CSF protein dynamics in murine models of α-synucleinopathy and cerebral β-amyloidosis

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Für Elisabeth, meine Eltern und Elena

"*No great mind has ever existed without a touch of madness.*"

- Aristotele

Summary

Parkinson's (PD) and Alzheimer's disease (AD) are the two most common neurodegenerative diseases and of growing importance for the rapidly aging population of industrialized countries. A common feature of both diseases is the progressive accumulation of proteins in insoluble aggregates, which are considered to play a fundamental role in the pathogeneses ultimately resulting in marked neuronal loss. The histopathological hallmark features of PD are called Lewy bodies (LBs) and Lewy neurites (LNs), both consisting predominantly of aggregated α-synuclein (αSyn), while neuropathological diagnosis of AD relies on the presence of neurofibrillary tangles and senile plaques comprised of hyperphosphorylated tau-protein or amyloid-β (Aβ), respectively. However, the implicated pathological mechanisms and pathways contributing to these diseases are still not fully understood and numerous studies emphasize the need for early diagnosis before major neuron-loss occurs. Cerebrospinal fluid (CSF) is in close contact with the central nervous system and therefore a valuable source of biochemical markers reflecting pathological changes in the brain and spinal cord. For AD, three core CSF biomarkers (Aβ, total-tau & phospho-tau) have been identified and extensively validated over the last years, while established biochemical markers are virtually absent for the diagnosis of PD.

The aim of the thesis was to investigate proteome alteration in the presence of different types of protein aggregates and ultimately to identify novel biochemical markers of disease. Therefore, transgenic mouse models of α-synucleinopathy or β-amyloidosis, the hallmark neuropathological aspects of PD or AD, were used. These mice express mutated human genes initially identified in patients suffering from familial forms of PD or AD.

The first set of experiments focused on the identification of proteins altered in the CSF of these models. This was done in an unbiased mass spectrometry-based shotgun approach, which led to the quantification of 636 and 665 CSF proteins in aged A30P-aSyn and APPPS1 cohorts, respectively. Both datasets contained transgene-related CSF protein changes that have already been associated with PD or AD, such as amyloid precursor protein (APP)-derived peptides, TREM2, ApoE or neurofilament light (NfL), but also yielded novel insights in protein alterations, such as LAG-3, CART and

lysosomal proteins. Next, both datasets generated from A30P-αSyn and APPPS1 CSF were compared and revealed a marked overlap of proteins deregulated in both models.

The second part of the thesis focused on NfL, which plays a key role in axonal stabilization and gained attention as biomarker of axonal injury in multiple neurological disorders. A validated immunoassay was used for the quantification of NfL in CSF and plasma of A30P-aSyn, APPPS1 and a third mouse line expressing an A53T mutation in αSyn. Markedly elevated levels were found in CSF and plasma of the mice at the same age as the respective brain lesions became apparent. This demonstrates that CSF and blood NfL increases are not specific for aggregated αSyn or β-amyloid and emphasizes its potential as marker of axonal damage upon neurodegeneration.

In conclusion, hundreds of proteins were quantified in the CSF of mouse models for α-synucleinopathy and β-amyloidosis. The datasets at hand provide novel and unbiased insights in pathological processes on molecular level and reveal common and distinct features of the respective pathology. The high proportion of hits related to PD, AD and other neurodegenerative diseases, as evaluated in human-based studies, substantiates the confidence in the high quality of the datasets and the translational value of the mouse models. Taken together, these findings provide a rich resource for the identification of novel biomarkers and their value concerning differential diagnosis.

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Contents

Nomenclature

αSyn	α-synuclein
A30P	alanine-to-proline substitution at codon 30 of the gene encoding for α-synuclein
A53T	alanine-to-threonine substitution at codon 53 of the gene encoding for α-synuclein
Aβ	amyloid-β
AA	amino acid
AD	Alzheimer's disease
APP	amyloid precursor protein
APS	atypical parkinsonian syndromes
CART	cocaine-and amphetamine-regulated transcript protein
CNS	central nervous system
CSF	cerebrospinal fluid
DLB	dementia with Lewy bodies
ECL	electrochemiluminescence

EDTA	ethylenediamine tetraacetic acid
ESI	electrospray ionization
FAD	familial Alzheimer's disease
FDR	false discovery rate
FPD	familial parkinson's disease
GO	gene onthology
GRN	granulin
IPA	Ingenuity pathway analysis
KEGG	kyoto encyclopedia of genes and genomes
KO	knockout
LAG3	lymphocyte activation gene 3 protein
LBs	Lewy bodies
LC	liquid chromatography
LFQ	label-free quantification
LNs	Lewy neurites
MAPT	microtubule associated protein tau
mo	months
MS	mass spectrometry
MSA	multiple system atrophy

NfL/NfM/NfH	neurofilament light/medium/heavy
NFTs	neurofibrillary tangles
NSE	neuron-specific enolase
PBS	phosphate-buffered saline
PD	Parkinson's disease
PET	positron emission tomography
PFA	paraformaldehyde
PGRN	progranulin
PS1	presenilin 1
RCF	relative centrifugal force
Serpin A3N	serine protease inhibitor A3N
SNpc	substantia nigra pars compacta
TG	transgenic
TREM2	triggering receptor expressed on myeloid cells 2
WT	wildtype / control

1 Introduction

1.1 Aging and neurodegeneration

From a biological point of view, aging reflects the process in which a variety of stressors are no longer adequately counteracted by the body's protective functions (Whalley et al., 2004). For the brain, aging also means damage from oxidative stress, mitochondrial dysfunction, inflammatory processes and accumulation of potentially injurious proteins. All of those can lead to irreversible changes, such as altered metabolic pathways, destabilization of neuronal membranes and cell death (Hipkiss, 2006; Leuner et al., 2007; Morgan et al., 2007; Tosato et al., 2007). Therefore it is not surprising that age is a major risk factor for a variety of neurodegenerative diseases, ranging from Alzheimer's disease to Parkinson's disease and amyotrophic lateral sclerosis.

According to a study from the United Nations Population Fund (UNFPA), 11% of the world's population is aged 60 and older. By the year 2050, the share is estimated to reach 22% (UNFPA, 2012). This will inevitably lead to a boost of people suffering from age-associated neurodegenerative diseases if no countermeasures are taken. Therefore it is of utmost importance to investigate and understand the molecular and cellular mechanisms causing dementia, so that patients can be identified in early stage of disease and successful treatments can be established.

1.2 Parkinson's disease

Parkinson's is a progressive multi-system neurodegenerative disease, affecting mostly the elderly population. The disease was named after James Parkinson, who, in 1817,

published "*An Essay on the Shaking Palsy*", describing six individuals suffering from a disease he called "paralysis agitans". He described it as "*Involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported; with a propensity to bend the trunk forwards, and to pass from a walking to a running pace: the senses and intellects being uninjured*" (Parkinson, 1817). Nowadays we know that "*the senses and intellects*" can be affected and that besides mentioned motor symptoms, non-locomotor symptoms can become apparent. Those symptoms may include cognitive, sensory and behavioral deterioration, culminating in dementia, depression, visual hallucinations, anxiety and sleep disturbances (Sveinbjornsdottir, 2016).

The incidence and prevalence of PD are on the rise, in concurrence with the aging population in industrialized countries (Pringsheim et al., 2014). PD is estimated to affect 0,3% of the general population, or 1% of people over the age of 60, making it the second most-common neurodegenerative disease after Alzheimer's (de Lau and Breteler, 2006). The average age of onset is between 50 and 70 years, while prevalence further increases with age, reaching 3,5% in 85-89 year old europeans (De Rijk et al., 1997; de Lau and Breteler, 2006; Clarke and Moore, 2007). In 2015, approximately 6.2 million people were suffering from this incurable disease worldwide (Vos et al., 2016).

Idiopathic –or sporadic– PD is the most common form of parkinsonism, lacking an identifiable external cause, such as a family history of the disorder. Sporadic PD is affected by numerous factors influencing age of onset and course of disease. Hereditary PD, on the contrary, has dominant genetic causes and is therefore often also referred to as familial form of PD (FPD) accounting for 5 - 10% of all PD cases (Thomas and Flint Beal, 2007; Lesage and Brice, 2009). Over the last years, genetic analyses have identified a set of mutations causing FPD, such as point-mutations, duplications and triplications of the α-synuclein (SNCA) gene. In addition, mutations in the gene coding for leucine-rich repeat kinase 2 (LRRK2), or loss-of-function mutations in Parkin, PINK1, DJ-1 and ATP13A2 can cause FPD (Lesage and Brice, 2009; Ferreira and Massano, 2017).

Neuropathology of Parkinson's disease

Parkinson's Disease is typically characterized by a pronounced loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the development of distinctive

eosinophilic, proteinaceous inclusion bodies, named Lewy bodies (LBs) and dystrophic Lewy neurites (LNs) (Gibb and Lees, 1988; Fearnley and Lees, 1991). LBs are considered a pathologically characteristic feature of PD and other synucleinopathies, such as dementia with Lewy bodies (DLB) and multiple system atrophy (MSA) (Goedert et al., 2013). The composition of LBs, which were first described by Fritz Heinrich Lewy in 1912, were later identified as a mix of filamentous αSyn, neurofilaments and ubiquitin (Spillantini et al., 1997). Although neuronal loss is pronounced in the SNpc, deposition of αSyn can also be observed in other parts of the central nervous system, including the amygdala and cortical areas (Braak et al., 2003; Braak and Del Tredici, 2009). Spreading typically happens along interconnected neuronal pathways, starting in the anterior olfactory nucleus and the dorsal motor nuclei of the vagal and glossopharyngeal nerves in the brainstem, spreading through the medulla oblongata and the amygdala, before reaching the SNpc. In later stages of disease, pathology spreads to the temporal mesocortex and finally reaches the neocortex (Braak et al., 2003). The loss of dopaminergic neurons in the SNpc leads to striatal dopamine deficiency, which accounts for most sensory-motor symptoms (Pires et al., 2017). To date it is still not completely clear which molecular pathways lead to this pathological picture, but both environmental and genetic factors appear to play major roles in disease susceptibility (Thomas and Flint Beal, 2007; Shulman et al., 2011).

Despite intensive research, a cure for PD remains to be found, while current treatment aims at improving and delaying motor symptoms through pharmacologic agents, such as levodopa and dopamine agonists (Pires et al., 2017). Considering the longstanding presymptomatic phase required to reach the full extent of disease, the need for early diagnosis and treatment aiming at initial pathological processes is substantial.

α-synuclein genetics in PD

As the main component of Lewy bodies, αSyn plays a pivotal role in PD. Mutations, duplications and triplications of the SNCA gene locus have been identified to cause rare familial forms of PD (Lesage and Brice, 2009). While duplications and triplications increase the amount of produced α-synuclein causing a dosage-dependent disease phenotype (Ikeuchi et al., 2008), point mutations are more complex, altering propensities of αSyn to oligomerize and form inclusions (Lázaro et al., 2014). Six mutations falling

into this category have been found over the last years: A30P, E46K, H50Q, G51D, A53T & A53E, as depicted in figure 1 (Polymeropoulos et al., 1997; Krüger et al., 1998; Zarranz et al., 2004; Lesage and Brice, 2009; Proukakis et al., 2013; Appel-Cresswell et al., 2013; Pasanen et al., 2014).

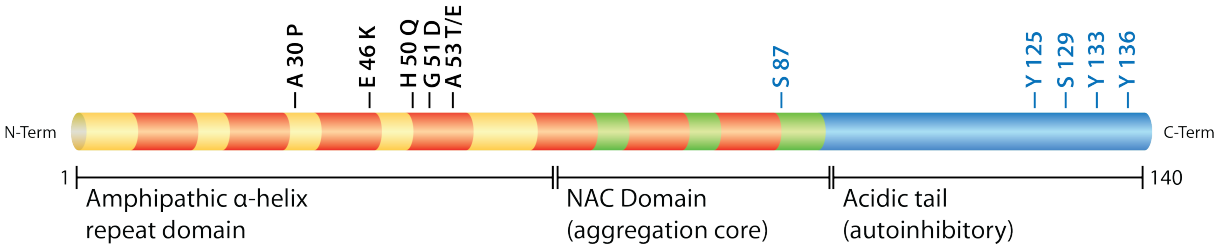

Figure 1 – The structure of α-synuclein and mutations linked to familial Parkinson's disease The human α-synuclein protein consists of 140 amino acids and is encoded by the SNCA gene. Residues 1-60 form the amphipathic region, containing four 11-residue repeats, composed of hexameric KTKEGV domains (red), which have structural alpha helix propensity. Residues 61-95 form the central region which includes the hydrophobic non-amyloid-β component (NAC) region able to form β-sheet structures. The highly acidic tail, spanning from AA 96 to 140 likely has auto-inhibitory functions, maintaining the natively unfolded structure of αSyn. All mutations linked to familial PD are located in the N-terminal region (marked in black), while phosphorylation sites (marked in blue) are situated towards the C-term (modified from Breydo et al., 2012 and Lázaro et al., 2014).

1.3 Alzheimer's disease

Alzheimer's Disease (AD) is a progressive neurodegenerative disease characterized by advancing dementia and deficits in cognitive abilities. Patients typically present with a progressive loss of episodic memory, difficulties in word finding, disorientation in time and place, but individuals can also suffer from emotional changes, depression, anxiety, apathy and delusions (Burns and Iliffe, 2009; Alzheimer's Association, 2013). The disease was first identified by Alois Alzheimer in 1901, who followed the case of the 51-year old Auguste Deter (Alzheimer, 1907). Suffering from severe dementia, she described her condition as "*I have lost myself, so to say*" when she was asked to write her name. After Auguste Deter died in 1906, Alzheimer examined her brain histologically and found two types of microscopic lesions he referred to as "senile plaques" and "neurofibrillary tangles" (Fig. 2, Illustrations 2-5). These lesions were later characterized as proteinaceous deposits and considered to be hallmark pathological features of AD, which still remain a valid and definite diagnosis for AD (Glenner and Wong, 1984b; Goedert and Spillantini, 2006; Scheltens et al., 2016).

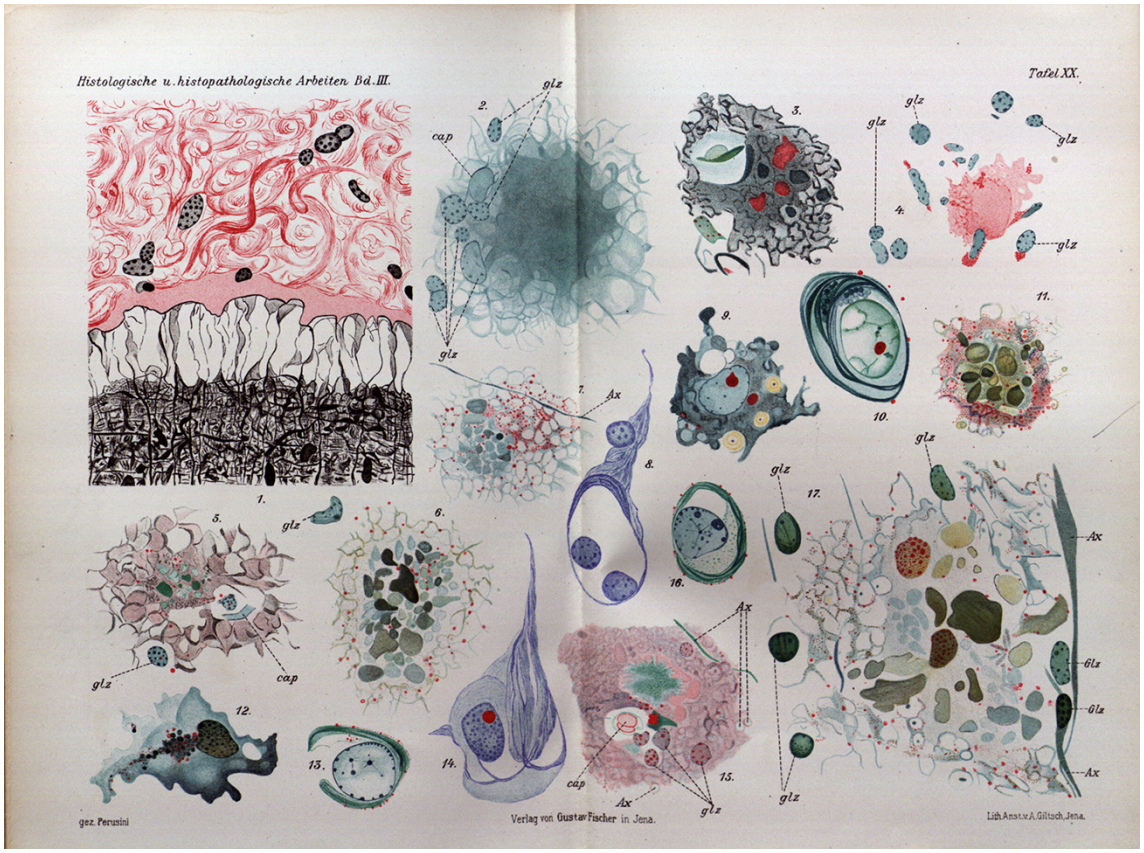

Figure 2 – Historic illustrations of plaques from the brain of Auguste Deter
Illustrations 2-5 show histologically stained plaques from the brain of Auguste Deter. Drawn and published by Gaetano Perusini in 1909.

While initially considered a rather rare disease, AD became the most common form of dementia due to an increasing life-expectancy over the last century. In 2015, 46.8 million people were estimated to suffer from AD, which also affects millions of relatives and caregivers. The economic burden of dementia, whereof AD has the largest contribution, is supposed to reach a trillion US dollars by 2018 (Prince et al., 2015). With age being a major risk-factor, AD is therefore often seen as a disease of the elderly. However, early onset forms of the disease exist in 4-5% of patients with an onset before 65 years of age (Mendez, 2012).

Neuropathology of Alzheimer's disease

Senile plaques and intracellular neurofibrillary tangles (NFTs), as initially described by Alois Alzheimer, remained an obscure observation of unknown composition for many

decades. In the 1960, technical advance in form of electron microscopy helped to shed first light on the characteristics of both structures. NFTs appeared to have a paired helical filamentous structure, whereas the core of senile plaques was composed of amyloid structures (Terry, 1963; Kidd, 1963; Wiśniewski et al., 1976). Years later, a 39-42 amino acid peptide named amyloid-β (Aβ) was identified as the main component of senile- or amyloid-plaques, whereas the microtubule associated protein tau (MAPT, Tau) was identified as the primary constituent of the paired helical structures forming NFTs (Grundke-Iqbal et al., 1986a; Kosik et al., 1986; Goedert et al., 1988). Tau was later found to be hyperphosphorylated in NFTs, while phosphorylation impairs its binding to microtubules (Grundke-Iqbal et al., 1986b). It has been suggested that the progressive trait of AD is due to increasing numbers of brain region-specific Aβ aggregates and NTFs (Braak and Braak, 1991; Thal et al., 2002; Jucker and Walker, 2013). Both of these lesions follow a defined propagation pattern throughout the course of disease, which can be used for post-mortem staging (Braak and Braak, 1991, 1995), but also sparked the idea of proteinaceous seeds with prion-like properties in AD and other neurodegenerative diseases (reviewed in Jucker and Walker, 2013; Nussbaum et al., 2013 and Walker and Jucker, 2015).

APP processing, amyloid-β peptide and genetic causes

Aβ peptide, the main constituent of amyloid plaques found in the brains of AD patients, is an approximately 4.3 kDa fragment proteolytically cleaved from the amyloid precursor protein (APP). APP is a single-pass trans-membrane protein that can have various isoforms generated by alternative splicing: APP_{751} and APP_{770} are expressed in non-neuronal tissues, while APP_{695} is abundantly produced and metabolized in neurons (Masters et al., 1985; Kang et al., 1987; Tanaka et al., 1988; Lee et al., 2008). The gene encoding for APP is located on chromosome 21, which also leads to a genetic predisposition and high prevalence of AD in people with Down's syndrome (Kang et al., 1987; Robakis et al., 1987; Schupf and Sergievsky, 2002). Therefore it is not surprising that the first isolation of Aβ from brain tissue succeeded from brains of deceased trisomy 21 patients (Glenner and Wong, 1984a). The Aβ peptide is derived from cerebral APP_{695} through sequential secretase cleavage and consists of 36-43 amino acids (Sisodia, 1992; O'Brien and Wong, 2011; Hamley, 2012). Depending on the secretases mediating

proteolytic cleavage, APP can either be processed in the "non-amyloidogenic" pathway or the "amyloidogenic" pathway.

In the non-amyloidogenic pathway (see Fig. 3 A), an α-secretase (ADAM10) cleaves APP within the sequence of Aβ and thereby prevents the production of (pathogenic) Aβ. This results in the generation of extracellular soluble APPα (APPsα) and a membrane-spanning c-terminal fragment (CTF-α) (Esch et al., 1990; Sisodia et al., 1990; Sisodia, 1992). Subsequent cleavage of CTF-α by γ-secretase results in the release of a soluble peptide, named P3 and the left-over APP intracellular domain (AICD).

The γ-secretase, a multi-subunit protease complex, consists of four proteins: Nicastrin, APH-1 (anterior pharynx-defective 1), PEN-2 (presenilin enhancer 2) and PSEN1 (presenilin-1). Mutations in the PSEN1 subunit have been shown to increase Aβ42 production in transgenic mouse models (further elucidated in section 1.4; Duff et al., 1996). This is easily explained by the role of γ-secretase in the amyloidogenic pathway (see Fig. 3 B), where APP is sequentially cleaved by β-secretase (BACE1) and γ-secretase, resulting in the production of Aβ. As the initial step of the amyloidogenic pathway, APP is cleaved by BACE1 at a cleavage site in the extracellular domain, 99 amino acids from the C-terminus (Vassar, 1999). This generates soluble APPsβ which is released from the cell and the hydrophobic membrane-spanning 99 amino acid c-terminal fragment (CTF-β or C99) (Selkoe, 1991). CTF-β is then cleaved by γ-secretase into AICD and a Aβ peptide (Vassar, 1999; Haass et al., 2012). Depending on the exact cleavage site of the γ-secretase and progressive cleavage steps, various Aβ peptides (ranging from 37 to 43 amino acids) can be generated from the initially released Aβ48 and Aβ49 peptides (Takami et al., 2009). In healthy subjects, the 40 amino acid peptide of APP (Aβ40) is the most abundant Aβ species, whereas the more hydrophobic and presumably pathogenic Aβ42 is less abundant with an Aβ42:Aβ40 ratio of approximately 1:9 (Vigo-Pelfrey et al., 1993; Yan and Wang, 2006; De Strooper and Annaert, 2010; Pauwels et al., 2012). Aβ42, in comparison to Aβ40, is more susceptible to aggregate and form Aβ fibrils that may further aggregate into oligomers and form amyloid-plaques (Jarrett et al., 1993; Yan and Wang, 2006; Thinakaran and Koo, 2008; O'Brien and Wong, 2011). This goes in line with the frequent observation of an increased Aβ42:Aβ40 ratio in the brains of AD patients, where this shift leads to altered aggregation kinetics and an enhanced toxicity of Aβ (Pauwels et al., 2012). Whether Aβ has a normal physiological function is not well understood and knockout-studies in mice have demonstrated that

neither APP nor Aβ are necessary for physiological functions (Hiltunen et al., 2009).

