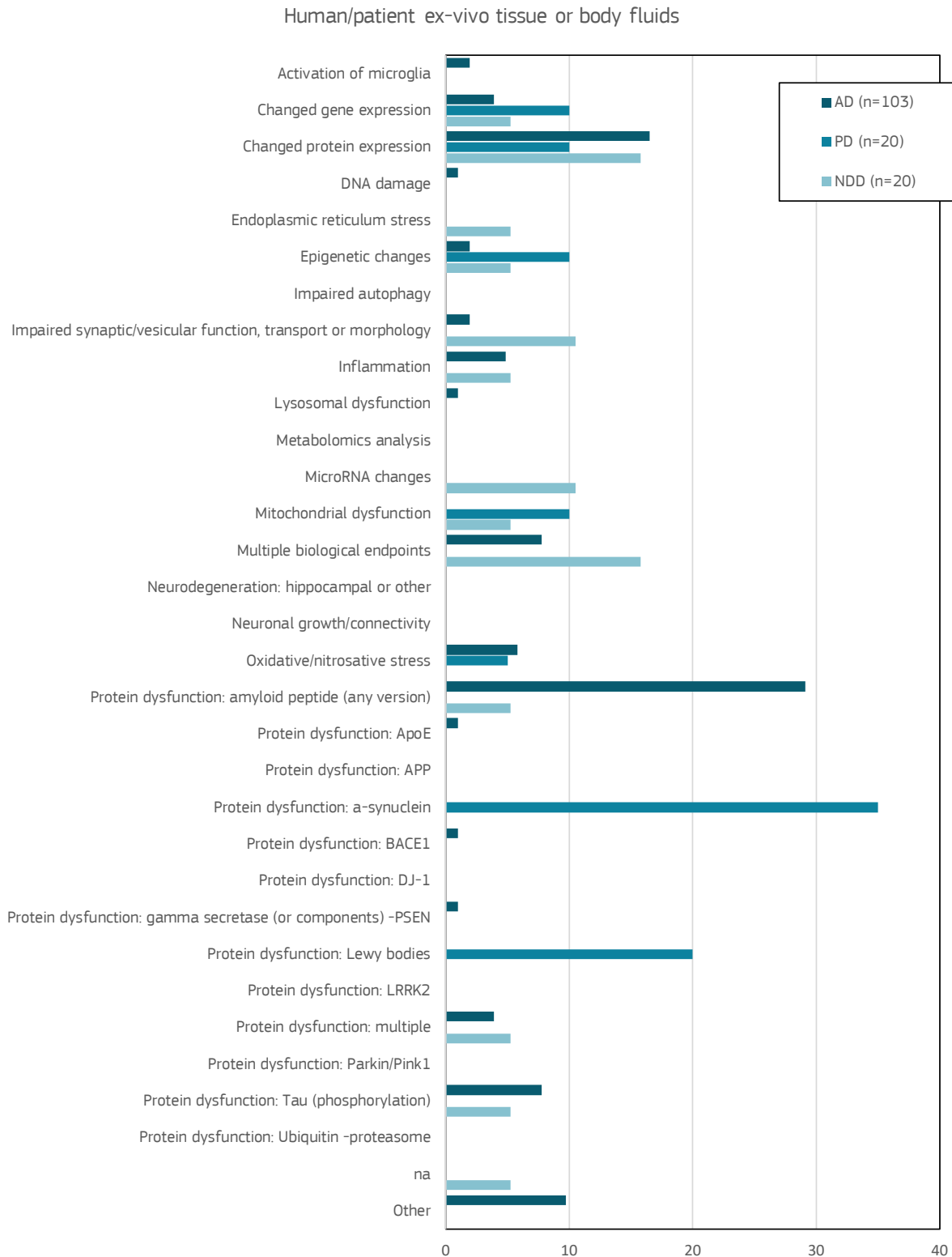
Figure 3.5:   Relative distribution of biological endpoints for Human/patient *ex vivo* tissue or body fluids model (% of models).

3.2.5 Throughput/content

This parameter on throughput and content was only included for the paper evaluation of AD, thus no comparison is possible with other areas. Overall these models/methods have a throughput, as well as content which is in many instances rather low.

Table 3.6:   Throughput/content for Human/patient *ex vivo* tissue or body fluids model category for three disease areas.

Throughput/content	AD (n=103)		PD (n=20)		NDD (n=20)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
High (automatic)/high	0	0	nd	nd	nd	nd
High (automatic)/medium	0	0	nd	nd	nd	nd
High (automatic)/low	3	3	nd	nd	nd	nd
Low-medium/high	3	3	nd	nd	nd	nd
Low-medium/medium	30	26	nd	nd	nd	nd
Low-medium/low	67	60	nd	nd	nd	nd

nd: no data available because this field was added after the evaluation of PD papers was finished.
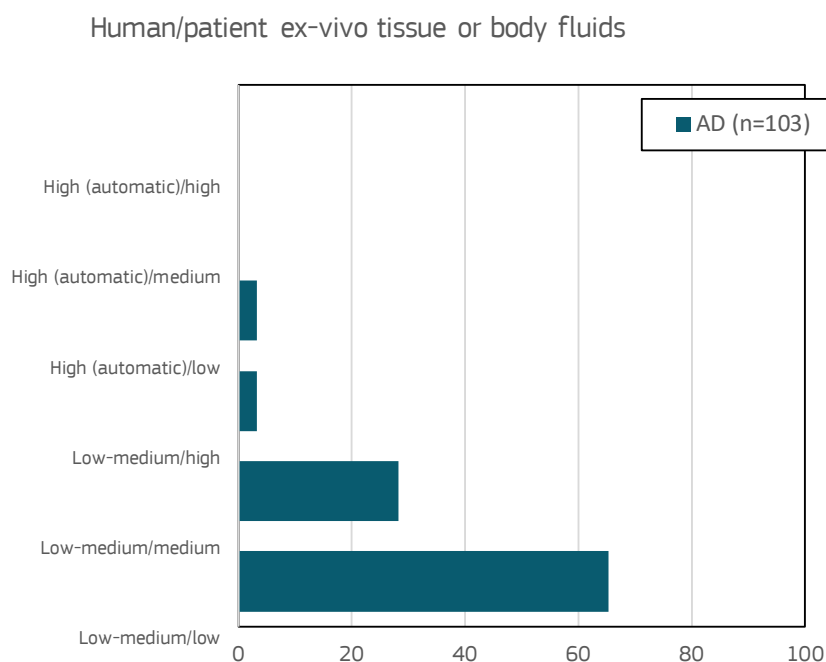
Figure 3.6:   Relative distribution of throughput/content for Human/patient *ex vivo* tissue or body fluids model (% of models).

███ 3.3 Human/patient primary or stem cells

A total of 98 publications were retained after full text evaluations of papers, resulting in respectively 39 relevant models for AD, 50 models for PD and 10 models for NDD.

███ *3.3.1 Application/aim*

The majority of AD papers do focus on disease mechanisms (80%), while diagnosis and therapy development present a minority of papers. On the contrary, the PD papers with the primary or stem cell model aim to contribute to disease therapy (approx. 40%), while also model development and validation work is included in respectively seven and five studies.

The overall number of NDD models is low, but model development is an important aim, next to disease therapy, drug development and study of disease mechanism.

Table 3.7:   Applications/aims for Human/patient primary or stem cells model for three disease areas.

Application/aim	AD (n=39)		PD (n=50)		NDD (n=10)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
Diagnosis of disease	3	3	0	0	0	0
Disease mechanism (exp/theor)	32	31	13	12	2	2
Disease therapy developm	3	3	21	18	2	2
Drug developm/ testing	1	1	2	2	2	2
Model/method development – experim	0	0	8	7	4	4
Model/method validation	0	0	6	5	0	0
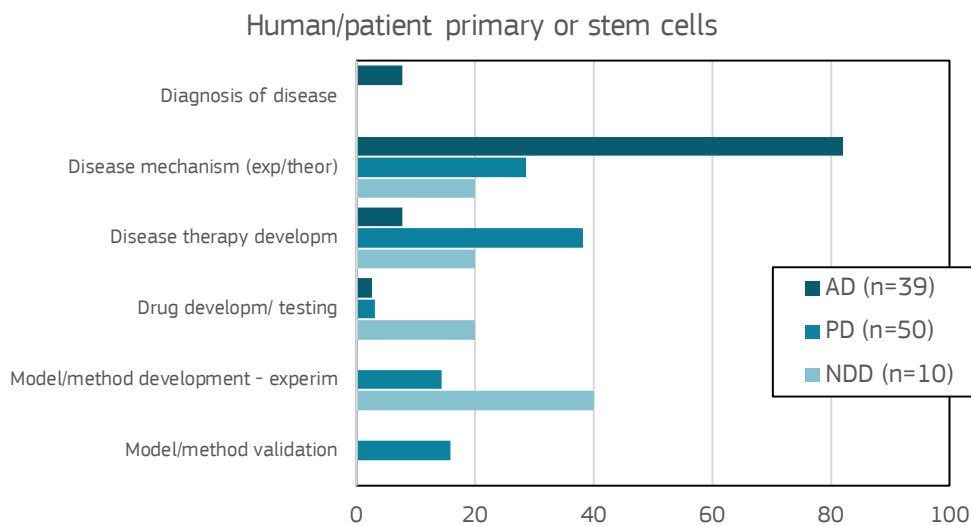
Figure 3.7:   Relative distribution of applications/aims for Human/patient primary or stem cells model (% of models).

3.3.2 Model system

In the AD and PD area, non-differentiated embryonic stem cells and iPSCs represent the largest group of models, respectively 20% and 40% of this category. These cellular models have been explored for reprogramming and offer potential for cell replacement therapy, linking to the aforementioned area of application. Furthermore, multipotent stem cells both of neuronal and non-neuronal origin are models of interest in the PD area. Differentiated primary cells of neuronal use are especially of interest in the AD field.

Table 3.8: Model systems for Human/patient primary or stem cells model for three disease areas.

Type	AD (n=39)		PD (n=50)		NDD (n=10)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
Co-culture model (multiple cells)	0	0	1	1	0	0
Differentiated human cells: neuronal	12	11	2	2	2	2
Differentiated human cells: non-neuronal CNS	1	1	6	6	1	1
Differentiated human cells: non-neuronal, non-CNS	1	1	0	0	0	0
Multipotent human stem cells: neuronal	1	1	4	4	1	1
Multipotent human stem cells: non-neuronal CNS	0	0	6	6	0	0
Non-differentiated human stem cells: embryonic	4	4	10	10	2	2
Non-differentiated human stem cells: iPSC	20	20	20	20	4	4
Other	0	0	1	1	0	0
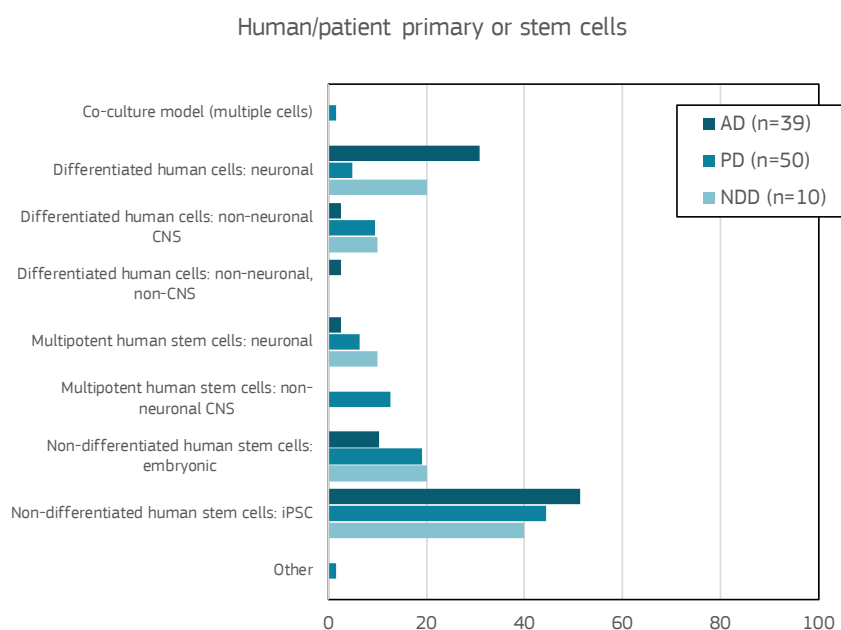
Figure 3.8: Relative distribution of model systems for Human/patient primary or stem cells model (% of models).

3.3.3 Disease feature

The majority of AD models/methods using primary cells or stem cells focused on protein aggregation, while 30% of the studies were rather exploratory with no specific focus on a disease hallmark. Neuro-inflammation, and to lesser extent neuronal loss and energy metabolism, are important subjects for research with the Human/patient primary or stem cells model.

When it comes to PD, the primary and stem cell model research primarily focuses on dopaminergic degeneration, while energy metabolism is of secondary importance as a disease hallmark, followed by protein aggregation. Both in the PD and the NDD group, a significant number of papers (approx. 30%) is exploratory at the level of disease research, or rather focused on model exploration, characterization and optimization (na) instead of considering specific disease features.

Table 3.9:   Disease features for Human/patient primary or stem cells model for three disease areas.

Disease feature	AD (n=39)		PD (n=50)		NDD (n=10)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
Dopaminergic neurodegeneration	0	0	20	19	1	1
Energy metabolism	2	2	10	9	0	0
Exploratory/ no specific feature	12	12	7	4	3	3
Neuronal loss	3	3	1	1	0	0
Neuro-inflammation	6	5	1	1	0	0
Protein aggregation	16	16	7	6	1	1
Not applicable	0	0	17	15	5	5
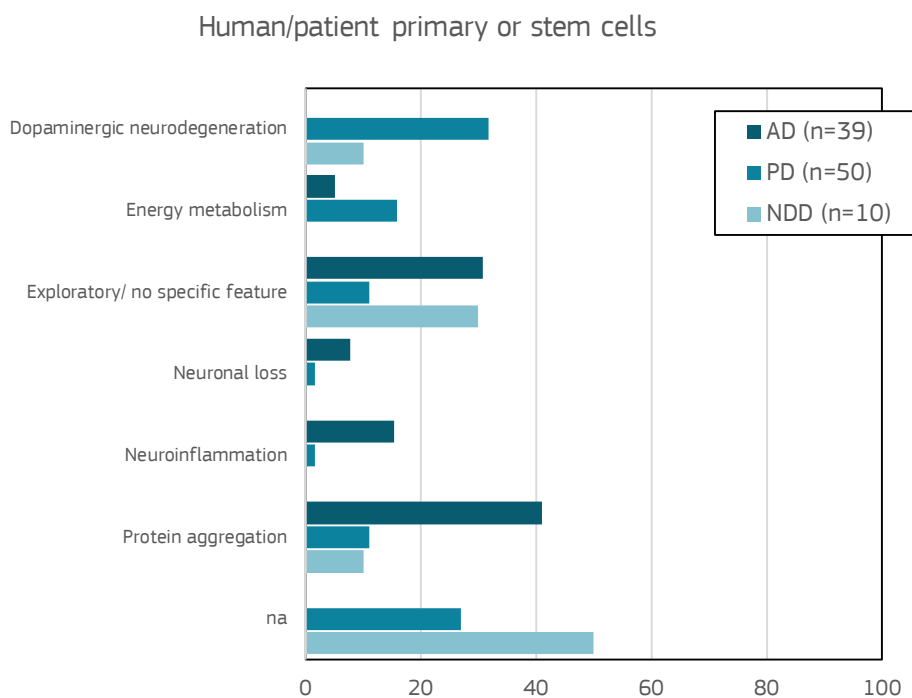
Figure 3.9:   Relative distribution of disease features for Human/patient primary or stem cells model (% of models).

