KTH ROYAL INSTITUTE
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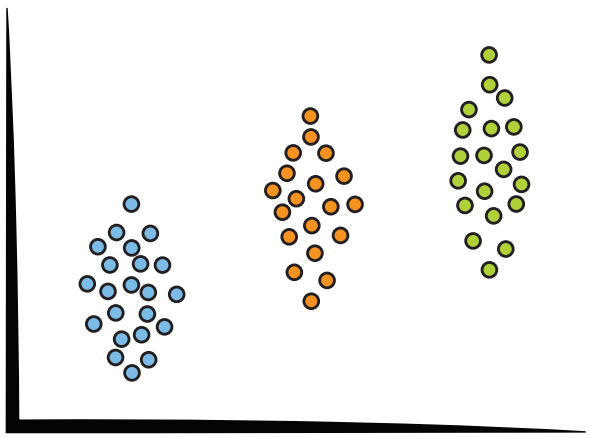
Doctoral Thesis in Biotechnology

Multiplexed antibody-based protein profiling in the pursuit of CSF biomarkers for neurodegenerative diseases

SOFIA BERGSTRÖM



Stockholm, Sweden 2021

Multiplexed antibody-based protein profiling in the pursuit of CSF biomarkers for neurodegenerative diseases

SOFIA BERGSTRÖM

Academic Dissertation which, with due permission of the KTH Royal Institute of Technology, is submitted for public defence for the Degree of Doctor of Philosophy on Friday the 12th of November 2021, at 10.00 in Air & Fire, SciLifeLab, Tomtebodavägen 23A, Solna.

Doctoral Thesis in Biotechnology
KTH Royal Institute of Technology
Stockholm, Sweden 2021

ISBN 978-91-8040-020-6
TRITA-CBH-FOU-2021:44

Printed by: Universitetsservice US-AB, Sweden 2021

Abstract

There is a desire for a transition from generic treatments designed for the average patient, towards more individual-based precision medicine. An increased knowledge about disease pathophysiology on a molecular level would be beneficial for this transition. The study of proteins can contribute with valuable insights into etiology and pathogenesis of different diseases and thereby aid the clinical assessment of patients and guide future treatments.

Neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, and frontotemporal dementia, are characterized by a progressive loss of function, and eventually death of neurons. Neurons allow the brain to communicate with the rest of the body, and a deteriorated function of neurons can result in problems with mobility or mental functions. Neurodegenerative diseases progress slowly over many years, with a long silent asymptomatic phase before symptom onset. It is hard to rebuild what is already lost, but disease-modifying treatments might be able to slow down or halt the deterioration of the brain. Therefore, there is a major research focus on investigating the early stages of disease pathogenesis in order to elucidate this critical phase in disease progression.

The four papers included in this thesis focus on identifying altered protein profiles in cerebrospinal fluid from patients with neurodegenerative diseases. For this purpose, multiplexed antibody-based suspension bead arrays have been used. This method allows for hundreds of proteins to be analyzed in hundreds of samples in the same assay. **Paper I** focuses on Alzheimer's disease and investigates the profiles of 200 proteins when comparing patients with controls. Six proteins were identified at altered levels and were further investigated in relation to the progression from mild cognitive impairment to Alzheimer's disease. **Paper II** explores 100 protein profiles in relation to the core Alzheimer's disease biomarkers in asymptomatic 70-year-olds to elucidate patterns preceding potential disease onset. **Paper III** investigates the transition to cognitive impairment in patients with Parkinson's disease and explores potential associations between protein profiles and cognitive assessment tests. Finally, **Paper IV** explores panels of proteins in the context of frontotemporal dementia. Panels of proteins, instead of single biomarkers, have an increased potential to capture the range of biological processes within these types of complex and multifactorial diseases.

Neurodegenerative diseases are often heterogeneous which puts high demands on the study design including an appropriate selection of study population. However, significant similarities are also present which makes it advantageous to have a broad perspective and work with several neurodegenerative disorders. This thesis presents the results from multiplexed antibody-based protein profiling as a contribution to a better understanding of neurodegenerative diseases.

Sammanfattning

Individbaserade behandlingar med skräddarsydda medicinska insatser är ofta mer fördelaktiga än generiska behandlingar framtagna för en genomsnittlig patient. En förutsättning för detta är emellertid en ökad förståelse för sjukdomarnas patofysiologi på molekylär nivå. Studier av proteiner kan bidra till den förståelsen och kan därigenom också i förlängningen bistå den kliniska bedömningen av patienter och vägleda vad för slags behandling som den enskilda patienten bör erbjudas.

Neurodegenerativa sjukdomar såsom Alzheimers sjukdom, Parkinsons sjukdom och frontallobsdemens karakteriseras av en fortskridande försämring av nervcellernas funktion vilket i sin tur resulterar i försämrad rörlighet eller nedsatta mentala funktioner. Ovannämnda sjukdomar utvecklas långsamt under många år och inleds med en lång tyst asymtomatisk fas. Det som förloras kan för det mesta aldrig återfås, men sjukdomsmodifierande läkemedel har möjligheten att bromsa eller i bästa fall helt stoppa en fortsatt nedbrytning av hjärnan. Det är mot den här bakgrunden som en stor del av forskningen kring dessa sjukdomar just avser klarlägga dess tidiga faser.

Följande avhandling innefattar fyra artiklar vilka fokuserar på att identifiera proteiner vars nivåer är kopplade till neurodegenerativa sjukdomar. Proteinerna analyseras med hjälp av en antikroppsbaserad teknik vilket möjliggör analys av hundratals proteiner i hundratals prover i samma analysomgång. **Artikel I** fokuserar på Alzheimers sjukdom och undersöker profilerna av 200 proteiner i patienter jämfört med friska individer. Sex proteiner med förhöjda nivåer identifierades i patienterna och undersöktes vidare i relation till progressionen från mild kognitiv svikt till Alzheimers sjukdom. **Artikel II** utforskar 100 proteiner i relation till de vedertagna biomarkörerna för Alzheimers sjukdom i asymtomatiska 70-åringar för att klarlägga potentiella mönster som är mätbara innan eventuell start av symptom. **Artikel III** utforskar övergången till kognitiv svikt i patienter med Parkinsons sjukdom och undersöker potentiella associationer mellan proteinprofiler och kognitiva testresultat. **Artikel IV** fokuserar på att identifiera paneler av proteiner som tillsammans kan urskilja patienter med frontallobsdemens och dess progression. Paneler av proteiner, i stället för enskilda biomarkörer, har en ökad potential att återspegla dessa typer av komplexa och multifaktoriella sjukdomar.

Neurodegenerativa sjukdomar är ofta heterogena vilket ställer höga krav på studieutformningen och urvalet av individer till studien. Det finns dock även likheter mellan sjukdomarna vilket gör det fördelaktigt att ha ett brett perspektiv och att arbeta tvärvetenskapligt med flera neurodegenerativa sjukdomar. Avhandlingen presenterar resultaten från antikroppsbaserade proteinanalyser som ett bidrag till en bättre förståelse för dessa sjukdomar.

Popular scientific summary

Neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and frontotemporal dementia are characterized by a progressive loss of function and eventually death of nerve cells in the brain. An increased understanding of the brain and neurodegenerative diseases may enable us to improve the treatments and thereby lessen the burden for the patients. The following thesis aims to contribute to the understanding of neurodegenerative diseases by studying the levels of proteins in relation to the progression of these conditions.

Proteins are important for many functions in our bodies. They provide structural support, transport and store molecules, regulate processes and protect our bodies from pathogens. Hence, proteins play a vital role, both when you are healthy but also when you are sick. Proteins are built up by amino acids, following a blueprint provided by our DNA. The amino acid sequences are folded into complex, three-dimensional structures determining the function of the protein. The proteome, the complete set of proteins in your body, varies depending on the state of the body. This in turn allows proteins to be used as indicators for diseases. If we for instance observe a sudden increase of structural proteins usually present in nerve cells, this might indicate that nerve cells have been injured. Protein profiling is the quantitative assessment of the levels of proteins at a certain point. The protein profiles are often compared between two different groups of individuals, for example healthy individuals and patients with Alzheimer's disease. Protein profiling can allow for identification of protein biomarkers, which can be used as objective indicators of health and disease. Furthermore, protein profiling can increase the understanding of different diseases.

Protein levels can be studied using different methods. The work presented in this thesis is based on measurements of proteins using antibodies. Antibodies have an inherent ability to bind specifically to certain proteins, making them valuable tools in the study of proteins. The proteins studied in this thesis have been studied using a multiplexed method allowing for many proteins to be measured in parallel. Proteins are most often measured in blood, a common and widely available sample material. However, in the following thesis the proteins have been measured in cerebrospinal fluid, CSF, an attractive material when studying neurodegenerative diseases due to its proximity to the brain. By studying proteins that are altered in CSF from patients with neurodegenerative diseases, we aim to shed light upon the different processes that are present in the brains of these patients. This has the potential to provide the foundation for identifications of different subgroups of patients, diagnostic tools, as well as tailored treatment strategies.

Thesis defense

This thesis will be defended November 12th, 2021, at 10.00 for the degree of *Teknologie doktor*, Doctor of Philosophy, PhD in Biotechnology.

Air & Fire, SciLifeLab, Tomtebodavägen 23A, Solna, Sweden

Respondent

Sofia Bergström, MSc Eng in Biotechnology

Division of Affinity Proteomics, Department of Protein Science, KTH Royal Institute of Technology, SciLifeLab, Stockholm, Sweden

Faculty opponent

Professor Markus Otto

Department of Neurology, University clinic, Martin Luther University Halle-Wittenberg, Halle, Germany

Evaluation committee

Associate professor Ann Brinkmalm

Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden
Clinical Neurochemistry Laboratory Sahlgrenska University Hospital, Mölndal, Sweden

Associate professor Alexander Santillo

Department of Clinical Sciences, Clinical Memory Research Unit, Lund University, Lund, Sweden
Memory Clinic, Skåne University Hospital, Sweden

Anna Matton, PhD

Division of Neurogeriatrics and Division of Clinical Geriatrics, Center for Alzheimer Research, NVS, Karolinska Institutet, Stockholm, Sweden

Chair of the thesis defense

Professor Jochen M Schwenk

Division of Affinity Proteomics, Department of Protein Science, KTH Royal Institute of Technology, SciLifeLab, Stockholm, Sweden

Main supervisor

Professor Peter Nilsson

Division of Affinity Proteomics, Department of Protein Science, KTH Royal Institute of Technology, SciLifeLab, Stockholm, Sweden

Co-supervisor

Anna Månberg, PhD

Division of Affinity Proteomics, Department of Protein Science, KTH Royal Institute of Technology, SciLifeLab, Stockholm, Sweden

List of publications and manuscripts

The presented thesis is based on the following four papers. Full versions of the papers can be found in the appendix at the end of the thesis.

Paper I **Sofia Bergström**, Julia Remnestål, Jamil Yousef, Jennie Olofsson, Ioanna Markaki, Stephanie Carvalho, Jean-Christophe Corvol, Kim Kultima, Lena Kilander, Malin Löwenmark, Martin Ingelsson, Kaj Blennow, Henrik Zetterberg, Bengt Nellgård, Frederic Brosseron, Michael T. Heneka, Beatriz Bosch, Raquel Sanchez-Valle, Anna Månberg, Per Svenningsson, Peter Nilsson
Multi-cohort protein profiling reveals higher levels of six brain-enriched proteins in Alzheimer's disease patients
Annals of Clinical and Translational Neurology, 2021 Jul;8(7):1456-1470,
doi: 10.1002/acn3.51402

Paper II Julia Remnestål, **Sofia Bergström**, Jennie Olofsson, Evelina Sjöstedt, Kaj Blennow, Henrik Zetterberg, Anna Zettergren, Silke Kern, Ingmar Skoog, Anna Månberg, Peter Nilsson
Association of CSF proteins with tau and amyloid β levels in asymptomatic 70-year-olds
Alzheimer's Research & Therapy, 2021 Mar 2;13(1):54,
doi: 10.1186/s13195-021-00789-5

Paper III Ioanna Markaki, **Sofia Bergström**, Panagiota Tsitsi, Julia Remnestål, Anna Månberg, Ellen Hertz, Wojciech Paslawski, Kimmo Sorjonen, Mathias Uhlén, Graziella Mangone, Stephanie Carvalho, Olivier Rascol, Wassilios G. Meissner, Eloi Magnin, Ullrich Wüllner, Jean-Christophe Corvol, Peter Nilsson, Per Svenningsson
Cerebrospinal fluid levels of kininogen-1 indicate early cognitive impairment in Parkinson's disease
Movement Disorders, 2020 Nov;35(11):2101-2106, doi: 10.1002/mds.28192

Paper IV **Sofia Bergström**[#], Linn Öijerstedt[#], Julia Remnestål, Jennie Olofsson, Abbe Ullgren, Harro Seelaar, John C van Swieten, Matthis Synofzik, Raquel Sanchez-Valle, Fermin Moreno, Elizabeth Finger, Mario Masellis, Carmela Tartaglia, Rik Vandenberghe, Robert Laforce, Daniela Galimberti, Barbara Borroni, Chris R Butler, Alexander Gerhard, Simon Ducharme, Jonathan D Rohrer, Anna Månberg, Caroline Graff[‡], Peter Nilsson[‡], on behalf of the Genetic Frontotemporal Dementia Initiative (GENFI)
A panel of CSF proteins separates genetic frontotemporal dementia from presymptomatic mutation carriers: a GENFI study
Manuscript in resubmission to Molecular Neurodegeneration

[#] The authors contributed equally to the work
[‡] Shared senior authorship

Respondent's contribution to the included work

Paper I
Main responsible for project design, experimental design, data analysis, interpretations as well as manuscript writing.