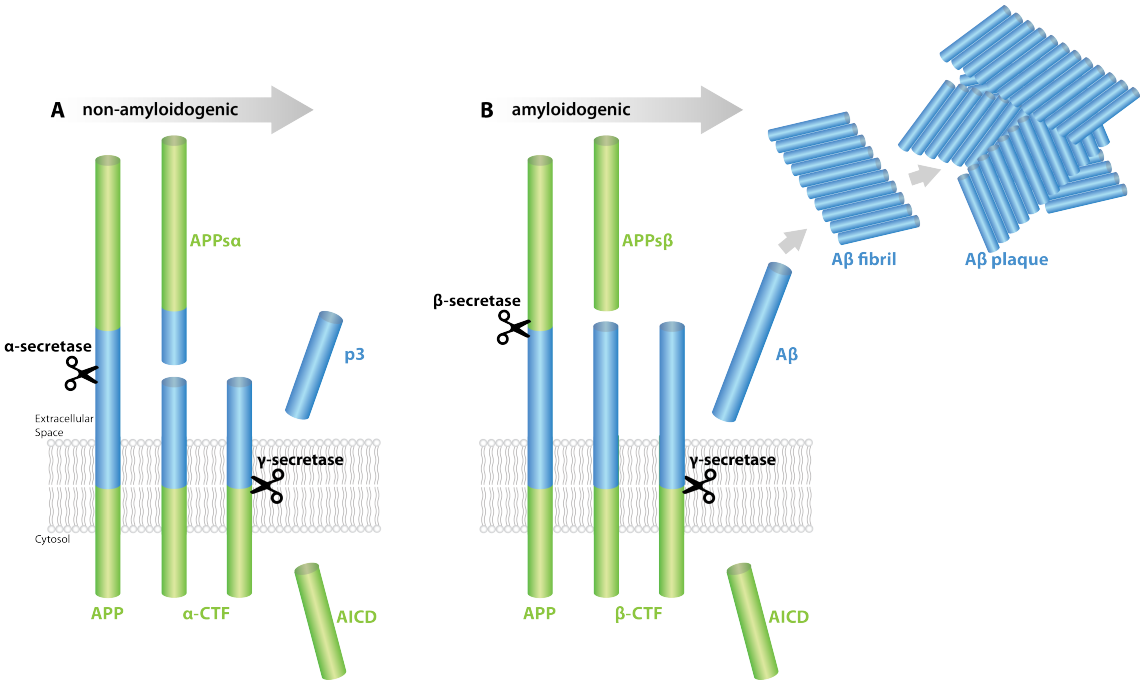

Figure 3 – Sequential cleavage of APP occurs by two pathways
(A) Non-amyloidogenic processing of APP involves sequential cleavage by α-secretase and γ-secretase. Aβ production is abolished by α-secretase cleavage in the Aβ region (blue). Instead a truncated peptide (p3) is released. (B) Amyloidogenic processing of APP involves β-secretase and γ-secretase cleavage, resulting in the release of Aβ, which then tends to form oligomeric fibrils accumulating in plaques. Both pathways produce soluble extracellular domains (sAPPα and sAPPβ) and identical intracellular C-terminal fragments (AICD) (figure adapted from Thinakaran and Koo, 2008).

In 1991, John Hardy and David Allsop formulated the amyloid cascade hypothesis, trying to explain the underlying pathology inevitably leading to neuronal death and the clinical phenotype known as Alzheimer's disease. In short: Amyloid deposition is a consequence of surplus Aβ which is the result of Aβ overproduction (e.g. trisomy 21) and/or diminished clearance (e.g. ineffective breakdown of Aβ). This is then followed by abnormal phosphorylation of tau protein and the formation of NFTs, ultimately resulting in neuroinflammation, neuronal death and the manifestation of disease (Hardy and Allsop, 1991; Hardy and Higgins, 1992).

The amyloid cascade hypothesis is further supported by pathogenic mutations in APP leading to familial forms of AD (FAD, Fig. 4). Such mutations are often autosomal dominantly inhehenrited in specific families, where first signs of dementia usually occur rather early in life (http://www.alzforum.org/early-onset-familial-ad, last accessed July 4th, 2017). In these families, three major genes have been identified that can

cause FAD when mutated: APP (51 mutations, 121 families), Presenilin-1 (PSEN1, 219 mutations, 480 families) and Presenilin-2 (PSEN2, 16 mutations, 34 families) (http://-www.molgen.ua.ac.be/ADmutations/ last accessed may 25th, 2017).

In APP, most pathogenic mutations cluster near the γ-secretase cleavage site (Fig. 4), except the "Swedish" and some other mutations that are located adjacent to the β-secretase cleavage site (O'Brien and Wong, 2011; Haass et al., 2012). Mutations in the presenilin gene generally have the same effect as APP mutations and lead to an increased production of Aβ42 (De Strooper, 2007; Shen and Kelleher, 2007). However, the substitution of alanine-to-threonine at codon 673 (A673T) confers protection against development of AD by making APP a less favorable substrate for cleavage by BACE1, therefore reducing production of Aβ (Jonsson et al., 2012; Maloney et al., 2014).

In contrast to FAD, the causes of late-onset sporadic AD (SAD), accounting for more than 95% of all AD cases, are largely unknown (Goedert and Spillantini, 2006). Numerous studies have been conducted and led to the identification of several risk-factors, such as age, diabetes, depression, hyptertension, traumatic brain injuries and genetic disposition (Burns and Iliffe, 2009). The genetic component of SAD is mainly conferred by mutations in TREM2 or variations in apolipoprotein E (APOE) alleles. Mutations in TREM2 have been associated with a 3 to 5 fold increased risk to develop AD (Guerreiro et al., 2013; Jonsson et al., 2013), whereas the APOE-E4 allele, present in 10%–20% of various populations (Singh et al., 2006), triples the risk to develop AD when expressed heterozygously, while homozygous expression increases the risk by a factor of 15 (O'Brien and Wong, 2011).

Despite very distinct causes, the underlying pathology of familial AD, including amyloid deposition, the buildup of NFTs and neuroinflammation, is strikingly similar to sporadic AD. Their similarity is further supporting the amyloid cascade hypothesis, as Aβ deposition is the primary event in sporadic AD (Shepherd et al., 2009).

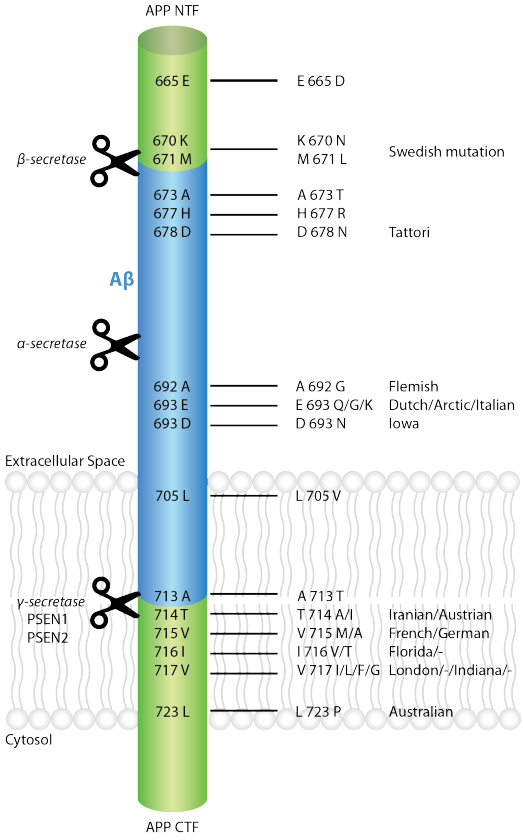

Figure 4 – Mutations in APP associated with familial Alzheimer's disease
Various APP mutations are known to cause early-onset or familial forms of AD. Known mutations and their respective amino-acid modification are indicated in the scheme. Mostly clustered in close proximity of secretase cleavage sites, these mutations thereby influence APP processing. Said mutations can be found both in- and outside the Aβ sequence (highlighted in blue) and are usually named after the origin of identified families. Mutations in the genes coding for Presenilin 1 and 2 (PSEN1, PSEN2) alter γ-secretase activity and therefore increase the production of the highly amyloidogenic Aβ42 isoform (modified from Van Dam and De Deyn, 2006).

1.4 From familial cases to mouse models of PD and AD

Over the last years, transgenic mouse models have been used in various studies to gain better understanding of the characteristics and underlying mechanisms of neurodegenerative disorders, including PD and AD. Such genetically altered mice may never reflect the full extent of human pathology, but have proven to hold great translational value in the search for biomarkers suitable for diagnosis in pre-clinical stages of disease (Antony et al., 2011; Maia et al., 2015; Bacioglu et al., 2016).

For PD, the first identified mutations causing familial PD were located in the gene

encoding αSyn (Polymeropoulos et al., 1997; Krüger et al., 1998), which gave rise to the generation of numerous mouse-lines overexpressing αSyn under several promotors and with various mutations (Fernagut and Chesselet, 2004), such as mice expressing either A30P or A53T mutated human αSyn (Krüger et al., 1998; Polymeropoulos et al., 1997). Yet it must be mentioned that mutated αSyn is only found in a small subset of PD patients. However, a common feature of αSyn transgenic models and human pathology is the advancing accumulation of αSyn in LBs, which is a hallmark feature of PD and other synucleinopathies (Forno, 1996; Antony et al., 2011; Goedert et al., 2013).

The first generated mouse line mimicking familial PD was the Thy1-hA30P-αSyn line, carrying a A30P mutation in αSyn, expressed under a neuron-specific murine Thy-1 promoter (Krüger et al., 1998; Kahle et al., 2000). In homozygous mutants, as used here, the level of mutated αSyn in the brain is two-fold over levels of endogenous murine αSyn (Kahle et al., 2000). A Lewy-like deposition of αSyn inclusions can predominantly be found in the substantia nigra and the spinal cord. Progressive motor impairments, such as spastic hind-limb paralysis and hunchback posture usually occur between 16 and 20 months of age, which is delayed by approximately one year when the transgene is expressed heterozygously (Neumann et al., 2002).

Another mouse line expressing a mutated human αSyn gene is the Thy1-hA53T-αSyn model. The A53T αSyn mutation was initially described in families suffering from FPD (Polymeropoulos et al., 1997; van der Putten et al., 2000). These mice show a very early onset of symptoms usually between 2 to 6 months of age with a marked deterioration of motor functions, even with heterozygous transgene expression. The features of the induced α-synucleinopathy are strikingly similar to those observed in human brains with Lewy pathology and neuronal degeneration with a particular vulnerability of motor neurons and the brain stem (van der Putten et al., 2000).

Similar to the AD models, transgenic generation of mice mimicking the hallmark pathologies of AD was also inspired by genetic profiling in families suffering from early-onset FAD. Three main genes promoting early-onset FAD were identified. Besides mutations in the genes coding for presenilin 1 & 2 (PSEN1, PSEN2) (Sherrington et al., 1995; Levy-Lahad et al., 1995), numerous mutations were found in the APP gene (Van Broeckhoven et al., 1990; Goate et al., 1991). As depicted in Figure 4, most pathogenic

APP mutations happen to be next to β and γ-secretase cleavage sites and have the same effect as mutations in presenilin 1 & 2, which alter APP processing and thereby increase production of Aβ42 (De Strooper, 2007; Shen and Kelleher, 2007).

The APPPS1 mouse line is co-expressing two mutated human genes: APP and Presenilin-1. The APP harbors the "Swedish" mutation, where lysine and methionine at codon 670 and 671 are substituted with a asparagine and leucine (KM670/671NL). In the transgenic presenilin-1 gene, leucine is substituted with proline at codon 166. The model was developed by Radde et al. in 2006 and is commonly used as models of β-amyloidosis, as they show age-related deposition of Aβ in the brain (Radde et al., 2006). The line has also proven to be useful in biomarker research , despite the absence of neurofibrillary tangles and only modest overall neuron loss (Jucker, 2010; Maia et al., 2013, 2015; Bacioglu et al., 2016).

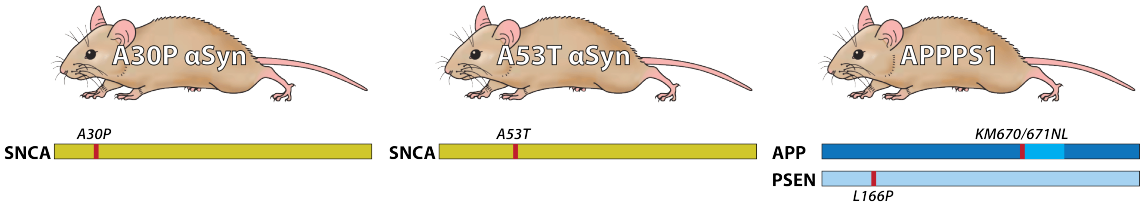

Figure 5 – Mouse models of neurodegenerative diseases used for CSF proteome analysis A30P-αSyn and A53T-αSyn are single-transgenic models, expressing the gene for human αSyn with an alanine-to-proline substitution at codon 30, or an alanine-to-threonine substitution at position 53. APPPS1 co-expresses the "Swedish" mutated gene for human APP and a mutated gene for Presenilin 1 with a leucine-to-proline substitution at codon 166.

1.5 Biomarkers of PD and AD

Chronic neurodegenerative disorders, such as AD and PD, typically have slow progression and partly overlapping symptomatology. Tissue sampling for direct visualization is impossible in a clinical setting, urging methods for accurate – and, ideally, also early – diagnosis, before widespread neuronal death occurs. Thus it is a major aim of biomarker research to provide clinicians with biochemical or imaging tools suited for timely diagnosis and tracking of disease progression (Olsson et al., 2011; Blennow et al., 2016).

Over the last years, neuroimaging has proven to be useful for the diagnosis of AD

and is routinely done with positron emission tomography (PET), a cost-intensive nuclear imaging technique showing in-vivo ligand binding and metabolic processes. The imaging-agents used in AD PET scans typically aim at fibrillar Aβ and aggregated tau protein (Klunk et al., 2004; Mintun et al., 2006; Villemagne et al., 2015; Brier et al., 2016). For the diagnosis of PD, such scans have proven challenging, albeit they can be used to assess dopaminergic function in the basal ganglia and exclude other diseases (Brooks, 2010; Loane and Politis, 2011).

Cerebrospinal fluid (CSF) is an ultrafiltrate of blood, produced in the ventricles and around the blood vessels of the central nervous system (CNS). It circulates and encompasses brain and spinal cord, draining into blood with a turnover time of approximately 8 h in human (Hühmer et al., 2006). Due to its close contact with the central nervous system, it is a valuable source for biochemical markers, reflecting pathological changes in brain and spinal cord (Halbgebauer et al., 2016). For AD, three core CSF biomarkers have been identified and extensively validated in various studies. These core-biomarkers include total tau (T-tau), phosphorylated tau (P-tau) and Aβ42, which reflect key elements of AD pathophysiology (Hampel et al., 2004). Reduced CSF levels of Aβ42 are thought to be a result of amyloid deposition, where Aβ42 binds and thereby reduces its diffusion to the CSF (Strozyk et al., 2003). Increased amounts of T-tau reflect cortical neuronal loss, while a reduced CSF P-tau levels coincide with cortical tangle formation (Hesse et al., 2001; Buerger et al., 2006; Tapiola et al., 2009). Those biomarkers have high diagnostic accuracy for AD, with sensitivity and specificity reaching 85–90% (Blennow et al., 2010). In addition various other proteins have been proposed as biomarkers for AD, such as TREM2 and neuron-specific enolase (NSE), but did not reach sensitivity and specificity of the mentioned core biomarkers (Blennow et al., 1994; Kleinberger et al., 2014; Schmidt et al., 2014; Suárez-Calvet et al., 2016).

To date there are no validated biochemical markers for PD and diagnosis is still reliant on clinical criteria, when the disease is already advanced. For successful therapy, diagnosis needs to happen as early as possible. Biochemical markers are key for a timely detection of PD and a prerequisite for the development of novel disease-modifying treatment strategies, which are currently not available (Halbgebauer et al., 2016).

In analogy to the core biomarkers of AD, various studies have investigated the diagnostic and prognostic value of α-synuclein, the major component of Lewy bodies and Lewy

neurites (Tokuda et al., 2010; Wennström et al., 2013; Parnetti et al., 2014b). These studies investigated the different α-synuclein species – total, oligomeric and phosphorylated – , but data from these published studies are partly conflicting. CSF total αSyn is usually reported as decreased, while oligomeric αSyn is often found to be increased in PD patients (Tokuda et al., 2010; Wennström et al., 2013; Parnetti et al., 2014b,a; Blennow et al., 2016). However, the prognostic value of CSF αSyn is questionable since no correlation has been found between total or oligomeric CSF αSyn and disease progression (Parnetti et al., 2011; Compta et al., 2014). Other CSF biomarkers, mainly in the context of oxidative stress, neuroinflammatory processes and neuronal injury, have also been considered by the field. Amongst the particularly promising candidates are: DJ-1, neurofilament light (NfL), synaptic proteins and lysosomal enzymes, due to their importance in αSyn degradation (Webb et al., 2003; Lee et al., 2004; Magdalinou et al., 2014; Parnetti et al., 2014a; Blennow et al., 2016).

1.6 The proteome

The term "proteome" describes the entire set of proteins expressed in an organism, organ, cell, tissue or bodily fluid at a given time and condition. The science of global protein composition and modifications in complex biological samples is usually referred to as "proteomics". Protein abundance, protein-protein interactions and the localization of proteins are of particular interest when it comes to understanding molecular processes and mechanisms in disease and models of disease.

Mass spectrometry in proteomics and label-free quantification

Mass spectrometry-based approaches represent the ideal tool to address the technically challenging tasks of proteomic research in a fast, antibody-independent and unbiased way. These approaches can roughly be split into bottom-up (shotgun) and top-down strategies. While top-down proteomics typically aim at fragmentation and measurement of isolated and undigested proteins to identify and quantify unique proteoforms through the analysis of intact proteins (Durbin et al., 2016), bottom-up approaches involve proteolytic digestion and can be used for analysis of complex mixtures from

crude protein extracts (Aebersold and Mann, 2003). After initial protein extraction and digestion, bottom-up experiments require separation of peptides by liquid chromatography and measurement by mass spectrometry. Mass spectrometers are capable of detecting the mass-to-charge ratio (m/z) of charged molecules with high precision and accuracy, which can later be used to calculate the mass of a respective molecule.

An established apparatus often used in proteomic research is the combination of a nano-LC system, an ion source and an Orbitrap mass spectrometer. In this array, peptides are gradually eluted from the LC column and ionized subsequently by electrospray ionization (ESI). The charged peptides are then transferred through the ion optics, the quadrupole mass filter, gathered in the c-trap and injected into the orbitrap mass-analyzer (Fig. 6). The Orbitrap mass-analyzer contains a spindle-shaped electrode, forcing peptides to oscillate in their characteristic axial oscillation frequency which is proportional to the square root of m/z ($\omega = \sqrt{\frac{k}{m/z}}$). This allows very precise measurement of peptides m/z with a deviation of ± 0.001 Da (Makarov, 2000). In the so-called MS or MS1 mode, the signal intensity allows to quantify the proportion of peptides in the sample. MS signal intensity correlates with concentration and can therefore be used for quantification (Bantscheff et al., 2007). To determine the amino acid sequence and thus identify the peptides and proteins, charged peptides of a specific m/z are sequentially collected in the c-trap, forwarded into the HCD cell and fragmented by collision with an inert gas (CID, collision induced dissociation). Resulting fragments are then analyzed in the orbitrap mass-analyzer to gain spectra from fragments of different size. Discrepancies in the fragment masses can then be matched to the specific molecular masses of amino acids. This procedure, and spectra generated from it, are usually referred to as MS/MS or MS2. In the last step, acquired MS1 and MS2 spectra are matched to peptide and protein databases, resulting in a list of proteins identified by their respective peptides (Michalski et al., 2012).

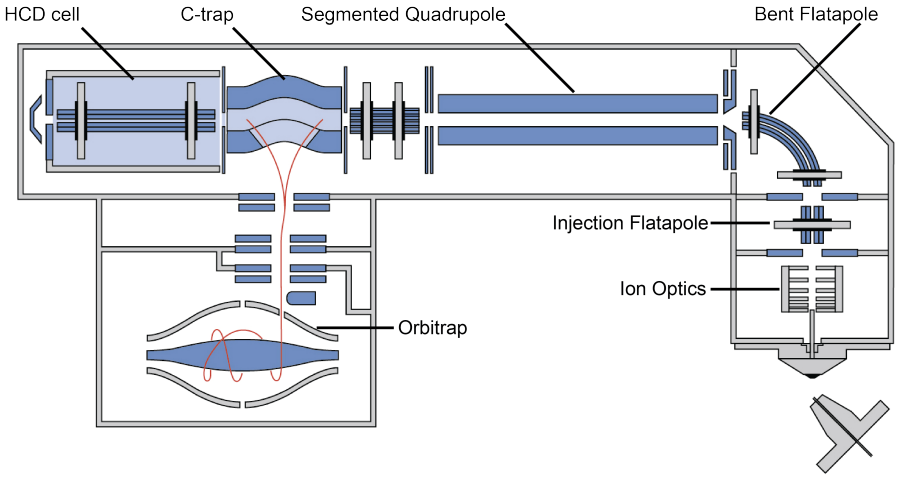

Figure 6 – Schematic design of a Q-Exactive mass spectrometer
The Q-Exactive is a state-of-the-art MS device, capable of detecting the mass-to-charge ratio of ionized peptides in the Orbitrap operated at MS1 and MS2 mode. This allows the quantification and identification of peptides which can later be assigned to proteins. Figure adapted from www.thermofischer.com

2 Material & Methods

2.1 Mice

A variety of transgenic mouse models were used to analyze differences in CSF proteome composition and for validation of the measurements (see also Fig. 5):

[A30P]αSYN (Thy1-hA30P-αS) mice (Kahle et al., 2000; Neumann et al., 2002), over-expressing human A30P-mutated α-synuclein under the control of a murine Thy1-promoter, were kindly provided by Prof. P. J. Kahle (University of Tübingen). In this model of α-synucleinopathies, a severe and lethal motor phenotype rapidly develops in aged homozygous mice (18±2 months), but not in heterozygous animals of the same age.

[A53T]αSYN (Thy1-hA53T-αS) mice (van der Putten et al., 2000) also express human α-synuclein under the same murine Thy1-promoter, but with an A53T mutation in α-synuclein. This model was kindly provided by Dr. D.R. Shimshek (Novartis). Heterozygous mice develop severe and fatal motor-symptoms very rapidly at young age (8±1 months).

The αSyn-transgenic mice were routinely monitored, occurrence and severity of motor symptoms was assessed based on a score sheet. In addition, the body weight was tracked constantly as clinical parameter to define humane endpoints.

As a model for beta-amyloidosis, we used APPPS1 (Thy1-hKM670/671NL-APP; Thy1-hL166P-PS1) mice (Radde et al., 2006), which co-express KM670/671NL mutated human APP and L166P mutated human PS1 under the control of the same neuron-specific murine Thy1 promoter element used in both α-Syn-transgenic models.

For validation of MS results, Lag3 knockout (KO) mice (B6.129S2-Lag3tm1Doi/J) (Miyazaki et al., 1996), were purchased from Jackson Laboratories (Bar Harbor, ME, USA), kept and bred in the animal facility of the Hertie institute prior to sample collection. Those mice are deprived of Lag3 expression by a null-mutation of the endogenous Lag3 gene.

All mice were maintained on a C57BL/6 background and kept under specific pathogen-free conditions in a 12 h light/dark cycle. The experimental procedures were carried out in accordance with the veterinary office regulations of Baden-Württemberg (Germany) and approved by the local Animal Care and Use Committees.

2.2 CSF and blood sampling from mice

CSF and blood were collected from anesthetized transgenic A30P-αSyn, APPPS1, Lag3-KO mice and age-matched non-tg wildtype (WT) littermates. The CSF collection was done according to standards set by the Alzheimer's Association external quality control program for human CSF biomarkers (Mattsson et al., 2011). In order to minimize effects by circadian proteome fluctuations, CSF sampling was always performed between 1 and 7 PM. Mice were deeply anesthetized with a mixture of ketamine (100 mg/kg bodyweight) and xylazine (10 mg/kg bodyweight) and placed on a heat pad to ensure a stable body temperature during CSF sampling.

After incision of the overlying skin and retraction of posterior neck muscles, the Dura mater, covering the Cisterna magna, was carefully cleaned with cotton swabs soaked with PBS or ethanol. In case of bleedings in the surrounding tissues, hemostyptic gauze (Tabotamp, Ethicon) was used to keep the Dura mater spared from blood contamination (Fig. 7). Next, the Dura mater was air-dried and subsequently lanced with a 30G needle (BD Biosciences). CSF was collected with a 20 µL gel loader tip (Eppendorf, shortened), transferred into polypropylene tubes (Eppendorf) and placed on ice. CSF was always collected immediately after puncturing of the Dura and up to three minutes later. Typically, a total volume of 15-25 µL was collected per mouse. The samples were then centrifuged for 10 min at 2000 RCF, assessed macroscopically for blood contamination, aliquoted and stored at -80°C until further usage. Samples affected by blood contamination were discarded.