3.3.4 Biological endpoints

A large variety of biological endpoints is simultaneously used, either for characterisation of cellular models upon differentiation or maturation, or at the level of biological responses in relation to disease hallmark (e.g. neurodegeneration). In case of parallel measurements, the category 'multiple' is included for respectively cellular characterisation, or for biological endpoints.

These get the highest scores for AD and PD models, respectively. Other important and specific endpoints in the context of PD are dopaminergic cell functions, electrophysiological measurements, apoptosis and protein aggregation (α-synuclein). Protein aggregation (amyloid) and inflammation are endpoints of high relevance for AD models. In the NDD group, a multitude of biological endpoints is used, but less specific.

Table 3.10: Biological endpoints for Human/patient primary or stem cells model for three disease areas.

Biological endpoints	AD (n=39)		PD (n=50)		NDD (n=10)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
Activation of microglia	1	1	0	0	0	0
Apoptosis/cell death	0	0	6	5	0	0-
Changed gene expression	1	1	0	0	0	0
Dopamine release and/or uptake in cells	0	0	5	5	0	0
Electrophysiological characterisation	0	0	6	6	0	0
Inflammation	5	4	1	1	0	0
Mitochondrial dysfunction	1	1	0	0	0	0
Multiple biological endpoints	19	19	10	8	4	4
Multiple: cell characterisation/ differentiation/maturation	0	0	20	17	4	4
Oxidative/nitrosative stress	2	2	0	0	1	1
Protein dysfunction: amyloid peptide (any version)	6	6	0	0	0	0
Protein dysfunction: APP	3	3	0	0	0	0
Protein dysfunction: α-synuclein	0	0	7	6	1	1
Protein dysfunction: multiple	1	1	0	0	0	0
Protein dysfunction: Tau (phosphorylation)	2	2	0	0	0	0
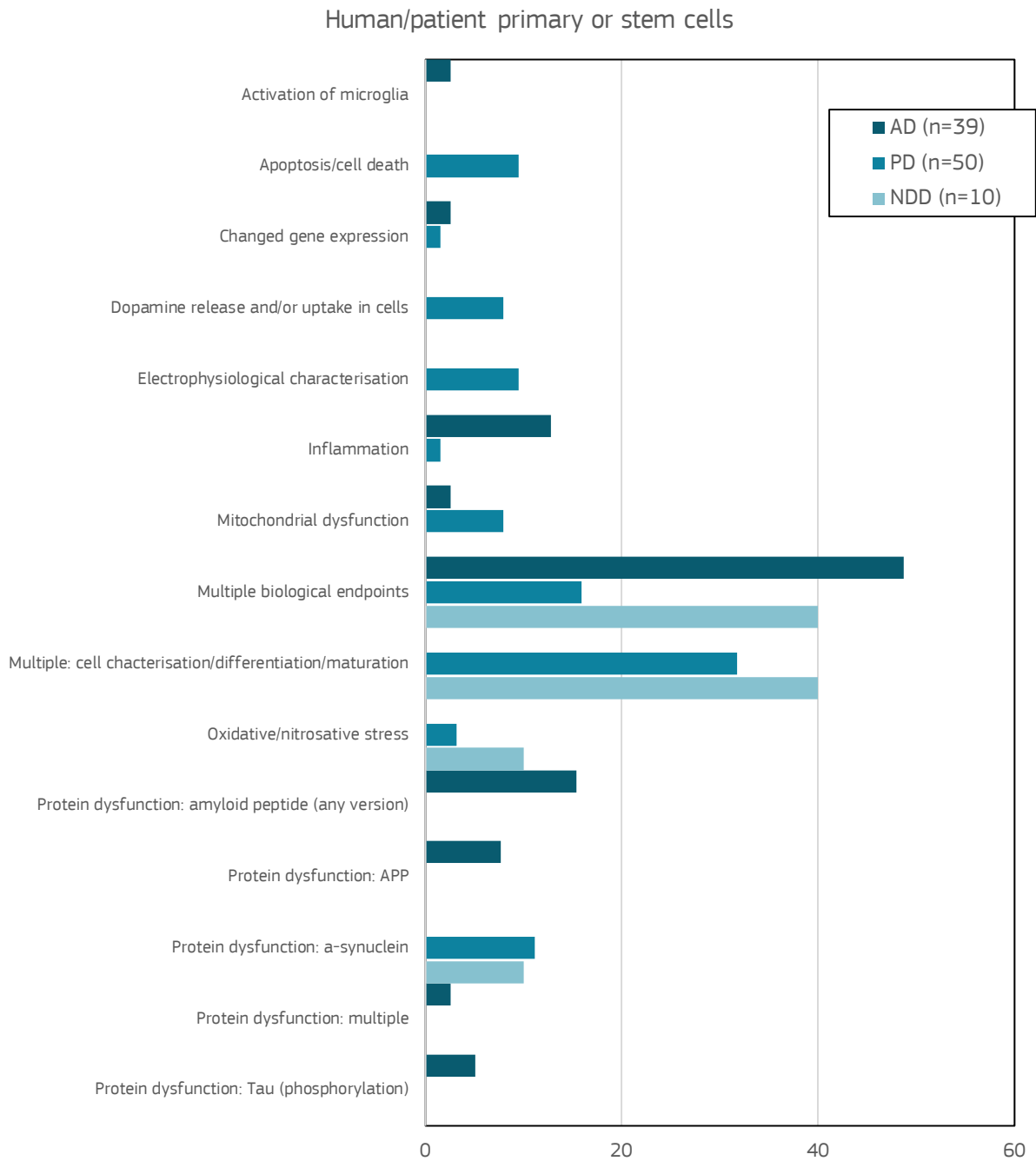
Figure 3.10: Relative distribution of biological endpoints for Human/patient primary or stem cells model (% of models).

▬▬ 3.3.5 *Throughput/content*

This parameter on throughput and content was only included for the paper evaluation of AD, thus no comparison is possible with other areas. These cell models/methods have a throughput that is overall low because of large time efforts to maintain cultures. Content score is low to medium because of the primary nature of cells resembling better physiological conditions compared to immortalised models.

Table 3.11: Throughput/content for Human/patient primary or stem cells model for three disease areas.

Throughput/content	AD (n=39)		PD (n=50)		NDD (n=10)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
High (automatic)/high	0	0	nd	nd	nd	nd
High (automatic)/medium	1	1	nd	nd	nd	nd
High (automatic)/low	0	0	nd	nd	nd	nd
Low-medium/high	1	1	nd	nd	nd	nd
Low-medium/medium	35	34	nd	nd	nd	nd
Low-medium/low	2	2	nd	nd	nd	nd

nd: no data available because this field was added after the evaluation of PD papers was finished.
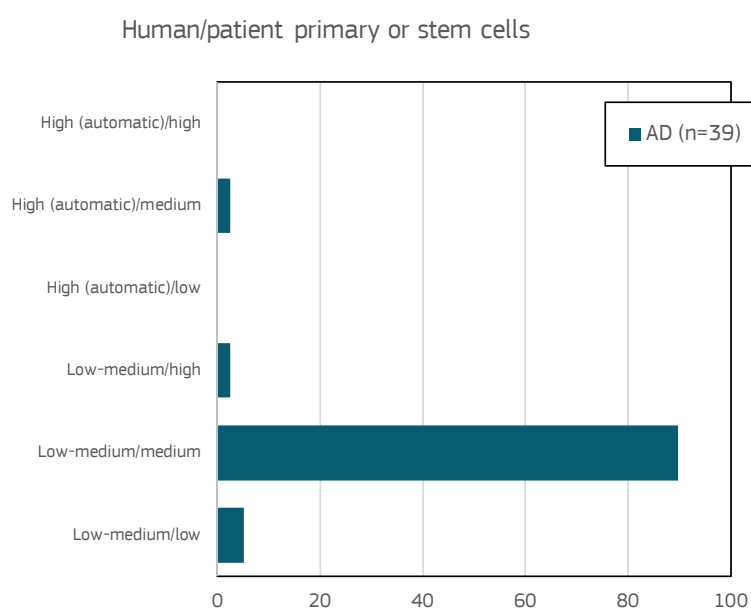
Figure 3.11: Relative distribution of throughput/content for Human/patient primary or stem cells model (% of models).

3.4 Human-derived cell lines

51 publications was retained from full text evaluations of papers, which resulted into respectively 31 relevant models for AD, 14 models for PD and eight models for NDD.

3.4.1 Application/aim

Cell line models are applied to study disease mechanisms, both for AD and for PD. In the area of NDD (with a limited number of models included), the focus in terms of aims is less clear.

Table 3.12: Applications/aims for Human-derived cell lines model for three disease areas.

Application/aim	AD (n=31)		PD (n=14)		NDD (n=8)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
Diagnosis of disease	3	3	0	0	1	1
Disease mechanism (exp/theor)	21	19	8	8	2	2
Disease therapy developm	3	3	1	1	0	0
Drug developm/ testing	0	0	0	0	2	2
Model/method development – experim	1	1	0	0	1	1
Model/method validation	1	1	2	2	1	1
Neuroprotection/neurotoxicity	2	2	3	3	1	1
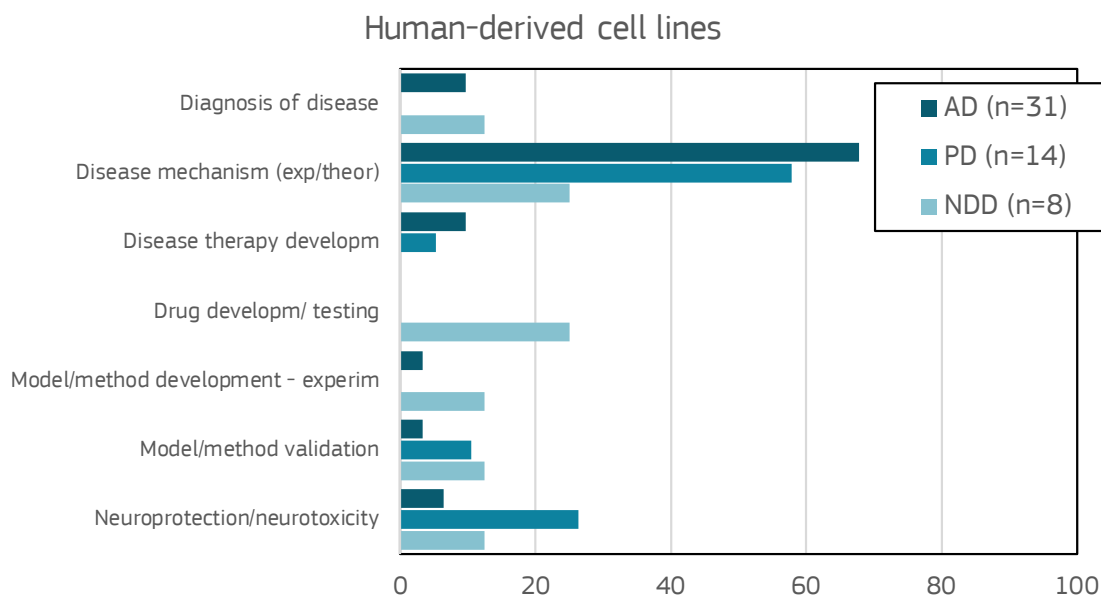
Figure 3.12: Relative distribution of applications/aims for Human-derived cell lines model (% of models).

3.4.2 Model system

It is evident that for brain disease research, the most appropriate immortalised cell line models are those derived from brain or CNS tissue, which is reflected in the high fraction of that model type for the three disease areas (Figure 3.13).

However, in exceptional cases, other non-neuronal, non-CNS models are used as well. It concerns in most cases the development of cell reporter systems used as a detection system, or the study of mutations. An often-used non-neuronal cell line model for such purposes is HEK-293.

Table 3.13: Model systems for Human/patient primary or stem cells model for three disease areas.

Type	AD (n=31)		PD (n=14)		NDD (n=8)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
Co-culture model (multiple cells)	1	1	0	0	0	0
Human-derived immortalised cell lines: neuronal	26	26	12	12	6	6
Human-derived immortalised cell lines: non-neuronal CNS	0	0	2	2	0	0
Human-derived immortalised cell lines: non-neuronal, non CNS	4	4	0	0	2	2

Human-derived cell lines
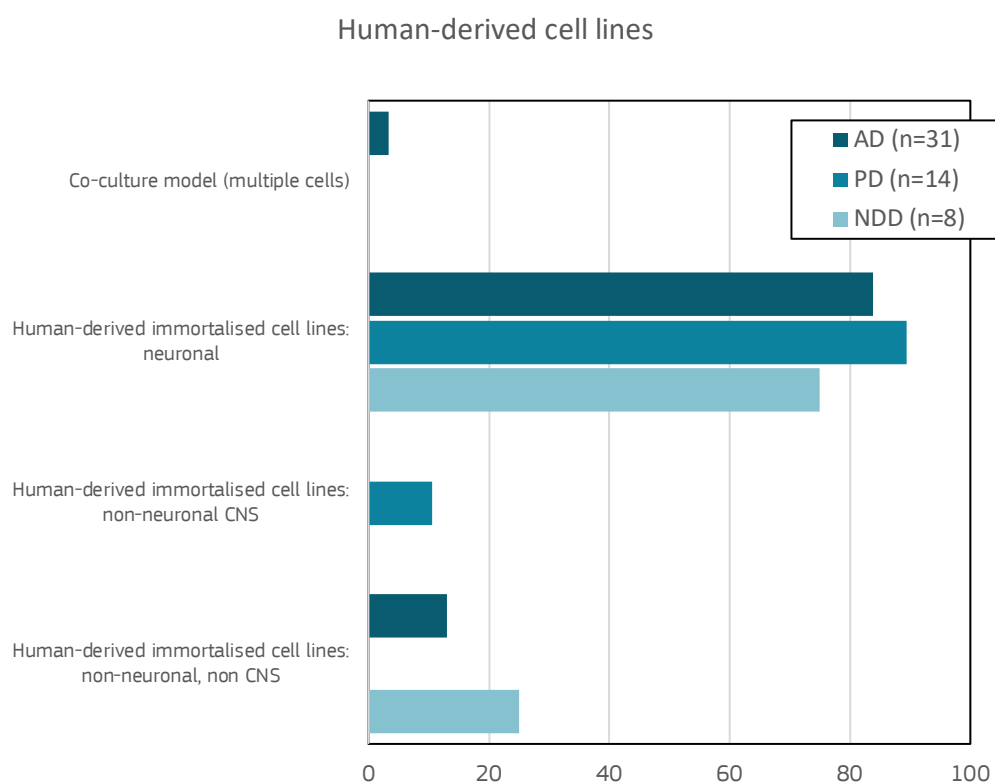
Figure 3.13: Relative distribution of model system for Human-derived cell lines model (% of models).

3.4.3 *Disease feature*

The majority of AD models/methods (55%) using cell line models are focused to protein aggregation studies, while studies on neuro-inflammation (13%), neuronal loss (10%) and exploratory studies (13%) with no specific focus to a disease hallmark, and are less common. In the group of the PD papers, protein aggregation is also one of the main topics. In line with the main hallmarks of PD, dopaminergic neurodegeneration as well as energy metabolism are prominent among the studied disease features when it comes to cellular models. The non-specific character (and limited number of models) of the NDD group makes that a diversity of disease features are of interest for model development.