Paper II
Contributed during project design, data analysis, interpretations, and manuscript writing.

Paper III
Main responsible for project design, experimental design, and subsequent quality control of the data. Contributed to the data analysis, interpretations, and manuscript writing.

Paper IV
Main responsible for experimental design and data analysis. Shared main responsible for project design, interpretations, and manuscript writing.

Related publications not included in thesis

Systematic evaluation of SARS-CoV-2 antigens enables a highly specific and sensitive multiplex serological COVID-19 assay

Sophia Hober, Cecilia Hellström, Jennie Olofsson, Eni Andersson, **Sofia Bergström**, August Jernbom Falk, Shaghayegh Bayati, Sara Mravinacova, Ronald Sjöberg, Jamil Yousef, Lovisa Skoglund, Sara Kanje, Anna Berling, Anne-Sophie Svensson, Gabriella Jensen, Henric Enstedt, Delaram Afshari, Lan Lan Xu, Martin Zwahlen, Kalle von Feilitzen, Leo Hanke, Ben Murrell, Gerald McInerney, Gunilla B. Karlsson Hedestam, Christofer Lendel, Robert G. Roth, Ingmar Skoog, Elisabet Svenungsson, Tomas Olsson, Anna Fogdell-Hahn, Ylva Lindroth, Maria Lundgren, Kimia T. Maleki, Nina Lagerqvist, Jonas Klingström, Rui Da Silva Rodrigues, Sandra Muschiol, Gordana Bogdanovic, Laila Sara Arroyo Mühr, Carina Eklund, Camilla Lagheden, Joakim Dillner, Åsa Sivertsson, Sebastian Havervall, Charlotte Thålin, Hanna Tegel, Elisa Pin, Anna Månberg, My Hedhammar, Peter Nilsson

Clinical and Translational Immunology, 2021 July 19, doi: 10.1002/cti2.1312

HIV-exposed seronegative sex workers express high levels of regulatory T cells, low levels of T cell activation and an intact ectocervical tissue microenvironment

Maria Röhl, Annelie Tjernlund, Julie Lajoie, Gabriella Edfeldt, Frideborg Bradley, **Sofia Bergström**, Vilde Kaldhusdal, Alexandra Åhlberg, Anna Månberg, Kenneth Omollo, Geneviève Boily-Larouche, Muhammad Asghar, Douglas S. Kwon, Julius Oyugi, Joshua Kimani, Peter Nilsson, Keith R. Fowke, Kristina Broliden

Vaccines, 2021 March 4, doi: 10.3390/vaccines9030217

Cerebrospinal fluid proteins altered in corticobasal degeneration

Wojciech Paslawski, **Sofia Bergström**, Xiaoqun Zhang, Julia Remnestål, Yachao He, Adam Boxer, Anna Månberg, Peter Nilsson, Per Svenningsson

Movement Disorders, 2021 March 4, doi: 10.1002/mds.28543

Antibodies to SARS-CoV-2 and risk of past or future sick leave

Joakim Dillner, K Miriam Elfström, Jonas Blomqvist, Carina Eklund, Camilla Lagheden, Sara Nordqvist-Kleppe, Cecilia Hellström, Jennie Olofsson, Eni Andersson, August Jernbom Falk, **Sofia Bergström**, Emilie Hultin, Elisa Pin, Anna Månberg, Peter Nilsson, My Hedhammar, Sophia Hober, Johan Mattsson, Laila Sara Arroyo Mühr, Kalle Conneryd Lundgren

Scientific Reports, 2021 March 4, doi: 10.1038/s41598-021-84356-w

High amounts of SARS-CoV-2 precedes sickness among asymptomatic healthcare workers
Joakim Dillner, K Miriam Elfström, Jonas Blomqvist, Lars Engstrand, Mathias Uhlén, Carina Eklund, Fredrik Boulund, Camilla Lagheden, Marica Hamsten, Sara Nordqvist-Kleppe, Maike Seifert, Cecilia Hellström, Jennie Olofsson, Eni Andersson, August Jernbom Falk, **Sofia Bergström**, Emilie Hultin, Elisa Pin, Ville N Pimenoff, Sadaf Hassan, Anna Månberg, Peter Nilsson, My Hedhammar, Sophia Hober, Johan Mattsson, Laila Sara Arroyo Mühr, Kalle Conneryd Lundgren
The Journal of Infectious Diseases, 2021 Feb 13, doi: 10.1093/infdis/jiab099

SARS-CoV-2 exposure, symptoms and seroprevalence in healthcare workers in Sweden
Ann-Sofie Rudberg, Sebastian Havervall, Anna Månberg, August Jernbom Falk, Katherina Aguilera, Henry Ng, Lena Gabrielsson, Ann-Christin Salomonsson, Leo Hanke, Ben Murrell, Gerald McInerney, Jennie Olofsson, Eni Andersson, Cecilia Hellström, Shaghayegh Bayati, **Sofia Bergström**, Elisa Pin, Ronald Sjöberg, Hanna Tegel, My Hedhammar, Mia Phillipson, Peter Nilsson, Sophia Hober, Charlotte Thålin
Nature Communications, 2020 Oct 8, doi: 10.1038/s41467-020-18848-0

Altered levels of CSF proteins in patients with FTD, presymptomatic mutation carriers and non-carriers
Julia Remnestål[#], Linn Öijerstedt[#], Abbe Ullgren, Jennie Olofsson, **Sofia Bergström**, Kim Kultima, Martin Ingelsson, Lena Kilander, Mathias Uhlén, Anna Månberg, Caroline Graff[‡], Peter Nilsson[‡]
Translational Neurodegeneration, 2020 Jun 23, doi: 10.1186/s40035-020-00198-y

Newborn proteomic screening enables presymptomatic diagnosis of complement deficiencies and phagocytic disorders
Mahya Dezfouli, **Sofia Bergström**, Lillemor Skattum, Hassan Abolhassani, Maja Neiman, Clara Franco Jarava, Juana M. Ferrer Balaguer, Charlotte A Slade, Anja Roos, Luis M. Fernandez Pereira, Margarita López Trascasa, Luis Ignacio Gonzalez Granado, Yumi Mizuno, Yusuke Yoshi, Vanda Friman, Åsa Lundgren, Anna-Carin Norlin, Zahra Chavoshzadeh, Nima Rezaei, Asghar Aghamoahammadi, Andrea Martin Nalda, Roger Colobran, Pere Soler-Palacin, Manuel Hernández-Gonzalez, Ingrid Winship, Vanessa Bryant, Drick Roos, Heleen van Velzen-Blad, Luis M. Allende, Ulrika von Döbeln, Stefan Berg, Anders Fasth, Lennart Truedsson, Toshiro Hara, Shigeaki Nonoyama, Jochen M. Schwenk, Peter Nilsson, Lennart Hammarström
Frontiers in Immunology, 2020 Mar 17, doi: 10.3389/fimmu.2020.00455

Array-Based Profiling of Proteins and Autoantibody Repertoires in CSF
Elisa Pin, Ronald Sjöberg, Eni Andersson, Cecilia Hellström, Jennie Olofsson, August Jernbom Falk, **Sofia Bergström**, Julia Remnestål, David Just, Peter Nilsson, Anna Månberg
Methods in Molecular Biology, 2019 August 21, doi: 10.1007/978-1-4939-9706-0_19

Levels of human proteins in plasma associated with acute paediatric malaria
Philippa Reuterswärd[#], **Sofia Bergström**[#], Judy Orikiiriza, Elisabeth Lindquist, Sven Bergström, Helene Andersson Svahn, Burcu Ayoglu, Mathias Uhlen, Mats Wahlgren, Johan Normark[‡], Ulf Ribacke[‡], Peter Nilsson[‡]
Malaria Journal, 2018 Nov 15, doi: 10.1186/s12936-018-2576-y

[#] The authors contributed equally to the work
[‡] Shared senior authorship

Aims of the thesis

To utilize multiplexed antibody-based protein profiling and develop data analysis strategies to explore novel CSF biomarkers within neurodegenerative diseases.

Paper I

The aim with the study was to contribute to the long-term goal of a better understanding of the Alzheimer's disease continuum by investigating CSF levels of 200 proteins in patients with Alzheimer's disease, individuals with mild cognitive impairment and controls.

Paper II

The aim with the study was to bring further insight to the processes preceding the development of Alzheimer's disease by exploring the association between 100 CSF proteins and the core biomarkers for Alzheimer's disease in a cohort of asymptomatic 70-year-olds.

Paper III

The aim of the study was to contribute to the assessment of potential CSF markers for cognitive decline in Parkinson's disease. We investigated levels of 200 proteins in CSF from patients with early Parkinson's disease in relation to cognitive impairment.

Paper IV

The aim of the study was to contribute to the identification of CSF biomarkers for frontotemporal dementia. Here, patterns of 100 CSF proteins were explored to identify a panel of proteins that together has the potential to distinguish symptomatic patients from presymptomatic mutation carriers and mutation non-carriers. Furthermore, we explored the potential to identify a panel of proteins able to separate presymptomatic mutation carriers from mutation non-carriers.

Preface

I started as a PhD student in 2017 and have since then analyzed a large number of proteins in samples from many different individuals. The aim has been to identify and characterize protein profiles that might reflect what is going on in our bodies when we develop or contract a disease.

I have a Master of Science in Engineering degree in biotechnology from KTH Royal Institute of Technology. The education enabled me to acquire an understanding of different technologies that are available to analyze biological samples, and different aspects related to data analysis including interpretations of results. This set of skills is the foundation that I have continued to extend during the last four years, where I have focused on the study of proteins in different neurodegenerative diseases. I have been a PhD student at the Affinity Proteomics division within the Protein Science department at KTH Royal Institute of Technology. The division is located at the Science for Life Laboratory, SciLifeLab, which is a Swedish national center for large-scale life science research. SciLifeLab is a joint effort between four Swedish universities: KTH Royal Institute for Technology, Karolinska Institutet, Stockholm University and Uppsala University. The SciLifeLab community enables networking and an exchange of ideas between different research groups. During my PhD studies, I have focused on neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and frontotemporal dementia.

During these years I have also been a part of other projects including, among others, amyotrophic lateral sclerosis, HIV, COVID-19, immunodeficiency, and malaria. All these additional projects have had the multiplexed bead-based assays as a fundamental component where I have especially been focusing on the technology and different strategies to analyze the data. My work in these areas have further broadened my perspective and enabled me to be a part of a large network and to collaborate with many different researchers.

This thesis covers different aspects related to the pursuit of CSF biomarkers for neurodegenerative disorders. Given the large field of neuroproteomics, I have restricted the thesis to the concepts that I find most interesting. Part I covers different aspects of biomarkers, sample materials, antibodies and technologies frequently used to study proteins. Part II covers neurodegenerative diseases and examples of relevant biomarkers. Part III includes a discussion around different challenges regarding study design, including the selection of an appropriate cohort, experimental

design, and data analysis strategies. Finally, part IV includes the present investigations and summaries of the four included papers. The full versions of these papers can be found in the appendix.

The study of disease-related protein profiles is complex and requires united efforts from many different groups. This thesis aims to provide a few pieces to the large puzzle that I hope we will continue to solve together.

Enjoy!

Sofia Bergström, October 2021

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Part I: Proteomics

Biomarkers

A biomarker is defined as an objective and measurable indicator of the state of the body. Consequently, biomarkers provide insight into normal physiological states, pathological processes as well as biological responses to therapeutic interventions (Biomarkers Definitions Working Group, 2001). Well known biomarkers include body temperature, blood pressure and oxygen saturation. Proteins however, serve especially well as biomarkers (Anderson, 2014). The proteome, the complete set of expressed proteins at a certain time, is dynamic and changes in response to various factors, including disease. For instance, the blood concentration of C-reactive protein (CRP) increases dramatically upon an infection and can be used as a biomarker for ongoing inflammation.

Proteins

Proteins are found throughout our bodies and cover a broad range of different functions. Proteins can give structural support to cells, transport and store molecules, act as messengers to coordinate processes, and proteins in the form of enzymes can facilitate chemical reactions. Antibodies are another type of proteins, and they protect our bodies from pathogens. See section *Proteomics: Antibodies for protein profiling applications* for more details about antibodies. Proteins are composed of 20 different types of amino acids that are attached together in long chains. Our DNA holds approximately 20,000 protein-coding genes (20,442 according to Ensembl, database version 104.38, updated March 2021 and 20,386 according to UniProt, release 2021-03 of June 2021) which can be translated into proteins via mRNA. However, another level of complexity emerges when taking into account products of alternative splicing, allelic variations and post translational modifications (PTMs). This means that the same gene can give rise to many different proteoforms (Smith, 2013). Although other forms of molecular biomarkers exist, such as DNA, RNA or metabolite biomarkers, this thesis focuses on protein biomarkers.