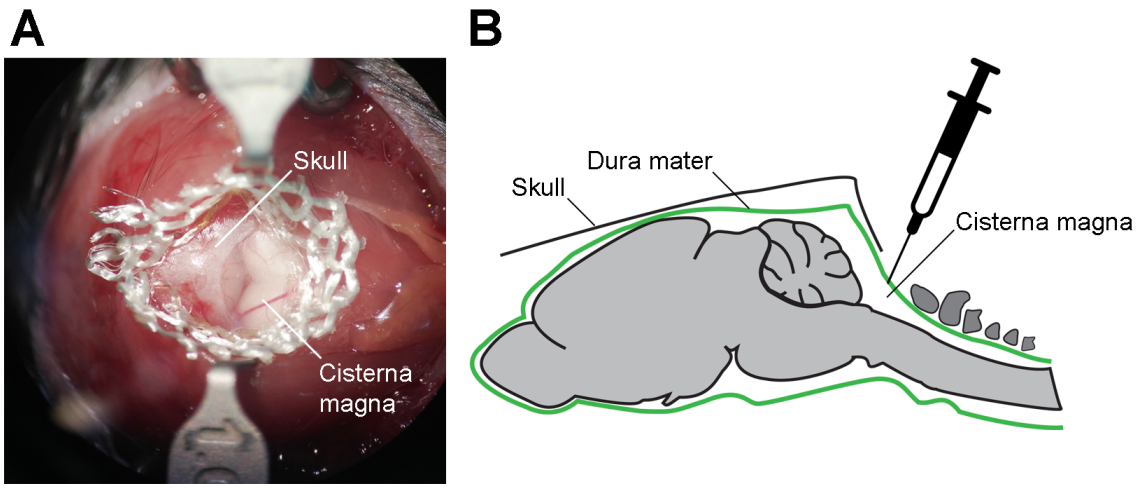

Figure 7 – CSF preparation in mice
After incision of the skin, retraction of neck muscles and cleaning, the Dura mater can be punctured and CSF can be collected. (A) Dorsal view on a mouse Cisterna magna shortly before CSF collection and (B) sagittal illustration of the CSF collection procedure, note the shallow depth of tip-insertion to avoid tissue and vessel ruptures.

Next, blood was collected from the right cardiac chamber with a syringe containing EDTA to prevent coagulation. To obtain serum, samples were centrifuged at 2000 RCF for 10 min, aliquoted and stored at -80°C until use.

Sampling of A30P-αSyn mice, and respective control animals, was done in young (3 months of age), presymptomatic/medium aged (11 months) and symptomatic/aged animals (19±1 months) of both sexes, while CSF and blood of APPPS1 mice were collected from young (3 months of age) and aged (18 months) males and age-matched littermates.

2.3 Brain sampling from mice

After CSF and blood sampling, heart and vessels were perfused to flush remaining blood from brain- and other arteries. For this purpose, the left cardiac chamber was punctured with a needle and slowly flushed with 12 mL of sterile PBS (4°C, Lonza, Switzerland). The brain was then removed, split into hemispheres and subsequently frozen in liquid nitrogen, or fixed by incubation in PFA (4%, in PBS, 48h) and cryoprotected in sucrose (30% in PBS).

2.4 CSF proteome analysis

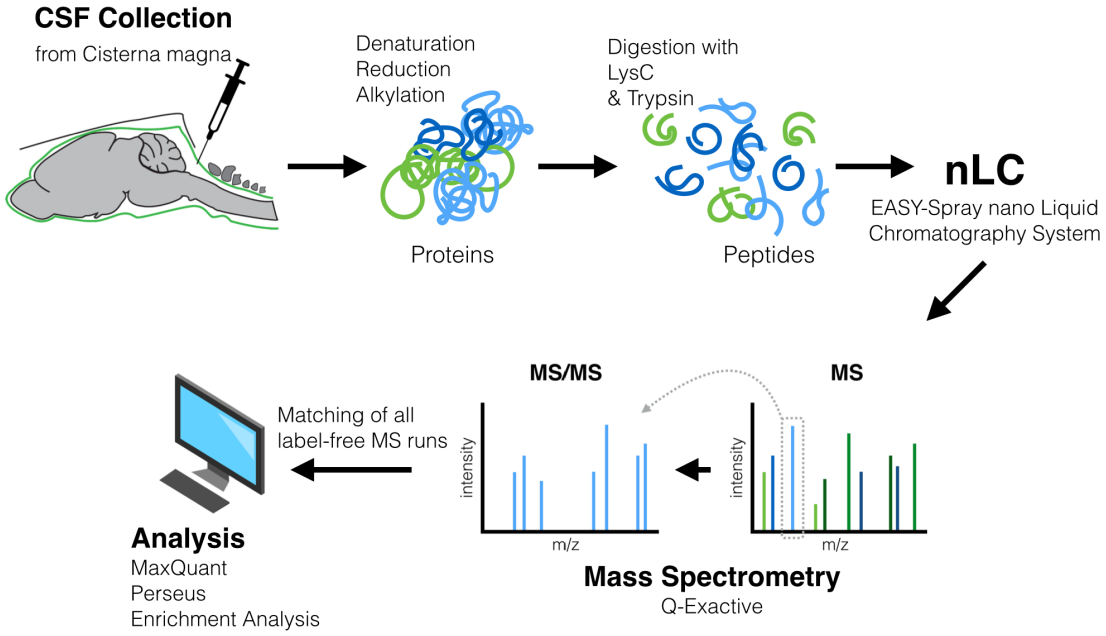

Figure 8 – Workflow and methods utilized for the analysis of individual A30P-αSyn, APPPS1 and WT CSF proteomes
CSF was collected from the Cisterna magna of anesthetized mice; proteins were digested with Lys-C and Trypsin, before wash and desalting steps on C18 STAGE-Tips. Resulting peptides were eluted from the STAGE-Tip before subsequent loading and elution from a nano-LC system. Peptides were ionized by electrospray-ionization before injection into an Q-Exactive Orbitrap mass spectrometer. Resulting spectra were loaded in MaxQuant and searched against a Uniprot database.

Sample processing

Denaturation Per mouse, 5 µL of CSF were diluted in 45 µL of denaturation buffer (6 M urea, 2 Thiourea in 10 mM HEPES pH 8.0). 5 µL of a 10 mM Dithiothreitol (DTT) solution was added and the sample incubated for 30 min to reduce disulfide bonds on proteins. Cysteines were alkylated by adding 5 µL of 55 mM Iodoacetamide (IAA) solution and incubation for 20 min in the dark. Excess of IAA was removed by addition of 5 µL 10 mM DTT and 30 min of incubation. All incubation steps were conducted at room temperature.

Enzymatic digestion The samples were pre-digested by the endopeptidase LysC and incubated for 3 h, prior to dilution in 100 µL of 50 mM ammonium bi-carbonate (ABC)

and digestion with Trypsin over night. After digestion, samples were desalted, washed using in-house prepared C18 (3M, USA) STAGE-Tips (Rappsilber et al., 2003), dried by vacuum centrifugation and re-dissolved in 20 µL of 0.1% formic acid.

LC-MS/MS analysis

For the sake of reducing sample complexity, 8 µL of previously digested and re-dissolved CSF samples were separated in a nano-LC system coupled online via a nanospray flex ion source or via an Easy-spray system (all from Proxeon - Thermo Scientific, US) to a Q-Exactive mass spectrometer (Thermo Scientific, US). The scan resolution in full scan mode was set to 70'000 (m/z range: 300-1400). The top 10 precursor ions (determined by intensity, exceeding an intensity of $2x10^4$), were further fragmented by collision induced dissociation with nitrogen gas. Spectra of resulting fragments were acquired at a resolution of 17'500. Precursor ions chosen for dissociation were included in a dynamic exclusion list for 60 seconds and blocked from repeated peptide fragmentation.

Data analysis & statistics

The spectra generated by MS were analyzed in the MaxQuant software-suite version 1.5.3.12 (Cox et al., 2014). This program searches and matches the generated spectra to a reviewed canonical FASTA database containing 16725 entries for mouse (*Mus musculus*) proteins; downloaded from UniProt (downloaded: August 26th 2015). The database was furthermore supplemented with a list of typical contaminants and the transgenic proteins of the mouse models used. Trypsin was defined as protease to assure correct *in silico* digestion of the proteins. A maximum of two missed cleavages were allowed for the database search. Peptide masses were pre-calibrated within a window of 20 ppm, by activating "first-search", leading to a more precise main search. Main search mass tolerance was set to 4.5 ppm for peptides and peptide fragment mass tolerance was set to 20 ppm. Acetylation of the protein N-term and oxidation of methionine were chosen as variable modifications. The false-discovery rate (FDR) for protein and peptide identification was set to $\leq 1\%$, using a target-decoy approach searching an integrated forward/reverse database. For label-free quantification (LFQ) of proteins, ratio counts of at least two unique peptides were required and quantification was done

solely on unique peptides. For the APPPS1 dataset, samples were measured in two separate LC-MS/MS sessions and batches were normalized in order to combine them. Therefore we normalized LFQ intensities of each protein group and sample to the respective mean LFQ intensities of aged (18 months) control animals. Normalization of the LFQ algorithm was disabled to account for age dependent CSF protein concentration differences, thus preventing global effects on quantification. The Perseus software package (Cox and Mann, 2012; Tyanova et al., 2016) version 1.5.2.6 was used to import and further analyze the data from MaxQuant. Correlation of technical or biological replicates were used to estimate the quality of each single measurement and helped to identify LS-MS/MS runs with technical issues, which were manually reviewed for proper LC-elution and excluded from further analysis otherwise. LFQ intensities of technical replicated were averaged, when possible, otherwise the LFQ intensity of a single technical replicate was used for the following steps. Potential contaminants, reverse hits or only hits only identified by site were filtered from the dataset and excluded from analysis. Next, LFQ intensities were log2 transformed and two-sided student's t-tests were carried out for each protein group to evaluate statistically significant regulated proteins. This was done for control mice and transgenic mice of the same age group. A p-value of 5% was set as initial significance threshold, while a permutation-based FDR estimation (250 randomizations, S0 = 0.1) was performed in addition to compensate for false positive hits generated by the large number of t-tests. For more reliable results, a minimum of three valid LFQ intensities per protein and experimental group was reuired for statistical tests.

Ontological enrichment analysis

In order to identify subsets of altered proteins in our filtered datasets, various enrichment analyses were conducted. Ingenuity Pathway Analysis IPA® (Quiagen, Germany) and DAVID 6.8 (http://david.ncifcrf.gov) (Huang et al., 2009a,b) provided deep insight into the datasets changed protein composition. For IPA, a p-value $< 5\%$ and a log2 fold-change $> \pm 0.5$ were set as thresholds. For DAVID analysis, significantly enriched proteins were searched for Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) annotations, while all reliably quantified proteins (quantified in ≥ 3 animals in each transgenic and WT groups) were set as background. The following

GO databases were searched: "CC_DIRECT", "BP_DIRECT" and "MF_DIRECT". Thresholds for functional annotation were set to a minimum count of 2 and an EASE score of 0.1 (a modified Fisher Exact p-value; smaller $\triangleq$ more enriched).

2.5 SDS-PAGE and immunoblotting

Samples were analyzed on 4-12% NuPAGE Bis-Tris mini gels, using NuPAGE LDS sample buffer (Invitrogen, USA). For immunoblotting, samples were transferred onto nitrocellulose membranes, followed by blocking in 3% BSA and 5% skim milk, then probed over night in monoclonal mouse antibody raised against murine LAG3 (4-10-C9 MABF954; Merck Millipore). Proteins were visualized using horseradish peroxidase-conjugated secondary antibodies and the SuperSignal West Dura chemiluminescence system (Thermo) exposed on Amersham Hyperfilm ECL (GE Life Sciences). Quantification of bands was done in ImageJ software v1.50i (Schneider et al., 2012). Statistical analysis was performed using GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA). Groups were compared using Bonferroni's post hoc test. Statistical significance level was set as follows: * if $p < 0.05$, ** if $p < 0.01$, *** if $p < 0.001$.

2.6 Electrochemiluminescence immunoassay for NfL

NfL levels were measured in duplicates using an electrochemiluminescence (ECL) immunoassay with some modifications (capture monoclonal antibody: 47:3; biotinylated detector monoclonal antibody: 2:1, Uman-Diagnostics AB, Sweden) (Gaiottino et al., 2013). Murine CSF (5 µL) and plasma were pre-diluted (1:14 & 1:2) in sample buffer (TBS containing 1% BSA, 0.1% Tween 20). For brain NfL measurements, fresh frozen brain hemispheres were homogenized to 10% (w/v) in homogenization buffer (50 mM Tris [pH 8.0], 150 mM NaCl, 5 mM EDTA, Pierce protease and phosphatase inhibitor cocktail) and subsequently centrifuged (25'000 x g; 4°C, 1 hr). Supernatant was diluted 1:1'000 in sample buffer. NfL specificity of the assay was confirmed with samples from NfL-deficient mice. CSF and blood samples were measured on the same plate. Samples with coefficients of variation (CVs) $> 20\%$ between duplicates were re-analyzed. All measurements were performed blinded. Statistical analysis was done in GraphPad

Prism 6.0 (GraphPad Software, San Diego, CA, USA). Differences between age groups or transgenics and WT of the same age group were compared using Bonferroni's post hoc test for multiple comparisons. Statistical significance level was set as follows: * if $p < 0.05$, ** if $p < 0.01$, *** if $p < 0.001$.

3 Results

3.1 Shotgun proteomics in the search for CSF biomarkers

Mouse models of disease, such as α-synucleinopathies and β-amyloidosis, provide the chance to discover protein alterations solely caused by underlying core pathologies and associated processes. These mice hold great translational value for the search of biomarkers, not only because they are spared from concomitant diseases, but can also be kept in controlled environments, unaffected from medication and other treatment. These advantages result in a clear reflection of disease associated events and facilitate identification of common and pathology-specific protein alterations.

3.2 CSF proteome complexity

CSF proteome is a complex mixture of proteins

The number of spectra, identified peptides and protein groups allows an initial estimation of the respective dataset quality. This was done separately for both LC-MS/MS experiments:

A30P-αSyn In total, the label free LC-ESI-MS/MS shotgun analysis yielded over 2.6 million MS/MS spectra from 42 mouse CSF samples. Those spectra led to the detection of 11609 peptide sequences originating from 1333 protein groups. After filtering out potential contaminants, reverse hits and hits only assigned by site, 1261 protein

groups were identified and quantified in the CSF of 3, 11 and 18 months old A30P-αSyn transgenic and age-matched control mice (Tab. 1). Those 1261 proteins were used for further analysis. This corresponds to an average of 598 ± 87 (mean ± SD) protein groups per mouse, quantified in just 2 µL of CSF.

APPPS1 Close to four million MS/MS spectra were generated from APPPS1 and corresponding control samples, the 31 CSF samples were measured in duplicates, which resulted in 11591 peptides that were assigned to 1256 protein groups (Tab. 1). After filtering, 1168 protein groups were left for analysis. On average 568 ± 106 (mean ± SD) protein groups were quantified per mouse.

Table 1 – CSF proteomes by numbers

		A30P αSyn	APPPS1
MS/MS Spectra submitted		2 604 601	3 964 242
MS/MS Spectra identified (% of submitted)		166 267 (6,4%)	223 322 (5,6%)
Peptides identified		11609	11591
Protein Groups identified		1333	1256
Protein Groups identified without C,R,S		1261	1168
Proteins quant. in ≥ 3 mice in TG and WT	at 18 mo	636	665
	at 11 mo	526	-
	at 3 mo	545	580

CSF proteome mainly consists of secreted and membrane-associated proteins

We sought to obtain a general overview of the proteome composition represented in the CSF of mice analyzed. With the CSF being in close proximity to the brain and blood vessels, cell degeneration or blood contamination may obscure biomarker changes and affect further analysis. Therefore, we used the subcellular location, assigned by UniProt SL-terms, as a proxy to untangle the origin of proteins detected in the CSF.

Consistently in both models and the respective controls, “Secreted”, “Membrane” and “Cytoplasm” were the most frequently assigned SL-terms, with “Secreted” being represented in 31 - 37% of all proteins. Devoid of any quantitative data, the actual proportion

Table 2 – Representation of UniProt terms "subcellular location"

	A30P αSyn dataset		APPPS1 dataset	
	A30P αSyn [%]	WT [%]	APPPS1 [%]	WT [%]
Cytoplasm	30,4	32,0	28,7	29,0
Membrane	31,5	31,0	29,7	29,9
Nucleus	13,0	13,5	11,1	12,8
Mitochondrion	3,9	3,7	2,8	3,8
Secreted	32,6	31,4	36,8	35,2
Unknown	16,0	17,5	15,6	16,5

of secreted protein in CSF is assumed to be higher. The protein annotation "Mitochondrium" makes up the smallest share (2,8 - 3,9%) of all SL-terms considered (Tab. 2). A low fraction of mitochondrion and nucleus affiliated proteins demonstrate minor, if any, effects of tissue, cell and blood contamination. Since proteins often have complex functions and can be found in multiple compartments, SL terms are not exclusive and values add up to more than 100%.

3.3 CSF proteome of αSyn transgenic mice (A30P-αSyn)

After surveying overall CSF proteome composition, we looked for proteins distinctly shifted from their corresponding level in non-transgenic age-matched controls. As a prerequisite for statistical testing, respective protein groups had to be quantified in at least three biological replicates in A30P-αSyn and control animals.

In young animals of 3 months, CSF proteome composition of transgenic animals is quite akin to controls, whereas peroxiredoxin-2 is closest to reach significance. While ongoing formation of α-synuclein lesions in presymptomatic mice has little influence on most protein levels, CSF concentration of cocaine- and amphetamine-regulated transcript protein (CART) -or derived peptides- is 2-fold higher than in age-matched controls ($p = 0{,}0001$, Fig. 10 B). In 18 month old A30P-αSyn mice however, levels were back to baseline and control levels.

In symptomatic animals, at approximately 18 months of age, a total of 220 protein

groups passed the stringent significance cutoff (Fig. 9). Amongst the most altered CSF proteins were complement C4, serotransferrin, triggering receptor expressed on myeloid cells 2 (TREM2), gamma-enolase (ENO2), chitinase-3-like protein 1 (YKL-40), lymphocyte activation gene 3 protein (LAG3), clusterin (Clu), granulin (Grn), apolipoprotein E (ApoE) and neurofilament medium polypeptide (NfM) (Figs. 9, 10 C-G & Tab. 3). NfM is the most significantly altered protein with levels approximately 1500-fold those found in control animals. All of those proteins had elevated levels in A30P-αSyn, while only six out of the 220 proteins had reduced levels compared to controls (Fig. 9 & Tab. 4). Both tables 3 & 4 are ranked according to p-value of A30P-αSyn versus controls at 18 months.

Another noteworthy trend is a general increase in various cathepsins and components of the complement system (Fig. 16). Proteins not detected in any of the groups to be compared, likely due to restrictions with the lower detection limit, cannot be integrated in this analysis. This concerns a small subset of proteins including NfL, which had considerably high LFQ-intensities in symptomatic A30P-αSyn, but was not quantified in non-transgenic littermates (Fig. 10 H-L).

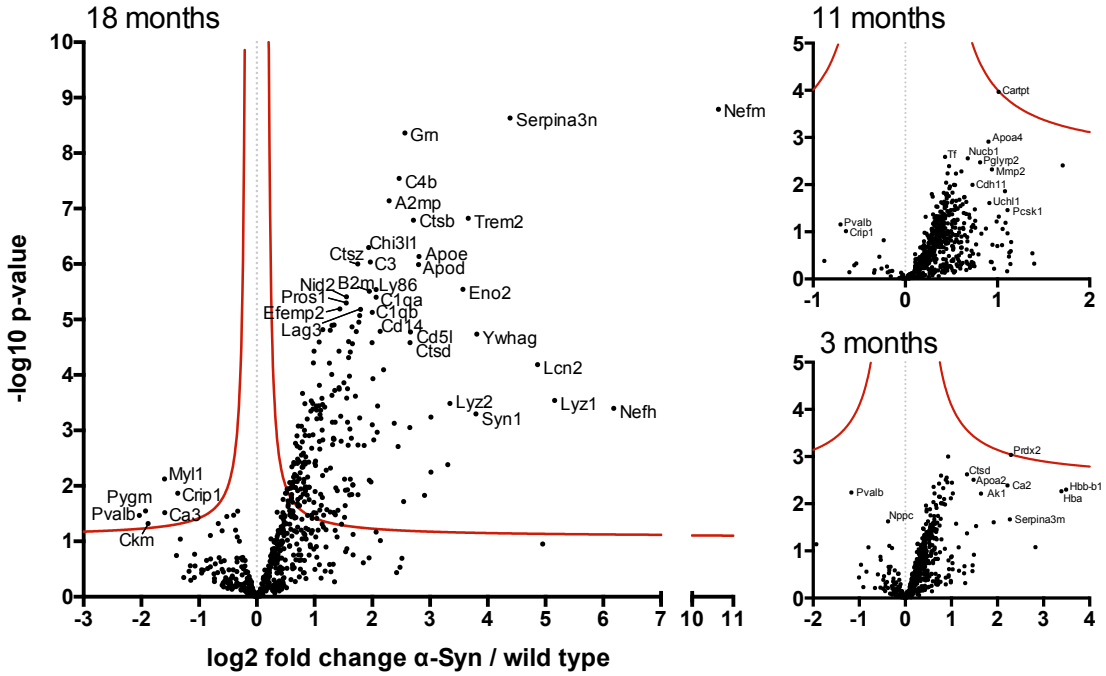

Figure 9 – CSF proteome changes in A30P-αSyn compared to age-matched controls at 18, 11 and 3 months of age

The figure illustrates both, fold change difference of A30P-αSyn to WT and the p-value of pairwise comparisons for each protein. Fold change difference is depicted on a log 2, p-value on a negative log 10 scale. Each dot represents a protein quantified in at least three A30P-αSyn and three control replicates per group. The cutoff for significance is indicated by a red curve, depicting the significance cutoff of permutation based FDR correction. Most differentially expressed proteins are labeled with their respective gene name. Comparisons of transgenic versus control mice are based on 5 to 10 animals for each age group. Proteins below detection limit and proteins not quantified in at least three biological replicates of both groups compared, can not be considered in this figure.

We then chose a subset of proteins from the dataset and examined them in more detail, including fluctuations across different age groups (Fig. 10). As a proof of concept, we quantified levels of αSyn-derived peptides and detected it almost exclusively in transgenic mice, with a marked 18 to 26-fold increase in symptomatic animals. Due to high similarity of endogenous murine and human transgenic α-Syn (94% AA sequence homology), the analysis did not differentiate between transgenic and endogenous α-Syn.

While TREM2 was not detected in young A30P-αSyn mice and is only slightly above control levels at 11 months, it is considerably elevated at 18 months, exceeding WT-levels by a factor of 12 (Fig. 10 C). A consistent and almost 4-fold increase of TREM2 is also detectable during "normal" aging in control animals. Levels of serpin A3N doubled during normal aging, while its amount in 18 mo αSyn-transgenic mice was 32-times

higher than in 3 month old A30P-αSyn and 20-times more than in 18 mo controls. Quantification of LAG3 revealed quadrupling concentrations in control CSF during normal aging and a 12-fold increase during aging in A30P-αSyn, resulting in an approximately 3.5 fold difference in the oldest age group (Fig. 10 E). In contrast to the previously described proteins, aging has hardly any effect on granulins, albeit granulin builds up considerably in CSF from symptomatic αSyn mice. In general, variance in CSF NfM amounts is quite prominent in young and intermediate age, but surprisingly consistent at 18 months, while the average levels are increased by a factor of approximately 1500 in transgenic animals. For NfL, LFQ intensities are missing in all groups, expect for symptomatic A30P-αSyn. Albeit this prevents any relative quantification, high LFQ intensities indicate large amounts of NfL. As another member of the neurofilament group, α-internexin shows a similar LFQ intensity-pattern, namely high LFQ intensities in aged αSyn transgenic animals. In the other age groups identification and quantification of peptides, allegedly originating from α-internexin, are exclusively based on "match between runs", a computational algorithm increasing the number of quantifiable peptides. Unfortunately, the algorithm can result in incorrect identification and quantification. The same is true for depicted intensities of syntaxin-B1 in the 11-month groups. Furthermore, tau and the synaptic proteins syntaxin and SNAP-25 were elevated in CSF of symptomatic A30P-αSyn (Fig. 10 J-L).