Table 3.14: Disease features for Human-derived cell lines model for three disease areas.

Disease feature	AD (n=31)		PD (n=14)		NDD (n=8)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
Cholinergic dysfunction/degeneration	2	2	0	0	0	0
Dopaminergic neurodegeneration	0	0	5	5	0	0
Energy metabolism	1	1	7	7	1	1
Exploratory/ no specific feature	4	4	1	1	1	1
Neuronal loss	3	3	1	1	0	0
Neuroinflammation	4	3	0	0	1	1
Protein aggregation	17	16	4	4	4	4
Not applicable	0	0	1	1	1	1
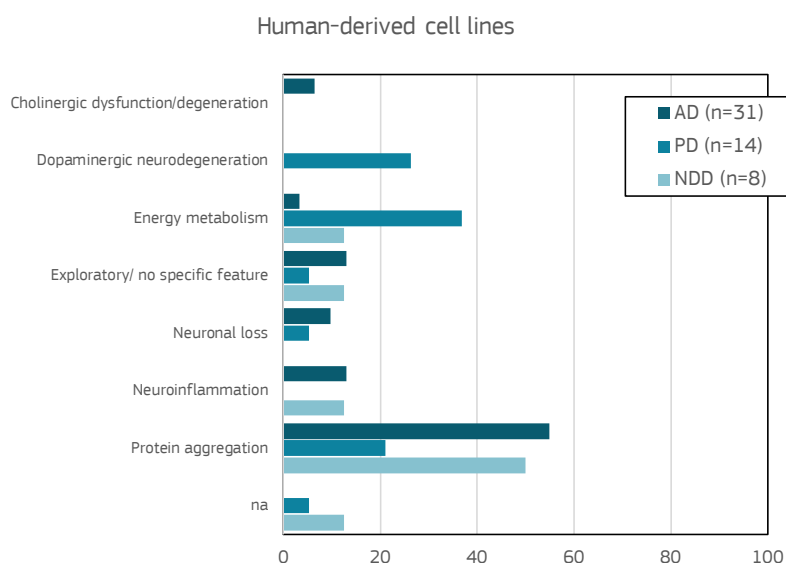
Figure 3.14: Relative distribution of disease features for Human-derived cell lines model (% of models).

3.4.4 *Biological endpoints*

It is clear from the following figure that numerous endpoints are chosen to study cell line models for different disease areas. This variety of endpoints implies that many contribute at about 20% or less. This is next to the grouped biological endpoints 'multiple biological endpoints', which is scored separately for a few papers.

On the other hand, when we group the four last endpoints related to protein aggregation (Table 3.15), then the total number of hits (models/papers) reaches approximately 30 to 40% with amyloid, tau and APP for the AD area, α-synuclein for PD models, and for the NDD group there are hits for tau and α-synuclein.

Many other disease-relevant endpoints (microglia activation, cholinergic function, mitochondrial function) are part of studies with model systems but represent only a handful of models or papers within the same disease area.

Table 3.15: Biological endpoints for Human-derived cell lines model for three disease areas.

Biological endpoints	AD (n=31)		PD (n=14)		NDD (n=18	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
Activation of microglia	1	1	0	0	0	0
BBB (dys-)function	2	2	0	0	0	0
Calcium (dys-)regulation	0	0	0	0	1	1
Changed gene expression	2	1	1	1	0	0
Changed protein expression	2	2	0	0	0	0
Cholinergic signalling/esterases	1	1	0	0	0	0
DNA damage	0	0	1	1	0	0
Impaired autophagy	0	0	1	1	0	0
Inflammation	3	2	0	0	1	1
Metabolomics analysis	0	0	0	0	1	1
Mitochondrial dysfunction	1	1	3	3	0	0
Multiple biological endpoints	3	3	4	4	1	1
Neurodegeneration: hippocampal or other	1	1	0	0	0	0
Oxidative/nitrosative stress	0	0	1	1	1	1
Protein dysfunction: amyloid peptide (any version)	5	5	0	0	0	0
Protein dysfunction: APP	4	4	0	0	0	0
Protein dysfunction: α-synuclein	0	0	4	4	1	1
Protein dysfunction: Tau (phosphorylation)	6	6	0	0	2	2
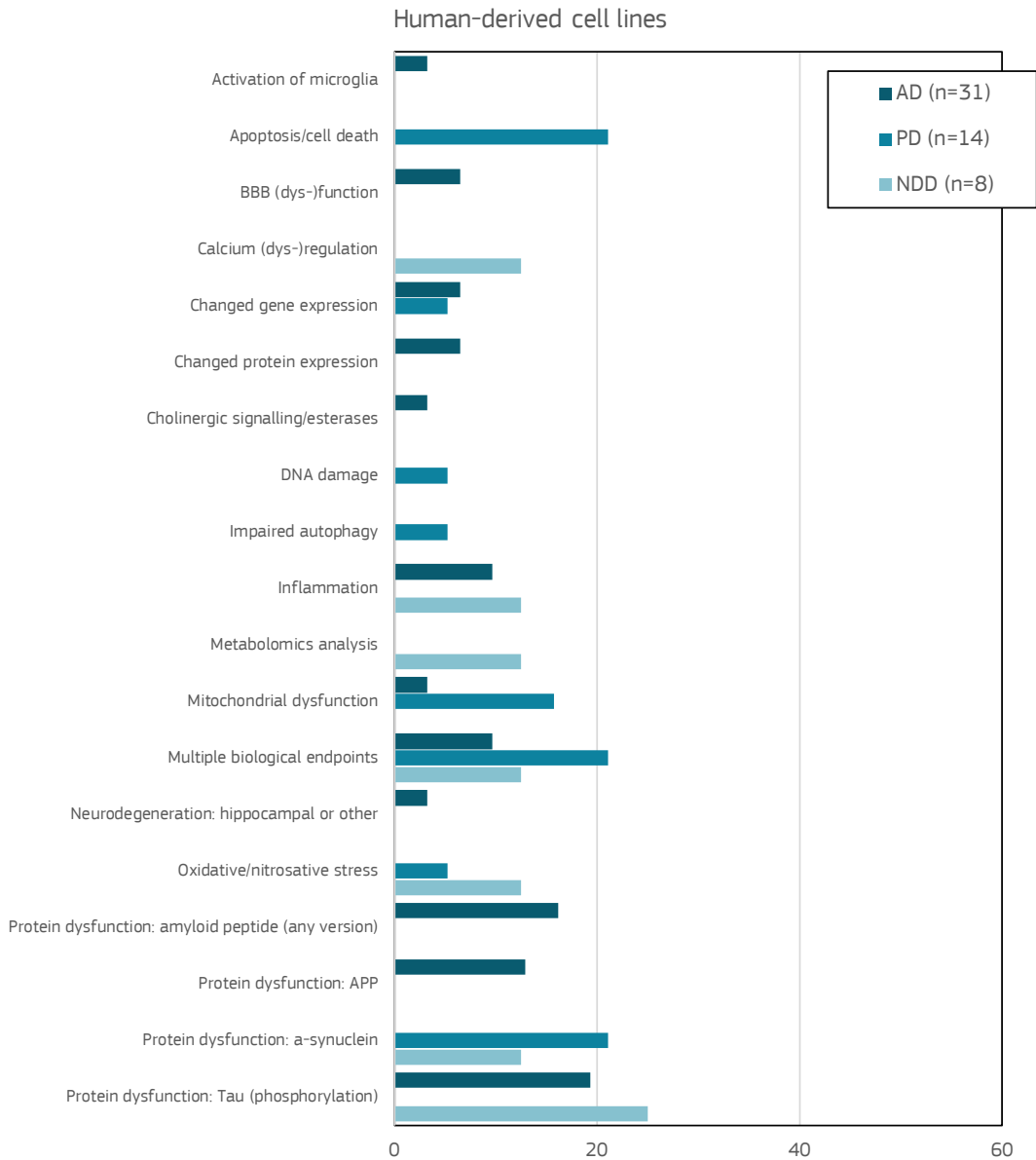
Figure 3.15: Relative distribution of biological endpoints for Human-derived cell lines model (% of models).

▬▬ *3.4.5 Throughput/content*

This parameter on throughput and content was only included for the paper evaluation for AD, thus no comparison is possible with other areas. The throughput of these cell line methods is low to medium, and never fully automated. At the level of content, this model system has poor biological relevance to represent CNS or ND disease specific features. While the current evaluation pertains to AD papers, it holds true for other NDD research areas as well.

Table 3.16: Throughput/content for Human-derived cell lines model for three disease areas.

Throughput/content	AD (n=31)		PD (n=14)		NDD (n=8)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
High (automatic)/high	0	0	nd	nd	nd	nd
High (automatic)/medium	0	0	nd	nd	nd	nd
High (automatic)/low	0	0	nd	nd	nd	nd
Low-medium/high	0	0	nd	nd	nd	nd
Low-medium/medium	6	6	nd	nd	nd	nd
Low-medium/low	25	23	nd	nd	nd	nd

nd: no data available because this field was added after the evaluation of PD papers was finished

Human-derived cell lines
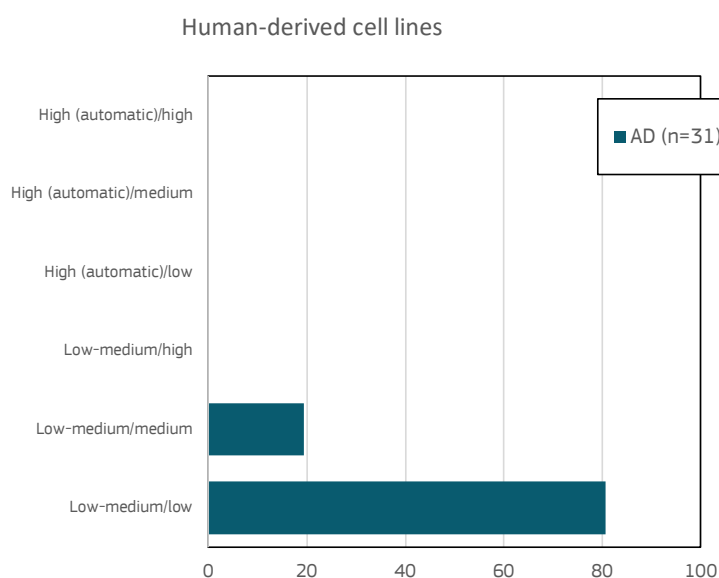
Figure 3.16: Relative distribution of throughput/content for Human-derived cell lines model (% of models).

3.5 2D or 3D co-cultures

22 publications was retained after full-text evaluations of papers, resulting in 23 model systems, with 16 models in the AD area, 3 models in the PD area and 4 models in the NDD area. Taking into account these low numbers, one should be sufficiently critical when evaluating the models in relation to a specific disease area, especially for PD and NDD in general.

3.5.1 *Application/aim*

Considering the most important AD group with sufficient models/papers (n=16), it appears that study of disease mechanisms as well as model/method development are more or less equally important. There is also a hit for disease therapy development (n=3) and study on neuroprotection of neurotoxicity (n=1).

Table 3.17: Applications/aims for 2D or 3D co-culture model for three disease areas.

Application/aim	AD (n=16)		PD (n=3)		NDD (n=4)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
Disease mechanism (exp/theor)	7	7	1	1	1	1
Disease therapy developm	3	3				
Model/method development – experim	4	4	2	1	2	2
Model/method development – theoret	1	1				
Model/method validation					1	1
Neuroprotection/neurotoxicity	1	1	0	0	0	0
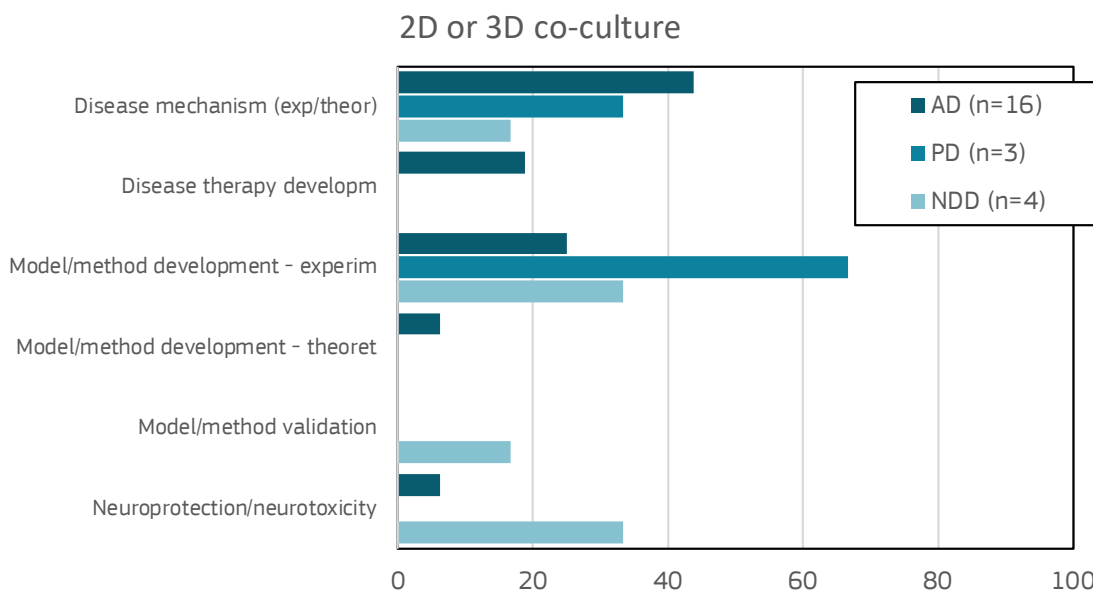
Figure 3.17: Relative distribution of applications/aims for 2D or 3D co-culture model (% of models).

███ *3.5.2 Model system*

In a 2D co-culture or in a 3D model, different cell types or combinations thereof can be used. It will depend on the purpose of the culture model, which type of cells will be included and whether differentiated primary cells, iPSCs or embryonic stem cells, or even immortalised cell line models are part of it.

Table 3.18: Model system for 2D or 3D co-culture model for three disease areas.