Different types of biomarkers

Biomarkers can be divided into different groups depending on their application (FDA-NIH Biomarker Working Group, 2016). Diagnostic biomarkers indicate a presence of a disease or a condition, while prognostic biomarkers assess the probable progression of the disease. Susceptibility or risk biomarkers indicate the potential for developing a specific disease.

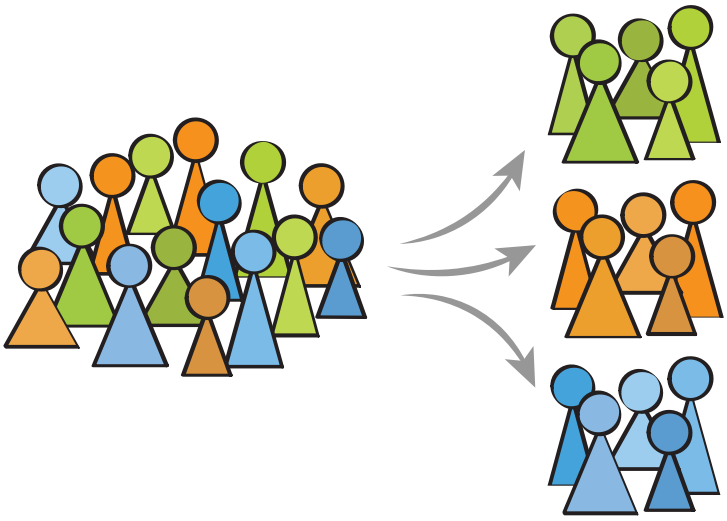

Figure 1: Biomarkers can be used to divide individuals into different groups.

Other biomarkers are related to medical interventions: Predictive biomarkers can identify individuals that are likely to respond to a certain treatment. Pharmacodynamic or response biomarkers indicate a positive response connected to a medical product while safety biomarkers detect or predict an adverse effect related to a medical intervention. Lastly, repeated measurements of monitoring biomarkers are used as an indication of the status of the disease. Identifying protein profiles with pathophysiological importance is vital for precision medicine and its efforts to improve the ways we predict, prevent, diagnose, and treat different diseases (Hampel, 2018b).

From clinical symptoms to underlying pathological processes

The transition of diseases from a syndromal to a biological construct, or more precisely the shift from studying clinical symptoms and signs to study their underlying pathological processes, enables the stratification of patients into distinct subgroups. A syndrome is a clinical consequence that can be caused by one or more diseases which makes the definition and stratification of patients complicated. In order to increase the understanding of the mechanisms underlying the clinical presentations, a biological definition is necessary. Alzheimer's disease (AD) is an example of a disease where the identification of a set of biomarkers has enabled a transition from a syndrome-

based to a biologically defined disease (Jack, 2018). Without biomarkers, AD could only be diagnosed in living individuals as *possible* or *probable* AD based on clinical symptoms (McKhann, 1984). An autopsy was needed to confirm the underlying pathological processes associated with AD. However, the diagnosis of AD based on clinical assessment was found to only moderately concord with postmortem examination (sensitivity 71-87%, specificity 44-71%) (Beach, 2012), highlighting the need for a more distinct definition of the disease which can be obtained by the use of biomarkers.

Furthermore, the use of biomarkers can be beneficial in drug trials. In earlier AD drug trials, only clinical syndromes were used as a selection criteria and up to 25% of mild AD participants lacked biomarker evidence of AD related pathology (Karran, 2014). Another phase 3 trial had similar problems where 36% of the participants with mild-to-moderate AD at baseline did not have detectable biomarker evidence of pathological processes associated with AD (Salloway, 2014). To overcome this high misclassification rate, more recent AD drug trials used different types of biomarkers to screen for suitable participants to include in the study which increases the potential to show efficacy (Honig, 2018; Mattsson, 2015; Sevigny, 2016; Sperling, 2014). When it comes to neurodegenerative diseases, AD is the successful exception. No other brain proteinopathy has a biomarker profile as well-defined, extensively characterized, and precise as the core AD biomarkers.

Thresholds for biomarkers on the continuous scale

There are some types of qualitative biomarker tests designed to only specify the presence or absence of a particular indicator. For instance, the standard pregnancy test measures a specific hormone produced by cells surrounding the embryo upon fertilization, thus providing a simple positive or negative result. However, most biomarkers are already present in normal states, but their levels are altered upon a disease state. This means that the biomarker will be measured on a continuous scale, but it will often be classified into a positive or negative result to facilitate communication and understanding. However, the continuous scale may reveal additional information that could not be captured using the dichotomous threshold-based thinking. A threshold indicating the range of normal states can still be determined in order to relate the observed values to this reference level, which can be useful as long as values close to the cut-off is treated with care and interpreted properly. Furthermore, the selected threshold can be tailored to individual studies depending on the application and circumstances.

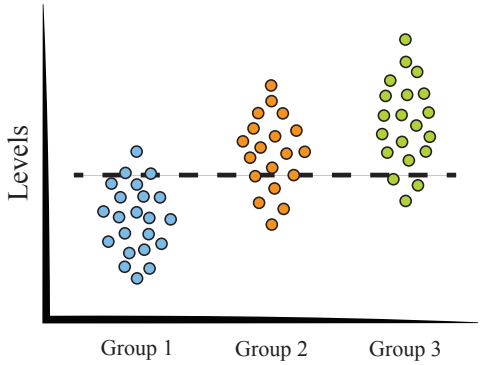

Figure 2: Schematic results of an analysis of the levels of a biomarker in a set of individuals divided into three groups. The y-axis represents the measured concentration of the protein, and each dot represents the result from one individual. The dashed line indicated the pre-selected threshold which divides the samples into positive or negative for a specific marker. It is common to dichotomize biomarker results to facilitate communication, but additional information might be included in the continuous scale.

It is however not a trivial task to determine suitable thresholds. The suitable reference level may vary based on for example age and sex. Hospitals may use different reference levels depending on the method that was used to measure the protein. Additionally, the threshold is sometimes updated within the same hospital due to drifts in the measurements. To overcome these types of variabilities, the way the protein is measured needs to be standardized. Moreover, suitable reference materials can be used to homogenize the results obtained from different hospitals.

In conclusion, biomarkers are tools that have the ability to track changes that occur in our bodies. They provide invaluable insight into health and disease and can inform decision-making and improve clinical practice. Furthermore, panels of several biomarkers have the potential to reflect the complex and multifactorial nature of neurodegenerative disorders. Panels may accommodate an increased resolution between related diseases and different disease phenotypes. In addition to the discovery of biomarkers that are useful in the clinics or during drug development, identifying proteins with altered levels in different disease groups might shed light on the underlying biology and pathological processes preceding the symptom onset. The aim to identify proteins that can aid in the understanding of a disease is an important part in the search for disease-associated proteins.

Cerebrospinal fluid for biomarker research

Disease-associated proteins can be secreted or leaked from affected tissues and end up in body fluids where they can be measured. Body fluids include, among others, blood derived samples (plasma and serum), cerebrospinal fluid (CSF), urine (Marimuthu, 2011), tear fluid (Adigal, 2021), and saliva (Boroumand, 2021). As many as 15,000 different proteins have been detected in different types of body fluids (Huang, 2021) which highlights the potential to find disease-associated proteins in this type of sample material. The suitable body fluid should ideally accommodate sufficient levels of the protein of interest in relation to the sample matrix to enable detection, and the collection procedure should be minimally invasive. The collection of body fluids is often less invasive than tissue biopsies, another biological media suitable for biomarker measurements, though the degree of invasiveness varies between different types of fluids.

Blood is a widely used sample material

Blood-derived samples are easily accessible and a widely used sample material for biomarker research. Biomarkers that are detectable in blood can be used as screening tools in primary care and are suitable for longitudinal sampling (Blennow, 2018). Two common types of blood-derived samples include plasma and serum. Plasma is prepared by the addition of an anticoagulant, such as EDTA, heparin, or citrate. Serum on the other hand is prepared by allowing the blood to clot, followed by a centrifugation where the blood clots are removed. One obstacle with protein analysis in blood derived samples is that some proteins are present in high concentrations while others are present at very low concentrations. The dynamic range of proteins in plasma is at least 10 orders of magnitude which constitutes challenges for detection of low abundant proteins (Anderson, 2002). Moreover, blood is a systemic fluid, and its circulatory system spans the entire body. The substantial blood volume may extensively dilute potential biomarkers and thereby prevent their detection. To enable detection of disease-related proteins, a body fluid in proximity to the tissue or organ of interest can be preferable.

Advantages of CSF as sample material

CSF is an attractive biofluid to study in the context of neurodegenerative diseases due to its proximity to the brain. There is a free exchange of molecules between the brain and the CSF but only a fraction of these proteins enters the blood stream. The main functions of CSF, a colorless fluid surrounding the brain and the spinal cord, are to protect the central nervous system from

injury by shielding the brain from external force, remove waste products, and provide the brain with nutrients. CSF is mainly formed in the choroid plexus of the ventricles (Johanson, 2008) and its volume in the body is estimated to be approximately 150 ml (Blennow, 2012). The total protein concentration in CSF is approximately 0.5 mg/ml, making it 100-fold lower compared to plasma.

A single layer of ependymal cells forms the brain-CSF barrier (Brat, 2018). The barrier is highly permeable and enables proteins to enter the CSF from the extracellular space in the brain (Blennow, 2012). Blood-derived proteins are also present in CSF, but both the blood-CSF barrier and the blood-brain barrier limit the passage of proteins from blood to CSF. It is estimated that approximately 20% of the proteins in CSF are synthesized in the brain, and the remaining 80% are blood-derived (Thompson, 1995), though this ratio might change upon pathological conditions. The brain-derived proteins present in CSF are especially interesting to study in the context of neurodegenerative disorders. These proteins have the potential to reflect the status of the brain, and their concentration in CSF will change upon acute brain injury, but also as an effect of pathophysiological processes in the brain, as the ones observed in dementia (Blennow, 2012). Brain-derived proteins can be present in blood, but often at lower concentrations compared to CSF. Furthermore, if the protein is expressed outside the central nervous system as well, it may be challenging to determine whether the observed altered levels in blood reflects what is happening in the brain or not (Zetterberg, 2019a).

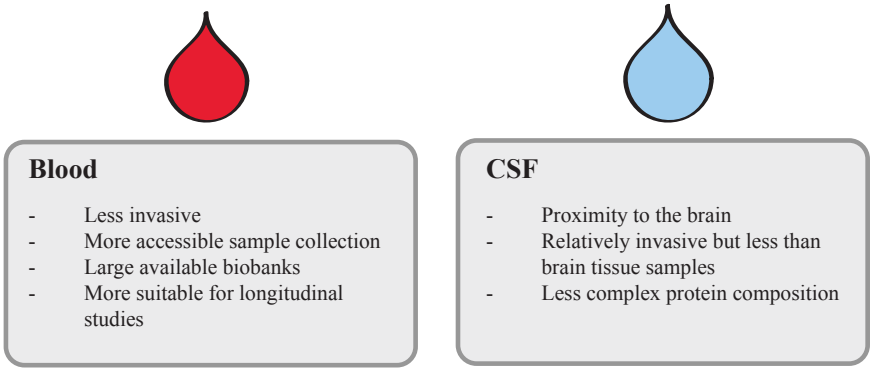

Figure 3: Advantages with blood and CSF as sample material for neuroproteomics.

The CSF proteome has been investigated in several studies and over 6000 proteins have been identified in total (Huang, 2021). However, the number of identified proteins varies between different studies. The variability may be connected to several factors including the selected experimental procedure as well as to the chosen degree of certainty. For example, one study investigated the normal CSF proteome using a sample pool of 11 individuals and a mass spectrometer-based assay which included depletion of abundant proteins. They observed 2630 non-redundant proteins and more than half were based on at least two peptide identifications (Schutzer, 2010).

Challenges with CSF as sample material

CSF is collected by lumbar puncture which is regarded as a relatively invasive procedure (Niemantsverdriet, 2015). However, a multicenter study determined that lumbar puncture can be safely performed in memory clinics, and with a low complication rate (Duits, 2016). Serious complications were very rare, they observed complications requiring medical intervention in 1% of the individuals that underwent lumbar puncture, while most complaints were mild and commonly included post lumbar puncture headache or back pain. Furthermore, consensus guidelines and recommendations for lumbar puncture procedures have been compiled, to further reduce complication rates (Engelborghs, 2017). Some patients may be reluctant towards a lumbar puncture, but the procedure is often more tolerable than expected. Moreover, a high acceptance rate of lumbar puncture has been observed (92%) (Duits, 2016). However, due to its invasive nature, repeated lumbar puncture for CSF collection may be restricted which makes longitudinal studies difficult.