Next, to pinpoint enriched subsets of altered proteins, we performed enrichment analysis searching Gene Ontology (GO) databases and KEGG annotations. Therefore 220 significantly altered proteins were submitted to DAVID, of which 198 were included in the analysis. The results are shown in table 5 (described in 2.4 & 3.3). "Extracellular region" was the most commonly found GO-CC (GO Cellular Component) term, associated with 125 out of 198 proteins included in the analysis. "Innate immune response", "inflammatory response" and "proteolysis", amongst others, were found to be enriched biological processes (GO-BP), while "Lysosome" was the most altered pathway according to KEGG annotations, reflecting markedly increased levels of 16 lysosomal proteins in the CSF of symptomatic A30P-αSyn mice.

We furthermore analyzed the whole dataset by IPA. It found "Autophagy", "Atherosclerosis Signaling" and an increased "LXR/RXR Activation" to be the top three deregulated pathways in symptomatic A30P-αSyn mice, amongst "Acute Phase Response Signaling", "Complement System" and others (Fig. 11 on top). In CSF from presymp-

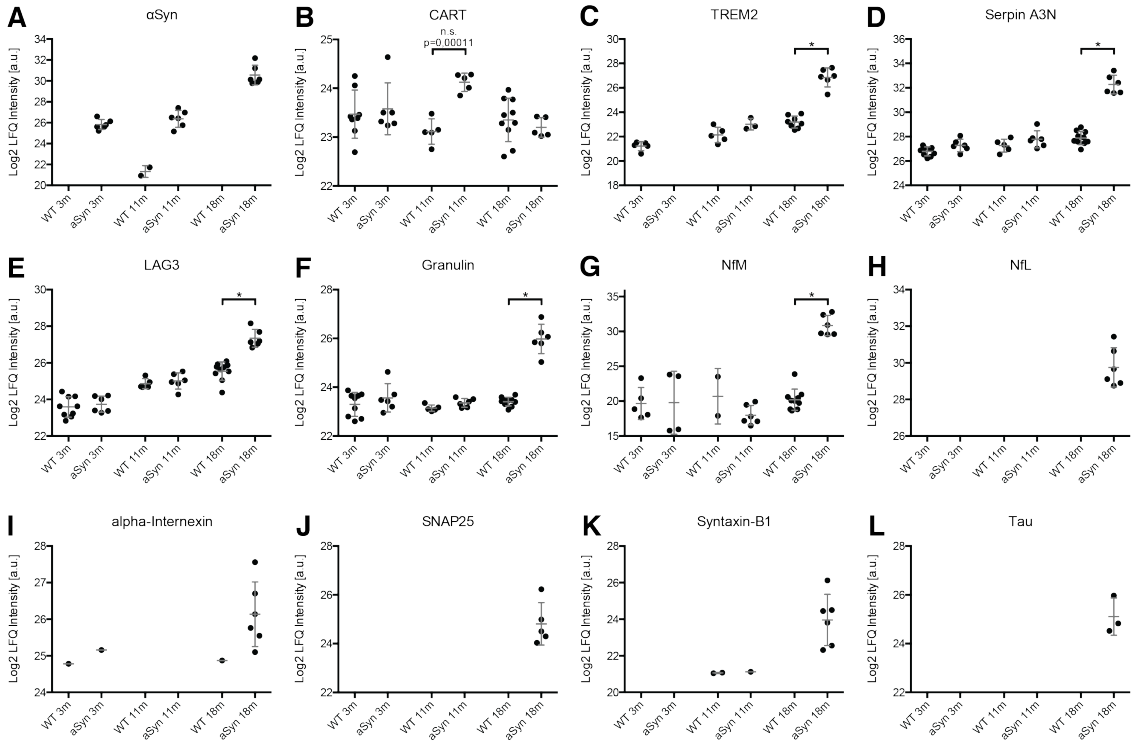

Figure 10 – Selected proteins in CSF of A30P-αSyn
Proteins quantified in CSF from A30P-αSyn mice and controls at 3, 11 and 18 months of age demonstrate varying protein levels during aging in transgenics and controls. (A) Due to high homology of murine and human transgenic αSyn distinction was not possible. (B) CART exhibits a distinct peak in A30P-αSyn mice at 11 months, while TREM2, Serpin A3N, LAG3, Granulin and NfM have their highest levels in symptomatic mice (C-G). (H-L) NfL, Tau, SNAP-25, Syntaxin-B1 and α-Internexin were exclusively or predominantly quantified in symptomatic A30P-αSyn, with levels below detection limits or unreliable quantification by spectral matching in the other groups. LFQ intensities are log2 transformed, n = 5-10 mice/group, Means ± SD are shown.

tomatic mice, only three pathways had a p-value slightly smaller than 5%, while a surprisingly high number of pathways were altered in young mice at 3 months of age (Fig. 11 mid and lower part). A small set of proteins (Apo-A1, Apo-A2, Apo-A4, Apo-E, RBP4, Serpin A1) were represented in most of the pathways listed, highlighting to these unexpected pathways.

3.4 CSF proteome of APP transgenic mice (APPPS1)

In analogy to the previously described CSF proteome analysis of A30P-αSyn mice, we searched the CSF of APPPS1 mice for markers of cerebral protein deposition and downstream pathological events. As mentioned earlier, a requirement for the analysis was protein quantification in at least three mice per group to be compared.

As shown in figure 12 (right panel), statistical testing found no markedly altered proteins in young animals. In aged mice however, eight proteins differed significantly in APPPS1 and WT mice and passed the strict FDR-corrected significance cutoff (Fig. 12, left panel). All of those eight proteins, including NF-M, TREM2 and LAG3, had increased CSF levels in transgenic mice. A list of the 25 most altered protein groups, including the eight significant hits, is given in table 6 (significant hits are highlighted in italics).

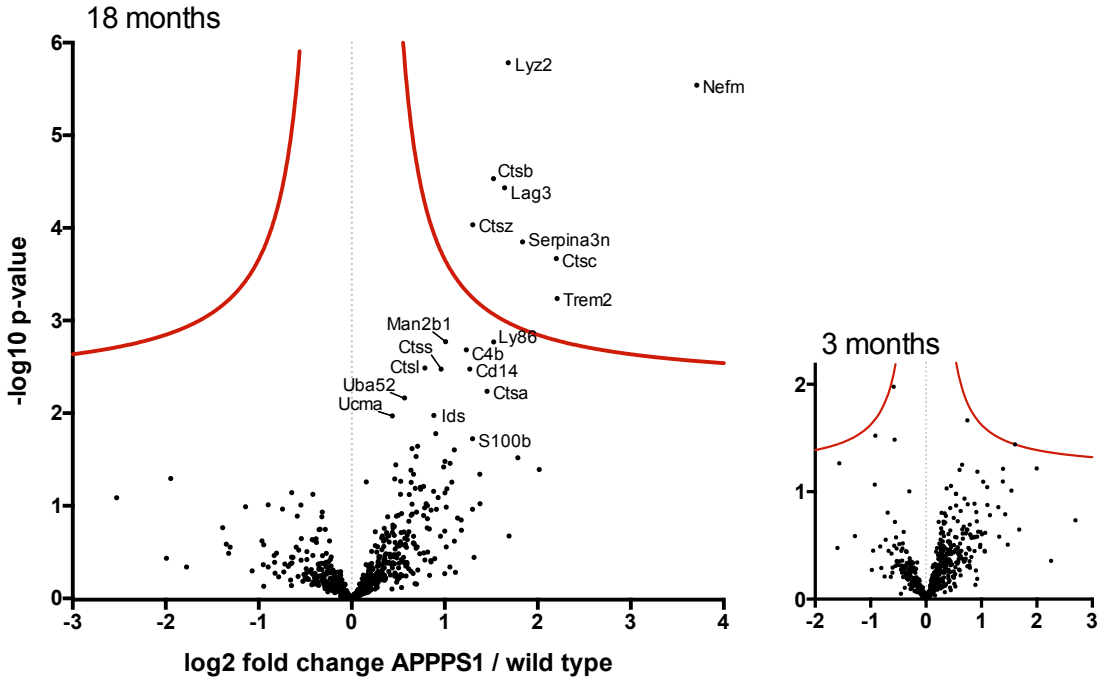

Figure 12 – CSF proteome changes in APPPS1 compared to age-matched controls at 18 and 3 months of age

Each dot represents a single protein quantified in APPPS1 and control animals. Log2-transformed fold difference of mean APPPS1 to WT is plotted to the negative log 10 p-value of the pairwise comparison. Significance cutoff (red line) is based on permutation based FDR corrected t-tests and factors in p-value and fold change. Most differentially expressed proteins were labeled with the respective gene name. Comparisons of transgenic versus control mice are based on 7 to 8 males per age group. Proteins below detection limit or not quantified in at least three biological replicates of both groups are not displayed in this graph.

As expected, we detected transgenic human APP harboring the "Swedish" mutation in APPPS1 mice exclusively (Fig. 13 A), while presenilin-1 was not quantified in any of the samples. We also observed stable levels of murine APP independent of age and transgene (Fig. 13 B). Notably, TREM2, serpin A3N, LAG3, granulin and NfM (Fig. 13 C-G) show the same, but less pronounced profile described in A30P-αSyn mice. All of said proteins have substantially increased concentrations in aged APPPS1 mice, with NfM showing the strongest effect with a more than 10-fold increase. Lysozyme C-2 has the smallest p-value of all proteins quantified in aged APPPS1 and WT mice, increased by a factor of 3.2 (Fig. 13 H).

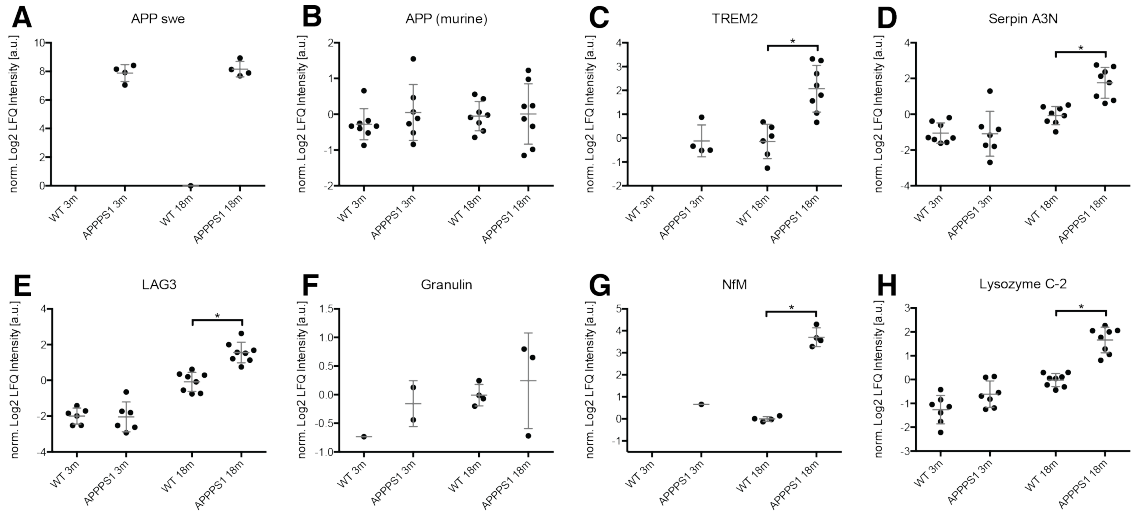

Figure 13 – Selected proteins in CSF of APPPS1
Proteins quantified in CSF of APPPS1 mice and controls at 3 and 18 months of age show increased protein concentrations in aged APPPS1, age-dependent increase is often also apparent in controls. (A,B) As expected, transgenic human APP with Swedish mutation was exclusively found in APPPS1 mice, while endogenous murine APP is stably found independent of age and transgene. (C-H) TREM2, Serpin A3N, LAG3, NfM and Lysozyme C2 levels were considerably elevated in APPPS1 at 18 months of age, while Granulin was quantified in insufficient numbers to allow definitive conclusions. LFQ intensities are log2 transformed and normalized separately for each of both batches (see 2.4), n = 7-8 mice/group, Means ± SD are shown.

Similar to the other dataset, eight significant hits were submitted to the DAVID functional annotation tool, searching previously mentioned GO and KEGG databases. Unfortunately, four out of the eight proteins were not represented in any of the enriched annotation clusters, which left cathepsins B, C, Z and TREM2 for the analysis. This led to the identification of cathepsin- and lysosome-associated GO-Terms (not shown). To make the analysis more conclusive, we also uploaded the top 25 proteins and repeated the analysis. However, this did not change the outcome considerably. A considerable proportion of cathepsins led to a set of very similar terms that can be summarized as lysosomal and peptidase affiliated processes.

IPA did not find enriched pathways in young mice, but found an enrichment of "Autophagy", "Phagosome Maturation" and "Macropinocytosis Signaling" associated proteins in aged APPPS1 (Fig. 14).

3.5 Shared and specific characteristics of αSyn and APP transgenic mouse CSF

Combining results from both experiments revealed pathology-related changes in the composition of the CSF proteome and facilitated discrimination of general neurodegeneration and neuroinflammation markers. We compared significance and direction of protein changes between A30P-αSyn and APPPS1 datasets (Fig. 15). Interestingly we found a correlative trend of up/up and down/down among the 550 proteins overlapping from both datasets, indicating a more general and unspecific disease profile (Pearson correlation, $p < 0.0001$). Among those were 24 proteins with $p < 0.05$ in both datasets, representing CSF proteins altered in due to secondary events, such as neurodegeneration or inflammation, but does not allow the two datasets -and respective pathologies- to be distinguished (Fig. 15, green box). Aside from those, we also found 208 and 4 proteins reflecting αSyn- or APPPS1-related CSF protein changes respectively (Fig. 15, yellow and blue boxes). TREM2 was considerably altered in both models of β-amyloidosis and synucleinopathies. The same can also be concluded for NfM, LAG3, various cathepsins, serpin A3N and complement C4B. Intriguingly, granulins had a considerably low p-value in A30P-αSyn mice, but not in APPPS1 mice (see also Figs. 10 G & 13 G).

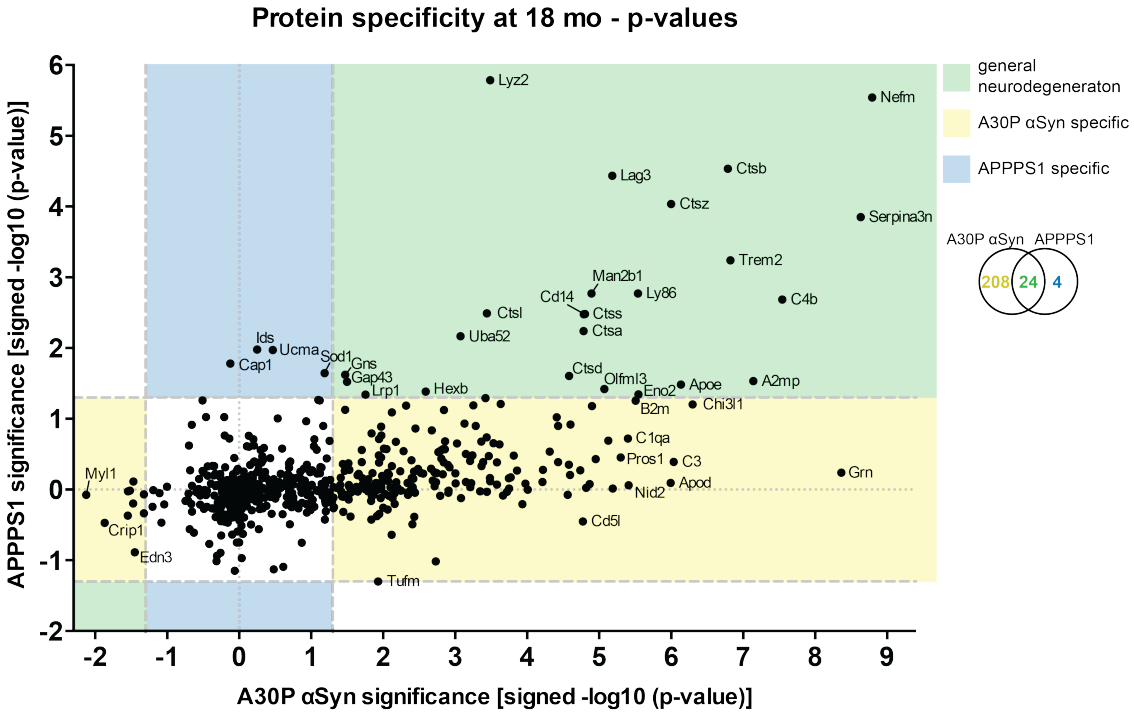

Figure 15 – Specificity of CSF proteome alterations
Proteome changes in αSyn and APPPS1 were compared in order to assess pathology-specific proteins. -log10 p-values (higher value = more significant) were transformed to negative values when the proteins fold change was negative. Proteins with p1 & p2 < 0.05 (approximately 1.3 on -log10 scale) in both datasets reflect general effects present in both models of neurodegenerative diseases (green), while p1 $<$ 0.05 and p2 > 0.05 of either of both datasets point protein alterations specific for either of the pathologies.

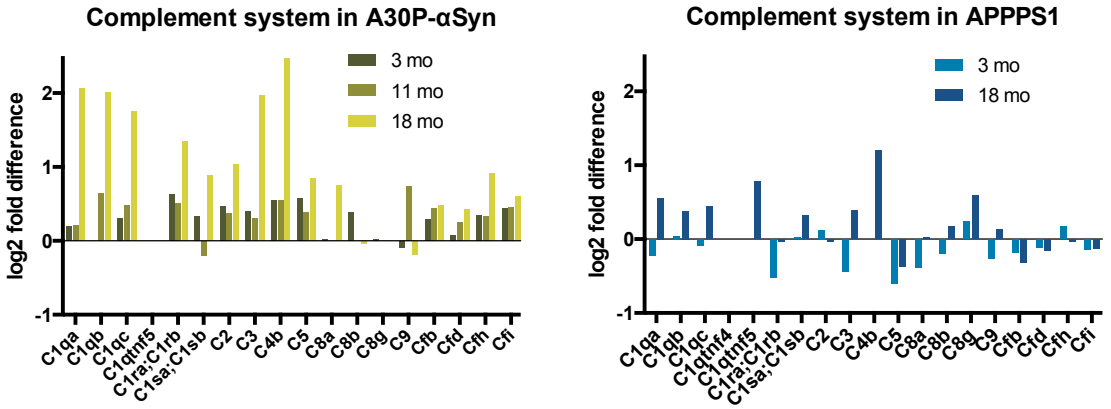

Figure 16 – Proteins of the complement system enriched in A30P-αSyn CSF
Distinct increase in proteins from the complement system was observed in symptomatic A30-αSyn. In aged APPPS1 mice only minor increases were observed.

Another interesting outcome of matching both datasets is depicted in Figure 16. The fold-change of complement-system associated proteins is shown for both models. A noticeable 2-4 fold increase over the levels of controls was found for complements C1q to C4-B in aged A30P-αSyn, but not in APPPS1.

3.6 LAG3 immunoblotting

As already highlighted by LC-MS measurements, LAG3 was clearly increased in aged transgenics in both models under investigation (Figs. 10 E & 13 E). Its supposed contribution in cell-to-cell transport of preformed α-synuclein fibrils made it a particularly interesting target for validation of our MS datasets. For that purpose, we used antibody-based western blot techniques (described in 2.5) and detected bands corresponding to a molecular weight of 52-55 kDa, which were absent in CSF from a homozygous Lag3-knockout mouse (Fig. 17). We therefore concluded that this signal stems from a 54 kDa fragment of LAG3, termed soluble-LAG3 (sLAG3), which contains all extracellular domains, but lacks the transmembrane and cytoplasmic domains. Quantification of the signals detected in A30P-αSyn and corresponding controls (Fig. 17 A) revealed the strongest difference in end-stage symptomatic A30P-αSyn, exceeding the average signal detected in aged controls by a factor of 4. The difference to young mice of the same genotype is even more obvious. A similar, but less pronounced trend is also present in APPPS1 mice and controls (Fig. 17 B), where the intensities in aged APPPS1 are doubled compared to controls of the same age. Consistently for both models, levels of LAG3 tripled also during normal aging of control animals.

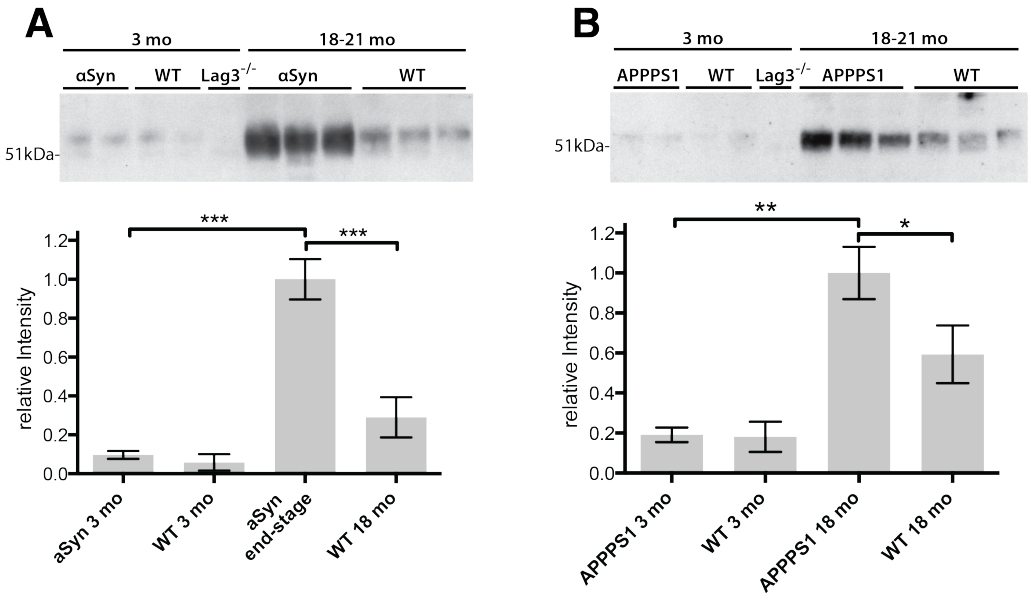

Figure 17 – Levels of (soluble) LAG3 in CSF, determined by western blot
This antibody based method was used to validate LC-MS/MS based results and discriminate soluble LAG3 (54 kDa) from full length LAG3. Quantification of signals from A30P-αSyn (A) and APPPS1 (B) confirm the quantitative outcome of the LC-MS/MS analysis. Differences between age groups, or transgenics and WT of the same age group, were compared using Bonferroni's post hoc test.

3.7 Neurofilament Light

This section is taken and/or adopted from the published manuscript (Bacioglu et al., 2016).

In recent times, NfL has been increasingly discussed as CSF and blood biomarker for the progression of neurodegenerative disorders. After analysis of the LC-MS/MS proteome data, we aimed at an absolute measurement of CSF and serum NfL levels in the examined mouse models and in an additional α-synuclein transgenic mouse line expressing human A53T-mutated αSyn. This much more aggressive transgenic model shows severe motor-symptoms already at 8-10 months of age.

In all three examined mouse models increased CSF NfL levels were already observed in the intermediate age groups and plasma NfL levels reflected only 1/30–1/40 of CSF levels (Fig. 18). Measurements of NfL in both, CSF and plasma, from A30P-αSyn mice revealed a very robust age-related increase in magnitude similar to that of 6-8 month old

A53T-aS mice. In the A53T-αSyn mice, a significant, more than 10-fold increase over WT controls was already observed in non-symptomatic 2-4 month-old mice. Thereafter NfL levels increased dramatically; in 8-10 month-old symptomatic mice NfL levels were approximately 1.000-fold higher than in non-transgenic age-matched controls. Plasma NfL did not yet exhibit a significant increase at 2–4 months of age, but a greater than 100-fold increase was found in the A53T-αSyn mice at the symptomatic stage. Consistently, such findings were also observed in APPPS1 mice with significantly higher CSF NfL levels over non-tg controls at both 3 and 12 months of age. At 18 months of age, the increase in APPPS1 mice was 10-fold over the age-matched WT controls. Plasma NfL also increased compared to non-tg mice at 18 months of age (Figure 18). However, the absolute NfL increase in APPPS1 was less than that observed in the A30P-αSyn mice, which goes in line with the LC-MS/MS results, where NfL was exclusively quantified in aged A30P-αSyn, the group with the highest amount of NfL (Fig. 10 H).