Type	AD (n=16)		PD (n=3)		NDD (n=4)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
Co-culture model (multiple cells)	9	9	1	1	1	1
Differentiated human cells: neuronal	2	2	0	0	0	0
Differentiated human cells: non-neuronal, non-CNS	1	1	0	0	0	0
Multipotent human stem cells: neuronal	2	2	0	0	0	0
Neurospheres/3D model	2	2	0	0	1	1
Non-differentiated human stem cells: embryonic	0	0	1	1	0	0
Non-differentiated human stem cells: iPSC	0	0	1	1	2	2
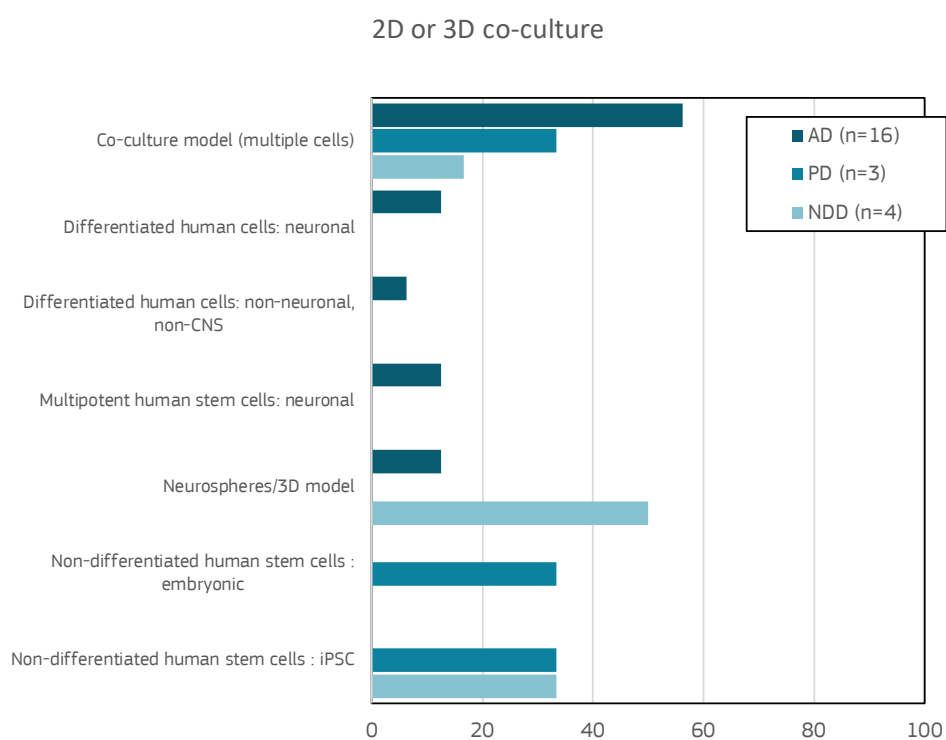
Figure 3.18: Relative distribution of model system for 2D or 3D co-culture model (% of models).

3.5.3 *Disease feature*

One third of AD models/methods using a co-culture or 3D approach are focused on protein aggregation studies (n=6), while other features such as neuroinflammation or energy metabolism are each covered by only one model/paper. The majority of AD co-culture models are still used for exploratory research. The NDD and PD models have an exploratory nature as well, or pertain to developments that even are not associated to disease specific topics. One of the PD models does focus on protein aggregation as well.

Table 3.19: Disease features for 2D or 3D co-culture model for three disease areas.

Disease feature	AD (n=16)		PD (n=3)		NDD (n=4)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
Dopaminergic neurodegeneration	0	0	0	0	1	1
Energy metabolism	1	1	0	0	2	2
Exploratory/ no specific feature	8	8	0	0	3	3
Neuroinflammation	1	1	0	0	0	0
Protein aggregation	6	6	1	1	0	0
Not applicable	0	0	2	1	0	0
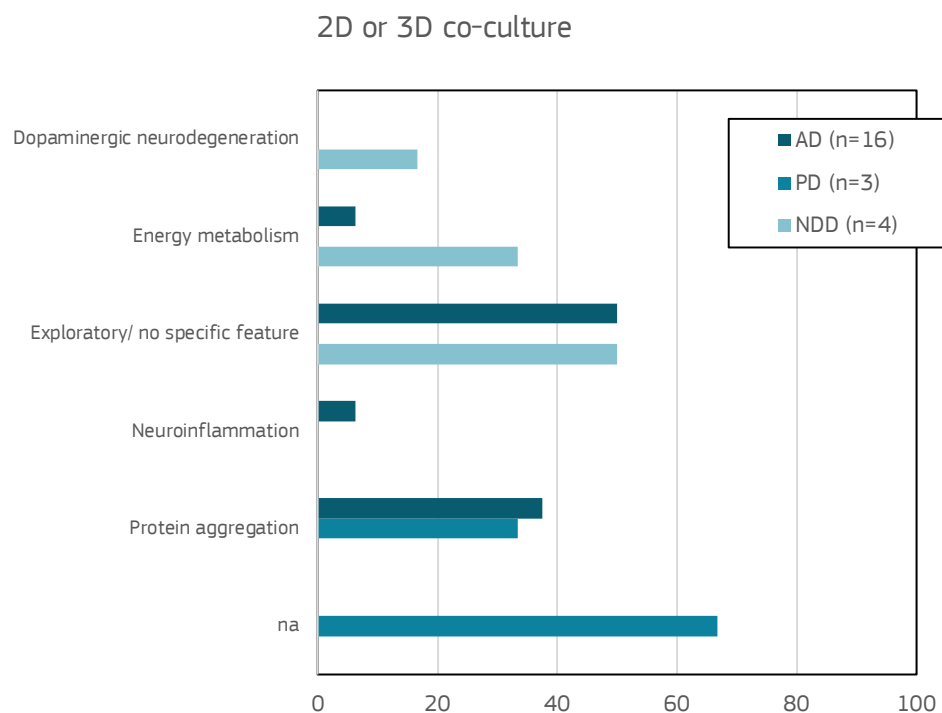
2D or 3D co-culture

Figure 3.19: Relative distribution of disease features for 2D or 3D co-culture model (% of models).

3.5.4 *Biological endpoints*

A limited number of biological endpoints were used, compared to the cellular models covered in category Human/patient primary or stem cells model, or Human-derived cell lines. The AD models are mainly applied in the context of BBB function, or in the context of protein aggregation (amyloid and tau).

The other NDD and PD models also cover aspects in relation to either BBB function or protein aggregation in the context of brain diseases, or more exploratory work including multiple endpoints and approaches for better cell characterisation upon differentiation and maturation.

Table 3.20: Biological endpoints for 2D or 3D co-culture model for three disease areas.

Biological endpoints	AD (n=16)		PD (n=3)		NDD (n=4)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
Apoptosis/cell death	0	0	0	0	1	1
BBB (dys-)function	4	4	0	0	1	1
Changed protein expression	1	1	0	0	0	0
Inflammation	1	1	0	0	0	0
Mitochondrial dysfunction	1	1	0	0	0	0
Multiple biological endpoints	2	2	0	0	0	0
Multiple: cell characterisation/ differentiation/ maturation	0	0	2	1	0	0
Protein dysfunction: amyloid peptide (any version)	5	5	0	0	0	0
Protein dysfunction: α-synuclein	0	0	1	1	0	0
Protein dysfunction: Tau (phosphorylation)	1	1	0	0	0	0
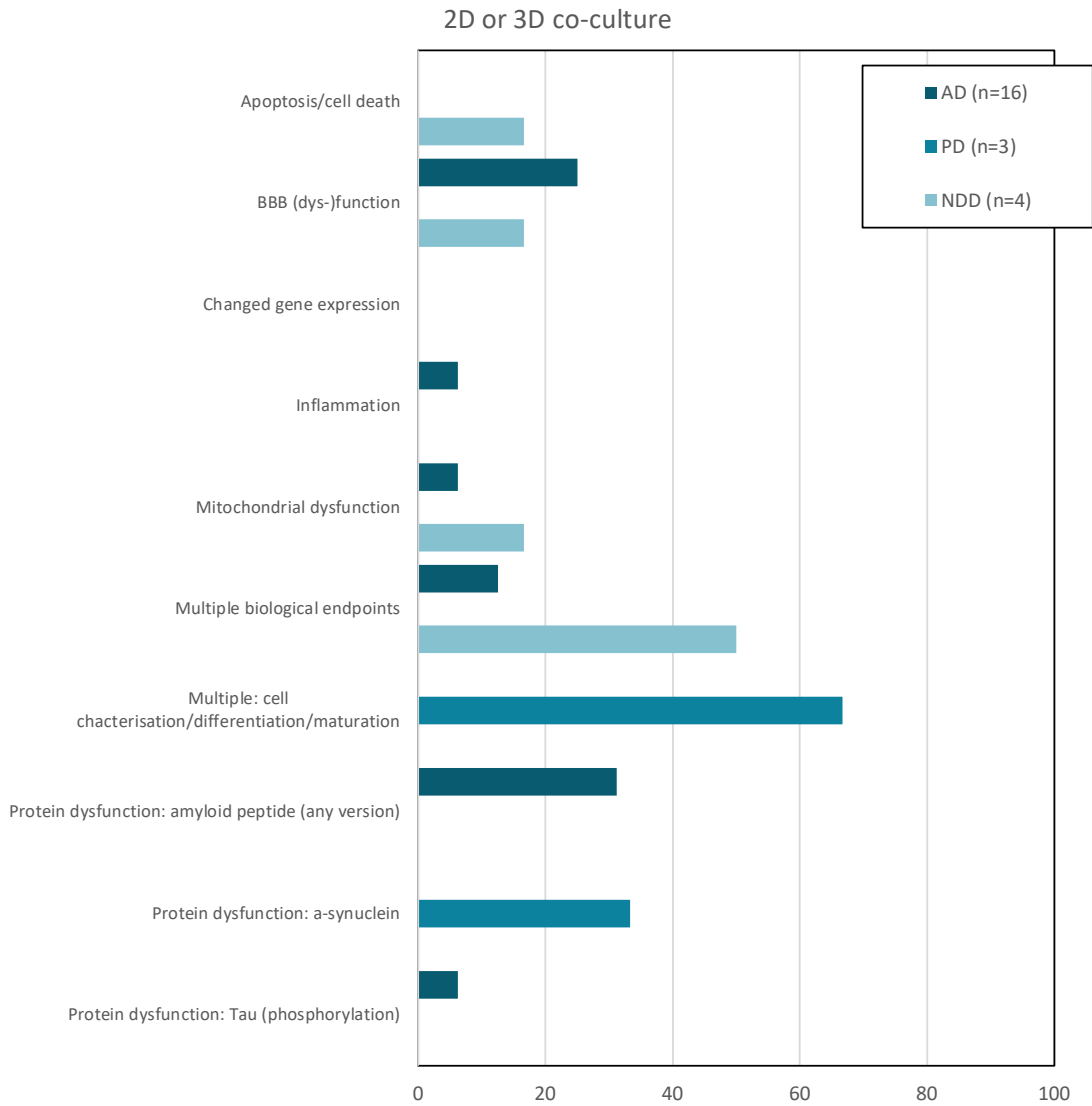
Figure 3.20: Relative distribution of biological endpoints for 2D or 3D co-culture model (% of models).

▬ *3.5.5 Throughput/content*

For the AD relevant models, we conclude that the 3D or co-culture approaches gain on relevance for biological content due to the multiple cellular models/functions and the architectural organisations. However, the throughput for most of these models remains low, except for two models.

Table 3.21: Throughput/content for 2D or 3D co-culture model for three disease areas.

Throughput/content	AD (n=16)		PD (n=3)		NDD (n=4)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
High (automatic)/high	0	0	nd	nd	nd	nd
High (automatic)/medium	2	2	nd	nd	nd	nd
High (automatic)/low	0	0	nd	nd	nd	nd
Low-medium/high	7	7	nd	nd	nd	nd
Low-medium/medium	7	7	nd	nd	nd	nd
Low-medium/low	0	0	nd	nd	nd	nd

nd: no data available because this field was added after the evaluation of PD papers was finished.
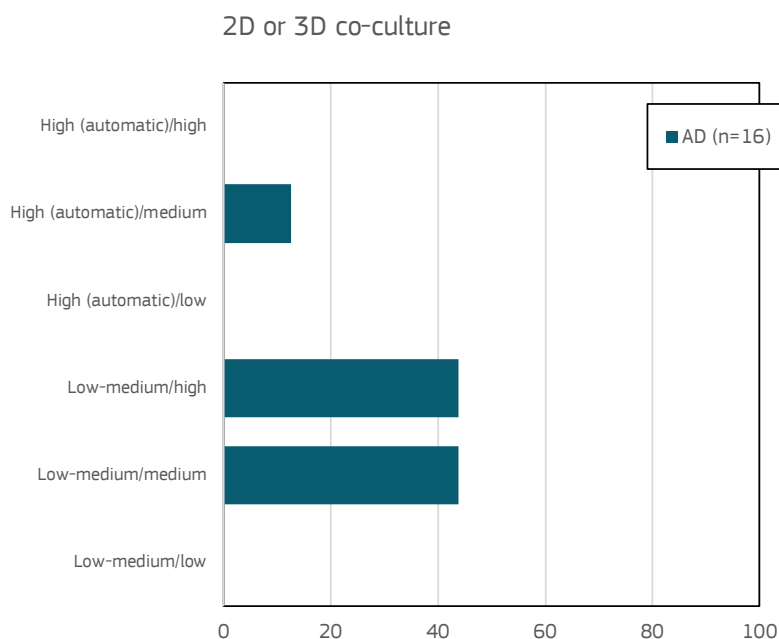
Figure 3.21: Relative distribution of throughput/content for 2D or 3D co-culture model (% of models).

3.6 Organoid model

After full text evaluation, two publications on PD resulted in two relevant models/ methods, and one publication on AD resulted in one relevant model/method. Because of the limited number of models and lack of benefit, the data presentation will be limited to tables for this method category.

3.6.1 Application/aim

Of the Organoid models captured across our entire screening (AD, PD) two were categorised

with the aim of experimental model/method development, and a single model had the application of drug development/testing. Both organoids applied to model development focused on Parkinson's disease and recapitulation of midbrain-like cell constructs. The organoid model categorised under the application of drug development was a useful tool to model the effect of secretase inhibitors on amyloid and tau pathology in AD.

Table 3.22: Applications/aims for Organoid model for two disease areas.

Application/aim	AD (n=1) & PD (n=2)	
	#Models	#Papers
Drug developm/ testing	1	1
Model/method development – experim	2	2

3.6.2 Model system

All these models were derived from stem cells, however, a different type of stem cell

was used for each model, highlighting the use of embryonic stem cells (n=1 for PD), neuroepithelial stem cells (n=1 for PD) and iPSCs (n=1 for AD).

Table 3.23: Model system for Organoid model for two disease areas.

Type	AD (n=1) & PD (n=2)	
	#Models	#Papers
Multipotent human stem cells: neuronal	1	1
Non-differentiated human stem cells: embryonic	1	1
Non-differentiated human stem cells: iPSC	1	1

3.6.3 Disease feature

The organoid disease features were defined as dopaminergic neurodegeneration (n=2 for PD) and protein aggregation (n=1 for AD). These model disease features reflect the pathological features recapitulated in organoids associated with the disease state of PD and AD, respectively.

Table 3.24: Disease features for Organoid model category for two disease areas.

Disease feture	AD (n=1) & PD (n=2)	
	#Models	#Papers
Dopaminergic neurodegeneration	2	2
Protein aggregation	1	1

3.6.4 Biological endpoints

The biological or disease endpoints described for the organoid models were related to dopaminergic neurodegeneration in substantia nigra and protein dysfunction. The measurement of these target disease features was performed by methods including (non-exhaustively) fluorescence-activated cell sorting (FACS) analysis, and quantification of protein aggregation.

Table 3.25: Biological endpoints for Organoid model for two disease areas.

Biological endpoint	AD (n=1) & PD (n=2)	
	#Models	#Papers
Dopaminergic neurodegeneration in substantia nigra	2	2
Protein dysfunction: multiple	1	1

3.6.5 Throughput/content

Throughput/content was assessed only for models tagged during the AD phase of the project (1 of 3 organoid models). For organoid models, the amount of labour and time required to generate functional models limits the ability to produce high-throughput assays. However, the model classified here is high content, reflecting the value of complex self-patterning organoids.

Table 3.26: Throughput/content for Organoid model for two disease areas.

Throughput/content	AD (n=1) & PD (n=2)	
	#Models	#Papers
Low-medium/high	1	1

3.7 Biochemical or cell-free assays

97 publications was retained from full text evaluations of papers, which resulted in 98 model systems, with 65 models in the AD area, 16 models in the PD area and 17 models in the other NDD area.