When designing a study, it is important to select an appropriate control group. Different types of comparison groups can be used but they affect which conclusions that can be drawn from the analysis. Sometimes the disease group is compared to a group of healthy individuals. It can be hard to recruit healthy individuals to CSF studies for example due to the relative invasiveness of the collection procedure which can constitute obstacles connected to ethical concerns. Furthermore, it is not uncomplicated to define "healthy normal individuals", and this is often not the group of individuals which is the most interesting to compare with. Individuals with neurodegenerative disorders are often old, and a comparison with young healthy 20-year-olds will probably not reveal the most interesting disease-relevant differences. Consequently, the control group in CSF studies often consists of individuals with other types of complaints or neurological diseases, different from the investigated disease group. Different types of comparison groups can be used, to facilitate

analysis regarding different parts of the research question. The properties of the control subjects, as well as the disease group of interest, should preferably be clearly described, including potential inclusion and exclusion criteria. It is preferred that all individuals are well-characterized, and that extensive demographic and clinical information is available. This is important in order to control and be aware of potential confounding factors, see section *Study design: Experimental design* for more details. Cognitively unimpaired individuals can be followed for a couple of years after sample collection to investigate whether signs of cognitive decline appear which might be relevant for the study.

The study of the CSF proteome has potential to increase the knowledge of the pathophysiological abnormalities present in patients with neurological disorders. The collection procedure of CSF is more invasive than a blood draw but there is a major advantage of the proximity to the organ of interest. There are several available platforms to analyze proteins in biofluids in a high-throughput manner, see the section *Proteomics: Protein profiling technologies* for more details. Consequently, the main limiting factor for protein profiling is not the sample throughput but the availability of large and appropriate sample sets.

Antibodies for protein profiling applications

Antibodies are a part of the adaptive immune system that aims to protect the body from pathogens. The Y-shaped proteins recognize and bind to encountered pathogens in order to neutralize or eliminate them. The inherent ability of antibodies to specifically bind to certain proteins makes them a frequently used tool when exploring the human proteome. Antibodies are used as affinity reagents in many different biotechnological applications, including immunoassays.

The structure of antibodies

In mammals, there are five different classes of antibodies, also known as immunoglobulins (Ig): IgG, IgA, IgD, IgE and IgM, where both IgG and IgA can be further divided into subclasses. IgG is the most abundant antibody isotype in blood and is the most frequently used antibody class for research. Antibodies are composed of two copies of identical heavy chains and two copies of identical light chains. The chains are connected by disulfide bonds and each chain can be divided into constant and variable regions. The constant region depends on the class of the antibody, while the variable region varies depending on which specific antigen it will bind. Three hypervariable regions (complementarity-determining regions, CDRs) constitute the actual antigen-binding site that recognizes the epitope on the targeted protein (Lipman, 2005). The strength of the interaction between the antibody and the antigen is referred to as the affinity of the antibody.

The recognized epitope can be continuous (linear) or discontinuous (conformational). A linear epitope consists of a consecutive stretch of amino acids, while a conformational epitope is located at different parts of the sequence and is brought into proximity when the protein is folded. The distinction is not absolute, for example a conformational epitope may include short segments of adjacent residues (Van Regenmortel, 2009). Antibody-based protein assays often include some type of denaturation procedures. Therefore, the antibodies generated for such applications often have to recognize the unfolded version of the protein. This might indicate that linear epitopes play a more prominent role under these circumstances compared to for example antibodies used for therapeutic purposes where the native protein should be recognized (Berglund, 2008).

Monoclonal and polyclonal antibodies

There are two main types of antibodies available for researchers: monoclonal and polyclonal antibodies. Polyclonal antibodies are a mixture of several antibodies recognizing different epitopes

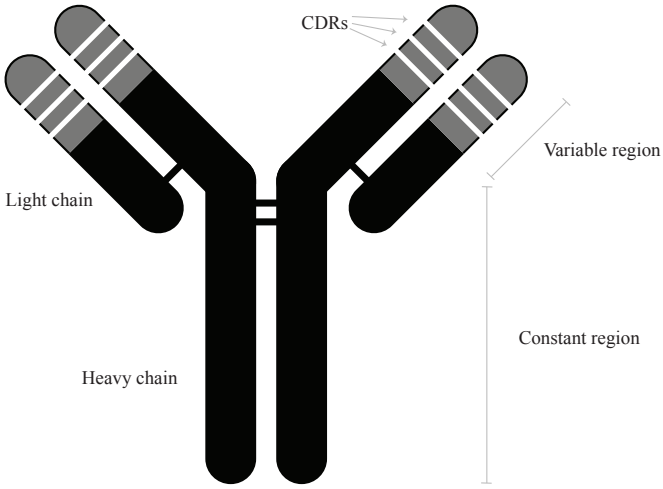

Figure 4: A schematic view of an antibody.

on the same protein, while monoclonal antibodies constitute one single species that recognizes the same epitope. Polyclonal antibodies are produced by the immune system of a host animal such as rabbit, goat, or sheep, as a response to an injected immunogen. A mixture of B-cell clones contributes to the response and give rise to the certain batch of polyclonal antibodies which can be obtained from the blood of the animal. The antibodies can be purified using bacterial proteins with antibody-binding properties, such as Protein A and Protein G. Another option is to purify the antibodies using the same immunogen that gave rise to the response, which results in a polyclonal mixture of mono-specific antibodies (Hjelm, 2011). The polyclonal antibodies are predisposed to vary between batches, since the immune response will vary between different animals. Repeated immunizations in separate animals can generate similar but not identical epitope patterns (Hjelm, 2012). To overcome this variability, Köhler and Milstein developed the foundation for monoclonal antibody production using the hybridoma technology. Here, the antibodies are derived from a single B-cell parent clone, immortalized by the fusion with myeloma cells which enables long-term generation of antibodies recognizing one single epitope (Köhler, 1975). Monoclonal antibodies have a low batch-to-batch variability and generally lower background noise. However, the production of polyclonal antibodies is faster and less expensive. Furthermore, the success rate of polyclonal antibodies seems to be higher in applications where denaturation of proteins is involved, for example by heat or detergents.

The Human Protein Atlas

The advantages, feasibility, and wide range of possible applications of polyclonal antibodies have been demonstrated by the Swedish-based Human Protein Atlas project (HPA) (Uhlén, 2005; Uhlén, 2010). This proteome-scale initiative has the aim to map expression levels and localizations of all human proteins. HPA uses various omics technologies, including antibody-based methods. HPA produces (Lindskog, 2005; Nilsson, 2005), characterizes and verifies (Sjoberg, 2016; Sjoberg, 2012; Stadler, 2012) polyclonal rabbit antibodies in a gene-centric and systematic manner. The HPA antibodies are validated based on the validation principles proposed by The International Working Group for Antibody Validation (Uhlén, 2016). The current version of HPA (version 20.1, released 2021-02-24) contains 26,941 antibodies targeting 17,165 unique proteins. The antibodies are subsequently used in different types of immunoassays to explore the human proteome, both levels and localizations, in different tissues, cells, and organs. The efforts by the HPA program have resulted in several open-access knowledge resources available at www.proteinatlas.org, including The Tissue Atlas (Uhlén, 2015), The Cell Atlas (Thul, 2017), The Pathology Atlas (Uhlén, 2017), The Blood Atlas (Uhlén, 2019b), The Human Secretome (Uhlén, 2019a), and The Brain Atlas (Sjöstedt, 2020). Furthermore, The Single Cell Type Atlas was published earlier this year which enables researchers to explore the expression of protein-coding genes in different cell types (Karlsson, 2021).

The HPA antibody production pipeline starts with the in silico identification of an amino acid sequence within the protein of interest that has a low sequence homology to other proteins. These 50-150 amino acid long sequences are called Protein Epitope Signature Tags (PrESTs). The PrEST is fused with a dual tag containing an albumin-binding protein (ABP) and a polyhistidine-tag (His_6) and produced in *Escherichia coli*. After purification and control procedures, the protein fragment is used for rabbit immunization. The resulting polyclonal antibody serum is purified by depletion of antibodies specific for the His_6ABP-tag and capture of antibodies specific for the PrEST (Hober, 2008). The specificity of the generated antibodies is evaluated using protein microarrays comprised of an array of PrESTs spotted onto an epoxy coated glass slide (Nilsson, 2005). Antibodies that pass the protein microarray evaluation are further evaluated using Western blot and are subsequently used to map protein localization in cells and tissues. Several PrESTs can be designed for each protein, resulting in different polyclonal antibody sets targeting different parts of the protein of interest.

Antibody validation

A functional antibody must bind the intended target and have a low cross-reactivity. However, antibodies are context dependent making it important to identify the right antibody for the right type of application. Therefore, it is important that antibody validation is caried out in an application and context dependent manner (Uhlén, 2016). An antibody can be extensively validated for one or several applications, but still need additional validation procedures if the intended use is too different from the validated applications (Taussig, 2018). An antibody that works in Western blot, does not necessarily work in immunoprecipitation, or immunohistochemistry, just to give a few examples. Additionally, the relative concentration difference between the intended target and potential off-target proteins will affect the specificity of an antibody and thereby its performance (Algenäs, 2014). This means that an antibody-pair which identifies the intended protein in CSF in a sensitive and specific manner when using a sandwich assay setup, does not necessarily perform as well in plasma. Furthermore, every new batch of antibodies might need to go through validation procedures, as observed in an immunofluorescence setting (Skogs, 2017). This makes antibody validation a complex and laborious task that needs to be tailored for each intended application. Several online resources are available that catalogue the available antibodies, including validation data. One such portal is the Antibodypedia (Björling, 2008), available at www.antibodypedia.com, where both antibody providers and users can contribute with validation results. The resource currently contains information about more than four million antibodies from 95 providers (updated September 2021).

Protein profiling technologies

The advances in high-throughput and multiplexed technologies for measuring proteins in biological samples including CSF or blood have resulted in numerous suggested biomarkers. However, most of these biomarkers are *potential biomarker candidates*. There is a long process from the initial discovery of a candidate marker to a clinical setting, and this procedure will result in failure for most candidates. Even when the biomarker has been established, further evaluation and characterization of the biomarker is needed. Some proteins that are not suitable to act as clinical biomarkers may still be of importance to elucidate the pathophysiological processes of a disease.

Identification of a biomarker is usually divided into several phases, with an initial discovery phase followed by a long validation phase. The processes typically begin with the analysis of many proteins and continues with further assessment using a targeted approach of a few selected proteins during the validation phase. This emphasizes the need for different types of methods to measure proteins, depending on the specific requirements for the specific stage of biomarker development.

The large-scale study of proteins, proteomics, is a promising approach for the discovery of potential biomarker candidates. Proteomic technologies can be divided into two main approaches: mass spectrometry-based proteomics and antibody-based proteomics. My work, as the title indicates, focuses on antibody-based proteomics where antibodies are used to capture and measure proteins. Mass spectrometry-based proteomics, on the other hand, measure the mass-to-charge ratio of protein-derived ions. However, the fields are not completely separated, antibodies can be used in combination with mass spectrometry, for example as an enrichment step. Proteomic approaches can further be divided into targeted knowledge-based approaches and an untargeted discovery approaches. Mass spectrometry-based proteomics is leading when it comes to the untargeted discovery approach, but several antibody-based methods have enables analysis of large protein panels, which increases the ability to do broad screenings.

Mass spectrometry

Simplified, a mass spectrometer consists of three parts: an ion source, a mass analyzer, and an ion detector. The generation of ions can be done in different ways, and the two main methods for ionization (Nadler, 2017) are electrospray ionization (ESI) (Fenn, 1989) and matrix-assisted laser desorption ionization (MALDI) (Karas, 1988). There are different types of mass analyzers, including the quadrupole, orbitrap, and time-of-flight (TOF) among others. A tandem mass spectrometry workflow is often utilized (MS/MS) and different types of mass analyzers can be

combined to take advantage of their different strengths (Aebersold, 2003). The complexity of the sample is often reduced prior to the analysis in the mass spectrometer to increase the multiplexing capacity. A coupled liquid chromatography MS system is widely used within proteomics to enable detection of a higher number of proteins. To further reduce the complexity of the sample, additional procedures can be performed prior to the analysis. This can be done on a protein level for example by size or by using antibodies for either depletion of high-abundant proteins or for enrichment of the proteins of interest. The sample can also be pre-fractionated on a peptide level by liquid chromatography.

Shotgun mass spectrometry can be used to discover new potential biomarker candidates, but the approach has limitations connected to its sensitivity. A typical workflow includes enzymatic protein digestion, often by trypsin, followed by separation using liquid chromatography and MS/MS measurements of the peptides (Picotti, 2013). When using a data-dependent acquisition (DDA) a stochastic selection of a limited number of precursor peptides is made. The selection is usually intensity-based which means that it is difficult to consistently measure low abundant proteins and different proteins can be identified between different runs. Data-independent acquisition (DIA) on the other hand performs MS2 quantification of the entire MS1 spectrum. This makes the data more difficult to analyze but this strategy enhances the coverage and overcomes some of the problems with missing values present in DDA proteomics. Targeted mass spectrometry approaches, including selected or multiple reaction monitoring (SRM/MRM) as well as parallel reaction monitoring (PRM), can be used for quantification of a predefined list of protein targets, which enables the detection of proteins with lower abundance (Borràs, 2017). Heavy protein or peptide standards can be analyzed in parallel to the endogenous peptides to enable absolute quantification. Every target needs some assay development, but this approach enables a more robust quantification.