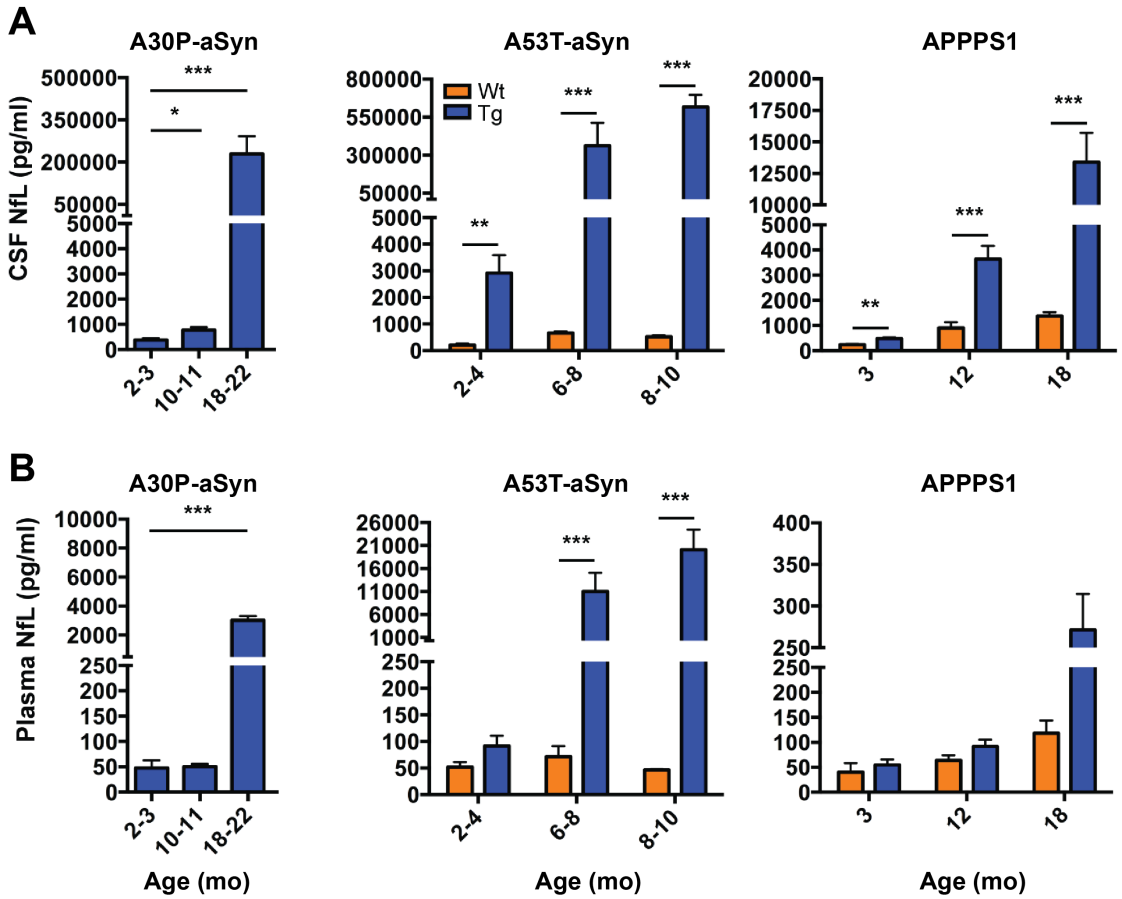

Figure 18 – Increase of NfL in CSF and plasma of α-Syn and APP transgenic mice
(A) CSF NfL and (B) plasma NfL levels in A30P-αSyn, A53T-αSyn, APPPS1 and WT control mice at different ages. Differences between age groups or transgenics and WT of the same age group were compared using Bonferroni's post hoc test for multiple comparisons. Note the markedly elevated levels of NfL in aged A30P-αSyn compared to APPPS1 mice of the same age. n = 4-6 mice per group, means ± SEM are shown. Figure adapted from Bacioglu et al., 2016.

Table 3 – Top 25 increased proteins in CSF of A30P-αSyn mice

Protein group	Gene	Protein ID	log2 fold change aSyn /Control 18 mo	11 mo	3 mo	-log10 p-value aSyn vs Control 18 mo	11 mo	3 mo
Neurofilament medium	Nefm	P08553	10,6		0,1	8,8		0,0
Serine protease inhibitor A3N	Serpina3n	Q91WP6	4,4	0,5	0,5	8,6	0,7	1,3
Granulins	Grn	P28798	2,6	0,2	0,3	8,4	1,3	0,4
Complement C4	C4b	P01029	2,5	0,5	0,5	7,5	2,2	1,6
Alpha-2-macroglobulin-P	A2mp	Q6GQT1	2,3	0,4	0,3	7,1	1,4	0,6
Triggering receptor expressed on myeloid cells 2	Trem2	Q99NH8	3,7	0,9		6,8	1,1	
Cathepsin B	Ctsb	P10605	2,7	0,4	0,4	6,8	1,8	1,2
YKL-40 / Chitinase-3-like protein 1	Chi3l1	Q61362	1,9	0,2	0,1	6,3	0,5	0,1
Apolipoprotein E	Apoe	P08226	2,8	0,7	0,8	6,1	1,4	2,3
Complement C3	C3	P01027	2,0	0,3	0,4	6,0	0,5	0,9
Cathepsin Z	Ctsz	Q9WUU7	1,7	0,4	0,2	6,0	1,2	0,5
Apolipoprotein D	Apod	P51910	2,8	0,5	0,6	6,0	1,5	0,9
Gamma-enolase	Eno2	P17183	3,6	0,7	-0,2	5,5	0,5	0,1
Lymphocyte antigen 86	Ly86	O88188	2,1	0,4	0,5	5,5	1,3	0,8
Beta-2-microglobulin	B2m	P01887	1,9	0,3	0,5	5,5	1,1	1,2
Nidogen-2	Nid2	O88322	1,6	0,3	0,7	5,4	0,6	2,2
Complement C1q subunit A	C1qa	P98086	2,1	0,2	0,2	5,4	0,5	0,4
Vitamin K-dependent protein S	Pros1	Q08761	1,5	0,5	0,4	5,3	1,5	1,0
EGF-containing fibulin-like extracellular matrix protein 2	Efemp2	Q9WVJ9	1,4	0,3	0,4	5,2	0,8	1,0
Lymphocyte activation gene 3 protein	Lag3	Q61790	1,8	0,1	0,2	5,2	0,3	0,2
Complement C1q subcomponent subunit B	C1qb	P14106	2,0	0,6	0,0	5,1	1,7	0,0
Olfactomedin-like protein 3	Olfml3	Q8BK62	1,8	0,6	0,0	5,1	1,1	0,0
Platelet-activating factor acetylhydrolase	Pla2g7	Q60963	1,8	0,4	0,7	5,0	1,4	1,6
Plexin domain-containing protein 2	Plxdc2	Q9DC11	1,3	0,2	0,3	4,9	0,3	0,5
Lysosomal alpha-mannosidase	Man2b1	O09159	1,3	0,5	0,3	4,9	1,8	1,1

Table 4 – Significantly decreased proteins in CSF of A30P-αSyn

			log2 fold change aSyn/Control			-log10 p-value aSyn vs Control		
Protein group	Gene	Protein ID	18 mo	11 mo	3 mo	18 mo	11 mo	3 mo
Myosin light chain 1/3, skeletal muscle isoform	Myl1	P05977	-1,6		-0,8	2,1		0,6
Cysteine-rich protein 1	Crip1	P63254	-1,4	-0,6	-0,3	1,9	1	0,4
Glycogen phosphorylase, muscle form	Pygm	Q9WUB3	-1,9	0,7	0,9	1,5	0,3	0,5
Carbonic anhydrase 3	Ca3	P16015	-1,6	0,6	-0,5	1,5	0,8	0,8
Parvalbumin alpha	Pvalb	P32848	-2	-0,7	-1,2	1,5	1,2	2,2
Creatine kinase M-type	Ckm	P07310	-1,9	0,7	-0,2	1,3	1,3	0,2

Table 5 – Enrichment analysis of 220 significantly altered proteins in CSF from 18 month old A30P-αSyn. Each category is ranked by EASE Score

Category	Term	Count	% of proteins	EASE Score
Cellular Component				
	extracellular region	125	56,8	0,000012
	lysosome	27	12,3	0,000044
	blood microparticle	37	16,8	0,00013
	extracellular exosome	158	71,8	0,00066
	extracellular space	110	50	0,00073
	basement membrane	17	7,7	0,0061
	extracellular matrix	39	17,7	0,018
	basal lamina	7	3,2	0,086
Biological Process				
	innate immune response	24	10,9	0,011
	proteolysis involved in cellular protein catabolic process	9	4,1	0,024
	extracellular matrix organization	14	6,4	0,039
	tissue regeneration	5	2,3	0,053
	proteolysis	23	10,5	0,055
	immune system process	20	9,1	0,062
	lipid metabolic process	12	5,5	0,073
	inflammatory response	14	6,4	0,078
Molecular Function				
	hydrolase activity	48	21,8	0,020
	cysteine-type peptidase activity	8	3,6	0,027
	hydrolase activity, acting on glycosyl bonds	9	4,1	0,027
	peptidase activity	24	10,9	0,045
	serine-type carboxypeptidase activity	5	2,3	0,056
KEGG Pathway				
	Lysosome	16	7,3	0,0066
	Phagosome	9	4,1	0,03
	Tuberculosis	9	4,1	0,03
	ECM-receptor interaction	11	5	0,046
	PI3K-Akt signaling pathway	17	7,7	0,071
	Pertussis	10	4,5	0,08

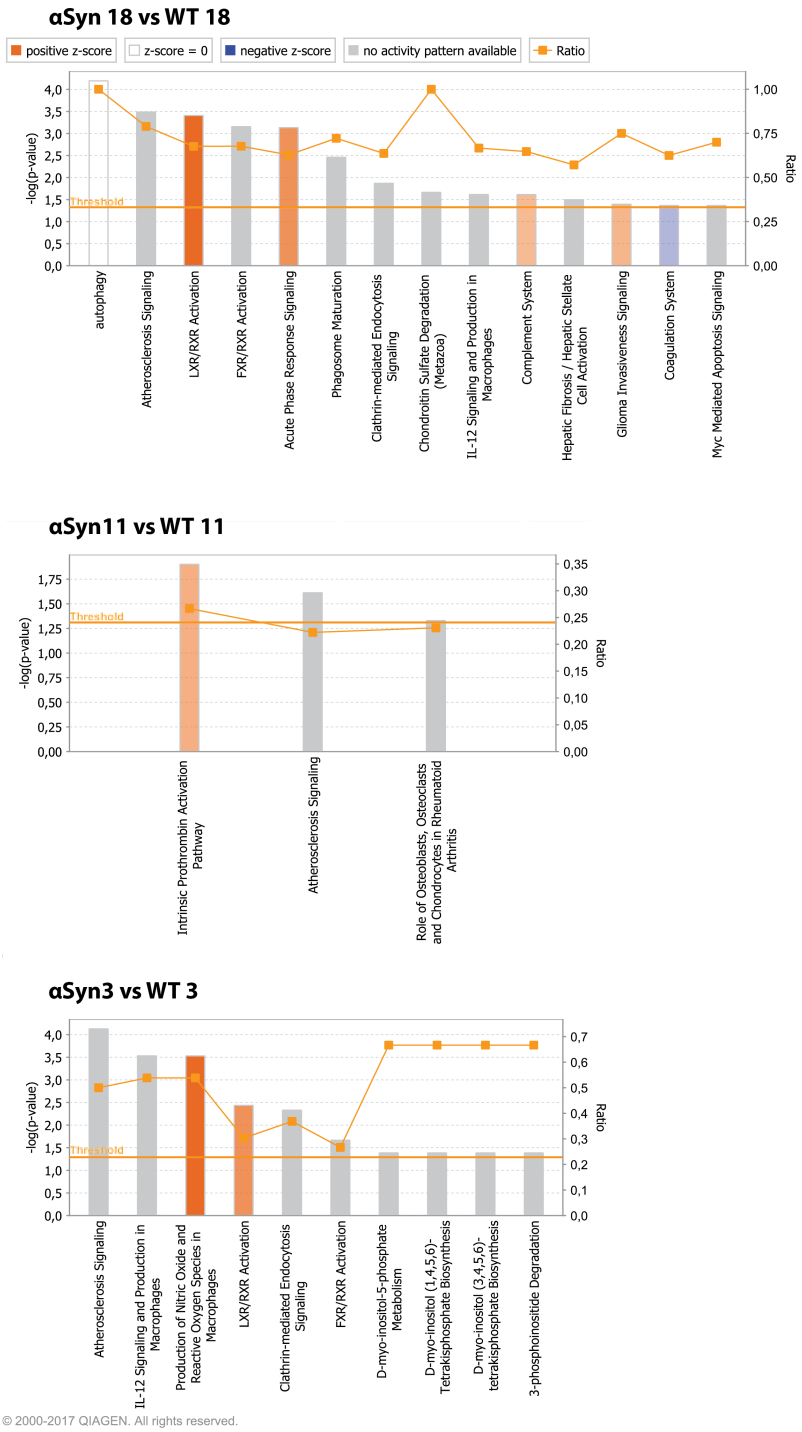

Figure 11 – Pathways altered in the CSF of A30P-αSyn
Pathways changed in the CSF of A30P-αSyn mice at different ages. Up- or downregulation of the respective protein subset is color-coded according to z-score.

Table 6 – Top 25 altered proteins in CSF of APPPS1 mice

			log2 fold change in APPPS1 / Controls		-log10 p-value APPPS1 vs Control	
Protein name	Gene	Protein ID	18 mo	3 mo	18 mo	3 mo
Lysozyme C-2	*Lyz2*	*P08905*	*1,7*	*0,6*	*5,8*	*1,3*
Neurofilament medium polypeptide	*Nefm*	*P08553*	*3,7*		*5,5*	
Cathepsin B	*Ctsb*	*P10605*	*1,5*	*0,3*	*4,5*	*0,3*
Lymphocyte activation gene 3 protein	*Lag3*	*Q61790*	*1,6*	*0,0*	*4,4*	*0,0*
Cathepsin Z	*Ctsz*	*Q9WUU7*	*1,3*	*0,3*	*4,0*	*0,5*
Serine protease inhibitor A3N	*Serpina3n*	*Q91WP6*	*1,8*	*0,0*	*3,9*	*0,0*
Cathepsin C; Dipeptidyl peptidase 1	*Ctsc*	*P97821*	*2,2*	*-0,6*	*3,7*	*2,0*
Triggering receptor expressed on myeloid cells 2	*Trem2*	*Q99NH8*	*2,2*		*3,2*	
Lysosomal alpha-mannosidase	Man2b1	O09159	1,0	0,0	2,8	0,0
Lymphocyte antigen 86	Ly86	O88188	1,5	0,1	2,8	0,1
Complement C4	C4b	P01029	1,2	0,0	2,7	0,0
Cathepsin L1	Ctsl	P06797	0,8	0,1	2,5	0,2
Cathepsin S	Ctss	O70370	1,0	0,3	2,5	0,5
Monocyte differentiation antigen CD14	Cd14	P10810	1,3	0,2	2,5	0,1
Cathepsin A; Lysosomal protective protein	Ctsa	P16675	1,5	-0,1	2,2	0,1
Ubiquitin	Uba52;[...]	P62984[...]	0,6	0,3	2,2	0,5
Iduronate 2-sulfatase	Ids	Q08890	0,9	-0,3	2,0	0,4
Unique cartilage matrix-associated protein	Ucma	Q14BU0	0,4	0,4	2,0	0,8
Adenylyl cyclase-associated protein 1	Cap1	P40124	0,9		1,8	
Protein S100-B	S100b	P50114	1,3	0,8	1,7	0,4
Superoxide dismutase [Cu-Zn]	Sod1	P08228	0,7	0,2	1,6	0,3
N-acetylglucosamine-6-sulfatase	Gns	Q8BFR4	0,6	0,1	1,6	0,2
Cathepsin D	Ctsd	P18242	1,1	0,4	1,6	0,3
Alpha-2-macroglobulin-P	A2mp	Q6GQT1	0,7	0,2	1,5	0,3
Neuromodulin	Gap43	P06837	1,8	0,1	1,5	0,2

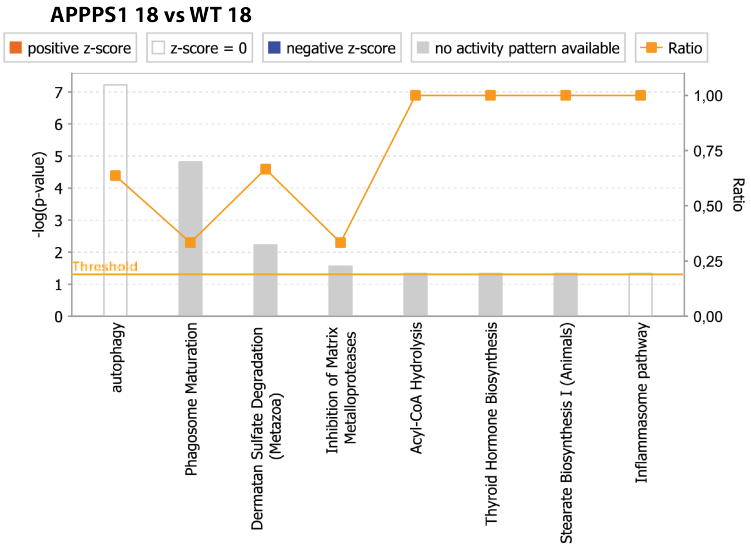

Figure 14 – Pathway altered in the CSF of APPPS1 at 18 months of age
Pathways changed in the CSF of APPPS1 mice at 18 months of age show a strong involvement of "Autophagy" and "Phagosome Maturation" due to a strong deregulation of cathepsins. No enrichment clusters were found at 3 months of age.

4 Discussion and Conclusions

Although this year marks the 200th anniversary of James Parkinson's first description of "the shaking palsy" and the 110th year after Alois Alzheimer's initial report on Auguste Deter, researchers have yet not fully understood the underlying pathomechanisms and prevention strategies remain to be found. Overlapping symptomatology and slow progression of those and other neurodegenerative diseases further contribute to diagnostic difficulties and emphasize the need for specific biochemical markers (Blennow et al., 2016). New approaches for the treatment of PD and AD would greatly profit from novel and more reliable biomarkers that can facilitate diagnosis in early stages of disease and tracking of its progression.

In AD research, the quest for respective biomarkers started only in 1995, when the first enzyme-linked immunosorbent assays (ELISA) for Aβ42, phosphorylated tau (P-tau) and total tau (T-tau) protein were published (Blennow et al., 1995; Motter et al., 1995), whereby a new field of research was born. Currently Aβ42, P-tau and T-tau, due to their interplay with the pathophysiological lesions, are still considered the most reliable biomarkers for AD. Regarding PD, such biochemical markers remain to be found and validated (Blennow et al., 2016).

Over the last years, numerous studies tried to identify novel biomarkers fulfilling the criteria for clinical diagnosis. However, those studies typically focused on single candidates or small subsets of proteins and peptides, while the variety of sampling and analysis procedures often led to conflicting results (Andersen et al., 2017). With the technological progress of mass spectrometry-based proteomics, antibody-independent methods were available and led to the development of the first fully validated single reaction monitoring (SRM) mass spectrometry method for targeted quantification of Aβ42 (Leinenbach et al., 2014). Progress of MS-instrumentation also gave rise to a

broader approach, the so-called shotgun proteomics, where complex mixtures of proteins can be analyzed in an un-biased manner and result in quantification of several hundreds or thousands of proteins, making it an ideal tool for the discovery of novel biomarkers.

4.1 CSF proteome alterations

Mice are the most commonly used animal model in clinical research of neurological diseases (Chesselet and Carmichael, 2012). As a result of a mutated human transgene, the models used here recapitulate many aspects of disease. The resemblance to transgenic protein deposition and core-biomarker changes in familial cases of PD and AD is remarkable (Maia et al., 2013, 2015), which makes these mice suitable tools for the investigation of protein dynamics in CSF and thereby search for altered molecular processes and novel biomarkers of disease.

Hence the goals of this thesis were two-fold: First we wanted to quantify the CSF proteome in an unbiased way to unveil alterations in proteins and biological processes as a result of proteopathic lesions. Second, we sought to highlight both, common and specific, -α-synucleinopathy or β-amyloidosis derived- changes and emphasize novel potential biomarkers. Regarding the first goal, 1261 and 1168 proteins were identified in as little as 2 µL CSF per technical replicate from A30P-αSyn and APPPS1 mice. This underlines the remarkable sensitivity achieved by the label-free LC-ESI-MS/MS approach for mouse CSF. The resulting datasets unveiled 220 protein groups in A30P-αSyn and 8 protein groups in APPPS1 with significantly altered levels at 18 months of age, when protein deposition is clearly recognizable in both models. As anticipated, significant changes were missing in the CSF proteome of 3 month old mice, which demonstrates that the mere presence of the transgenic protein does not inevitably lead to substantial proteome alterations. Concerning the second goal, our approach revealed a myriad of proteins associated with neuroinflammation, the autophagy-lysosomal system and other processes, but also shed light on proteins that may serve as novel biomarkers. This emphasizes the value of αSyn and APP transgenic mouse models in the search for new disease markers.

4.2 CSF core biomarkers

α-synuclein The diagnostic value of αSyn has been investigated in many studies and led to often conflicting results, while most studies report CSF total αSyn as being decreased in PD patients when compared to controls (Blennow et al., 2016). Intriguingly, when compared to levels of young or presymptomatic mice, we measured 18-26 fold increased levels of αSyn in the CSF of late-stage A30P-αSyn transgenic mice. At such an advanced stage of disease, symptoms typically occur as a result of the death of dopaminergic neurons in the substantia nigra (Parkkinen et al., 2008; Blennow et al., 2016), which in turn leads to a release of intracellular oligomeric αSyn. Due to tryptic digestion, which is essential for a shotgun mass-spec based approach, the experimental setup can not differentiate between the various αSyn species, such as monomers or oligomers. We must therefore assume that oligomeric αSyn gets digested by trypsin and thereby boosts the amount of αSyn peptides quantified by MS. The αSyn peptides detected in 11mo WT animals either originate from carry-over of prior LC runs, are artifacts of the match between runs algorithm, or simply reflect murine αSyn. Murine αSyn was not discriminated from A30P-human αSyn due to a high degree of homology.

APP We were able to distinguish between the endogenous murine APP and the transgenic APP with the Swedish mutation (APPswe) expressed in APPPS1 mice. As a proof of concept, we detected relatively stable levels of the murine APP over age, but also similar expression levels in the control animals. The analysis also revealed that APPswe-derived peptides were exclusively quantified in CSF from APPPS1 mice and that the levels were not influenced by the age of the transgenic mice.

Tau Although the microtubule-associated protein tau is considered a core biomarker for AD, we did not quantify it in the model of β-amyloidosis. Also one would not necessarily expect to find substantial levels of tau in a model of β-amyloidosis, spared from considerable tau-pathology and NFTs . Nevertheless, we exclusively quantified tau protein in three of the symptomatic A30P-αSyn mice. Tau is known to bind αSyn and positive correlation between CSF αSyn and tau levels has been observed (Jensen et al., 1999; Slaets et al., 2014), which might explain its CSF abundance in aged αSyn-overexpressing mice.