3.7.1 Application/aim

The majority of models/methods (n=29, 45%) in the AD area were focused on generating greater insight into the disease mechanism, followed by experimental model development, disease therapy development, diagnosis of disease, drug development and testing and theoretical model development.

The majority of the applications/aims of the methods for Parkinson's disease (n=14, 87.5 %) was used to improve our current understanding of the disease mechanism (both with a more experimental or theoretical approach). Instead, only in 12.5 % (n=2) of the applications/aims the major focus was given to the development of new therapies.

Also for non-disease focused methods, the large majority of methods (n=12, 71%) were focused on generating greater insight into the disease mechanism.

Only a single (or two) methods were aimed at disease diagnosis, therapy development, experimental model development or model validation.

Table 3.27: Applications/aims for Biochemical or cell-free assay for three disease areas.

Application/aim	AD (n=65)		PD (n=16)		NDD (n=17)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
Diagnosis of disease	6	6	0	0	2	2
Disease mechanism (exp/theor)	29	29	14	14	12	12
Disease therapy developm	11	11	2	2	1	1
Drug developm/ testing	3	3	0	0	0	0
Model/method development – experim	15	14	0	0	1	1
Model/method development – theoret	1	1	0	0	0	0
Model/method validation	0	0	0	0	1	1

Biochemical/cell-free assay
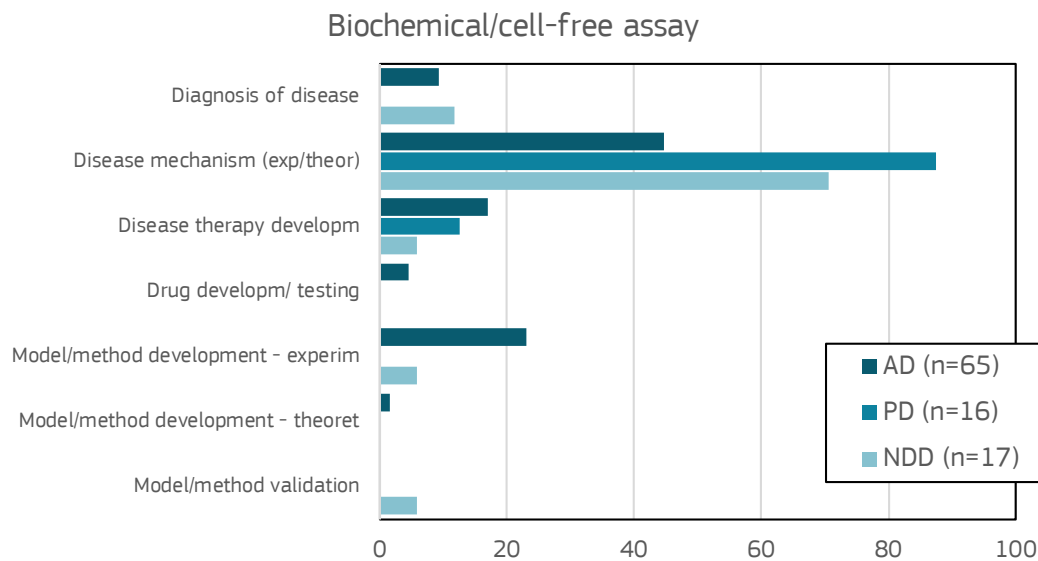
Figure 3.22: Relative distribution of applications/aims for Biochemical or cell-free assay model (% of models).

3.7.2 Model system

Biochemical and cell-free methods and models to study AD primarily focus on different protein species including, peptides, oligomers, fibrils and proteins. This is also reflected in the graph on disease features (see below), from which it is clear that nearly all methods are focused on protein aggregation.

In almost half of the methods evaluated for PD (43.75%, n=7) the main material/model type of interest were proteins. The remaining 56.25% (n=9) was instead divided as follows. In 18.75% of the publications, a new/existing polymer/hydrogel was studied to identify its potential use in non-animal methods for a better understanding of the disease mechanisms or therapy developments for PD. The remaining 37.5% (n=6) was instead focused on 2 more specific disease features like fibrils (25%, n=4) or oligomers (12.5%, n=2).

Both the model type, as well as disease feature (see below) attributed to the methods for NDD in general do underscore the large focus on protein aggregation of this group of methods. Not surprisingly, since this is a common aspect of all neurodegenerative diseases.

Table 3.28: Model system for Biochemical or cell-free assay model for three disease areas.

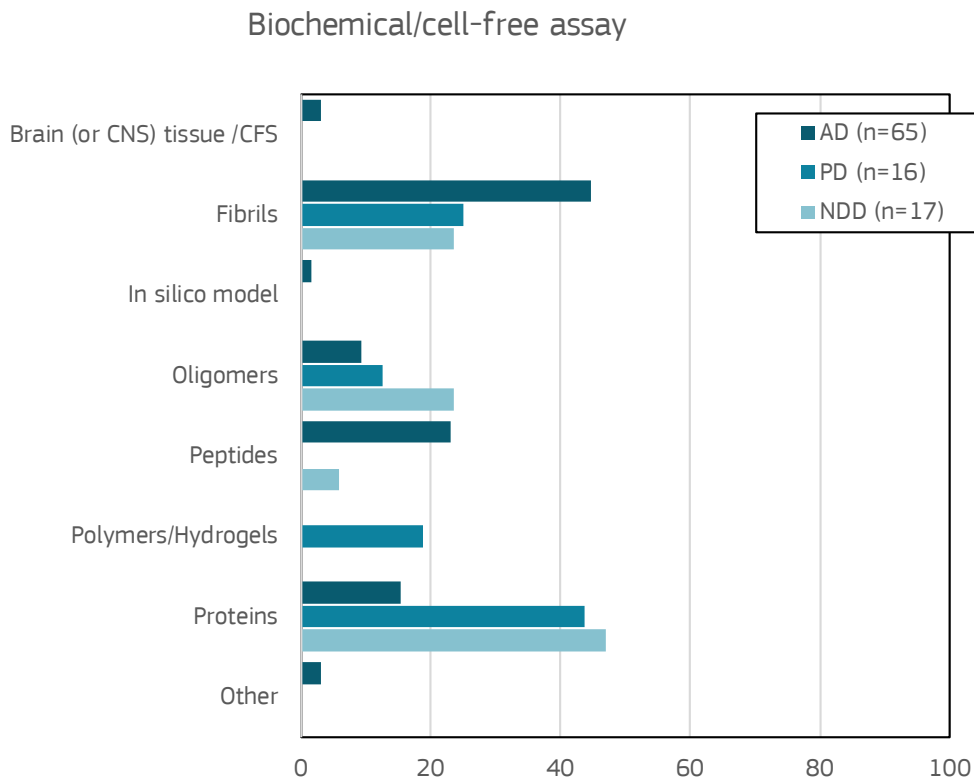| Type	AD (n=65)		PD (n=16)		NDD (n=17)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
Brain (or CNS) tissue/CFS	2	2	0	0	0	0
Fibrils	29	29	4	4	4	4
In silico model	1	1	0	0	0	0
Oligomers	6	6	2	2	4	4
Peptides	15	15	0	0	1	1
Polymers/Hydrogels	0	0	3	3	0	0
Proteins	10	10	7	7	8	8
Other	2	2	0	0	0	0

Figure 3.23: Relative distribution of model system for Biochemical or cell-free assay model (% of models).

3.7.3 *Disease feature*

Overall, there is a large focus on protein aggregation across the evaluated research fields. For PD, interestingly, these publications also correspond to the 87.5% whose application focused on the understanding of the disease mechanisms. It can therefore be underlined how the majority of the protein aggregation studies in biochemical/cell-free assays is devoted to better understand the mechanisms of development and action of PD (and to a similar extent, this is also true for AD).

The remaining papers (12.5%, n=2) are instead focused on dopaminergic neurodegeneration and their main application/aim is instead related to the development of new therapies for PD.

Table 3.29: Disease features for Biochemical or cell-free assay model for three disease areas.

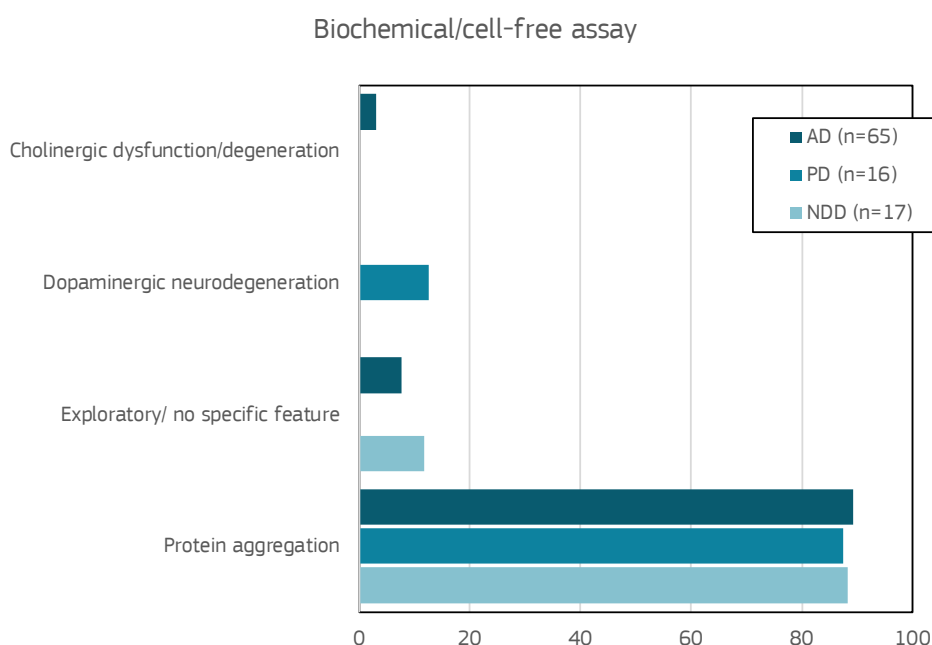| Disease feature	AD (n=65)		PD (n=16)		NDD (n=17)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
Cholinergic dysfunction/degeneration	2	2	0	0	0	0
Dopaminergic neurodegeneration	0	0	2	2	0	0
Exploratory/ no specific feature	5	5	0	0	2	2
Protein aggregation	58	57	14	14	15	15

Figure 3.24: Relative distribution of disease features for Biochemical or cell-free assay model (% of models).

▬▬ 3.7.4 Biological endpoints

When evaluating the biological endpoints in the AD field, it is again clear that the inventoried biochemical methods focused on protein aggregation, namely amyloid β and tau.

The same is true for the methods evaluated in the PD field. Of the 14 models focused on protein dysfunction, only one method includes multiple protein dysfunction features while all the remaining methods give major focus to α-synuclein. These papers have as main application the better understanding of the disease mechanisms. The remaining 12.5% of the methods (n=2) are instead either focused on dopamine dysregulation or, more specifically, on dopaminergic neurodegeneration in *substantia nigra* therefore again overlapping with the methods mainly focused on the development of new therapies for PD.

The same theme returns when looking at biological endpoints for the NDD area methods: amyloid β, α-synuclein, tau or a combination thereof are the prime focus of these methods.

Table 3.30: Biological endpoints for Biochemical or cell-free assay model for three disease areas.

Biological endpoints	AD (n=65)		PD (n=16)		NDD (n=17)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
Cholinergic signalling/esterases	2	2	0	0	0	0
Dopamine dysregulation	0	0	1	1	0	0
Dopaminergic neurodegeneration in substantia nigra	0	0	1	1	0	0
Inflammation	1	1	0	0	0	0
Multiple biological endpoints	2	2	0	0	0	0
Neurodegeneration: hippocampal or other	1	1	0	0	0	0
Protein dysfunction: amyloid peptide (any version)	44	44	0	0	1	1
Protein dysfunction: α-synuclein	0	0	13	13	7	7
Protein dysfunction: multiple	1	1	1	1	7	7
Protein dysfunction: Tau (phosphorylation)	9	9	0	0	2	2
Not applicable	1	1	0	0	0	0
Other	1	1	0	0	0	0
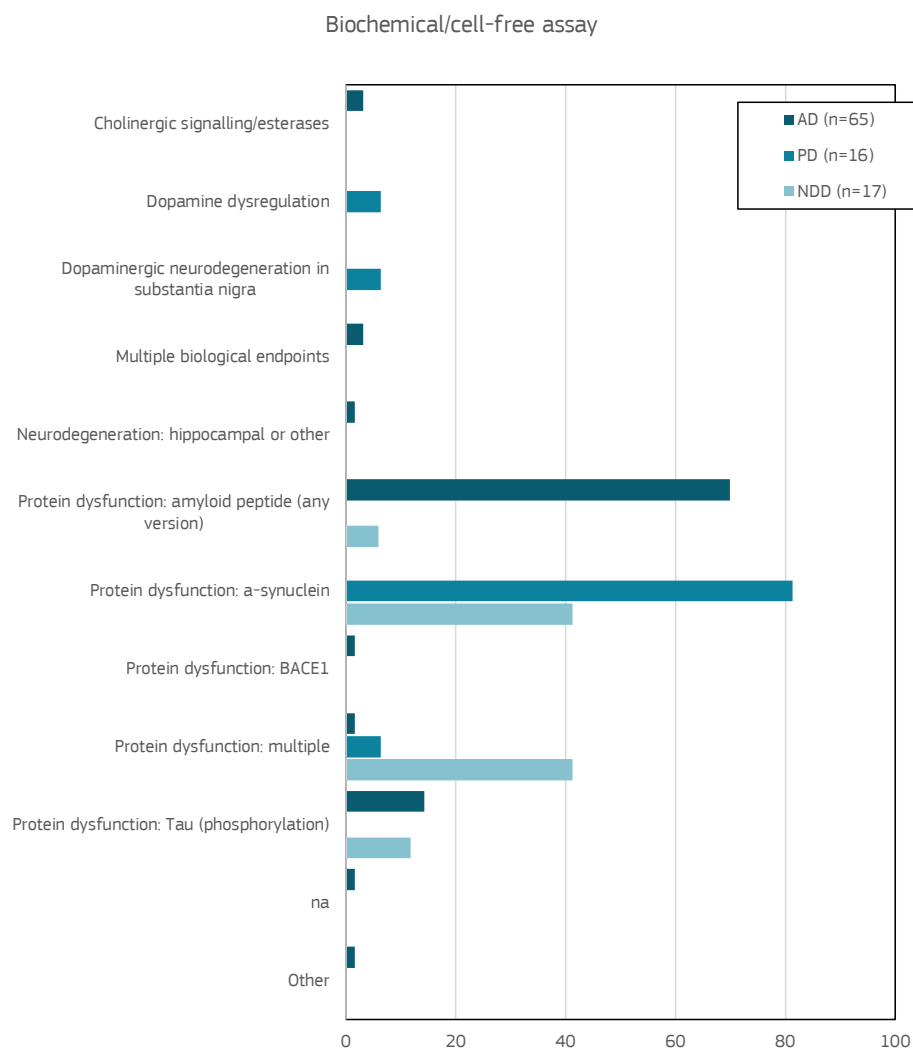
Figure 3.25: Relative distribution of biological endpoints for Biochemical or cell-free assay model (% of models).

3.7.5 Throughput/content

Compared to other categories (e.g. *ex vivo* material), the evaluated biochemical methods are more often high-throughput (automated; n=15); however, the content remains low in the majority of cases (n=56), or maximum medium (n=7), which is a limitation of these methods.