The main advantage with mass spectrometry is the more direct identification of proteins, compared to immunoassays which require affinity reagents. The ability to utilize an untargeted discovery approach in beneficial, but the sample throughput can be comparably low in these types of setups. Targeted mass spectrometry approaches are beneficial as validation procedures for both untargeted mass spectrometry findings as well as findings from antibody-based methods. Advances in mass spectrometry technologies have the potential to overcome some of its disadvantages and thereby further complement immunoassays. However, despite this, antibody-based protein profiling is still very attractive due to the easier transition into assays suitable for a clinical setting.

Important properties of immunoassays

The main advantage with antibody-based proteomics is the sensitivity, it is possible to identify low abundant proteins. Sensitivity relates to the assay's ability to identify the analyte within the sample matrix, while specificity relates to the assay's ability to distinguish between the intended target and other components. Different assays have different intrinsic detection limits. The limit of detection can be extremely low if methods able to detect single molecules are used (Dong, 2017). Additionally, the sensitivity of an immunoassay depends on the affinity of the antibody. Sensitive assays for protein detection commonly use two antibodies in one way or another. Usually one antibody captures the protein of interest, while a second antibody enables detection. The requirement of two antibodies recognizing the same protein enhances the specificity of the assay. However, potential cross-reactivity of secondary antibodies limits the number of proteins that can be analyzed simultaneously, and different platforms have tackled this problem in various ways.

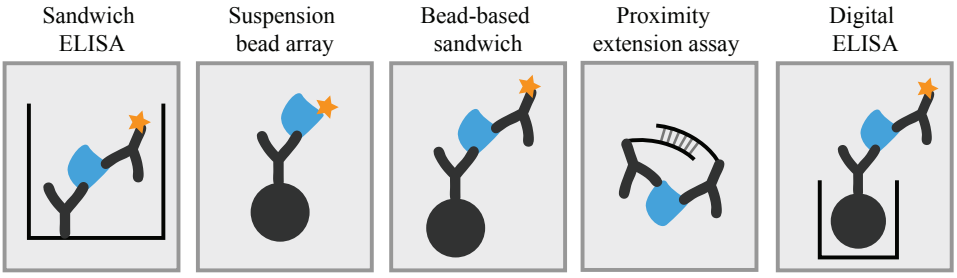

Figure 5: Different antibody-based protein profiling technologies. The blue object indicates the protein of interest.

Sandwich ELISA

The enzyme-linked immunosorbent assay (ELISA) was developed 50 years ago (Engvall, 1971) and is still widely used in many different formats in diagnostic and research laboratories. This immunoassay utilizes two different antibodies to measure the protein of interest, one capture antibody and one detection antibody. The capture antibodies are coated into the wells of a microtiter plate and diluted samples can subsequently be added. The intended target will bind to the capture antibodies and detection is enabled through e.g. biotinylated antibodies and a streptavidin-coupled enzyme. Next, a substrate is added which results in a color shift that can be measured. Commonly, one protein is measured per ELISA and the assay takes place in a 96-well microtiter plate. Some wells are dedicated for controls and a standard curve, which means that around 80 samples can be measured in the same assay. However, many commercial kits recommend all samples to be analyzed in duplicates, which further limits the throughput.

Suspension bead array

It is attractive to use multiplexed immunoassays which enables the analysis of a higher number of proteins in the same assay. Multiplexing increases the number of proteins analyzed per sample volume, it reduces expenses per measured protein, and it saves time. This is especially important for research, whereas a single plex assay may be sufficient in a clinical setting.

Using suspension bead arrays (Häggmark, 2013; Pin, 2019; Schwenk, 2008) hundreds of proteins can be analyzed in hundreds of samples in the same assay run. The dual-dimensional multiplexing capacity is enabled through direct labeling of whole samples with biotin, and a single binder setup. In a single binder assay, the generation of a signal only requires interaction between the capture antibody and the labelled protein, compared to sandwich assays where both a capture antibody and a detection antibody are required.

The suspension bead arrays use antibody coupled beads and a flow cytometer-based system to measure proteins in plasma, serum, CSF and other biofluids. Capture antibodies are coupled onto carboxylated magnetic color-coded beads using carbodiimide crosslinker chemistry and can subsequently be pooled to form the suspension bead array. Next, the bead array is incubated with biotinylated, diluted, and heat-treated samples in a microtiter plate and the read-out is performed using a streptavidin-coupled fluorophore as the reporter system in a FLEXMAP 3D instrument

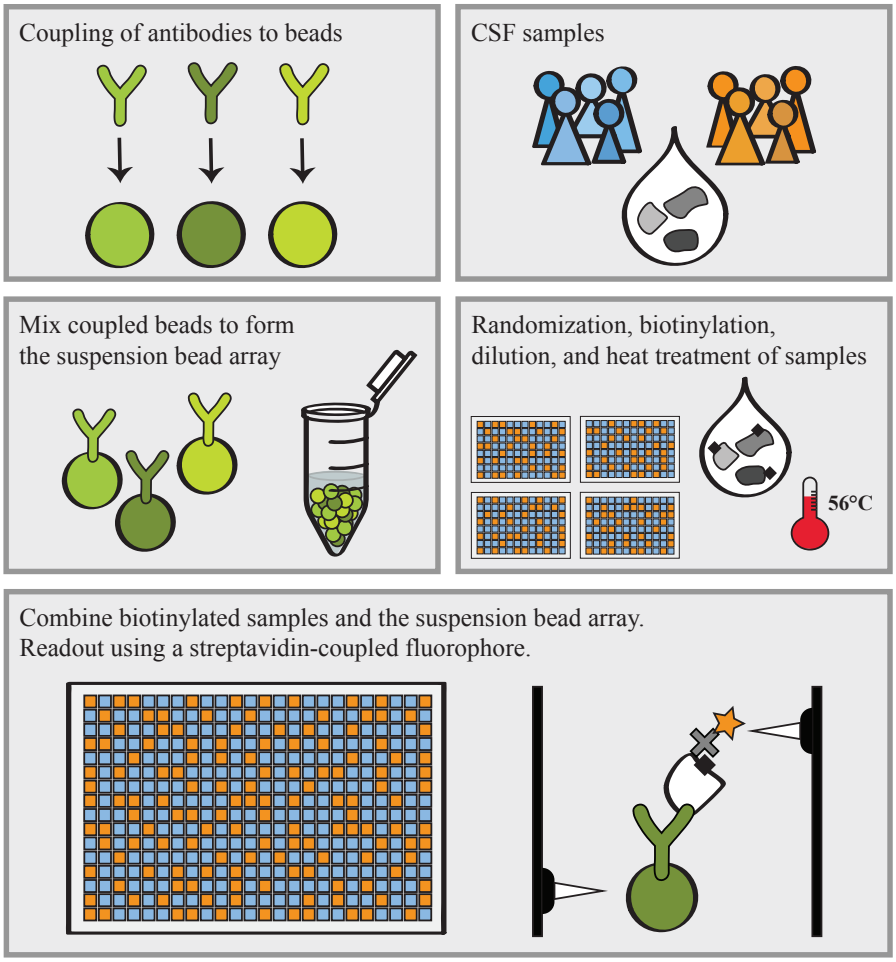

Figure 6: The suspension bead array workflow. The selected antibodies are coupled to color-coded magnetic beads, and subsequently mixed to form the suspension bead array. The suspension bead array is distributed into every well in a 384-well plate. The CSF samples are randomized into 96-well plates, and 15 µl per sample is labelled with biotin. The labelled samples are further diluted, heat treated and combined with the suspension bead array in the 384-well plate. A streptavidin conjugated fluorophore is added, and the readout is performed in a FLEXMAP 3D instrument (Luminex Corp). One laser identifies the color-coded beads, and one laser detects the reporter fluorophore. The median signal per bead identity per well results in the relative quantification of that protein.

(Luminex Corp). The beads are subjected to two different lasers, one that detects the bead identity and thereby the capture antibody and one laser that detects the reporter fluorophore and thereby the signal that corresponds to the relative quantification of the protein. This method allows for measurements of up to 500 proteins per bead array, restricted by the number of different color-coded beads available. The samples are analyzed in microtiter plates with 96 or 384 wells per analysis. The use of suspension bead arrays allows for a high degree of flexibility regarding the specific combinations of proteins that can be analyzed in the same assay. The protein selection can be tailored in detail to the specific research focus in each project via selection of capture antibodies to be included in the suspension bead array.

Due to its multiplexing capacity and high sample throughput, the investigations presented in this thesis all employed suspension bead array assays as the main antibody-based protein profiling method. Our standard operating procedure enables measurements of 384 proteins per bead array, in 384 CSF samples (Pin, 2019). Another advantage with the suspension bead arrays is the low sample consumption. For CSF protein profiling, 15 µl raw sample (3 µl for plasma) is biotinylated. This is enough for measurements of more than 2000 proteins, divided into six bead arrays.

Bead-based sandwich assays

Multiplexed and high-throughput protein profiling assays are attractive for discovery and screening applications. These types of proteomic technologies may result in potential biomarker candidates and thorough validation is needed to confirm the observed patterns and to further characterize the protein profiles.

A sandwich-based setup can be used to add support for on-target binding. However, the development of a working sandwich-assay is time consuming and laborious. It is not trivial to identify two antibodies able to bind to the intended protein at the same time. Furthermore, extensive optimization and characterization of the antibody-pairs are needed. The development of a working sandwich assay is specific for each potential biomarker candidate and the effort needed therefore multiplies when several proteins are to be validated (Bendes, 2021). Bead-based technology can be employed with a sandwich-based setup similar to sandwich ELISA. For bead-based sandwich assays, the capture antibody is immobilized onto magnetic beads instead of the wells in a microtiter plate. Optimization of bead-based sandwich assays include assessment of the optimal sample dilution, a suitable assay buffer, as well as sufficient incubation time and concentration of the detection antibody.

A procedure for systematic and parallel development of bead-based sandwich assays for a panel of proteins has been developed, using the single-binder suspension bead arrays as a foundation (Bendes, 2021). Recently, a large systematic effort to develop bead-based sandwich assays was presented (Haussler, 2019). Different combinations of 624 antibodies were investigated, targeting 209 proteins within the plasma secretome. Out of 1791 unique antibody pairs, 20% successfully detected the recombinant protein as well as the protein in a plasma sample in a dilution dependent manner. This highlights the need to assess different capture and detection antibody combinations when developing a working sandwich assay. The specificity is higher in a sandwich assay compared to a single binder assay, but the ability to multiplex is lower. Each well can only hold one sample and one or a few detection antibodies.

Proximity extension assay

Other immunoassays that also utilizes several antibodies to detect each protein include the proximity litigation assay (PLA) and the proximity extension assays (PEA) (Assarsson, 2014; Fredriksson, 2002). These assays enable quantification of hundreds of proteins simultaneously and circumvents the inherent cross-reactivity problems in other multiplex dual-recognition assays. Each of the used antibodies have a DNA oligonucleotide attached which enables amplification of the signal. If the oligonucleotides are in proximity after sample dilution, due to being bound to the same protein, they can undergo DNA ligation (PLA) or DNA polymerization (PEA). The objective is to form amplifiable reporter DNA strands for read-out via either a quantitative real-time PCR or by Next Generation Sequencing. Proximity assays are very sensitive. They can measure proteins at the subpicogram per ml range, despite the low sample volume needed (1µl) (Landegren, 2021). The standard panels from Olink, based on PEA technology, enable the analysis of 92 proteins in the same assay, with a sample throughput of approximately 90 samples. However, both the multiplexing capacity and the sample throughput is increased with the new Olink Explore platform.

Digital ELISA

The Simoa from Quanterix (Rissin, 2010) is an ultrasensitive assay that utilizes a single molecule counting technology which enables detection of proteins at subfemtomolar concentrations. The samples are incubated with antibody-coupled beads and biotinylated detection antibodies. Subsequently, a streptavidin-coupled enzyme capable of generating fluorescent product is added and detected via fluorescent imaging when the substrate has been mixed with the beads. The concentrated bead mixture is loaded onto the Simoa disk surface, where each well is only large

enough to hold one bead. The low reaction volume in each well enables detection of the fluorescent product, due to the high local concentration. The protein level is determined by counting the number of wells where a signal is detected relative to the number of wells that holds a bead. Usually, one protein is analyzed per Simoa assay, and the sample throughput is similar to a regular sandwich ELISA: 96 wells are available and should hold the standard curve, controls and the samples to be analyzed.