4.3 Inflammatory proteins

Microglial activation and neuroinflammation are increasingly recognized to play critical roles in the pathogenesis of PD, AD and other neurodegenerative diseases (Amor et al., 2010; Tansey and Goldberg, 2010; Heneka et al., 2015). Whether the activation of microglia is beneficial or adverse for the course of disease is still an open question (reviewed in greater detail in Frank-Cannon et al., 2009). In the work presented here we show that neuroinflammation is also reflected in the CSF proteome composition of transgenic mouse models.

Serpin A3N We found a remarkable increase in serine protease inhibitor A3N (Serpin A3N), the murine orthologue of human α1-antichymotrypsin (ACT) (Horvath et al., 2005) in both models at 18 months of age. ACT is a well established marker of inflammation; co-localized with amyloid-β in senile plaques (Abraham et al., 1988) and elevated levels were reported in CSF and plasma of AD patients (Licastro et al., 1995; Porcellini et al., 2008). Genetic association studies have come to conflicting results concerning the connection of ACT gene polymorphism and susceptibility for PD, but the age of disease onset seems to be influenced by ACT (Yamamoto et al., 1997; Wang et al., 2001). These findings, together with our results, propose an unspecific and inflammation-derived role for Serpin A3N, as a consequence of proteopathic deposition.

Granulin / Progranulin Strikingly, granulins (GRN) were increased in the A30P-αSyn dataset, but not in APPPS1 CSF. Granulins are cleaved from its precursor protein progranulin (PGRN) and typically involved in cellular processes such as wound healing and inflammation (He et al., 2003). In humans, PGRN mutations are a major inherited cause of frontotemporal dementia (FTD), and mutation-caused PGRN deficiency promotes neuroinflammation (Baker et al., 2006; Cruts et al., 2006; Martens et al., 2012). Intriguingly, mutations in PGRN were described in some rare cases of AD and PD (Brouwers et al., 2007, 2008; Kelley et al., 2010). PGRN is also connected to the dopaminergic system in mice, having a protective function for dopaminergic neurons, while PGRN deficiency promotes deregulated microglial activation and contributes to increased neuronal loss (Martens et al., 2012; Van Kampen et al., 2014). Further experiments are needed to identify the molecular mechanism underlying CSF granulin

increase observed in our mouse model of α-synucleinopathy, while it is likely linked to the dopaminergic system.

Complement system We also found increased levels of complement related proteins in A30P-αSyn CSF. The complement system is part of both, innate and adaptive immune system, while its inappropriate activation can contribute to disease (Mackay et al., 2001). Furthermore, Magdalinou and colleagues recently found marked increases of complement C9 and complement factor H in the CSF of PD patients (Magdalinou et al., 2017). However, they did not report an increase in other complement components, which are altered in A30P-αSyn, but not in APPPS1.

TREM2 While triggering receptor expressed on myeloid cells 2 (TREM2) has been intensively investigated as biomarker for AD (Guerreiro et al., 2013; Kleinberger et al., 2014; Suárez-Calvet et al., 2016), others highlight its role in inflammation and microglial activation (Hickman and El Khoury, 2014). TREM2 has been shown to be upregulated on amyloid-plaque-associated microglia in depositing APP23 mice, another APP-transgenic model for β-amyloidosis (Frank et al., 2008). We found considerably elevated levels of TREM2 in the CSF of both models and thereby characterize TREM2 as a general CSF marker for neuroinflammation or -degeneration, rather than a specific marker for amyloid-deposition.

4.4 LAG3

Mao et al. recently described lymphocyte activation gene 3 protein (LAG3) as a protein facilitating binding and transmission of preformed α-synuclein fibrils (PFF) on neurons. They also demonstrated a high specificity of LAG3 binding to αSyn-PFF (Mao et al., 2016). Intriguingly, our study shows increased levels of LAG3 not only in αSyn mice, but also in APPPS1 mice, devoid of any α-synucleinopathy. We therefore assume an αSyn-independent mechanism causing an increase of LAG3-derived peptides in CSF. LAG3 can be cleaved by α-secretases ADAM10 and ADAM17, resulting in a 16-kDa transmembrane and a 54-kDa fragment containing all extracellular domains while the 54-kDa fragment is released as soluble LAG3 (sLAG3) upon T-cell activation (Lammich

et al., 1999; Li et al., 2004). This matches the molecular weight of the signal detected in murine CSF on western blot. The quantitative results of both LC-ESI-MS/MS experiments were validated by western blot analysis of LAG3, hence demonstrating a high correlation of sLAG3 levels determined by immunoblotting and mass spectrometry.

4.5 Lysosomal proteins

Impairment of the autophagy-lysosomal pathway is increasingly regarded as a major pathogenic event in neurodegenerative diseases, including PD (Dehay et al., 2013). Our analysis revealed a high fraction of lysosomal proteins enriched in A30P-αSyn CSF, including 3-fold increased levels of phospholipase D3 (PLD3). However, in APPPS1 we did not measure such an increase, while others demonstrated inverse correlations of PLD3 expression with APP expression or Aβ-production in vitro (Cruchaga et al., 2014a). Furthermore, mutations in Pld3 confer risk to AD and an *in-vitro* knockdown leads to an increase of extracellular Aβ42 and Aβ40, while PLD3 was furthermore found to accumulate in senile plaques (Cruchaga et al., 2014b; Satoh et al., 2014). Fazzari and colleagues recently challenged PLD3 implication in APP metabolism or plaque-burden and emphasized its localization and implication in the lysosomal system suggesting alternative functions for PLD3 in neurodegeneration (Fazzari et al., 2017).

Lysosomal malfunction accompanies αSyn aggregation and is increasingly recognized as key part in pathogenesis of many neurodegenerative diseases, including PD (Meredith et al., 2002; Pan et al., 2008). Cathepsins, lysosomal alpha-mannosidase (Man2b1) and N-acetylglucosamine-6-sulfatase (Gns) are examples of lysosomal proteins deregulated in CSF of both models used in this study. Considering the marked increase of PLD3 in A30P-αSyn CSF and the rather unspecific elevation of other lysosomal proteins, our data support the concept of a disturbed lysosomal system as a result of proteopathic lesions.

4.6 CART

Motivated by the idea to find biomarkers that would cover the urgent need for early biomarkers of PD, we also analyzed presymptomatic A30P-αSyn mice, which have αSyn

deposition predominantly in the brainstem and spinal cord, but lack noticeable motor-symptoms, thereby mimicking PD patients in early stages of disease. Peptides derived from cocaine- and amphetamine-regulated transcript protein (CART) were elevated in CSF of presymptomatic αSyn mice, while being reduced to WT levels in symptomatic mice. CART is involved in various physiological processes mediated by dopamine, while administration of CART was shown to ameliorate dopaminergic neuronal loss in models of PD (Mao et al., 2013; Subhedar et al., 2014; Upadhya et al., 2016). While CSF CART levels were reduced by 30% in cases of dementia with Lewy bodies (DLB) (Schultz et al., 2009), we report the amount of CART to be doubled in the CSF of A30P-αSyn mice at 11 months of age. The exact mechanism influencing CART-level fluctuations in the CSF remains to be identified.

4.7 Synaptic proteins

Synaptic dysfunction represents a major event in PD, which is likely a consequence of abnormal αSyn aggregation. In a healthy physiological state, αSyn is a natively unfolded monomer involved in maintenance of neurotransmitter homeostasis by regulating vesicle fusion, clustering and trafficking via SNARE complexes, thereby proposing synapses as the primarily affected site in case of αSyn pathology (Calo et al., 2016; Blennow et al., 2016). SNARE-complexes are formed from synaptobrevin, syntaxin and SNAP-25 (Chandra et al., 2005; Burre et al., 2010). In this study we were able to quantify SNAP-25 and syntaxin-1B, the isoform of syntaxin necessary for the regulation of synaptic vesicle exocytosis (Mishima et al., 2014). However, SNAP-25 and syntaxin-1B were detected almost exclusively in the CSF of aged A30P-αSyn, while the few quantification events in younger mice are based on error-prone "match between runs" identification. It is also a valid assumption that the levels of both proteins were below detection limit in controls and younger A30P-αSyn mice. A recent study also reported a disease specific overall increase of SNAP-25 in CSF of PD patients related to cognitive and motor symptom severity (Bereczki et al., 2017). We can therefore assume that synaptic dysfunction is also occurring in our mouse models of α-synucleinopathy and that synaptic proteins, such as SNAP-25 and syntaxin-1B may potentially serve as future diagnostic biomarkers or predictive factors for the progression to dementia in PD (Blennow et al., 2016).

4.8 Neurofilament light

As the proteome dataset demonstrated, neurofilaments, such as α-internexin, neurofilaments light (NfL), medium (NfM) and heavy (NfH), are markedly increased in symptomatic A30P-αSyn and aged APPPS1 mice. From the group of neurofilaments, we chose NfL to confirm the increase not only in CSF, but also in plasma. We additionally used a third mouse-model, expressing the A53T-mutated form of human α-synuclein, to emphasize the link between αSyn deposits and NfL levels in CSF and blood.

We used a validated NfL assay (Gaiottino et al., 2013) adapted for murine samples and small volumes and therewith confirmed increased NfL levels in CSF and in blood of mouse models for β-amyloidosis and α-synucleinopathies. In all three models, the increase occurred at the same age as the respective brain lesions became apparent. Elevated CSF and blood NfL levels were recently also found in patients with α-synucleinopathies, tauopathies and β-amyloidosis (Zetterberg et al., 2015; Rohrer et al., 2016), which not only makes NfL an easily accessible blood biomarker, but also endorses the translational value of our mouse models. The study further demonstrated that neither CSF nor blood NfL increases are specific for aggregated αSyn, tau or β-amyloid. In turn, this means that the observed NfL boost stems from a downstream, or secondary, event in neurodegeneration. As neurofilaments play a key role in axonal stabilization and function as integral components of synapses, an increase in NfL blood or CSF levels is thought to reflect NfL release upon neuronal injury, axonal degeneration and synapse loss (Lee and Cleveland, 1996; Petzold, 2005; Yuan et al., 2015), all of which have been observed in αSyn and amyloid-β transgenic mice (van der Putten et al., 2000; Phinney et al., 2003; Wirths et al., 2006). The more pronounced increase of NfL in αSyn transgenic models, compared to APPPS1 mice, may be caused by a faster and more pronounced protein deposition and accumulation predominantly in brainstem and spinal cord. Both areas harbor thick-caliber myelinated motor neurons, rich in NfL (Petzold, 2005; Yuan et al., 2012). Consistently, CSF NfL levels of patients suffering from amyotrophic lateral sclerosis (ALS), a motor neuron-specific disease, are notably higher than in AD patients (Gaiottino et al., 2013). Albeit the exact mechanism by which NfL gets released into the CSF remains obscure, we can assume that the NfL-stock in blood is derived from the CSF. This is not only because neurofilaments are synthesized in the central nervous system, which is in closer contact to CSF than blood,

but also by the observation, that all three models show a more prominent and earlier NfL increase in CSF than in blood.

Despite the focus on NfL, the proteome dataset demonstrates that other neurofilament subunits may also be suited as CSF and blood marker of proteopathic neurodegenerative diseases. NfM, for example, was also found to be elevated in plasma and CSF of ALS and FTD patients (Pijnenburg et al., 2007; Häggmark et al., 2014). Nevertheless, NfL has proven to be an easily accessible blood biomarker that can be used to track CNS neurodegeneration and may serve as progression and treatment response marker for human neurodegenerative diseases.

4.9 Comparison to human CSF proteome studies

Another part of this work was the comparison of the murine CSF proteome datasets to published studies with similar proteomic approaches in human patients and neurologically healthy controls.

For PD, only few such studies have been conducted within the last years (Abdi et al., 2006; Zhang et al., 2008; Constantinescu et al., 2010; Ishigami et al., 2012; Lehnert et al., 2012; Magdalinou et al., 2017) and singling out promising candidate markers has generally proven to be difficult (Magdalinou et al., 2017). For the comparison, we focused on the most recent and technically most similar work published recently by Magdalinou and colleagues. The comparison of the A30P-αSyn dataset and their 27 significant hits demonstrates the high degree of similarity of our findings with results obtained from patients suffering from PD and atypical parkinsonian syndromes (APS). Of 27 hits, 25 could be translated to respective murine proteins of which 21 were also quantified in our mouse dataset. Intriguingly, four out of six hits being increased in PD and APS patients were also significantly elevated in the CSF of A30P-αSyn mice: Serine protease inhibitor A3N (Serpin A3N), chitinase-3-like protein 1 (YKL-40), complement factor H and zinc-alpha-2-glycoprotein. The remaining 15 proteins identified were unaffected in our mouse model. However, the same general cellular processes were found in patients and mice, namely inflammation markers and synaptic proteins.

Much of the CSF proteome work on AD has focused on the core biomarkers, T-tau, P-tau and Aβ42, while unbiased shotgun approaches are less common. The most comprehensive shotgun study of the last years is probably the work of Khoonsari et al., 2016. Despite similar instruments and analysis in MaxQuant, the overlap with the APPPS1 dataset is rather poor. Of 85 differentially altered proteins in the Khoonsari dataset, 61 were converted to the murine homologous proteins and matched to our dataset. Amongst those, only lysozyme C-2 and serpin A3N were significantly altered in both studies. However, in contrast to our results, they reported reduced levels of these proteins. Nevertheless, this is not very surprising, as one must note that AD patients included in the study were already at a late stage of disease, whereas APPPS1 mice model Aβ deposition, an early event in the cascade of AD pathology. Surprisingly, neither neurofilaments nor TREM2 were amongst the significant hits described in the human study.

4.10 Pathology specific and unspecific protein changes

In order to highlight specific CSF proteome alterations resulting from αSyn proteopathic lesions and discriminate these changes from general neurodegenerative processes, which would also occur in models of β-amyloidosis, we compared the CSF proteome from A30P-αSyn and APPPS1 mice (see 3.5). Amongst 24 unspecific proteins (with p-values $< 5\%$) we found a clear representation of inflammatory and microglia-related proteins. While we identified 208 A30P-αSyn specific proteins, we only found 4 proteins specific for APPPS1. The difference in numbers might be due to differences in the severity of pathology at 18 months of age. While APPPS1 mice aged 18 months are in a generally good condition, A30P-αSyn mice exhibit motor-symptoms and worse overall health condition.

In conclusion, we were able to quantify hundreds of proteins in CSF from αSyn and APPPS1 transgenic mouse models, granting deep insight in PD- and AD-associated CSF protein changes. The sheer number of proteins identified overlapping with PD and

AD literature, emphasizes the value and utility of transgenic mouse models especially in the context of CSF biomarkers and highlights the strong implication of neuroinflammatory processes and the lysosomal system in α-synucleinopathies. The datasets at hand will be a helpful reference for future CSF biomarker research in PD and AD.

Bibliography

Abdi, F., Quinn, J. F., Jankovic, J., McIntosh, M., Leverenz, J. B., Peskind, E., Nixon, R., Nutt, J., Chung, K., Zabetian, C., Samii, A., Lin, M., Hattan, S., Pan, C., Wang, Y., Jin, J., Zhu, D., Li, G. J., Liu, Y., Waichunas, D., Montine, T. J., and Zhang, J. (2006). Detection of biomarkers with a multiplex quantitative proteomic platform in cerebrospinal fluid of patients with neurodegenerative disorders. *Journal of Alzheimer's disease : JAD*, 9(3):293–348.

Abraham, C. R., Selkoe, D. J., and Potter, H. (1988). Immunochemical identification of the serine protease inhibitor alpha1-antichymotrypsin in the brain amyloid deposits of Alzheimer's disease. *Cell*, 52(4):487–501.

Aebersold, R. and Mann, M. (2003). Mass spectrometry-based proteomics. *Nature*, 422(6928):198–207.

Alzheimer, A. (1907). Uber eine eigenartige Erkrankung der Hirnrinde. *Allg Zeits Psychiatry Psychisch Y Gerichtlich Med*, 64:146–8.

Alzheimer's Association (2013). 2013 Alzheimer's disease facts and figures. *Alzheimer's and Dementia*, 9(2):208–245.

Amor, S., Puentes, F., Baker, D., and Van Der Valk, P. (2010). Inflammation in neurodegenerative diseases. *Immunology*, 129(2):154–169.

Andersen, A. D., Binzer, M., Stenager, E., and Gramsbergen, J. B. (2017). Cerebrospinal fluid biomarkers for Parkinson's disease - a systematic review. *Acta Neurologica Scandinavica*, 135(1):34–56.

Antony, P. M. A., Diederich, N. J., and Balling, R. (2011). Parkinson's disease mouse models in translational research. *Mammalian Genome*, 22(7-8):401–419.

Appel-Cresswell, S., Vilarino-Guell, C., Encarnacion, M., Sherman, H., Yu, I., Shah, B., Weir, D., Thompson, C., Szu-Tu, C., Trinh, J., Aasly, J. O., Rajput, A., Rajput, A. H., Jon Stoessl, A., and Farrer, M. J. (2013). Alpha-synuclein p.H50Q, a novel pathogenic mutation for Parkinson's disease. *Movement Disorders*, 28(6):811–813.

Bacioglu, M., Maia, L. F., Preische, O., Schelle, J., Apel, A., Kaeser, S. A., Schweighauser, M., Eninger, T., Lambert, M., Pilotto, A., Shimshek, D. R., Neumann, U., Kahle, P. J., Staufenbiel, M., Neumann, M., Maetzler, W., Kuhle, J., and Jucker, M. (2016). Neurofilament Light Chain in Blood and CSF as Marker of Disease Progression in Mouse Models and in Neurodegenerative Diseases. *Neuron*, 91(1):56–66.

Baker, M., Mackenzie, I. R., Pickering-Brown, S. M., Gass, J., Rademakers, R., Lindholm, C., Snowden, J., Adamson, J., Sadovnick, A. D., Rollinson, S., Cannon, A., Dwosh, E., Neary, D., Melquist, S., Richardson, A., Dickson, D., Berger, Z., Eriksen, J., Robinson, T., Zehr, C., Dickey, C. A., Crook, R., McGowan, E., Mann, D., Boeve, B. F., Feldman, H. H., and Hutton, M. (2006). Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. *Nature*, 442(7105):916–919.

Bantscheff, M., Schirle, M., Sweetman, G., Rick, J., and Kuster, B. (2007). Quantitative mass spectrometry in proteomics: A critical review. *Analytical and Bioanalytical Chemistry*, 389(4):1017–1031.

Bereczki, E., Bogstedt, A., Höglund, K., Tsitsi, P., Brodin, L., Ballard, C., Svenningsson, P., and Aarsland, D. (2017). Synaptic proteins in CSF relate to Parkinson's disease stage markers. *npj Parkinson's Disease*, 3(1):7.

Blennow, K., Biscetti, L., Eusebi, P., and Parnetti, L. (2016). Cerebrospinal fluid biomarkers in Alzheimer's and Parkinson's diseases-From pathophysiology to clinical practice. *Movement Disorders*, 31(6):836–847.

Blennow, K., Hampel, H., Weiner, M., and Zetterberg, H. (2010). Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. *Nature Reviews Neurology*, 6(3):131–144.

Blennow, K., Wallin, A., and Agren, H. (1995). tau Protein in Cerebrospinal Fluid. A Biochemical Marker. *Molecular and Chemical Neuropathology*, 26(3):231–245.

Blennow, K., Wallin, A., and Ekman, R. (1994). Neuron specific enolase in cerebrospinal fluid: A biochemical marker for neuronal degeneration in dementia disorders? *Journal of Neural Transmission - Parkinson's Disease and Dementia Section*, 8(3):183–191.

Braak, H. and Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathologica*, 82(4):239–259.

Braak, H. and Braak, E. (1995). Staging of alzheimer's disease-related neurofibrillary changes. *Neurobiology of Aging*, 16(3):271–278.

Braak, H. and Del Tredici, K. (2009). Neuroanatomy and pathology of sporadic Parkinson's disease. *Advances in anatomy, embryology, and cell biology*, 201:1–119.

Braak, H., Del Tredici, K., Rüb, U., De Vos, R. A. I., Jansen Steur, E. N. H., and Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of Aging*, 24(2):197–211.

Brier, M. R., Gordon, B., Friedrichsen, K., McCarthy, J., Stern, A., Christensen, J., Owen, C., Aldea, P., Su, Y., Hassenstab, J., Cairns, N. J., Holtzman, D. M., Fagan, A. M., Morris, J. C., Benzinger, T. L., and Ances, B. M. (2016). Tau and A-beta imaging, CSF measures, and cognition in Alzheimer's disease. *Science Translational Medicine*, 8(338):1–10.

Brooks, D. J. (2010). Imaging Approaches to Parkinson Disease. *Journal of Nuclear Medicine*, 51(4):596–609.

Brouwers, N., Nuytemans, K., van der Zee, J., Gijselinck, I., Engelborghs, S., Theuns, J., Kumar-Singh, S., Pickut, B. a., Pals, P., Dermaut, B., Bogaerts, V., De Pooter, T., Serneels, S., Van den Broeck, M., Cuijt, I., Mattheijssens, M., Peeters, K., Sciot, R., Martin, J.-J., Cras, P., Santens, P., Vandenberghe, R., De Deyn, P. P., Cruts, M., Van Broeckhoven, C., and Sleegers, K. (2007). Alzheimer and Parkinson diagnoses in progranulin null mutation carriers in an extended founder family. *Archives of neurology*, 64(10):1436–1446.

Brouwers, N., Sleegers, K., Engelborghs, S., Maurer-Stroh, S., Gijselinck, I., Van Der Zee, J., Pickut, B. A., Van Den Broeck, M., Mattheijssens, M., Peeters, K., Schymkowitz, J., Rousseau, F., Martin, J. J., Cruts, M., De Deyn, P. P., and Van Broeckhoven, C. (2008). Genetic variability in progranulin contributes to risk for clinically diagnosed Alzheimer disease. *Neurology*, 71(9):656–664.

Buerger, K., Ewers, M., Pirttilä, T., Zinkowski, R., Alafuzoff, I., Teipel, S. J., DeBernardis, J., Kerkman, D., McCulloch, C., Soininen, H., and Hampel, H. (2006). CSF phosphorylated tau protein correlates with neocortical neurofibrillary pathology in Alzheimer's disease. *Brain*, 129(11):3035–3041.

Burns, A. and Iliffe, S. (2009). Alzheimer's disease. *BMJ*, 338(feb05 1):b158–b158.

Burre, J., Sharma, M., Tsetsenis, T., Buchman, V., Etherton, M. R., and Sudhof, T. C. (2010). alpha-Synuclein Promotes SNARE-Complex Assembly in Vivo and in Vitro. *Science*, 329(5999):1663–1667.

Calo, L., Wegrzynowicz, M., Santivanez-Perez, J., and Grazia Spillantini, M. (2016). Synaptic failure and alpha-synuclein. *Movement Disorders*, 31(2):169–177.

Chandra, S., Gallardo, G., Fernandez-Chacon, R., Schluter, O. M., and Sudhof, T. C. (2005). alpha-synuclein cooperates with CSP alpha in preventing neurodegeneration. *Cell*, 123(3):383–396.

Chesselet, M.-F. and Carmichael, S. T. (2012). Animal Models of Neurological Disorders. *Neurotherapeutics*, 9(2):241–244.

Clarke, C. E. and Moore, A. P. (2007). Parkinson's disease. *BMJ clinical evidence*, 2007.

Compta, Y., Valente, T., Saura, J., Segura, B., Iranzo, Á., Serradell, M., Junqué, C., Tolosa, E., Valldeoriola, F., Muñoz, E., Santamaria, J., Cámara, A., Fernández, M., Fortea, J., Buongiorno, M., Molinuevo, J. L., Bargalló, N., and Martí, M. J. (2014). Correlates of cerebrospinal fluid levels of oligomeric- and total-α-synuclein in premotor, motor and dementia stages of Parkinson's disease. *Journal of Neurology*, 262(2):294–306.

Constantinescu, R., Andreasson, U., Li, S., Podust, V. N., Mattsson, N., Anckarsäter, R., Anckarsäter, H., Rosengren, L., Holmberg, B., Blennow, K., Wikkelsö, C., Rüetschi, U., and Zetterberg, H. (2010). Proteomic profiling of cerebrospinal fluid in parkinsonian disorders. *Parkinsonism and Related Disorders*, 16(8):545–549.