Table 3.31: Throughput/content for Biochemical or cell-free assay model for three disease areas.

Throughput/content	AD (n=65)		PD (n=16)		NDD (n=17)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
High (automatic)/high	0	0	nd	nd	nd	nd
High (automatic)/medium	2	2	nd	nd	nd	nd
High (automatic)/low	13	13	nd	nd	nd	nd
Low-medium/high	0	0	nd	nd	nd	nd
Low-medium/medium	5	5	nd	nd	nd	nd
Low-medium/low	44	43	nd	nd	nd	nd

nd: no data available because this field was added after the evaluation of PD papers was finished.
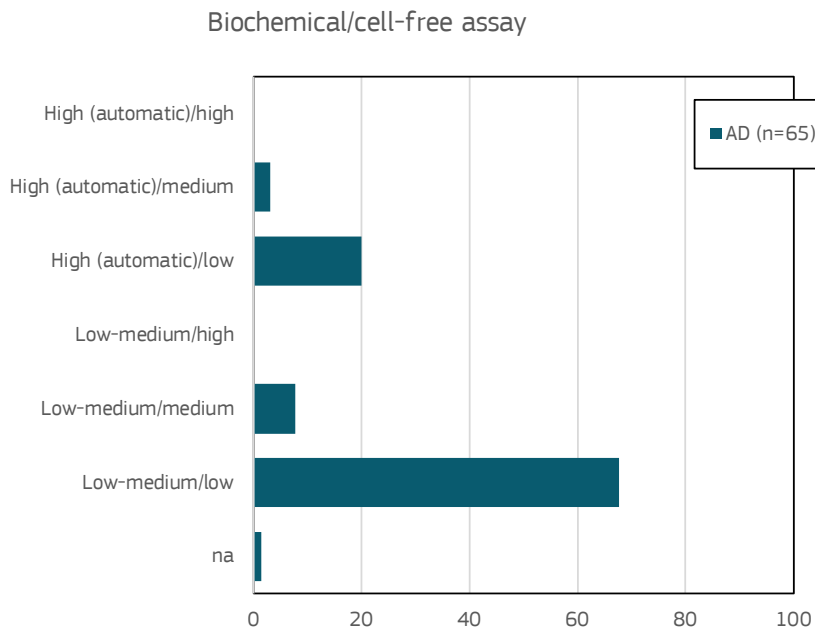

Figure 3.26: Relative distribution of throughput/content for Biochemical or cell-free assay model (% of models).

3.8 Lab/brain on chip or microfluidic systems

51 publications was retained after full text evaluations of papers, resulting in 51 model systems, with 40 models in the AD area, 2 models in the PD area and 9 models in the NDD area. Taking into account these low numbers, one should be sufficiently critical when evaluating the models in relation to a specific field of disease, especially for PD and NDD in general.

3.8.1 Application/aim

A majority of the inventoried methods for AD (n=18) were focused on diagnosis of disease, reflecting the abundance of research regarding the use of amyloid β (see biological or disease-specific endpoint) as a disease indicator.

A significant number of models (n=10) were categorised as devices focused on investigating specific disease mechanisms of AD, enabled by new device geometries and/or features. Some models (n=6) were designed to be used as tools to aid in drug development and testing, generally allowing high-throughput screening of potential drug candidates.

For models in the area of PD or NDD in general, the most frequently categorised application/aim focused on exploring disease mechanism (n=3 for NDD, n=2 for PD), emphasising the use of physical devices to probe cellular mechanisms and protein interactions associated with PD and NDDs.

The application category of experimental model/method development (n=3 for Several NDD) emphasises the use of lab on chip devices to create *in vitro* cell models. For models pertaining to several NDDs, publications were also categorised under the application of 'Diagnosis of disease' (n=2) and as 'Model/method validation' (n=1).

Table 3.32: Applications/aims for Lab/brain on chip or microfluidic systems model for three disease areas.

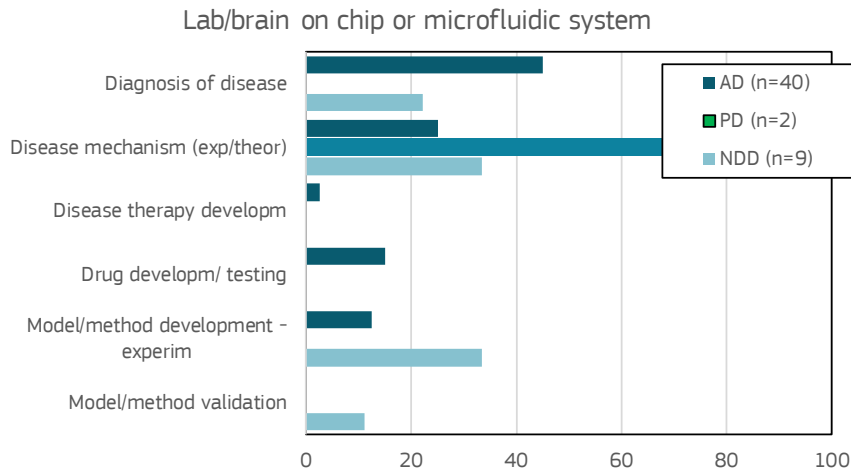| Application/aim	AD (n=40)		PD (n=2)		NDD (n=9)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
Diagnosis of disease	18	18	0	0	2	2
Disease mechanism (exp/theor)	10	10	2	2	3	3
Disease therapy developm	1	1	0	0	0	0
Drug developm/ testing	6	6	0	0	0	0
Model/method development – experim	5	5	0	0	3	3
Model/method validation	0	0	0	0	1	1

Figure 3.27: Relative distribution of applications/aims for Lab/brain on chip or microfluidic systems model (% of models).

3.8.2 Model system

For AD, the biology of lab-on-chip and microfluidic systems varied widely, non-exhaustively including synthesised proteins (n=23), co-culture models (n=4) and others (n=4). Some models in the categories 'co-culture model' or 'other' were included that featured the use of non-human cells but with the design-intent of being a universal tool, additionally applicable to the study of human cells. Next to that, a minority of papers mention the use of human-derived cell models.

When looking at models in the PD and NDD area, the biological component of the model was identified most often as proteins (n=4 for NDD), Dopamin. Neuronal precursor cell line (n=2 for NDD), co-culture models (n=1 for NDD, n=2 for PD), fibrils (n=1 for NDD) or 'other' (n=1 for NDD). In case of the latter, primary rat neurons were used, however, this method was nevertheless included because the microfluidic device developed allows visualization of mitochondria transport along axon, intended to be applicable to human cells.

Table 3.33: Model system for Lab/brain on chip or microfluidic systems model for three disease areas.

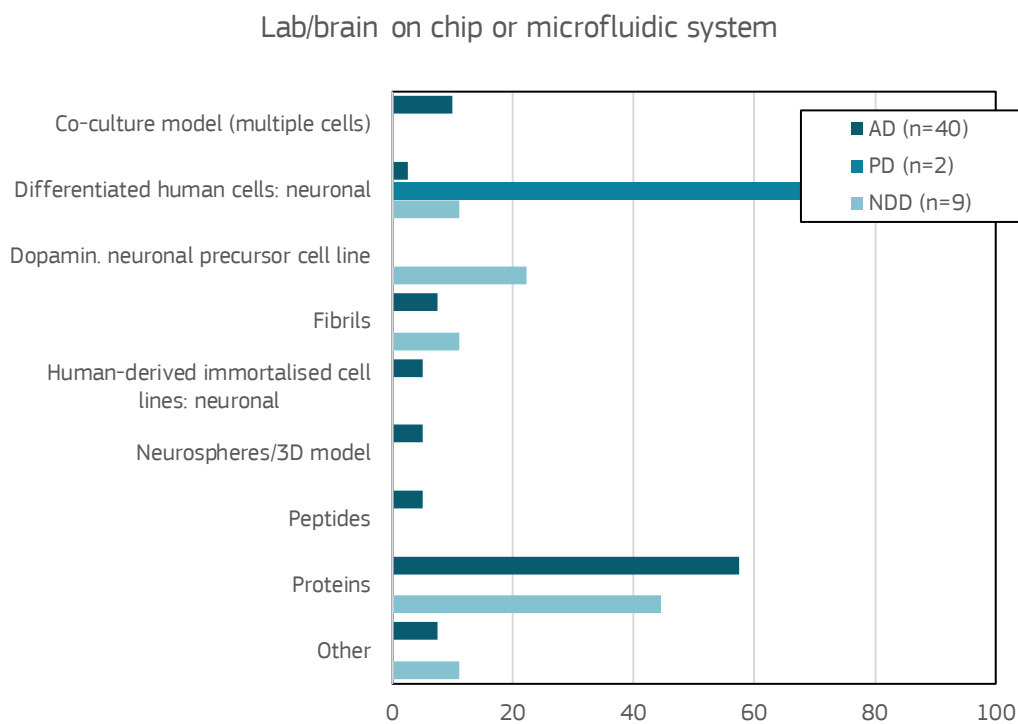| Type	AD (n=40)		PD (n=2)		NDD (n=9)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
Co-culture model (multiple cells)	4	4	0	0	0	0
Differentiated human cells: neuronal	1	1	2	2	1	1
Dopamin. neuronal precursor cell line	0	0	0	0	2	2
Fibrils	3	3	0	0	1	1
Human-derived immortalised cell lines: neuronal	2	2	0	0	0	0
Neurospheres/3D model	2	2	0	0	0	0
Peptides	2	2	0	0	0	0
Proteins	23	23	0	0	4	4
Other	3	3	0	0	1	1

Figure 3.28: Relative distribution of model system for Lab/brain on chip or microfluidic systems model (% of models).

3.8.3 *Disease feature*

An overwhelming majority of the AD models were categorised as focused on protein aggregation as a disease feature (n=32). Again, this reflects the emphasis of research on amyloid β aggregation, as well as the general importance of protein aggregation in definition of AD pathology.

The model disease features for the methods focusing on several NDD were relatively evenly split between Dopaminergic neurodegeneration (n=2), exploratory (n=3), and protein aggregation (n=3). The Several NDD model marked as 'na' was marked as such because the publication does not contain a specific disease feature but is instead a universal method of microfluidic construction for the development of neuronal cell networks.

For PD, disease features were classified as neuroinflammation (n=1) and protein aggregation (n=1).

Table 3.34: Disease features for Lab/brain on chip or microfluidic systems model for three disease areas.

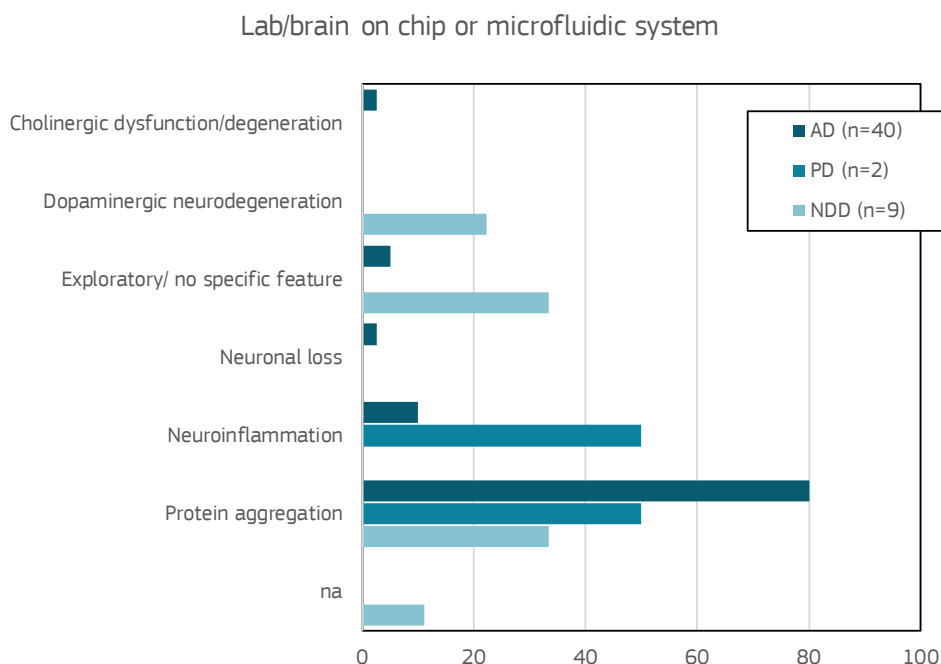| Disease feature	AD (n=40)		PD (n=2)		NDD (n=9)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
Cholinergic dysfunction/degeneration	1	1	0	0	0	0
Dopaminergic neurodegeneration	0	0	0	0	2	2
Exploratory/ no specific feature	2	2	0	0	3	3
Neuronal loss	1	1	0	0	0	0
Neuroinflammation	4	4	1	1	0	0
Protein aggregation	32	32	1	1	3	3
Not applicable	0	0	0	0	1	1

Figure 3.29: Relative distribution of disease features for Lab/brain on chip or microfluidic systems model (% of models).

3.8.4 Biological endpoints

The biological or disease endpoints described by this models applied in the AD field vary widely. In the context of the largest category of application (diagnosis of disease), the endpoints tended to be amyloid β concentration defined by a variety of means.

In the area of PD and NDD, the biological or disease endpoints described by these models vary widely but included, non-exhaustively tau and amyloid β dysfunction, oxidative stress, and mitochondrial dysfunction.

3.8.5 Throughput/content

Overall, compared with other model types, Lab/brain on chip and microfluidic models tend to

be better suited, or intentionally designed, to high throughput applications. Despite this, only a single inventoried AD model was identified as high throughput/high content. Combined, high throughput/medium content and high throughput/low content accounted for more than half of these categorised models for AD.

For lab/brain on chip models applied to several NDD and PD applications, the devices tended to be high throughput. For Several NDD, four of the five models were ranked as High throughput but with varying content levels, a single model was marked as low/medium throughput and low content, as it detailed a non-automated device for a limited content study of fibril growth rate. For PD, the two models were captured as high throughput and medium content.

Table 3.35: Throughput/content for Lab/brain on chip or microfluidic systems model for three disease areas.

Throughput/content	AD (n=65)		PD (n=16)		NDD (n=17)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
High (automatic)/high	1	1	nd	nd	nd	nd
High (automatic)/medium	12	12	nd	nd	nd	nd
High (automatic)/low	13	13	nd	nd	nd	nd
Low-medium/high	1	1	nd	nd	nd	nd
Low-medium/medium	8	8	nd	nd	nd	nd
Low-medium/low	5	5	nd	nd	nd	nd

nd: no data available because this field was added after the evaluation of PD papers was finished.
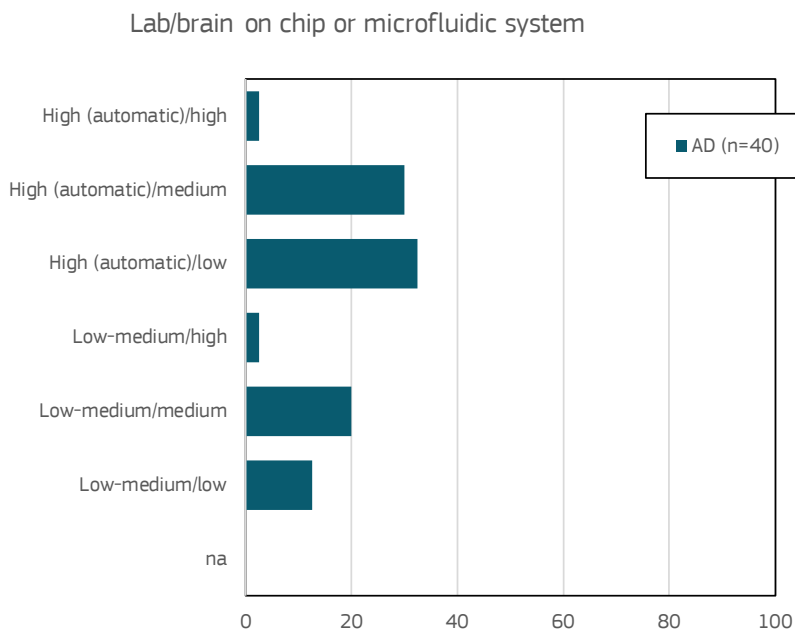

Figure 3.30: Relative distribution of throughput/content for Lab/brain on chip or microfluidic systems model (% of models).