Other protein profiling technologies

There are other protein profiling platforms available, all with their different advantages. *Simple Plex*, developed by ProteinSimple, is an automated sandwich immunoassay technology that consists of microfluidic cartridge-based assays that are analyzed using the Ella instrument (Aldo, 2016). *Meso Scale Discovery* uses electrochemiluminescence for detection and can be multiplexed by the use of distinct electrode regions with different capture antibodies. Furthermore, the aptamer-based proteomic platform called *SomaScan* from SomaLogic can measure up to 7000 proteins in a single sample by utilizing slow off-rate modified aptamers (Gold, 2010).

Selection of appropriate protein profiling technology

To summarize, there are various available tools and strategies which have the potential to facilitate the pursuit of biomarkers within neurodegenerative diseases. The suitable antibody-based platform depends on the desired sample throughput, the desired level of multiplexing, automation requirements, the available sample volume, whether absolute quantification is necessary or if relative quantification is enough and the desired degree of sensitivity. Furthermore, many of the commercially available multiplexed kits are comprised of a pre-selected panel of proteins, for example the Neurology panel from Olink which includes the analysis of 92 neuro-related proteins. A more flexible approach may be preferred to accommodate the analysis of the specific combination of proteins selected for a study.

Part II: Neurodegenerative diseases

Neurodegenerative disorders include, among others, Alzheimer's disease (AD), Parkinson's disease (PD), and frontotemporal dementia (FTD). These diseases are characterized by a progressive loss of function, and eventually death of neurons. The location of the atrophy in the brain is relevant and reflected in the presented clinical symptoms. Furthermore, the above-mentioned diseases all have aggregates of specific proteins in the brain, and neuroinflammation with more or larger glial cells may be present.

Neurodegenerative disorders have a profound impact on the quality. Symptoms include memory decline, impairment of cognitive abilities, problems with speech, swallowing and breathing, as well as impaired mobility and unvoluntary movements. Neurodegenerative disorders can also result in sleep problems and mood changes. These disorders generally reduce life expectancy.

Cognitive decline occurs in normal aging as well, but without impairing the ability to perform activities connected to daily living (Harada, 2013). The pathologic decline is characterized by a more prominent deterioration at a faster rate. Different types of cognitive tests can be used to assess the state of an individual, such as the widely used Mini Mental State Examination (MMSE) (Folstein, 1975), the Montreal Cognitive Assessment (MoCA) (Nasreddine, 2005), and the comprehensive Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) (Randolph, 1998). They all have different advantages and disadvantages, but there are also customs involved where one test may be used more frequently in a specific setting for consistency reasons.

An understanding of the studied diseases is necessary for biomarker research. This chapter will briefly cover some aspects of the brain, the studied diseases, and a few important CSF biomarkers.

The brain

The intrinsic complexity and large regional variability of the brain makes it especially challenging to fully comprehend. Different regions of the brain hold different functions. Neurodegenerative disorders can affect different areas of the brain which is reflected in the presented symptoms. Despite its complexity, the brain is predominantly comprised of two different cell types: neurons and glia. However, these two cell families consist of many members with highly specialized functions (Brat, 2018). An understanding of the different regions and different cell types in the brain can be advantageous for biomarker research, both when selecting which proteins to study but also when interpreting the obtained results.

Brain regions

The main regions of the brain are the brain stem, the cerebellum, and the cerebrum. The brain stem is vital for basic functions like breathing, swallowing, digestions, sleep, and heart rate. Additionally, the brain stem connects the brain to the spinal cord and is important for filtering and routing information from sensory and motor nerves. The cerebellum is located in the lower back of the brain and is primarily important for motor control and coordination.

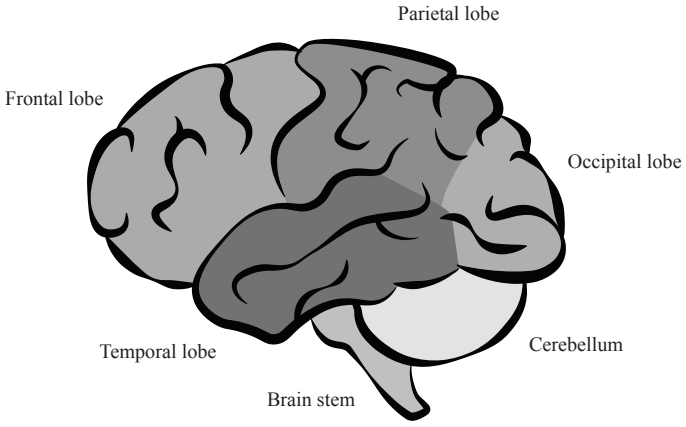

Figure 7: The brain can be divided into three major structures: the brain stem, cerebellum, and cerebrum. The cerebrum can be further divided into four major lobes: the frontal, temporal, parietal, and occipital lobes.

The cerebrum is important for integration and comprehension of all the received information. The cerebrum is comprised of the left and the right hemisphere. The hemispheres can in turn be divided into four lobes: the frontal, temporal, parietal, and occipital lobes. The frontal lobes are important for personality and emotions, but also for planning and executing movements, and higher level executive functions including attention, impulse control and critical thinking. The temporal lobes are important for sensory processing, including hearing, smell, and taste. Additionally, these lobes are important for language and speech. Like the temporal lobes, the parietal lobes are also involved with processing of sensory information. Finally, the occipital lobes are located in the back of the brain and are primarily involved in vision.

The brain can be further divided into different specialized regions where some are especially relevant for neurodegenerative diseases. The brain atrophy associated with Alzheimer's disease initially starts in the hippocampus. This area is important for memory functions and is located deep in the temporal lobes. The substantia nigra is a part of the basal ganglia which is important for motor control and affected in Parkinson's disease.

Neurons

Neurons, also called nerve cells, are a fundamental component of the brain and the rest of the nervous system. Neurons transmits information, such as sensory input from the external surrounding and send motor commands to our muscles. They are the effectors of the nervous system and communicate by chemical and electrical means.

Neurons are spindly looking with a long part called the axon leading away from the cell body, also known as the soma. The axon is responsible for transmitting the received information from one neuron to the next, or from the neuron to a muscle cell. Myelin sheaths located around the axon

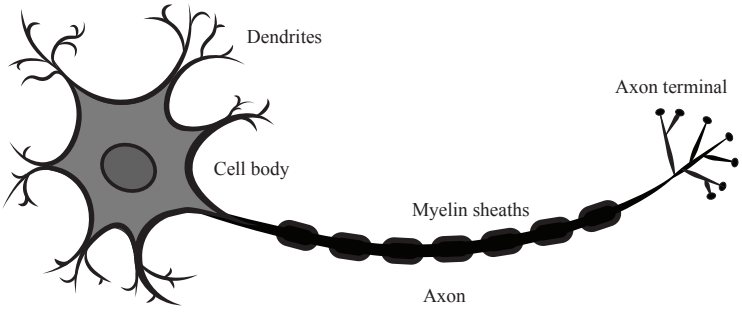

Figure 8: A schematic overview of a neuron.

allow quick transmission of signals along the nerve cell to the axon terminal where the information can be transmitted to other cells via synapses. Dendrites, the bushy part close to the cell body, are the receivers of synaptic impulses from other axons. The complex network of neuronal synapses is the functional unit of neurotransmission in the brain. There are many different subgroups of neurons that vary both in shape and in size, and they have different degrees of vulnerability to injury and pathological processes (Brat, 2018).

Glia

When the glial cells first were identified it was suggested that their function solely was to act as nerve glue. Since then, it has become clear that glial cells are more versatile than first assumed and that they possess a wide range of vital functions. The full spectra of their functions remain to be further explored. The main types of glial cells are astrocytes, oligodendrocytes, and microglia. Simplified, microglia can be regarded as the phagocytotic cells of the nervous system while oligodendrocytes produce the myelin that encapsulates the axon. Astrocytes have a star-like shape and a broad range of functions. Their most important functions include the maintenance of homeostatic balance of neurotransmitters and ions, contributions to the blood-brain barrier and interaction with neurons at tripartite synapses (Jäkel, 2017).

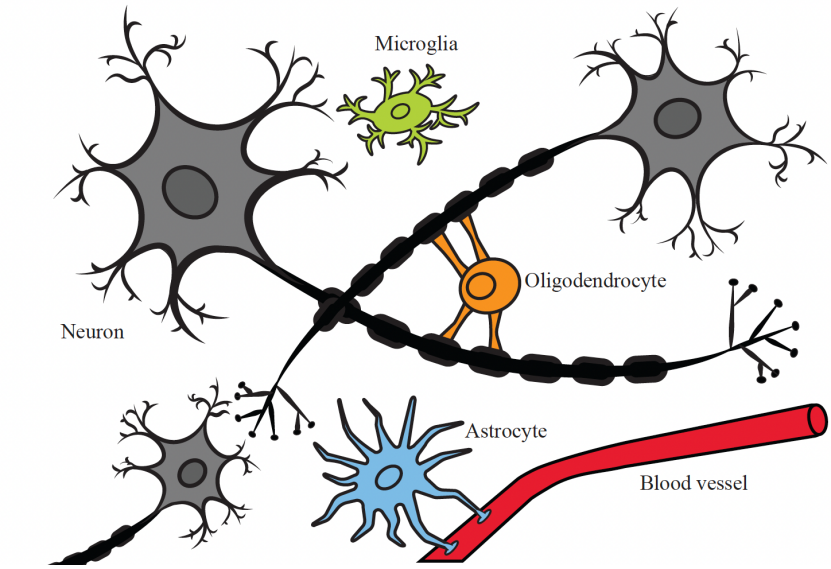

Figure 9: The brain is mainly comprised of two different cell types called neurons and glia. The glial cells can be further divided into different groups and the major types of glia includes astrocytes, microglia and oligodendrocytes.

Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disorder clinically characterized by progressive deterioration of memory and cognitive functions, until eventually becoming incapacitating. The pathogenesis is believed to start decades before onset of clinical symptoms (Aisen, 2017). The neuropathology in AD includes extracellular beta amyloid (Aβ) plaques, neurofibrillary tangles composed of truncated and phosphorylated forms of the protein tau (p-tau), and neuronal loss (Jack, 2018). Amyloid pathology and tau pathology are the pathological hallmarks of AD while neuronal injury or neurodegeneration are not specific for AD and may derive from a variety of diseases.

AD is mainly a sporadic condition with a multifactorial etiology, but there are also familial cases. Genes connected with dominantly inherited familial AD include amyloid beta precursor protein (*APP*), presenilin 1 (*PSEN1*), and presenilin 2 (*PSEN2*). This type of familial AD is very rare and represents less than 1 % of all AD cases (Masters, 2015). Furthermore, essentially all individuals with Down syndrome (trisomy 21) eventually develop AD probably due to the lifelong overexpression of *APP*, located on chromosome 21, which results in a constant overproduction of Aβ (McCarron, 2017). When AD affects younger individuals (usually below 65 years old) it is called early onset AD and this form of AD is not as common as late onset AD. Late onset (usually after 65 years old) AD is probably multifactorial and includes multiple susceptibility genes (Van Cauwenberghe, 2016). For many years the ε4 allele of apolipoprotein E (*APOE* ε4) was the only identified strong genetic risk factor in both early and late onset familial AD, but the knowledge regarding the genetic architecture of AD has made significant advances and an increasing number of other genetic risk loci have now been identified (Schwartzentruber, 2021; Van Cauwenberghe, 2016). However, these additional genetic risk loci are not associated with the same magnitude of risk increase as *APOE* ε4.

In 2018 the National Institute on Aging (NIA) and the Alzheimer's Association (AA) proposed a biological classification system for AD, *exclusively* based on biomarkers. The research framework was based on the amyloid, tau, neurodegeneration (ATN) system, utilizing AD biomarkers for the research definitions of the disease (Jack, 2018). Each pathologic process can be measured by both CSF and imaging biomarkers. Aβ biomarkers include amyloid positron emission tomography (PET) and CSF Aβ42 or Aβ42/ Aβ40 ratio. Tau pathology biomarkers include tau PET and CSF p-tau. Lastly, biomarkers for neurodegeneration or neuronal injury include CSF total tau (t-tau), fluoro-deoxyglucose (FDG) PET and anatomic magnetic resonance imaging (MRI), but these

biomarkers are not specific for AD. Almost 15 years ago, the International Working Group (IWG) proposed AD to be a clinical-biological entity and suggested diagnostic criteria for research to be revised in order to include in vivo biomarkers in combination with specific clinical phenotypes (Dubois, 2007). Additionally, the definition was extended to include prodromal stages. In 2010 the definitions were refined and extended to include a broader spectrum of the AD continuum (Dubois, 2010). The year after, working groups established by NIA-AA proposed revised *clinical* diagnostic criteria for both mild cognitive impairment and dementia due to underlying AD pathology (Albert, 2011; McKhann, 2011). Furthermore, a third working group from NIA-AA proposed criteria for three different stages of the preclinical phase of AD, intended for research settings (Sperling, 2011). In 2014 the IWG research diagnostic criteria were further refined (Dubois, 2014), but the classification systems proposed by NIA-AA and IWG still deviated on several points. In a preclinical AD paper from 2016, symptoms were no longer required for an AD diagnosis (Dubois, 2016). The same year an exclusively biomarker-based classification system was introduced which was independent from clinically defined diagnostic groups: the ATN system (Jack, 2016). This ATN system is the research framework that was further described in 2018 (Jack, 2018). The three binary categories within the ATN system results in eight possible profiles, e.g. A+T+N- or A+T-N-, where no assumptions regarding the temporal ordering of the AD neuropathological changes are made. However, this definition might not be ready for a clinical setting, especially when it comes to asymptomatic biomarker positive individuals (Dubois, 2021).