Cox, J., Hein, M. Y., Luber, C. A., Paron, I., Nagaraj, N., and Mann, M. (2014). Accurate proteome-wide label-free quantification by delayed normalization and maximal peptide ratio extraction, termed MaxLFQ. *Molecular Cellular Proteomics MCP*, 13(9):2513–2526.

Cox, J. and Mann, M. (2012). 1D and 2D annotation enrichment: a statistical method integrating quantitative proteomics with complementary high-throughput data. *BMC Bioinformatics*, 13(Suppl 16):S12.

Cruchaga, C., Karch, C. M., Jin, S. C., Benitez, B. A., ..., Kauwe, J. S. K., and Goate, A. M. (2014a). Rare coding variants in the phospholipase D3 gene confer risk for Alzheimer's disease. *Nature*, 505(7484):550–4.

Cruchaga, C., Kauwe, J. S. K., Harari, O., Jin, S. C., Cai, Y., Karch, C. M., Benitez, B., Jeng, A. T., Skorupa, T., Carrell, D., Bailey, M., Mckean, D., Shulman, J. M., and De, P. L. (2014b). GWAS of cerebrospinal fluid tau levels identifies novel risk variants for Alzheimer's disease. *Neuron*, 78(2):256–268.

Cruts, M., Gijselinck, I., van der Zee, J., Engelborghs, S., Wils, H., Pirici, D., Rademakers, R., Vandenberghe, R., Dermaut, B., Martin, J. J., van Duijn, C., Peeters, K., Sciot, R., Santens, P., De Pooter, T., Mattheijssens, M., Van den Broeck, M., Cuijt, I., Vennekens, K., De Deyn, P. P., Kumar-Singh, S., and Van Broeckhoven, C. (2006). Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. *Nature*, 442(August):920–924.

de Lau, L. M. and Breteler, M. M. (2006). Epidemiology of Parkinson's disease. *Lancet Neurology*, 5(6):525–535.

De Rijk, M. C., Tzourio, C., Breteler, M. M., Dartigues, J. F., Amaducci, L., Lopez-Pousa, S., Manubens-Bertran, J. M., Alpérovitch, A., and Rocca, W. A. (1997). Prevalence of parkinsonism and Parkinson's disease in Europe: the EUROPARKINSON Collaborative Study. European Community Concerted Action on the Epidemiology of Parkinson's disease. *Journal of neurology, neurosurgery, and psychiatry*, 62(1):10–5.

De Strooper, B. (2007). Loss-of-function presenilin mutations in Alzheimer disease. Talking Point on the role of presenilin mutations in Alzheimer disease. *EMBO reports*, 8(2):141–146.

De Strooper, B. and Annaert, W. (2010). Novel Research Horizons for Presenilins and gamma-Secretases in Cell Biology and Disease. *Annual review of cell and developmental biology*, (June):1–26.

Dehay, B., Martinez-Vicente, M., Caldwell, G. A., Caldwell, K. A., Yue, Z., Cookson, M. R., Klein, C., Vila, M., and Bezard, E. (2013). Lysosomal impairment in Parkinson's disease. *Movement Disorders*, 28(6):725–732.

Duff, K., Eckman, C., Zehr, C., Yu, X., Prada, C.-M., Perez-tur, J., Hutton, M., Buee, L., Harigaya, Y., Yager, D., Morgan, D., Gordon, M. N., Holcomb, L., Refolo, L., Zenk, B., Hardy, J., and Younkin, S. (1996). Increased amyloid-β42(43) in brains of mice expressing mutant presenilin 1. *Nature*, 383(6602):710–713.

Durbin, K. R., Fornelli, L., Fellers, R. T., Doubleday, P. F., Narita, M., and Kelleher, N. L. (2016). Quantitation and Identification of Thousands of Human Proteoforms below 30 kDa. *Journal of Proteome Research*, 15(3):976–982.

Esch, F. S., Keim, P. S., Beattie, E. C., Blacher, R. W., Culwell, a. R., Oltersdorf, T., McClure, D., and Ward, P. J. (1990). Cleavage of amyloid beta peptide during constitutive processing of its precursor. *Science*, 248(4959):1122–1124.

Fazzari, P., Horre, K., Arranz, A. M., Frigerio, C. S., Saito, T., Saido, T. C., and De Strooper, B. (2017). PLD3 gene and processing of APP. *Nature*, 541(7638):E1–E2.

Fearnley, J. M. and Lees, A. J. (1991). Ageing and Parkinson's Disease: Substantia Nigra Regional Selectivity. *Brain*, 114(5):2283–2301.

Fernagut, P.-O. and Chesselet, M.-F. (2004). Alpha-synuclein and transgenic mouse models. *Neurobiology of disease*, 17(2):123–130.

Ferreira, M. and Massano, J. (2017). An updated review of Parkinson's disease genetics and clinicopathological correlations. *Acta Neurologica Scandinavica*, 135(3):273–284.

Forno, L. S. (1996). Neuropathology of Parkinson's Disease. *Journal of Neuropathology amp; Experimental Neurology*, 55(3):259 LP – 272.

Frank, S., Burbach, G. J., Bonin, M., Walter, M., Streit, W., Bechmann, I., and Deller, T. (2008). TREM2 is upregulated in amyloid plaque-associated microglia in aged APP23 transgenic mice. *Glia*, 56(13):1438–1447.

Frank-Cannon, T. C., Alto, L. T., McAlpine, F. E., and Tansey, M. G. (2009). Does neuroinflammation fan the flame in neurodegenerative diseases? *Molecular Neurodegeneration*, 4(1):47.

Gaiottino, J., Norgren, N., Dobson, R., Topping, J., Nissim, A., Malaspina, A., Bestwick, J. P., Monsch, A. U., Regeniter, A., Lindberg, R. L., Kappos, L., Leppert, D., Petzold, A., Giovannoni, G., and Kuhle, J. (2013). Increased Neurofilament Light Chain Blood Levels in Neurodegenerative Neurological Diseases. *PLoS ONE*, 8(9):1–9.

Gibb, W. R. and Lees, A. J. (1988). The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. *Journal of Neurology, Neurosurgery Psychiatry*, 51(6):745–752.

Glenner, G. G. and Wong, C. W. (1984a). Alzheimer's disease and Down's syndrome: Sharing of a unique cerebrovascular amyloid fibril protein. *Biochemical and Biophysical Research Communications*, 122(3):1131–1135.

Glenner, G. G. and Wong, C. W. (1984b). Alzheimer's disease: Initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochemical and Biophysical Research Communications*, 120(3):885–890.

Goate, A., Chartier-Harlin, M. C., Mullan, M., Brown, J., Crawford, F., Fidani, L., Giuffra, L., Haynes, A., Irving, N., and James, L. (1991). Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature*, 349(6311):704–6.

Goedert, M. and Spillantini, M. G. (2006). A Century of Alzheimer's Disease. *Science*, 314(5800):777–781.

Goedert, M., Spillantini, M. G., Del Tredici, K., and Braak, H. (2013). 100 years of Lewy pathology. *Nature Reviews Neurology*, 9(1):13–24.

Goedert, M., Wischik, C. M., Crowther, R. A., Walker, J. E., and Klug, A. (1988). Cloning and sequencing of the cDNA encoding a core protein of the paired helical filament of Alzheimer disease: identification as the microtubule-associated protein tau. *Proceedings of the National Academy of Sciences of the United States of America*, 85(11):4051–5.

Grundke-Iqbal, I., Iqbal, K., Quinlan, M., Tung, Y.-C., Zaidi, M. S., and Wisniewski, H. M. (1986a). Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. *Journal of Biological Chemistry*, 261(13):6084—-6089.

Grundke-Iqbal, I., Iqbal, K., Tung, Y. C., Quinlan, M., Wisniewski, H. M., and Binder, L. I. (1986b). Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proceedings of the National Academy of Sciences*, 83(13):4913–4917.

Guerreiro, R., Ph, D., Wojtas, A., Bras, J., Carrasquillo, M., Rogaeva, E., Majounie, E., Cruchaga, C., Kauwe, J. S. K., Younkin, S., Hazrati, L., Lambert, J.-c., Amouyel, P., Goate, A., Singleton, A., Hardy, J., and Alzheimer, T. (2013). TREM2 variants in AD. *New England Journal of Medicine*, 368(2):117–127.

Haass, C., Kaether, C., Thinakaran, G., and Sisodia, S. (2012). Trafficking and proteolytic processing of APP. *Cold Spring Harbor Perspectives in Medicine*, 2(5).

Häggmark, A., Mikus, M., Mohsenchian, A., Hong, M.-G., Forsström, B., Gajewska, B., Barańczyk-Kuźma, A., Uhlén, M., Schwenk, J. M., Kuźma-Kozakiewicz, M., and Nilsson, P. (2014). Plasma profiling reveals three proteins associated to amyotrophic lateral sclerosis. *Annals of clinical and translational neurology*, 1(8):544–53.

Halbgebauer, S., Nagl, M., Klafki, H., Haußmann, U., Steinacker, P., Oeckl, P., Kassubek, J., Pinkhardt, E., Ludolph, A. C., Soininen, H., Herukka, S.-K., Wiltfang, J., and Otto, M. (2016). Modified serpinA1 as risk marker for Parkinson's disease dementia: Analysis of baseline data. *Scientific reports*, 6:26145.

Hamley, I. W. (2012). The amyloid beta peptide: A chemist's perspective. role in Alzheimer's and fibrillization. *Chemical Reviews*, 112(10):5147–5192.

Hampel, H., Mitchell, A., Blennow, K., Frank, R. A., Brettschneider, S., Weller, L., and Möller, H. J. (2004). Core biological marker candidates of Alzheimer's disease - Perspectives for diagnosis, prediction of outcome and reflection of biological activity. *Journal of Neural Transmission*, 111(3):247–272.

Hardy, J. and Allsop, D. (1991). Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends in Pharmacological Sciences*, 12(C):383–388.

Hardy, J. and Higgins, G. (1992). Alzheimer's disease: the amyloid cascade hypothesis. *Science*, 256(5054):184–185.

He, Z., Ong, C. H., Halper, J., and Bateman, A. (2003). Progranulin is a mediator of the wound response. *Nature Medicine*, 9(2):225–229.

Heneka, M. T., Carson, M. J., Khoury, J. E., Landreth, G. E., Brosseron, F., ..., and Kummer, M. (2015). Neuroinflammation in Alzheimer's disease. *The Lancet Neurology*, 14(4):388–405.

Hesse, C., Rosengren, L., Andreasen, N., Davidsson, P., Vanderstichele, H., Vanmechelen, E., and Blennow, K. (2001). Transient increase in total tau but not phospho-tau in human cerebrospinal fluid after acute stroke. *Neuroscience Letters*, 297(3):187–190.

Hickman, S. E. and El Khoury, J. (2014). TREM2 and the neuroimmunology of Alzheimer's disease. *Biochemical Pharmacology*, 88(4):495–498.

Hiltunen, M., van Groen, T., and Jolkkonen, J. (2009). Functional roles of amyloid-beta protein precursor and amyloid-beta peptides: evidence from experimental studies. *Journal of Alzheimer's disease : JAD*, 18(2):401–12.

Hipkiss, A. R. (2006). Accumulation of altered proteins and ageing: Causes and effects. *Experimental Gerontology*, 41(5):464–473.

Horvath, A. J., Irving, J. A., Rossjohn, J., Law, R. H., Bottomley, S. P., Quinsey, N. S., Pike, R. N., Coughlin, P. B., and Whisstock, J. C. (2005). The murine orthologue of human antichymotrypsin: A structural paradigm for clade A3 serpins. *Journal of Biological Chemistry*, 280(52):43168–43178.

Huang, D. W., Lempicki, R. a., and Sherman, B. T. (2009a). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature Protocols*, 4(1):44–57.

Huang, D. W., Sherman, B. T., and Lempicki, R. A. (2009b). Bioinformatics enrichment tools: Paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Research*, 37(1):1–13.

Hühmer, A. F., Biringer, R. G., Amato, H., Fonteh, A. N., and Harrington, M. G. (2006). Protein Analysis in Human Cerebrospinal Fluid: Physiological Aspects, Current Progress and Future Challenges. *Disease Markers*, 22(1-2):3–26.

Ikeuchi, T., Kakita, A., Shiga, A., Kasuga, K., Kaneko, H., Tan, C.-F., Idezuka, J., Wakabayashi, K., Onodera, O., Iwatsubo, T., Nishizawa, M., Takahashi, H., and Ishikawa, A. (2008). Patients homozygous and heterozygous for SNCA duplication in a family with parkinsonism and dementia. *Archives of neurology*, 65(4):514–9.

Ishigami, N., Tokuda, T., Ikegawa, M., Komori, M., Kasai, T., Kondo, T., Matsuyama, Y., Nirasawa, T., Thiele, H., Tashiro, K., and Nakagawa, M. (2012). Cerebrospinal fluid proteomic patterns discriminate Parkinson's disease and multiple system atrophy. *Movement Disorders*, 27(7):851–857.

Jarrett, J. T., Berger, E. P., and Lansbury Jr., P. T. (1993). The carboxy terminus of the β amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease. *Biochemistry*, 32(18):4693–4697.

Jensen, P. H., Hager, H., Nielsen, M. S., Højrup, P., Gliemann, J., and Jakes, R. (1999). alpha-synuclein binds to tau and stimulates the protein kinase A-catalyzed tau phosphorylation of serine residues 262 and 356. *Journal of Biological Chemistry*, 274(36):25481–25489.

Jonsson, T., Atwal, J. K., Steinberg, S., Snaedal, J., Jonsson, P. V., ..., and Stefansson, K. (2012). A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. *Nature*, 488(7409):96–99.

Jonsson, T., Stefansson, H., Steinberg, S., Jonsdottir, I., Jonsson, P. V., ..., and Stefansson, K. (2013). Variant of TREM2 Associated with the Risk of Alzheimer's Disease. *New England Journal of Medicine*, 368(2):107–116.

Jucker, M. (2010). The benefits and limitations of animal models for translational research in neurodegenerative diseases. *Nature Medicine*, 16(11):1210–1214.

Jucker, M. and Walker, L. C. (2013). Self-propagation of pathogenic protein aggregates in neurodegenerative diseases. *Nature*, 501(7465):45–51.

Kahle, P. J., Neumann, M., Ozmen, L., Müller, V., Jacobsen, H., Schindzielorz, A., Okochi, M., Leimer, U., van Der Putten, H., Probst, A., Kremmer, E., Kretzschmar, H. a., and Haass, C. (2000). Subcellular localization of wild-type and Parkinson's disease-associated mutant alpha -synuclein in human and transgenic mouse brain. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 20(17):6365–6373.

Kang, J., Lemaire, H.-G., Unterbeck, A., Salbaum, J., Masters, C., Grzeschik, K.-H., Multhaup, G., Beyreuther, K., and Müller-Hill, B. (1987). The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Alzheimer Disease Associated Disorders*, 1(3):206–207.

Kelley, B. J., Haidar, W., Boeve, B. F., Baker, M., Shiung, M., Knopman, D. S., Rademakers, R., Hutton, M., Adamson, J., Kuntz, K. M., Dickson, D. W., Parisi, J. E., Smith, G. E., and Petersen, R. C. (2010). Alzheimer disease-like phenotype associated with the c.154delA mutation in progranulin. *Archives of neurology*, 67(2):171–7.

Khoonsari, E., Häggmark, A., Lonnberg, M., Mikus, M., Kilander, L., Lannfelt, L., Bergquist, J., Ingelsson, M., Nilsson, P., Kultima, K., and Shevchenko, G. (2016). Analysis of the cerebrospinal fluid proteome in Alzheimer's disease. *PLoS ONE*, 11(3):1–25.

Kidd, M. (1963). Paired helical filaments in electron microscopy of Alzheimer's disease. *Nature*, 197:192–193.

Kleinberger, G., Yamanishi, Y., Suarez-Calvet, M., Czirr, E., Lohmann, E., ..., and Haass, C. (2014). TREM2 mutations implicated in neurodegeneration impair cell surface transport and phagocytosis. *Science Translational Medicine*, 6(243):243ra86–243ra86.

Klunk, W. E., Engler, H., Nordberg, A., Wang, Y., Blomqvist, G., Holt, D. P., Bergström, M., Savitcheva, I., Huang, G. F., Estrada, S., Ausén, B., Debnath, M. L., Barletta, J., Price, J. C., Sandell, J., Lopresti, B. J., Wall, A., Koivisto, P., Antoni, G., Mathis, C. A., and Långström, B. (2004). Imaging Brain Amyloid in Alzheimer's Disease with Pittsburgh Compound-B. *Annals of Neurology*, 55(3):306–319.

Kosik, K. S., Joachim, C. L., and Selkoe, D. J. (1986). Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease. *Proceedings of the National Academy of Sciences*, 83(11):4044—-4048.

Krüger, R., Kuhn, W., Müller, T., Woitalla, D., Graeber, M., Kösel, S., Przuntek, H., Epplen, J. T., Schols, L., and Riess, O. (1998). Ala30Pro mutation in the gene encoding α-synuclein in Parkinson's disease. *Nature Genetics*, 18(2):106–108.

Lammich, S., Kojro, E., Postina, R., Gilbert, S., Pfeiffer, R., Jasionowski, M., Haass, C., and Fahrenholz, F. (1999). Constitutive and regulated alpha-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. *Proceedings of the National Academy of Sciences of the United States of America*, 96(7):3922–3927.

Lázaro, D. F., Rodrigues, E. F., Langohr, R., Shahpasandzadeh, H., Ribeiro, T., Guerreiro, P., Gerhardt, E., Kröhnert, K., Klucken, J., Pereira, M. D., Popova, B., Kruse, N., Mollenhauer, B., Rizzoli, S. O., Braus, G. H., Danzer, K. M., and Outeiro, T. F. (2014). Systematic Comparison of the Effects of Alpha-synuclein Mutations on Its Oligomerization and Aggregation. *PLoS Genetics*, 10(11).

Lee, H.-J. J., Khoshaghideh, F., Patel, S., and Lee, S.-J. J. (2004). Clearance of alpha-synuclein oligomeric intermediates via the lysosomal degradation pathway. *J Neurosci*, 24(8):1888–1896.

Lee, J., Retamal, C., Cuitiño, L., Caruano-Yzermans, A., Shin, J. E., Van Kerkhof, P., Marzolo, M. P., and Bu, G. (2008). Adaptor protein sorting nexin 17 regulates amyloid precursor protein trafficking and processing in the early endosomes. *Journal of Biological Chemistry*, 283(17):11501–11508.

Lee, M. K. and Cleveland, D. W. (1996). Neuronal Intermediate Filaments. *Annual Review of Neuroscience*, 19(1):187–217.

Lehnert, S., Jesse, S., Rist, W., Steinacker, P., Soininen, H., Herukka, S. K., Tumani, H., Lenter, M., Oeckl, P., Ferger, B., Hengerer, B., and Otto, M. (2012). ITRAQ and multiple reaction monitoring as proteomic tools for biomarker search in cerebrospinal fluid of patients with Parkinson's disease dementia. *Experimental Neurology*, 234(2):499–505.

Leinenbach, A., Pannee, J., Dülffer, T., Huber, A., Bittner, T., Andreasson, U., Gobom, J., Zetterberg, H., Kobold, U., Portelius, E., and Blennow, K. (2014). Mass spectrometry-based candidate reference measurement procedure for quantification of amyloid-beta in Cerebrospinal fluid. *Clinical Chemistry*, 60(7):987–994.

Lesage, S. and Brice, A. (2009). Parkinson's disease: From monogenic forms to genetic susceptibility factors. *Human Molecular Genetics*, 18(R1):48–59.

Leuner, K., Hauptmann, S., Abdel-Kader, R., Scherping, I., Keil, U., Strosznajder, J. B., Eckert, A., and Müller, W. E. (2007). Mitochondrial Dysfunction: The First Domino in Brain Aging and Alzheimer's Disease? *Antioxidants Redox Signaling*, 9(10):1659–1676.

Levy-Lahad, E., Wasco, W., Poorkaj, P., Romano, D., Oshima, J., Pettingell, W., Yu, C., Jondro, P., Schmidt, S., and Wang, K. (1995). Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science*, 269(5226):973–977.

Li, N., Workman, C. J., Martin, S. M., and Vignali, D. a. a. (2004). Biochemical analysis of the regulatory T cell protein lymphocyte activation gene-3 (LAG-3; CD223). *Journal of immunology (Baltimore, Md. : 1950)*, 173(19):6806–6812.

Licastro, F., Parnetti, L., Morini, M. C., Davis, L. J., Cucinotta, D., Gaiti, A., and Senin, U. (1995). Acute phase reactant alpha 1-antichymotrypsin is increased in cerebrospinal fluid and serum of patients with probable Alzheimer disease. *Alzheimer Dis.Assoc.Disord.*, 9(0893-0341):112–118.

Loane, C. and Politis, M. (2011). Positron emission tomography neuroimaging in Parkinson's disease. *American Journal of Translational Research*, 3(4):323–341.

Mackay, I. R., Rosen, F. S., and Walport, M. J. (2001). Complement. *New England Journal of Medicine*, 344(15):1140–1144.

Magdalinou, N., Lees, A. J., and Zetterberg, H. (2014). Cerebrospinal fluid biomarkers in parkinsonian conditions: an update and future directions. *Journal of Neurology, Neurosurgery Psychiatry*, 85(10):1065–1075.

Magdalinou, N., Noyce, A., Pinto, R., Lindstrom, E., Holmén-Larsson, J., Holtta, M., Blennow, K., Morris, H., Skillbäck, T., Warner, T., Lees, A., Pike, I., Ward, M., Zetterberg, H., and Gobom, J. (2017). Identification of candidate cerebrospinal fluid biomarkers in parkinsonism using quantitative proteomics. *Parkinsonism Related Disorders*, pages 1–7.

Maia, L. F., Kaeser, S. A., Reichwald, J., Hruscha, M., Martus, P., Staufenbiel, M., and Jucker, M. (2013). Changes in Amyloid-β and Tau in the Cerebrospinal Fluid of Transgenic Mice Overexpressing Amyloid Precursor Protein. *Sci. Transl .Med.*, 5(194):194re2.

Maia, L. F., Kaeser, S. A., Reichwald, J., Lambert, M., Obermuller, U., Schelle, J., Odenthal, J., Martus, P., Staufenbiel, M., and Jucker, M. (2015). Increased CSF Abeta during the very early phase of cerebral Abeta deposition in mouse models. *EMBO Mol Med*, 7(7):895–903.

Makarov, A. (2000). Electrostatic axially harmonic orbital trapping: A high-performance technique of mass analysis. *Analytical Chemistry*, 72(6):1156–1162.

Maloney, J. A., Bainbridge, T., Gustafson, A., Zhang, S., Kyauk, R., Steiner, P., Van Der Brug, M., Liu, Y., Ernst, J. A., Watts, R. J., and Atwal, J. K. (2014). Molecular mechanisms of Alzheimer disease protection by the A673T allele of amyloid precursor protein. *Journal of Biological Chemistry*, 289(45):30990–31000.

Mao, P., Meshul, C. K., Thuillier, P., and Reddy, P. H. (2013). Neurotransmitter CART as a new therapeutic candidate for Parkinson's disease. *Pharmaceuticals*, 6(1):108–123.

Mao, X., Ou, M. T., Karuppagounder, S. S., Kam, T.-I., Yin, X., Xiong, Y., Ge, P., Umanah, G. E., Brahmachari, S., Shin, J.-H., Kang, H. C., Zhang, J., Xu, J., Chen, R., Park, H., Andrabi, S. A., Kang, S. U., Gonçalves, R. A., Liang, Y., Zhang, S., Qi, C., Lam, S., Keiler, J. A., Tyson, J., Kim, D., Panicker, N., Yun, S. P., Workman, C. J., Vignali, D. A. A., Dawson, V. L., Ko, H. S., and Dawson, T. M. (2016). Pathological α-synuclein transmission initiated by binding lymphocyte-activation gene 3. *Science (New York, N.Y.)*, 353(6307):aah3374–aah3374.

Martens, L. H., Zhang, J., Barmada, S. J., Zhou, P., Kamiya, S., Sun, B., Min, S. W., Gan, L., Finkbeiner, S., Huang, E. J., and Farese, R. V. (2012). Progranulin deficiency promotes neuroinflammation and neuron loss following toxin-induced injury. *Journal of Clinical Investigation*, 122(11):3955–3959.