3.9 Computational (*in silico*) models

98 publications was retained after full text evaluations of papers, resulting in 98 *in silico* or computational model systems, with 69 models in the AD area, 23 models in the PD area and 6 models in the NDD area.

3.9.1 Application/aim

The majority of *in silico* models were categorised with the aim of 'disease mechanism (exp/theor)' (n=40 for AD, n=9 for PD, n=5 for NDD). Here, the disease mechanism tends to be purely theoretical – the models include atomic, molecular, or protein interactions

using computer modelling. The second largest categorisation 'model/method development' (n=11 for AD, n=9 for PD), points to the ability of *in silico* methods in building models that encompass a significant portion of AD or PD pathology and used for further study.

A significant number of AD models were developed with the aim of either 'disease therapy development'(n=9) or drug development (n=6), pointing to the usefulness of *in silico* models to aid in early screening of potential drug candidates. In a few instances, computational methods in the PD area were applied for the evaluation/development of drugs as well (n=3).

Table 3.36: Applications/aims for Computational (*in silico*) model for three disease areas.

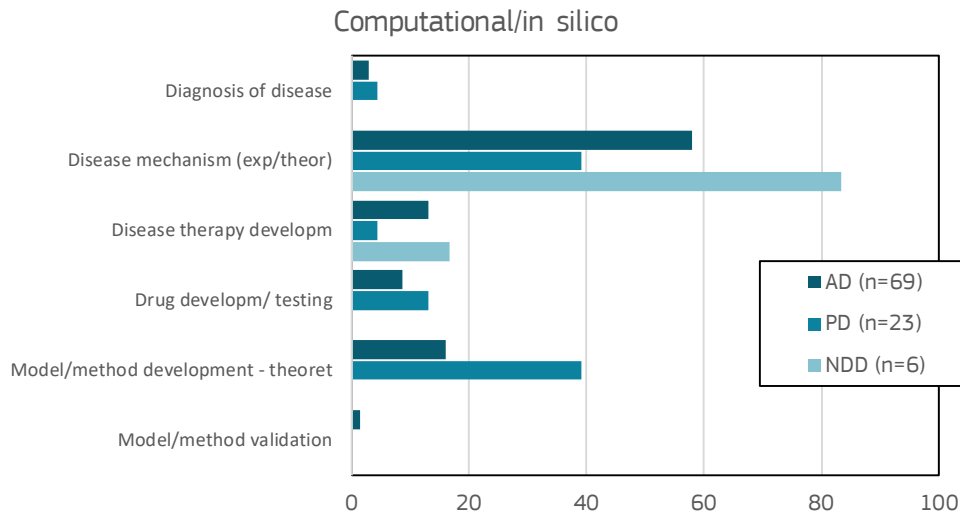| Application/aim	AD (n=69)		PD (n=23)		NDD (n=6)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
Diagnosis of disease	2	2	1	1	0	0
Disease mechanism (exp/theor)	40	40	9	9	5	5
Disease therapy developm	9	9	1	1	1	1
Drug developm/ testing	6	6	3	3	0	0
Model/method development – theoret	11	11	9	9	0	0
Model/method validation	1	1	0	0	0	0

Figure 3.31: Relative distribution of applications/aims for Computational (*in silico*) model (% of models).

3.9.2 Model system

Due to the fact that *in silico* methods are generally stand-alone computer-based models there is, in this case, no incorporation of physical biological material, the material/ model type was therefore classified as '*in silico* model' for all inventoried methods (69 of 69 publications, 23 of 23 publications, and 6 of 6 publications for AD, PD and several NDDs, respectively).

Table 3.37: Model system for Computational (*in silico*) model for three disease areas.

Type	AD (n=69)		PD (n=23)		NDD (n=6)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
In silico model	69	69	23	23	6	6

3.9.3 Disease feature

The most common computational (*in silico*) model disease feature for AD was overwhelmingly protein aggregation (n=55 or 80% for AD). This shows the value of molecular dynamics simulations to model protein-protein interactions, as well as the dynamics between aggregate inhibitors and amyloid β.

In silico models also focused on the specific dysfunction of certain neuronal subtypes, namely cholinergic dysfunction in the case of AD (n=6, mainly detailing the molecular behaviour of acetylcholinesterase (AChE) inhibitors) and dopaminergic neurodegeneration in the case of PD (n=16 or 70%).

Some models were exploratory with no specific feature (n=6 for AD, n=2 for PD and n=5 for several NDD) or defined by the disease feature of neuronal loss (n=2 for AD) or energy metabolism (n=1 for PD).

Table 3.38: Disease features for Computational (*in silico*) model for three disease areas.

Disease feature	AD (n=69)		PD (n=23)		NDD (n=6)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
Cholinergic dysfunction/degeneration	6	6	0	0	0	0
Dopaminergic neurodegeneration	0	0	16	16	0	0
Energy metabolism	0	0	1	1	0	0
Exploratory/ no specific feature	6	6	2	2	5	5
Neuronal loss	2	2	0	0	0	0
Protein aggregation	55	55	4	4	1	1
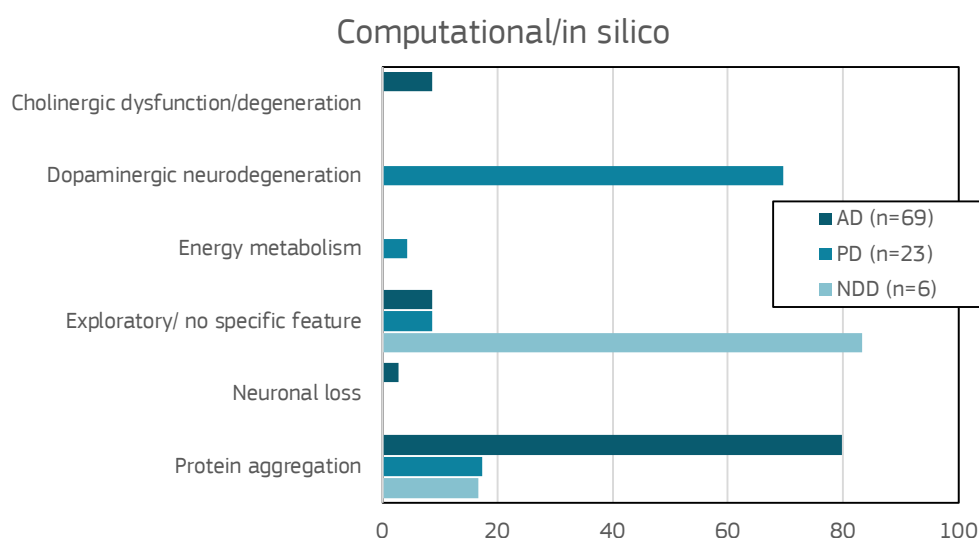
Figure 3.32: Relative distribution of disease features for Computational (*in silico*) model (% of models).

� 3.9.4 *Biological endpoints*

The same focus is reflected in the biological endpoints that are evaluated using the inventoried computational methods for the three disease area. For PD specifically for example, on the one hand, basal ganglia signalling in general is a large topic of interest, on the other hand, also dopaminergic metabolism more specifically is looked at very often. A minority of methods also models α-synuclein function and/or aggregation.

Because *in silico* models are simulations that feature no physical biological models, the endpoints were generally simulation outputs.

3.9.5 *Throughput/content*

Despite the use of computers for development of *in silico* methods, most models fell under 'low–medium' throughput – 'low–medium/medium' (n=19 for AD), 'low–medium/low' (n=37 for AD, n=2 for NDD).

Table 3.39: Throughput/content for Computational (*in silico*) model for three disease areas.

Throughput/content	AD (n=69)		PD (n=23)		NDD (n=6)	
	#Models	#Papers	#Models	#Papers	#Models	#Papers
High (automatic)/high	1	1	nd	nd	nd	nd
High (automatic)/medium	11	11	nd	nd	nd	nd
High (automatic)/low	0	0	nd	nd	nd	nd
Low-medium/high	0	0	nd	nd	nd	nd
Low-medium/medium	19	19	nd	nd	nd	nd
Low-medium/low	37	37	nd	nd	nd	nd

nd: no data available because this field was added after the evaluation of PD papers was finished.
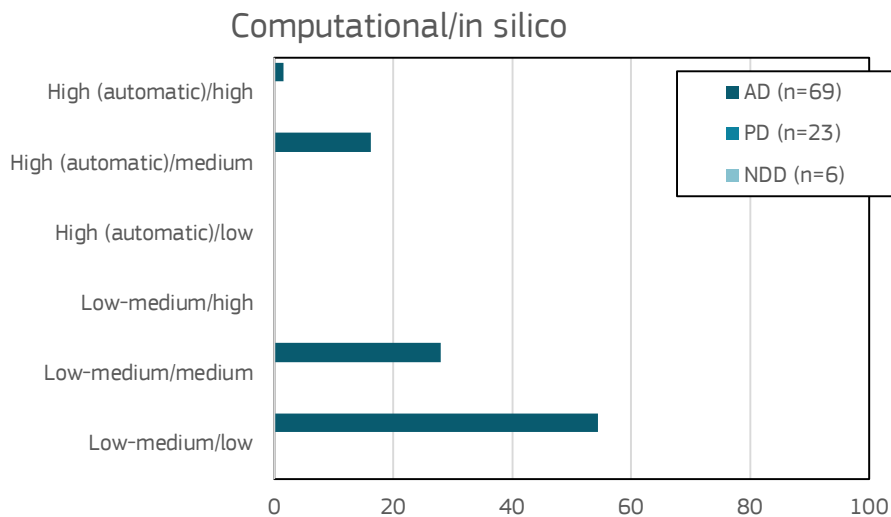

Figure 3.33:Relative distribution of throughput/content for Computational (*in silico*) model (% of models).

4 Discussion

TThis inventory resulting from the categorisation and scientific evaluation of innovative (human-based) non-animal models or approaches includes 568 models, focused either for AD or for PD specifically, or on NDD in general. The inventory details information about the context and use of a method, answering the central questions "Why?", "How?" and "What? as well as qualitative information related to the status, relevance and potential of a given method. As such, the inventory allows for straightforward browsing of existing published methods, which aims to contribute to their increased adoption and acceptance in neurodegeneration research and related fields. Sharing and comparing research tools and technologies in the context of a structured and transparent weight-of-evidence framework can only benefit these goals.

Ex vivo human/patient material is primarily used to evaluate aspects of protein aggregation, in the case of PD, α-synuclein and Lewy bodies, in the case of AD, Aβ and tau. Despite this strong focus on protein deposits, disease diagnosis is not the main aim of most of these methods/models, but rather developing a better understanding of disease mechanisms. Unsurprisingly, overall novelty, throughput and content of these methods are rather limited, as ex-vivo material by definition has limited accessibility as well. With the majority of studies in this category still focused on brain tissue, rather than CSF or blood, they remain applicable only to post-mortem cases. In that sense, their potential to replace the use of animal models is limited.

While human/patient derived primary or stem cell models are clearly the subject of intense research, this field is still very much under development. Perhaps unsurprisingly, the largest portion of the inventory consists of iPSC models. In many instances, there is a very broad focus on different biological endpoints,

but overall the content remains low-to-medium, considering the fact that cellular models (despite human and patient-derived) remain cellular models.

The most striking difference between the AD and PD fields is the relative shift in application. Where the majority of primary or stem cell models are used for mechanistic studies in AD, there is a relatively larger focus on treatment and therapy (dopaminergic cell replacement) when it comes to PD. Human-derived cell lines are less specific models for AD, PD and NDD in general, especially when compared to the aforementioned two categories (*ex vivo* material and primary or stem cells), however, they still represent an important part of the models and methods currently used in these fields. Disease mechanism studies represent the most important aim overall, with the range of biological endpoints considered being very broad. At the same time, throughput and information content are rather low.

The most known or common examples of 2D or 3D co-culture systems are models which represent blood brain barrier (BBB) function, but a standard method is apparently not yet available. As can be derived from the section 'disease feature', the majority of co-culture or 3D models is still of exploratory nature. There is a need for physiological, multifunctional and architectural complex systems representing the CNS or brain tissue, though improved characterisation of model systems is required in advance to mechanistic studies on disease features. These models are promising approaches to reduce animal use, but more standardisation and validation is required.

Despite the reduced number of papers screened for the organoids model, a consistent trend based on the use of human stem cells, although of different types, can be observed. According to the data from AD, these cells are

used in a way to maximise the content while the throughput is still low/medium. These models are mainly under development or optimization, therefore highlighting the research effort still needed to standardise and validate them. The limited number of data extracted from the screening performed in this work highlights the need for development or optimization of such organoid type experimental models with major focus on the neuronal degeneration of dopaminergic neurons as well as on protein aggregation. Although these models are extremely relevant for improving the prediction of current *in vitro* non-animal models, substantial work is still required not only to increase their standardisation and validation but also to widen the disease features that can benefit from them.

One clear observation made regarding biochemical cell free methods/assays is the focus on protein aggregation. This is not surprising, considering the high relevance of protein misfolding in the pathology of many different neurodegenerative diseases, and the suitability of biochemical and cell-free systems to study (aspects of) the physiological and pathological processes leading up to protein misfolding and aggregation. A striking difference across the two disease areas included is the status of the methods, with the AD field showing markedly more developmental efforts in this regard, compared to PD research. In relation to animal replacement, however, these in vitro methods represent very simplified, low content systems, which are more likely to precede rather than replace animal testing.

From the publications screened and the data presented here, it appears that many more organ-on-chip systems are dedicated to the study of AD as compared to PD or NDD in general. Many of the Lab/brain on chip or microfluidic system models in the area of AD focused on disease diagnosis, approximately 45% or 18 of 40 publications. There was a wide range of reported detection limits spanning fg/ml to hundreds of ng/mL, and variety in the biofluid in which amyloid β was isolated and quantified, including artificial CSF, PBS, human serum and patient CSF.

In the case of Lab/brain on chip or microfluidic system models related to the disease areas of several NDDs and PD, there was a relatively wide variety of application/aims, model types, and disease features. As with Lab/brain on chip or microfluidic system models applied to the study of AD, a majority of the devices were dedicated to the study or quantification of protein aggregation, combined across diseases areas making up 70%, or 36 of 51, of the specified disease features. Across all disease areas Lab/brain on chip or microfluidic system models tended to exhibit high throughput but low-medium or low content. The ability for these models to achieve high throughput may be one reason the model type is valuable in the context of augmenting or supplanting animal studies.

In silico models are highly focused in application to investigation of disease mechanisms or as disease models, with more than two thirds of the methods dedicated to these aims. As with other model types, in silico methods were overwhelmingly concerned with the disease feature of protein aggregation. Because these computational methods often use standardised software, most method status were categorised either as standardised or under optimization. The substantial number of publications describing the use of *in silico* methods in the study of AD, PD and NDD pathology may highlight the potential of the model type as an alternative to animal-based models.