The aim of the diagnostic advances throughout the years has been to expand the coverage to include criteria that capture the full AD continuum from asymptotic to the severe stages. Especially the preclinical stage of the disease progression has been the subject of an increasing interest due to its potential for effective preventive interventions. The rapid progress in the field, and the massive efforts to harmonize diagnostic definitions have made this transition possible and has improved the characterization of the different disease stages. However, it is not always feasible to measure the AD core biomarkers in participants in a study, and research on mild cognitive impairment and dementia without biomarker classifications will henceforth continue to play an important role in the efforts to elucidate the AD continuum (Jack, 2018). For biomarker research, it is important to be aware of which diagnostic criteria that has been applied in order to compare the results from different cohorts and to draw relevant conclusions. Furthermore, pure AD pathology is not common, instead multiple proteinopathies are usually present which complicates the definitions (Dubois, 2021).

Until recently, there have been two types of drugs for Alzheimer's disease: cholinesterase inhibitors and glutamate regulators. Acetylcholine is a chemical messenger in the brain that helps nerve cells to communicate. AD patients have decreased levels of acetylcholine, and cholinesterase inhibitors prevent the breakdown of this important chemical messenger. Glutamate is another messenger in the brain, but the death of nerve cells associated with AD results in excessive amount of this neurotransmitter. Glutamate regulators, N-methyl-D-aspartate (NMDA) receptor antagonists, regulate the activity of glutamate and blocks the toxic effects caused by the increased levels. These types of drugs may help to lessen or stabilize symptoms, but they do not affect the course of the disease (Kumar, 2015).

Aduhelm (aducanumab) was approved by the U.S. Food and Drug Administration (FDA) in June 2021 as the first new treatment for AD since 2003. Even more exceptional, it is the first drug to get approved which is directed towards the underlying pathophysiology of AD. Aducanumab is an immunotherapy that targets Aβ and aims to reduce the plaques in the brain. The new drug was approved under the FDA's accelerated approval pathway and requires a follow-up study to verify the anticipated clinical benefit (The Food and Drug Administration (FDA), 2021). However, the decision is controversial with an unusual route to approval based on the re-evaluation of data after two halted phase III trials. The approval of this putative disease-modifying therapy triggered an extensive debate in the scientific community regarding the limited evidence of efficacy, and the decision's impact on future drug trials for neurodegenerative diseases (Mullard, 2021; Perlmutter, 2021). Furthermore, this approval will generate a need for ATN classification of patients using widely assessable and inexpensive biomarkers (Blennow, 2021).

Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disease. The disorder is characterized by Lewy bodies, mainly composed of aggregated synuclein alpha, and degeneration of dopaminergic neurons in substantia nigra pars compacta. However, the pathology is believed to include other regions as well, various neurotransmitters and other protein aggregates (Kalia, 2015). Symptoms include parkinsonian motor symptoms with bradykinesia including either rest tremor, rigidity, or both, connected to the dopamine deficiency within the basal ganglia. Additionally, non-motor manifestations are often present and may include sleep dysfunction, autonomic dysfunction, and depression or anxiety (Postuma, 2015). The diagnosis requires motor symptoms, but a long prodromal phase prior to onset of motor manifestations may be present. This prodromal phase can include non-motor manifestations (Berg, 2015).

Furthermore, PD is associated with cognitive decline at a higher degree compared to the general elderly population (Aarsland, 2001). Cognitive impairment can be present at different levels, from PD with mild cognitive impairment to PD dementia (PDD). Several cognitive domains can be affected by the decline, including executive functions, problem solving, attention, and memory (Aarsland, 2021). However, the rate and pattern of cognitive decline in PD patients varies, from onset a few years after diagnosis up to several decades post PD symptom onset (Svenningsson, 2012). Coexisting AD pathology is common in PD patients with cognitive impairment. All PDD patients have Lewy pathology, but around 50% also have Aβ plaques. Moreover, out of the subjects with Aβ plaques, two-thirds have p-tau depositions (Aarsland, 2021).

Synuclein alpha is a presynaptic protein and the main component of the intraneuronal inclusions, the Lewy bodies, in PD patients. Dementia with Lewy bodies (DLB) is also characterized by Lewy body pathology, accompanied by dementia before or within the first year after onset of motor symptoms (McKeith, 2017). Hence, PDD and DLB are very similar, but separated based on the temporal dynamics of the occurrence of dementia and motor symptoms. However, this definition is somewhat debated.

Frontotemporal dementia

Frontotemporal dementia (FTD) is a group of neurodegenerative disorders characterized by a degeneration of the frontal and temporal lobes, typically with an earlier onset compared to AD. Approximately 70% of the cases are sporadic. The majority of the genetic cases are accounted for by autosomal dominant mutations in progranulin (*GRN*), microtubule-associated protein tau (*MAPT*) and the chromosome 9 open reading frame 72 (*C9orf72*). An increasing number of genes have been associated with autosomal dominant FTD, including mutations in TANK-binding kinase 1 (*TBK1*) (Freischmidt, 2015).

Both genetic and sporadic FTD include several neuropathological subtypes, where the protein aggregates most commonly are comprised of TAR DNA-binding protein 43 (TDP-43) or tau. The sporadic cases have approximately equal numbers of individuals with TDP-43 proteinopathies and taupathies. TDP-43 proteinopathies are most common in the genetic cases, associated with mutations in *GRN* or *C9orf72* expansions, as well as mutations in other less common genes. Taupathies (mutations in *MAPT*) are the second most common neuropathological subtype in genetic FTD, but other proteinopathies are also present (Greaves, 2019). Some patients have inclusions containing FUS protein, but this is less common compared to inclusions with tau or TDP-43. The pathogenic variants of *GRN* are associated with a 50% reduction of GRN which can be detected in both CSF and plasma (Swift, 2021). The presence of TDP-43 pathology has also been investigated in AD patients using immunohistochemistry in several studies, and inclusions were identified in approximately one-fourth to half of the patients (McGrowder, 2021).

The clinical presentations of FTD are heterogeneous and include behavioral variant FTD (bvFTD) (Rascovsky, 2011) with a progressive deterioration of personality including emotional blunting, apathy and loss of empathy, as well as primary progressive aphasia (PPA) (Gorno-Tempini, 2011) which includes progressive problems with speech production or comprehension difficulties. Furthermore, FTD can present itself together with problems in motor functions, for example as amyotrophic lateral sclerosis (FTD-ALS) (Strong, 2017) or different types of parkinsonism, for example corticobasal degeneration (CBD) (Armstrong, 2013) or progressive supranuclear palsy (PSP) (Höglinger, 2017). This large variability across the FTD spectrum requires large sample sets and creative approaches for efficient studies.

CSF biomarkers in clinics and research

Well-validated biomarkers that can aid the clinical assessment and improve the accuracy of differential diagnosis are desired. Additionally, proteins with altered disease associated levels might improve prognostic assessment and predict treatment response. Furthermore, identifying proteins with altered levels might elucidate the pathogenesis of different neurodegenerative diseases, and thereby provide further insights about similarities and differences.

AD has a set of well-established CSF biomarkers that successfully can indicate the presence of underlying pathology that are central to the biological definition of the disease: decreased CSF levels of Aβ42, and increased CSF levels of t-tau and p-tau (Jack, 2018). These core AD CSF biomarkers are incorporated into the research definition of the disease and can be used in clinical practice. Furthermore, CSF concentration of the core AD markers show high sensitivity and specificity in predicting conversion from mild cognitive impairment to AD (Hansson, 2006). Additionally, a fourth biomarker, neurofilament light polypeptide (NfL or NEFL), which indicates neuroaxonal damage can be used in clinical practice to support a diagnosis of AD or mild cognitive impairment (Zetterberg, 2021).

Biomarkers for Aβ pathology

The aggregation of Aβ42 in the brain of AD patients results in a reduced CSF concentration of the protein (Olsson, 2016; Strozyk, 2003). CSF Aβ42 is a widely accepted biomarker for AD (Blennow, 2010), and as mentioned previously incorporated into the research definition of the disease (Jack, 2018). To uniform thresholds and homogenize results obtained from different diagnostic assays, reference materials and methods for CSF Aβ42 have been standardized and are now used to calibrate several commercial immunoassays (Boulo, 2020).

An increasing body of evidence supports that the use of a ratio of Aβ42/Aβ40 is superior to Aβ42 concentration alone for detecting amyloidosis since it adjusts for between-individual differences in total Aβ production (Hansson, 2019). Aβ40 is the most abundant isoform of Aβ in CSF and the approach to normalize the aggregation prone Aβ42 based on this substitute for total Aβ concentration has been shown to improve the diagnostic capacity. The standardization and establishment of reference materials for CSF Aβ40 is ongoing and is hopefully completed soon (Zetterberg, 2021).

The amyloid PET scan was an important advance that enabled in vivo detection of fibrillar plaques by the use of radioactive tracers (Klunk, 2004). Amyloid PET scans are well-validated and a number of Aβ tracers are approved for clinical use in several countries (O'Brien, 2015; Rabinovici, 2019; Schmidt, 2015). However, CSF Aβ42/Aβ40 ratio concord well with PET results, independent on used ligand (Hansson, 2019). Furthermore, PET scans use radiation, are only available in specialized research centers, and are more expensive than CSF analysis and will therefore not be used as a screening tool of the general population. Moreover, CSF analysis enables future additional analyses due to the ability to store the samples which is an important advantage.

Blood collection is less invasive than lumbar puncture, and there has been extensive efforts to enable detection of amyloidosis in plasma or serum. The plasma concentration of Aβ42 in a healthy individual is around 20 pg/ml compared to the concentration in CSF which is around 800 pg/ml. The low concentration in plasma puts high demands on precision and sensitivity of a method, and this was fulfilled with the breakthrough on analytical capability using a SIMOA Aβ42 assay developed in 2011 (Zetterberg, 2011). Additionally, in 2014 two MS based methods (MALDI-TOF and SRM) were presented which successfully measured plasma Aβ after enrichment with an immunoprecipitation-based method (Pannee, 2014).

However, even though plasma Aβ42/Aβ40 levels accurately detect brain amyloidosis, the accuracy is higher in CSF (Schindler, 2019). The reduction of Aβ42/Aβ40 plasma levels in individuals with cerebral Aβ pathology is 10-20%, compared to 40-60% in CSF (Hansson, 2021). Nevertheless, there are several advantages of using a plasma-based test in addition to the lower cost and availability. For example, when assembling a cohort of participants with positive PET scans, individuals can be screened beforehand using Aβ42/Aβ40 plasma levels which may reduce the number of PET scans needed (Schindler, 2019).

Phosphorylated tau and total tau

The AD-related increase of CSF levels of tau phosphorylated at threonine 181 (p-tau181, often just referred to as p-tau) reflecting tau pathology is well established (Blennow, 2010; Olsson, 2016). The increased p-tau in CSF is highly specific and can differentiate AD from other non-AD dementias, including other tauopathies (Blennow, 2018; Hanes, 2020; Janelidze, 2020b).

Tau is important for the stability of neuronal axons and in microtubule binding, and the pathological alterations of tau lead to neuronal dysfunction (Holtzman, 2016; Mandelkow, 2012).

Tau is truncated and phosphorylated in the AD brain, which leads to aggregations of the protein into neurofibrillary tangles and increased levels of p-tau in CSF. Increased levels of t-tau in CSF on the other hand reflects neuroaxonal injury or degeneration (Zetterberg, 2017). Tau inclusions are present in neurons or glial cells in other neurodegenerative disorders as well, including some groups of FTD patients, and in atypical parkinsonism including progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) (Irwin, 2016). However, the changes in these non-AD tauopathies are not reflected in CSF by the currently used assays (Zetterberg, 2021).

An ultrasenstive assay for p-tau181 in plasma was published in 2017 based on a modified version of the t-tau assay from Quanterix Simoa (Tatebe, 2017) and increasing evidence supports that the AD-specific pattern for p-tau seen in CSF is also present in plasma (Janelidze, 2020a; Karikari, 2020; Lantero Rodriguez, 2020). Furthermore, plasma p-tau181 has been observed to increase with disease severity (Karikari, 2020) and enabled discrimination between AD and FTD (Benussi, 2020; Thijssen, 2021; Thijssen, 2020). Currently the studies of blood-based AD biomarkers are done in highly specialized research centers. Further studies are needed in consecutive patient populations and at primary care settings to reveal their utility and diagnostic accuracy in routine clinical care (Blennow, 2021). For plasma t-tau, only mild associations with AD hallmarks have been observed with large overlap between diagnostic groups (Mattsson, 2016) and t-tau does not correlate between plasma and CSF (Zetterberg, 2013) despite the use of ultrasensitive SIMOA assays.