Masters, C. L., Simms, G., Weinman, N. A., Multhaup, G., McDonald, B. L., and Beyreuther, K. (1985). Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proceedings of the National Academy of Sciences of the United States of America*, 82(12):4245–9.

Mattsson, N., Andreasson, U., Persson, S., ..., Zhang, J., Zetterberg, H., and Blennow, K. (2011). The Alzheimer's Association external quality control program for cerebrospinal fluid biomarkers. *Alzheimer's and Dementia*, 7(4):386–395.

Mendez, M. F. (2012). Early-onset Alzheimer's Disease: Nonamnestic Subtypes and Type 2 AD. *Archives of Medical Research*, 43(8):677–685.

Meredith, G. E., Totterdell, S., Petroske, E., Santa Cruz, K., Callison, R. C., and Lau, Y. S. (2002). Lysosomal malfunction accompanies alpha-synuclein aggregation in a progressive mouse model of Parkinson's disease. *Brain Research*, 956(1):156–165.

Michalski, A., Damoc, E., Lange, O., Denisov, E., Nolting, D., Muller, M., Viner, R., Schwartz, J., Remes, P., Belford, M., Dunyach, J.-J., Cox, J., Horning, S., Mann, M., and Makarov, A. (2012). Ultra High Resolution Linear Ion Trap Orbitrap Mass Spectrometer (Orbitrap Elite) Facilitates Top Down LC MS/MS and Versatile Peptide Fragmentation Modes. *Molecular Cellular Proteomics*, 11(3):O111.013698–O111.013698.

Mintun, M. A., Larossa, G. N., Sheline, Y. I., Dence, C. S., Lee, S. Y., MacH, R. H., Klunk, W. E., Mathis, C. A., Dekosky, S. T., and Morris, J. C. (2006). [11C]PIB in a nondemented population: Potential antecedent marker of Alzheimer disease. *Neurology*, 67(3):446–452.

Mishima, T., Fujiwara, T., Sanada, M., Kofuji, T., Kanai-Azuma, M., and Akagawa, K. (2014). Syntaxin 1B, but not syntaxin 1A, is necessary for the regulation of synaptic vesicle exocytosis and of the readily releasable pool at central synapses. *PLoS ONE*, 9(2).

Miyazaki, T., Dierich, A., Benoist, C., and Mathis, D. (1996). Independent modes of natural killing distinguished in mice lacking Lag3. *Science (New York, N.Y.)*, 272(5260):405–408.

Morgan, T., Wong, A., and Finch, C. (2007). Anti-inflammatory mechanisms of dietary restriction in slowing aging processes. *Interdisciplinary Topics in Gerontology*, 35:83–97.

Motter, R., Vigo-Pelfrey, C., Kholodenko, D., Barbour, R., Johnson-Wood, K., Galasko, D., Chang, L., Miller, B., Clark, C., and Green, R. (1995). Reduction of beta-amyloid peptide42 in the cerebrospinal fluid of patients with Alzheimer's disease. *Annals of neurology*, 38(4):643–648.

Neumann, M., Kahle, P. J., Giasson, B. I., Ozmen, L., Borroni, E., Spooren, W., Odoy, S., Fujiwara, H., Hasegawa, M., Iwatsubo, T., Trojanowski, J. Q., Kretzschmar, H. a., and Haass, C. (2002). Misfolded proteinase Kâresistant hyperphosphorylated. *Journal of Clinical Investigation*, 110(10):1429–1439.

Nussbaum, J. M., Seward, M. E., and Bloom, G. S. (2013). Alzheimer disease: a tale of two prions. *Prion*, 7(1):14–19.

O'Brien, R. and Wong, P. (2011). Amyloid precursor protein processing and alzheimer's disease. *Annual review of neuroscience*, 1987:185–204.

Olsson, B., Zetterberg, H., Hampel, H., and Blennow, K. (2011). Biomarker-based dissection of neurodegenerative diseases. *Progress in Neurobiology*, 95(4):520–534.

Pan, T., Kondo, S., Le, W., and Jankovic, J. (2008). The role of autophagy-lysosome pathway in neurodegeneration associated with Parkinson's disease. *Brain*, 131(8):1969–1978.

Parkinson, J. (1817). An essay on the shaking palsy. *The Journal of neuropsychiatry and clinical neurosciences*, 14(2):223–236.

Parkkinen, L., Pirttilâ, T., and Alafuzoff, I. (2008). Applicability of current staging/categorization of α-synuclein pathology and their clinical relevance. *Acta Neuropathologica*, 115(4):399–407.

Parnetti, L., Chiasserini, D., Bellomo, G., Giannandrea, D., de Carlo, C., Qureshi, M. M., Ardah, M. T., Varghese, S., Bonanni, L., Borroni, B., Tambasco, N., Eusebi, P., Rossi, A., Onofrj, M., Padovani, A., Calabresi, P., and El-Agnaf, O. (2011). Cerebrospinal fluid Tau/α-synuclein ratio in Parkinson's disease and degenerative dementias. *Movement Disorders*, 26(8):1428–1435.

Parnetti, L., Chiasserini, D., Persichetti, E., Eusebi, P., Varghese, S., Balducci, C., Paciotti, S., Tambasco, N., Bembi, B., and Bonanni, L. (2014a). Cerebrospinal Fluid Lysosomal Enzymes and Alpha-Synuclein in Parkinson ' s Disease. 29(8):1019–1027.

Parnetti, L., Farotti, L., Eusebi, P., Chiasserini, D., De Carlo, C., Giannandrea, D., Salvadori, N., Lisetti, V., Tambasco, N., Rossi, A., Majbour, N. K., El-Agnaf, O., and Calabresi, P. (2014b). Differential role of CSF alpha-synuclein species, tau, and Aβ42 in Parkinson's disease. *Frontiers in Aging Neuroscience*, 6(MAR).

Pasanen, P., Myllykangas, L., Siitonen, M., Raunio, A., Kaakkola, S., Lyytinen, J., Tienari, P. J., Pöyhönen, M., and Paetau, A. (2014). A novel alpha-synuclein mutation A53E associated with atypical multiple system atrophy and Parkinson's disease-type pathology. *Neurobiology of Aging*, 35(9):2180.e1–2180.e5.

Pauwels, K., Williams, T. L., Morris, K. L., Jonckheere, W., Vandersteen, A., Kelly, G., Schymkowitz, J., Rousseau, F., Pastore, A., Serpell, L. C., and Broersen, K. (2012). Structural basis for increased toxicity of pathological Aβ42:Aβ40 ratios in alzheimer disease. *Journal of Biological Chemistry*, 287(8):5650–5660.

Petzold, A. (2005). Neurofilament phosphoforms: Surrogate markers for axonal injury, degeneration and loss. *Journal of the Neurological Sciences*, 233(1-2):183–198.

Phinney, A. L., Horne, P., Yang, J., Janus, C., Bergeron, C., and Westaway, D. (2003). Mouse models of Alzheimer's disease: the long and filamentous road. *Neurol Res*, 25(6):590–600.

Pijnenburg, Y. A. L., Janssen, J. C., Schoonenboom, N. S. M., Petzold, A., Mulder, C., Stigbrand, T., Norgren, N., Heijst, H., Hack, C. E., Scheltens, P., and Teunissen, C. E. (2007). CSF neurofilaments in frontotemporal dementia compared with early onset Alzheimer's disease and controls. *Dementia and Geriatric Cognitive Disorders*, 23(4):225–230.

Pires, A. O., Teixeira, F., Mendes-Pinheiro, B., Serra, S. C., Sousa, N., and Salgado, A. J. (2017). Old and New Challenges in Parkinson's Disease Therapeutics. *Progress in Neurobiology*.

Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., Stenroos, E. S., Chandrasekharappa, S., Athanassiadou, A., Papapetropoulos, T., Johnson, W. G., Lazzarini, A. M., Duvoisin, R. C., Iorio, G. D., Golbe, L. I., and Nussbaum, R. L. (1997). Mutation in the α -Synuclein Gene Identified in Families with Parkinson ' s Disease. *Science*, 276(June):2045–2047.

Porcellini, E., Davis, E. J., Chiappelli, M., Ianni, E., Di, G., Forti, P., Ravaglia, G., and Licastro, F. (2008). Elevated plasma levels of alpha-1-anti-chymotrypsin in age-related cognitive decline and Alzheimer's disease: a potential therapeutic target. *Current pharmaceutical design*, 14(26):2659.

Prince, M., Wimo, A., Guerchet, M., Gemma-Claire, A., Wu, Y.-T., and Prina, M. (2015). World Alzheimer Report 2015: The Global Impact of Dementia - An analysis of prevalence, incidence, cost and trends. *Alzheimer's Disease International*, page 84.

Pringsheim, T., Jette, N., Frolkis, A., and Steeves, T. D. L. (2014). The prevalence of Parkinson's disease: A systematic review and meta-analysis. *Movement Disorders*, 29(13):1583–1590.

Proukakis, C., Dudzik, C. G., Brier, T., MacKay, D. S., Cooper, J. M., Millhauser, G. L., Houlden, H., and Schapira, A. H. (2013). A novel α-synuclein missense mutation in Parkinson disease. *Neurology*, 80(11):1062–1064.

Radde, R., Bolmont, T., Kaeser, S. a., Coomaraswamy, J., Lindau, D., Stoltze, L., Calhoun, M. E., Jäggi, F., Wolburg, H., Gengler, S., Haass, C., Ghetti, B., Czech, C., Hölscher, C., Mathews, P. M., and Jucker, M. (2006). Abeta42-driven cerebral amyloidosis in transgenic mice reveals early and robust pathology. *EMBO reports*, 7(9):940–6.

Rappsilber, J., Ishihama, Y., and Mann, M. (2003). Stop And Go Extraction tips for matrix-assisted laser desorption/ionization, nanoelectrospray, and LC/MS sample pretreatment in proteomics. *Analytical Chemistry*, 75(3):663–670.

Robakis, N., Ramakrishna, N., Wolfe, G., and Wisniewski, H. (1987). Molecular Cloning and Characterization of a cDNA Encoding the Cerebrovascular and the Neuritic Plaque Amyloid Peptides. *Proceedings of the National Academy of Sciences*, 84(12):4190–4194.

Rohrer, J. D., Woollacott, I. O. C., Dick, K. M., Brotherhood, E., Gordon, E., Fellows, A., Toombs, J., Druyeh, R., Cardoso, M. J., Ourselin, S., Nicholas, J. M., Norgren, N., Mead, S., Andreasson, U., Blennow, K., Schott, J. M., Fox, N. C., Warren, J. D., and Zetterberg, H. (2016). Serum neurofilament light chain protein is a measure of disease intensity in frontotemporal dementia. *Neurology*, 87(13):1329–1336.

Satoh, J.-I., Kino, Y., Yamamoto, Y., Kawana, N., Ishida, T., Saito, Y., and Arima, K. (2014). PLD3 is accumulated on neuritic plaques in Alzheimer's disease brains. *Alzheimer's research therapy*, 6(9):70.

Scheltens, P., Blennow, K., Breteler, M. M. B., de Strooper, B., Frisoni, G. B., Salloway, S., and aria Van der Flier, W. M. (2016). Alzheimer's disease. *Lancet (London, England)*, 388(10043):505–517.

Schmidt, F. M., Mergl, R., Stach, B., Jahn, I., Gertz, H. J., and Schönknecht, P. (2014). Elevated levels of cerebrospinal fluid neuron-specific enolase (NSE) in Alzheimer's disease. *Neuroscience Letters*, 570:81–85.

Schneider, C. a., Rasband, W. S., and Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9(7):671–675.

Schultz, K., Wiehager, S., Nilsson, K., Nielsen, J. E., Lindquist, S. G., Hjermind, L. E., Andersen, B. B., Wallin, A., Nilsson, C., and Petersén, Å. (2009). Reduced CSF CART in dementia with Lewy bodies. *Neuroscience Letters*, 453(2):104–106.

Schupf, N. and Sergievsky, G. H. (2002). Genetic and host factors for dementia in Down' s syndrome*. *British Journal of Psychiatry*, 180(5):405–410.

Selkoe, D. J. (1991). The Molecular Pathology of Alzheimer's Disease. *Neuron*, 6:487–498.

Shen, J. and Kelleher, R. J. (2007). The presenilin hypothesis of Alzheimer's disease: Evidence for a loss-of-function pathogenic mechanism. *Proceedings of the National Academy of Sciences*, 104(2):403–409.

Shepherd, C., McCann, H., and Halliday, G. M. (2009). Variations in the neuropathology of familial Alzheimer's disease. *Acta Neuropathologica*, 118(1):37–52.

Sherrington, R., Rogaev, E. I., Liang, Y., Rogaeva, E. A., Levesque, G., and Ikeda, M. (1995). Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature*, 375(6534):754–760.

Shulman, J. M., De Jager, P. L., and Feany, M. B. (2011). Parkinson's Disease: Genetics and Pathogenesis. *The Annual Review of Pathology: Mechanisms of Disease*, 6:193–224.

Singh, P. P., Singh, M., and Mastana, S. S. (2006). APOE distribution in world populations with new data from India and the UK. *Annals of Human Biology*, 33(3):279–308.

Sisodia, S., Koo, E., Beyreuther, K., Unterbeck, A., and Price, D. (1990). Evidence that β-Amyloid Protein in Alzheimer's Disease is not Derived by Normal Processing. *Science*, 248(4954):492–495.

Sisodia, S. S. (1992). Beta-amyloid precursor protein cleavage by a membrane-bound protease. *Proceedings of the National Academy of Sciences*, 89(13):6075–6079.

Slaets, S., Vanmechelen, E., Le Bastard, N., Decraemer, H., Vandijck, M., Martin, J. J., De Deyn, P. P., and Engelborghs, S. (2014). Increased CSF α-synuclein levels in Alzheimer's disease: Correlation with tau levels. *Alzheimer's and Dementia*, 10(5):S290–S298.

Spillantini, G. M., Schmidt, M. L., Lee, V. M.-Y., Trojanowski, J. Q., Jakes, R., and Goedert, M. (1997). a-Synuclein in Lewy bodies. *Nature*, pages 839–840.

Strozyk, D., Blennow, K., White, L. R., and Launer, L. J. (2003). CSF AÂ 42 levels correlate with amyloid-neuropathology in a population-based autopsy study. *Neurology*, 60(4):652–656.

Suárez-Calvet, M., Kleinberger, G., Araque Caballero, M. Á., Brendel, M., Rominger, A., Alcolea, D., ..., and Haass, C. (2016). sTREM2 cerebrospinal fluid levels are a potential biomarker for microglia activity in early-stage Alzheimer's disease and associate with neuronal injury markers. *EMBO molecular medicine*, 8(5):e201506123.

Subhedar, N. K., Nakhate, K. T., Upadhya, M. A., and Kokare, D. M. (2014). CART in the brain of vertebrates: Circuits, functions and evolution. *Peptides*, 54:108–130.

Sveinbjornsdottir, S. (2016). The clinical symptoms of Parkinson's disease. *Journal of Neurochemistry*, 139:318–324.

Takami, M., Nagashima, Y., Sano, Y., Ishihara, S., Morishima-Kawashima, M., Funamoto, S., and Ihara, Y. (2009). Beta-Secretase: Successive Tripeptide and Tetrapeptide Release from the Transmembrane Domain of Beta-Carboxyl Terminal Fragment. *Journal of Neuroscience*, 29(41):13042–13052.

Tanaka, S., Nakamura, S., Ueda, K., Kameyama, M., Shiojiri, S., Takahashi, Y., Kitaguchi, N., and Ito, H. (1988). Three types of amyloid protein precursor mRNA in human brain: their differential expression in Alzheimer's disease. *Biochemical and biophysical research communications*, 157(2):472–9.

Tansey, M. G. and Goldberg, M. S. (2010). Neuroinflammation in Parkinson's disease: Its role in neuronal death and implications for therapeutic intervention. *Neurobiology of Disease*, 37(3):510–518.

Tapiola, T., Alafuzoff, I., Herukka, S., and et Al (2009). Cerebrospinal fluid β-amyloid 42 and tau proteins as biomarkers of alzheimer-type pathologic changes in the brain. *Archives of Neurology*, 66(3):382–389.

Terry, R. D. (1963). The fine structure of neurofibrillary tangles in Alzheimer's disease. *Journal of Neuropathology Experimental Neurology*, 22(4):629—-642.

Thal, D. R., Rub, U., Orantes, M., and Braak, H. (2002). Phases of A beta-deposition in the human brain and its relevance for the development of AD. *Neurology*, 58(12):1791–1800.

Thinakaran, G. and Koo, E. H. (2008). Amyloid precursor protein trafficking, processing, and function.

Thomas, B. and Flint Beal, M. (2007). Parkinson's disease. *Human Molecular Genetics*, 16(R2):R183–94.

Tokuda, T., Qureshi, M. M., Ardah, M. T., Varghese, S., Shehab, S. A., Kasai, T., Ishigami, N., Tamaoka, A., Nakagawa, M., and El-Agnaf, O. M. (2010). Detection of elevated levels of α-synuclein oligomers in CSF from patients with Parkinson disease. *Neurology*, 75(20):1766–1772.

Tosato, M., Zamboni, V., Ferrini, A., and Cesari, M. (2007). The aging process and potential interventions to extend life expectancy. *Clinical interventions in aging*, 2(3):401–412.

Tyanova, S., Temu, T., Sinitcyn, P., Carlson, A., Hein, M. Y., Geiger, T., Mann, M., and Cox, J. (2016). The Perseus computational platform for comprehensive analysis of (prote)omics data. *Nature methods*, 13(9):731–40.

UNFPA (2012). Ageing in the Twenty-First Century: A Celebration and A Challenge. Technical report.

Upadhya, M. A., Shelkar, G. P., Subhedar, N. K., and Kokare, D. M. (2016). CART modulates the effects of levodopa in rat model of Parkinson's disease. *Behavioural Brain Research*, 301:262–272.

Van Broeckhoven, C., Haan, J., Bakker, E., Hardy, J. A., Van Hul, W., Wehnert, A., Vegter-Van der Vlis, M., and Roos, R. A. (1990). Amyloid beta protein precursor gene and hereditary cerebral hemorrhage with amyloidosis (Dutch). *Science (New York, N.Y.)*, 248(4959):1120–2.

van der Putten, H., Wiederhold, K. H., Probst, A., Barbieri, S., Mistl, C., Danner, S., Kauffmann, S., Hofele, K., Spooren, W. P., Ruegg, M. A., Lin, S., Caroni, P., Sommer, B., Tolnay, M., and Bilbe, G. (2000). Neuropathology in mice expressing human alpha-synuclein. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 20(16):6021–9.

Van Kampen, J. M., Baranowski, D., and Kay, D. G. (2014). Progranulin gene delivery protects dopaminergic neurons in a mouse model of Parkinson's disease. *PLoS ONE*, 9(5):e97032.

Vassar, R. (1999). Beta-Secretase Cleavage of Alzheimer's Amyloid Precursor Protein by the Transmembrane Aspartic Protease BACE. *Science*, 286(5440):735–741.

Vigo-Pelfrey, C., Lee, D., Keim, P., Lieberburg, I., and Schenk, D. B. (1993). Characterization of beta-amyloid peptide from human cerebrospinal fluid. *Journal of neurochemistry*, 61:1965–1968.

Villemagne, V. L., Fodero-Tavoletti, M. T., Masters, C. L., and Rowe, C. C. (2015). Tau imaging: Early progress and future directions. *The Lancet Neurology*, 14(1):114–124.

Vos, T., ..., and Zuhlke, L. J. (2016). Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990â2015: a systematic analysis for the Global Burden of Disease Study 2015. *The Lancet*, 388(10053):1545–1602.

Walker, L. C. and Jucker, M. (2015). Neurodegenerative Diseases: Expanding the Prion Concept. *Annual Review of Neuroscience*, 38(1):87–103.

Wang, Y.-c., Liu, H.-c., Liu, T.-y., and Hong, C.-j. (2001). Genetic Association Analysis of Polymorphism in Parkinson ' s Disease. (201):254–256.

Webb, J. L., Ravikumar, B., Atkins, J., Skepper, J. N., and Rubinsztein, D. C. (2003). α-synuclein Is Degraded by Both Autophagy and the Proteasome. *Journal of Biological Chemistry*, 278(27):25009–25013.

Wennström, M., Surova, Y., Hall, S., Nilsson, C., Minthon, L., Boström, F., Hansson, O., and Nielsen, H. M. (2013). Low CSF Levels of Both α-Synuclein and the α-Synuclein Cleaving Enzyme Neurosin in Patients with Synucleinopathy. *PLoS ONE*, 8(1).

Whalley, L. J., Deary, I. J., Appleton, C. L., and Starr, J. M. (2004). Cognitive reserve and the neurobiology of cognitive aging. *Ageing Research Reviews*, 3(4):369–382.

Wirths, O., Weis, J., Szczygielski, J., Multhaup, G., and Bayer, T. A. (2006). Axonopathy in an APP/PS1 transgenic mouse model of Alzheimer's disease. *Acta Neuropathologica*, 111(4):312–319.

Wiśniewski, H. M., Narang, H. K., and Terry, R. D. (1976). Neurofibrillary tangles of paired helical filaments. *Journal of the neurological sciences*, 27(2):173–81.

Yamamoto, M., Kondo, I., Ogawa, N., Asanuma, M., Yamashita, Y., and Mizuno, Y. (1997). Genetic association between susceptibility to Parkinson's disease and alpha1-antichymotrypsin polymorphism. *Brain research*, 759:153–155.

Yan, Y. and Wang, C. (2006). Abeta42 is more rigid than Abeta40 at the C terminus: implications for Abeta aggregation and toxicity. *Journal of molecular biology*, 364(5):853–62.

Yuan, A., Rao, M. V., Veeranna, and Nixon, R. A. (2012). Neurofilaments at a glance. *Journal of Cell Science*, 125(14):3257–3263.

Yuan, a., Sershen, H., Veeranna, Basavarajappa, B. S., Kumar, a., Hashim, a., Berg, M., Lee, J.-H., Sato, Y., Rao, M. V., Mohan, P. S., Dyakin, V., Julien, J.-P., Lee, V. M.-Y., and Nixon, R. a. (2015). Neurofilament subunits are integral components of synapses and modulate neurotransmission and behavior in vivo. *Molecular psychiatry*, 20(8):986–94.

Zarranz, J. J., Alegre, J., Gómez-Esteban, J. C., Lezcano, E., Ros, R., Ampuero, I., Vidal, L., Hoenicka, J., Rodriguez, O., Atarés, B., Llorens, V., Gomez Tortosa, E., Del Ser, T., Muñoz, D. G., and De Yebenes, J. G. (2004). The New Mutation, E46K, of alpha-Synuclein Causes Parkinson and Lewy Body Dementia. *Annals of Neurology*, 55(2):164–173.

Zetterberg, H., Skillback, T., Mattsson, N., Trojanowski, J. Q., Portelius, E., Shaw, L. M., Weiner, M. W., and Blennow, K. (2015). Association of Cerebrospinal Fluid Neurofilament Light Concentration With Alzheimer Disease Progression. *JAMA Neurol*, 73(1):1–8.

Zhang, J., Sokal, I., Peskind, E. R., Quinn, J. F., Jankovic, J., Kenney, C., Chung, K. A., Millard, S. P., Nutt, J. G., and Montine, T. J. (2008). CSF multianalyte profile distinguishes Alzheimer and Parkinson diseases. *American Journal of Clinical Pathology*, 129(4):526–529.

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Publications and Abstracts

Eninger, **T**., Müller, S. A., Bacioglu, M., Schweighauser, M., Lambert, M., Maia, L., Kahle, P. J., Lichtenthaler, S., Jucker, M., Kaeser, S. A. - CSF proteome profiling in murine models of α-synucleinopathy and cerebral β-amyloidosis reveals novel biomarker candidates.
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Matic*, K., **Eninger***, **T**., Bardoni, B., Davidovic, L., & Macek, B. (2014). Quantitative phosphoproteomics of murine Fmr1 -KO cell lines provides new insights into FMRP-dependent signal transduction mechanisms. Journal of Proteome Research, 13(10), 4388–4397.
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