5 Conclusions

From a general point of view, the wealth of information compiled in this report and in the inventory allows to identify areas of focus and interest in relation to methodological development for basic and translational research, some of which are more specific for AD rather than PD or vice versa. One clear example is the prominence of cellular models developed in light of therapeutic applications for PD. In contrast, for AD, there is a much larger focus on development of biochemical methods applied to study protein aggregation. These differences can also inform cross-field method adoption and development.

In addition, the inventory highlights promising but yet underdeveloped areas of methodological development. For example, the number of organoid models identified is still limited even considering the specific focus on publications from a recent five-year period, whereas a larger number of microfluidic systems have been retrieved.

Notwithstanding, it should be taken into account that often 3D co-cultures are used to create organoids and brain-on-chip devices and thus any future meta-analysis of model categories should consider these potential overlaps.

While we identified a larger number of microfluidic systems, their scope shows substantial room for improvement as well. For example, future developments should include the optimisation of microfluidic devices for the identification of NDD related biomarkers in clinical settings and as tools to monitor disease progression. As new in vitro amyloid β diagnostic devices continue to mature, they have the potential to enable broader implementation of disease screening and earlier diagnosis of asymptomatic AD patients. In addition, improved high throughput screening of drug candidate interactions could be achieved, for example in combination with organoids.
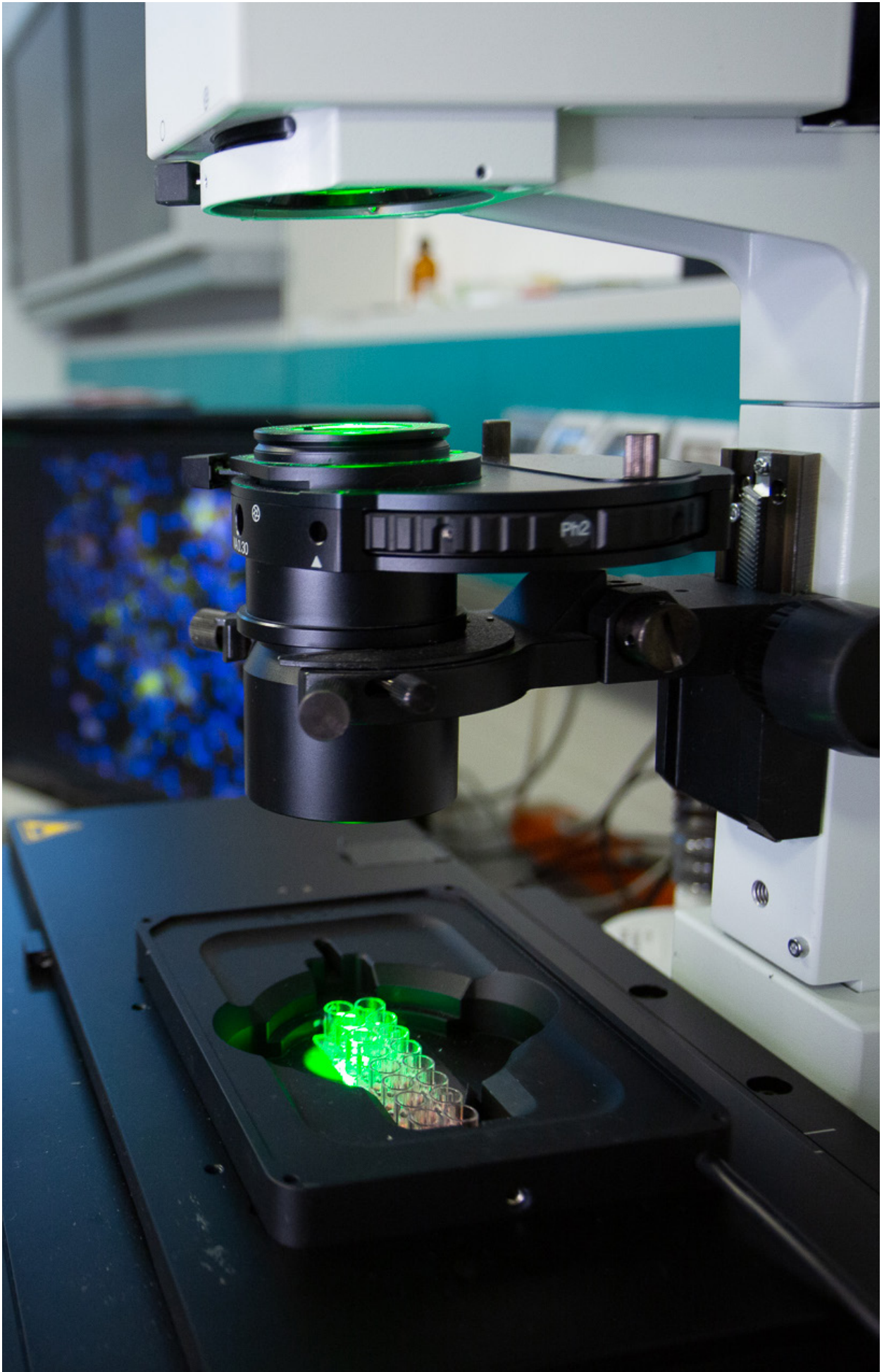
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7 Annex

Table 1: Inclusion criteria* used to retrieve scientific articles from literature.

1. Type of method[1]

a. Human-based 2D- or 3D-cellular models: e.g. cell lines, embryonic stem cells (ESC), induced pluripotent stem cells (iPSCs), organoid models relevant to the nervous system

b. Human-based samples such as cerebrospinal fluid or brain tissues, made available after surgical operations or post-mortem samples

c. Lab-on-chip/brain-on-chip approaches and/or microfluidic systems using e.g. human cells, tissue samples or proteins

d. Computational or *in silico* models, molecular docking tools

2. Neurodegenerative diseases

a. Brain or nervous system degeneration

b. Alzheimer's disease (AD), Parkinson's disease (PD)

c. NDD

3. Brain disease hallmarks, mechanisms or pathways for neurodegeneration, to be studied using non-animal methods

a. protein aggregation or related (misfolding, proteostasis failure, phosphorylation…)

b. amyloid-β

c. α-synuclein

d. Tau protein

e. Huntingtin

f. neuronal damage, neuronal cell death or apoptosis

g. neural circuit dysfunction or degeneration

h. neuroinflammation and microglial dysfunction

i. axonal transport defects and cytoskeletal disturbance

j. mitochondrial dysfunction and oxidative stress

k. excitotoxicity

l. synaptic abnormalities

m. autophagy, lysosomal defects

5. Information sources

a. Period 2013–2018

b. Peer reviewed

c. Original method papers (published) or protocols (database)

* A method/model was accepted based on a combination of at least one criterion from the list 'type of method ' and at least one criterion from the list 'neurodegenerative diseases'.

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1   The term 'method' is simultaneously used for non-animal approaches, models, techniques, bioassays, experimental or computational models.

Table 2: Exclusion criteria used to retrieve scientific articles from literature.

1. Type of method

a. Studies done in human by default

b. Mammalian rodent experimental models: *in vitro*, *ex vivo*, *in vivo*

c. Non-mammalian lower organisms: e.g. nematode, zebrafish, fruit fly, yeast, bacteria

d. Methods with restricted use (e.g. patent, ethical restrictions)

2. Neurodegenerative diseases

a. Other neurological or neurodegenerative diseases (e.g. stroke, spinal cord injury, prion diseases, Pick's disease, multiple system atrophy, Huntington's disease)

5. Information sources

a. Information in language other than English

b. Abstracts, papers in conference proceedings, review papers (review papers might be screened for original method papers in case there is apparent lack of certain methods)

c. Mandatory information to complete required parameters in the template model/method format is not available (e.g. identification of experimental system, data analysis)

Table 3: Search phrases for Parkinson's disease.

1. Run Search: "Parkinson_20180904": 4,740 hits	
	Cusotm year range 2013-2018; Language: English; Sources: Science Citation Index Expanded (SCI-EXPANDED) & Emerging Sources Citation Index (ESCI); Type of document: article, review
#4	#3 AND #2 AND #1 DocType=All document types; Language=All languages;
#3	(TS=("in vitro' OR "ex vivo" OR "cell model" OR "stem cell" OR "cell line" OR "primary cell*" OR "brain tissue*" OR "brain slice*" OR "brain sample*" OR "cerobrospinal fluid*" OR *chip* OR *microfluid* OR microdevice* OR organoid* OR *spheroid* OR "comput* model*" OR "comput* simulat* OR "comput* method*" OR "comput* tool*" OR "model* approach*" OR "in silico" OR QSAR OR "molecular field analy*" OR "molecul* dock*" OR bioinformat* OR "data acquisit*" OR "data mining" OR "enrichment analy*" OR "differential analy*" OR "predict* model")) AND LANGUAGE: (English) AND DOCUMENT TYPES: (Article OR Review) DocType=All document types; Language=All languages;
#2	(TS=("α synuclein" OR "alpha synuclein" "Lewy bod*" OR "dopamine*" OR "substantia nigra" OR striatum OR "synap* dysfunction*" OR "synap* defect*" OR "protein aggreg*" OR "protein misfold*" OR "MPP+" OR "neuron cell death" OR "oxidat* stress" OR "neuroinflammat*" OR "microglial dusfunction" OR apopto* OR "excitotoxicit*" OR "autophag*" OR "lysosom* defect" OR "mitochondr* dysfunction*" OR "axon* transport*")) AND LANGUAGE: (English) AND DOCUMENT TYPES: (Article OR Review) DocType=All document types; Language=All languages;
#1	(TS=Parkinson*) AND LANGUAGE: (English) AND DOCUMENT TYPES: (Article OR Review) DocType=All document types; Language=All languages;

Table 4: Search phrases for Alzheimer's disease.

	1. Run Search: "Alzheimer_20180904": 8,411 hits
	Cusotm year range 2013-2018; Language: English; Sources: Science Citation Index Expanded (SCI-EXPANDED) & Emerging Sources Citation Index (ESCI); Type of document: article, review
#4	#3 AND #2 AND #1 DocType=All document types; Language=All languages;
#3	(TS=("in vitro' OR "ex vivo" OR "cell model" OR "stem cell" OR "cell line" OR "primary cell*" OR "brain tissue*" OR "brain slice*" OR "brain sample*" OR "cerobrospinal fluid*" OR *chip* OR *microfluid* OR microdevice* OR organoid* OR *spheroid* OR "comput* model*" OR "comput* simulat* OR "comput* method*" OR "comput* tool*" OR "model* approach*" OR "in silico" OR QSAR OR "molecular field analy*" OR "molecul* dock*" OR bioinformat* OR "data acquisit*" OR "data mining" OR "enrichment analy*" OR "differential analy*" OR "predict* model")) AND LANGUAGE: (English) AND DOCUMENT TYPES: (Article OR Review) DocType=All document types; Language=All languages;
#2	(TS=("amyloid" OR "Aβ" "tau" OR "total tau" OR "phosphor-tau" OR "tau phosphoryl*" "neurofibrillary tangle" OR "NFT OR "oxidat* stress" OR "neuroinflammat*" OR "microglial dysfunction" OR "excitotoxicit*" OR "lysosom* defect" OR "mitochondrial defects" OR "mitochondr* dysfunction*" OR "axon* transport*")) AND LANGUAGE: (English) AND DOCUMENT TYPES: (Article OR Review) DocType=All document types; Language=All languages;
#1	(TS=Parkinson*) AND LANGUAGE: (English) AND DOCUMENT TYPES: (Article OR Review) DocType=All document types; Language=All languages;

Table 5: List of tags to rate the accepted abstracts in the dataset.

Field	Definition
1. Type of paper number	a. Review (focused to NDD alternative methods) b. New method (original paper using an alternative method development/validation considering alternative approaches) c. Method comparison or test strategy (alternative method/model developed and/or validated against an animal model/methods) d. Application (routine or existing, alternative method used for different purposes: e.g. drug development and testing, neuroprotection, toxicants, food, plant extracts, gene editing, mutant studies) e. Results comparison (findings obtained with different animal/ non-animal methods, in a study that is not dedicated to method development, optimisation or validation)
2. NDD disease	a. Alzheimer's disease (AD) b. Parkinson's disease (PD) f. Neurodegenerative diseases (NDD) (if endpoints are relevant for both PD and AD, and/or in general for neurodegenerative diseases)
3. Model systems	a. Human/patient ex-vivo tissue or body fluids (brain biopsy, CSF, post-mortem, blood, saliva, urine) b. Human/patient primary or stem cells c. Human-derived cell lines d. 2D or 3D models and/or co-cultures e. Organoid models f. Biochemical/cell-free assays g. Lab/brain on chip or microfluidic systems h. Computational (*in silico*) models i. Other
4. Cell types	a. Neuronal cells b. Glial cells (astrocytes; microglia, Schwann cells, oligodendrocytes) c. iPSCs (induced pluripotent stem cells, different sources) d. NSCs (neural stem cells or progenitor cells) e. Human embryonic cells f. SH-SY5Y cells g. Other h. na (= not applicable, method does not make use of cells)

5. Biological endpoint/read out	a. Gene expression (incl. Transcriptomics, real time RT-PCR)
	b. Protein expression (incl. Proteomics, Elisa)
	c. Lipidomics
	d. Metabolomics
	e. Neuroinflammation
	f. Oxidative stress
	g. Apoptosis
	h. Mitochondrial function
	i. Lysosomal function
	j. Axon function/structure/retraction
	k. Neurotransmission (incl. Acetylcholine, other transmitters)
	l. Excitotoxicity
	m. Autophagy
	n. Gliosis
	o. Tau (NFT, phosphorylated tau)
	p. Amyloid (Plaques, Aβ Aβ-peptides)
	q. Synuclein (Lewy bodies)
	r. Protein aggregation (NFT, plaques, Lewy bodies, proteasomal stress)
	s. Dopaminerg
	t. Blood brain barrier
	u. Morphology/ultrastructural changes
	v. Synapse function
	w. Cell death or degeneration
	x. Microglial dysfunction
	y. Oligodendrocyte dysfunction
	z. Astrocyte dysfunction
	aa. Neuron dysfunction
	ab. Schwann cell dysfunction
	ac. Substantia nigra
	ad. ApoE
	ae. TDP-43 protein
	af. Mutations (SOD1, FUS)
	ag. Phase separation or transition
	ah. Autoimmunity
	ai. Myelin, or demyelination
	aj. Nitrosative stress
	ak. Other (specify in notes)

Table 6: Agreed categories for the dataset.

Field	Definition
Model number	Model of neurodegenerative disease which is described in a paper
Disease area	One of main two neurodegenerative diseases (PD, AD), or other NDD
Disease features	The disease feature studied by the model
Category	The category of non-animal model assigned to the model
Type	More specifications of the model
Application/Aim	Main scientific aim or application of the model
Biological endpoints	List of potential biological endpoints used in a model system to describe the disease mechanism and/or study focus
Throughput/Content	Regarding productivity/automatisation of the model
Potential	Possible multiple model application in addressing disease features
Relevance	Biological relevance of the model for the disease feature in replacing animal models
DOI or link	Digital Object Identification number to retrieve the publication abstract. If not available, an alternative link is provided
First author name	Name of the first author of the peer-reviewed article
Year	Publication year from 2013 to 2018

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