Tau has many phosphorylation sites and other tau species in addition to p-tau181 have been investigated, including tau phosphorylated at threonine 217 (p-tau217) or tau phosphorylated at threonine 231 (p-tau231). They have been observed to be increased in CSF early in the disease progression and to discriminate between AD and other neurodegenerative disorders (Hanes, 2020; Janelidze, 2020b; Karikari, 2021; Suárez-Calvet, 2020). Recently, these trends of additional p-tau species have also been observed in plasma, including p-tau217 (Mattsson-Carlgren, 2020a; Palmqvist, 2020) and p-tau231 (Ashton, 2021b). Further studies performed in the same cohort by the same methods are needed to investigate the potential pathophysiological differences between the p-tau species (Blennow, 2021). Moreover, the introduction of PET ligands for pathological tau have enabled image-based biomarkers in addition to p-tau in CSF or plasma (Bischof, 2021; Cecchin, 2021; Villemagne, 2015).

Different ratios of the core AD biomarkers have also been investigated and may increase the diagnostic utility. CSF p-tau/ Aβ42, t-tau/Aβ42 or Aβ42/Aβ40 has a better concordance with amyloid PET than Aβ42 alone (Hansson, 2018b; Mattsson, 2017; Schindler, 2018; Wang, 2016)

However, a perfect concordance between CSF biomarkers and amyloid PET scans is not expected. Recent studies suggest that abnormal levels of core AD markers in CSF are present before amyloid PET positivity, and that these individuals likely have early AD brain pathology that eventually will result in a positive amyloid PET scan (Palmqvist, 2016; Vlassenko, 2016).

Furthermore, CSF profiling revealed evidence of AD pathology (either tau or amyloid) in almost half (46%) of the individuals in a population-based study, constituted of cognitively unimpaired 70-year-olds from Gothenburg (Kern, 2018). However, some of these individuals will not become symptomatic during their lifetime (Sperling, 2011).

Neurofilament light

Neurofilaments are specific cytoskeletal proteins of neurons. The cylindrical neurofilaments are important for neuronal morphology and axonal caliber and contain several subunits: neurofilament light polypeptide (NfL or NEFL), neurofilament medium polypeptide (NfM or NEFM) and neurofilament heavy polypeptide (NfH or NEFH) (Hoffman, 1987). When axonal degeneration or injury occurs, NfL is released to the extracellular space which results in an increase in CSF (Khalil, 2018). The light form is the backbone of neurofilaments and the most abundant subunit. NfL is the most studied subunit with increased levels in CSF in several neurological disorders, including AD, FTD, ALS, multiple sclerosis (MS), and in atypical parkinsonian disorders such as progressive supranuclear palsy (PSP), corticobasal syndrome (CBS) and multiple system atrophy (MSA), as well as in traumatic brain injury (TBI) (Bridel, 2019; Gaetani, 2019). The increased levels of NfL in plasma or CSF is a marker of neuroaxonal damage (Khalil, 2018; Norgren, 2003). However, NfL is also released from axons in healthy individuals, in an age dependent manner (Ashton, 2021a; Quiroz, 2020), highlighting the need for age related cut-offs.

The concentration of NfL in plasma is 50-100 times lower compared to CSF (Alagaratnam, 2021). Recent advances in ultrasensitive immunoassays enabled reliable quantification of NfL in plasma or serum due to the increased sensitivity gained from the Simoa assay (Disanto, 2017; Kuhle, 2016; Lewczuk, 2018; Sanchez-Valle, 2018; van der Ende, 2019a; Weston, 2017), and a recent systematic review and meta-analysis found a pooled correlation coefficient of 0.72 (Pearson correlation) between NfL in blood derived samples and CSF (Alagaratnam, 2021). The ability to measure NfL in this less invasive sample material further increases the usefulness of this marker in the initial screening of patients with a potential neurodegenerative disease. There are already available tests for plasma NfL in clinical practice in Sweden, France, and the Netherlands (Zetterberg, 2021).

For familial AD, NfL levels increase (both in CSF and in plasma) many years before expected symptom onset which might reflect the early neurodegenerative processes (Preische, 2019; Weston, 2019) which indicates its utility as a biomarker for predicting AD. Furthermore, elevated CSF NfL levels in elderly without cognitive impairment has been observed to be associated with an increased risk of developing mild cognitive impairment (Kern, 2019).

Increased NfL levels are not disease specific, but have a massive potential as a marker for neuronal injury to indicate or reject neurodegeneration for patients with cognitive symptoms, for example to differentiate depression in older adults from neurodegenerative disorders. This disease-unspecific marker is sometimes used in the clinical practice to provide support for a neurodegenerative condition (Mattsson-Carlgren, 2020b). Furthermore, the same increase is not observed in PD as in atypical parkinsonian groups (Ashton, 2021a) indicating its potential to distinguish between these conditions. Furthermore, monitoring NfL level changes has a potential in prognostic assessment and disease monitoring, for example as a predictor of a decrease in cognitive (AD, FTD and PD) or motor (ALS, atypical parkinsonian disorders) functions (Gaetani, 2019). FTD urgently needs diagnostic and prognostic markers, and NfL has been suggested to be a promising biomarker for assessing FTD severity and prognosis (Benussi, 2020) and that plasma NfL could be an important endpoint in clinical trials (Rojas, 2021).

To further improve NfL as a biomarker suitable for a clinical setting, a standardized reference material would be beneficial as well as large studies on healthy individuals to determine age specific reference values.

Expanding the biomarker toolbox

It would be advantageous to increase the number of biomarkers associated with neurodegenerative disorders to reflect additional ongoing pathological processes, such as CSF biomarkers able to indicate TDP-43 pathology. This has so far not been accomplished (Swift, 2021). TDP-43 is expressed by many tissues throughout the body, and it has been suggested that the TDP-43 detected in CSF mainly is blood-derived (Feneberg, 2014). Assays detecting the pathological form of TDP-43 is desired (Zetterberg, 2019b).

Some degree of dysfunction or alteration of the synapses is a pathogenic event in several neurodegenerative disorders (Taoufik, 2018; Torres, 2017). This might be one of the earliest features of disease pathology and progression and the reduction in synaptic activity and density

might even precede neuronal loss (Masliah, 2001). The study of synaptic proteins in CSF has the potential to reflect synaptic dysfunction and has therefore become an important area of research within neurodegenerative disorders (Camporesi, 2020). Among others, synaptic proteins studied in the context of neurodegenerative disorders include the postsynaptic neurogranin (NRGN) and presynaptic growth associated protein 43 (GAP43), synuclein alpha (SNCA), synaptosomal-associated protein 25 (SNAP25), and synaptotagmin-1 (SYT1), all with elevated levels in patients, as reviewed by (Camporesi, 2020; Colom-Cadena, 2020; McGrowder, 2021). Furthermore, neuronal pentraxins (NPTX1, NPTX2 and NPTXR) are emerging as promising synaptic markers, as reviewed by (Gómez de San José, 2021). Their levels are reduced in several neurodegenerative disorders, including AD (Galasko, 2019; Libiger, 2021; Lim, 2020; Nilsson, 2021), FTD (Barschke, 2020; Remnestål, 2020; van der Ende, 2019b; van der Ende, 2020) and DLB (Boiten, 2020; van Steenoven, 2020), which is an advantageous pattern in addition to the increased synaptic markers. Some of the synaptic markers are only observed in AD and not in other neurodegenerative disorders (Clarke, 2019), which may indicate that the pathology associated with AD results in the highest synaptic damage (Camporesi, 2020).

One neuropathological feature of AD is the activated glia and neuroinflammation. Proteins reflecting astrocytosis may thereby play an important role in the neurodegenerative biomarker toolbox. Astrocytes tend to localize in close proximity to amyloid plaques which may reflect the relevance of astroglial activation in the pathogenesis (Medeiros, 2013). Glial fibrillary acidic protein (GFAP) is an astrocytic cytoskeletal protein and may serve as a marker for astrocytic activation and neuroinflammation. Increased levels have been observed in both CSF and blood derived samples in AD (Asken, 2020; Chatterjee, 2021; Cicognola, 2021; Elahi, 2020; Oeckl, 2019; Pereira, 2021; Verberk, 2020). Furthermore, increased plasma GFAP has been observed in genetic FTD among GRN mutation carriers, but not in C9orf72 or MAPT mutation carriers (Heller, 2020). Other emerging glial markers include chitinase 3-like 1 (YKL-40) and soluble triggering receptor expressed on myeloid cells 2 (sTREM2), where YKL-40 is involved in activation of the innate immune system and TREM2 is expressed by microglia (Dhiman, 2019; McGrowder, 2021). Furthermore, glial cells are involved in the regulation of neuronal plasticity and a deepened understanding of how glial cells communicate with neurons, as well as how different glial cells interact with each other would be beneficial (Sancho, 2021).

Part III: Study design

Biomarker development is a long and complex process. Many published biomarker studies are not reproducible and only a few biomarkers have reached the clinics. The reproducibility challenge and the low number of identified clinically relevant biomarkers can often be explained by inadequate study designs, where some important factors and potential confounders are not taken into consideration. Here, the term *study design* is used to reflect the whole study process: from selection of study subjects, sample collection and handling, experimental procedures as well as data analysis. Recognition of factors that might lead to variations in the results (both random and systematic) is essential. The importance of well-designed studies to avoid proteomic efforts with inclusive results, and to improve reproducibility of findings has received increased attention (Caruso, 2020; Hansson, 2018a; Maes, 2015; Mattsson-Carlgren, 2020b; McShane, 2017). These are crucial considerations in order to better prioritize proteins for further biomarker assessment.

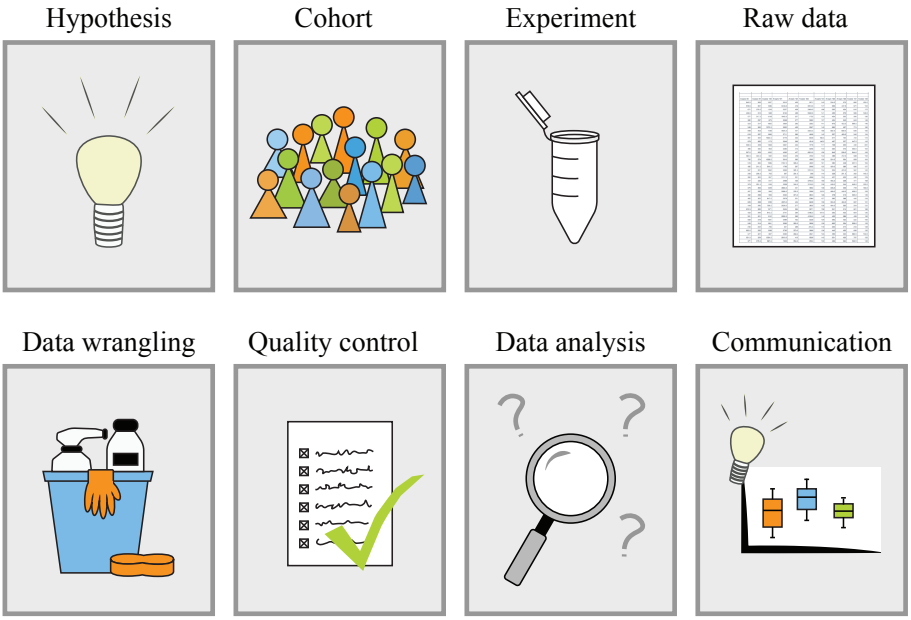

Figure 10: A study design includes several different aspects including experimental design and data analysis strategies. The experimental design starts with a hypothesis or a topic to be explored. A suitable cohort and sample material is selected as well as the preferred experimental setup which will result in raw data. After the raw data has been generated it is time for the data analysis. Data wrangling includes cleaning and standardization of the data which is followed by quality control. Results can be obtained during the subsequent data analysis which hopefully will lead to some research findings that will be communicated.

Experimental design

During the experimental design process a hypothesis is formulated, an appropriate study population is chosen and a suitable experimental setup is selected. The research question can be specific with a focused idea. However, proteomics enables the research question to be broad and unspecific: *Are there detectable altered levels of proteins between the compared groups of individuals?* (Cairns, 2011). These types of explorative studies can be used to create more specific research questions that will be further examined in additional studies.

Well-designed cohorts facilitate appropriate interpretations of the analyzed protein profiles. Both reproducibility and generalizability of the findings might be hampered if extensive inclusion and exclusion criteria are used, compared to a consecutive or random recruitment (Mattsson-Carlgren, 2020b). The control group should preferably be as similar to the studied group as possible, for example regarding age and sex. To avoid unknown systematic differences, it is desired to recruit all individuals in a cohort, both patients and controls, during the same period at the same center, utilizing consistent sample collection and handling procedures (Mattsson-Carlgren, 2020b). Several centers can recruit individuals to obtain a larger cohort, but preferably both patients and controls are recruited at each center. This is easier said than done in a real world setting with specialized clinics that handle patients with a specific disease, and where studies often are conducted retrospectively using stored samples collected in routine clinical care settings or as a part of another study addressing other research questions (McShane, 2017).

Another important consideration is the number of included samples and individuals, there is a relationship between the power of a study and the accuracy of the research finding. When pursuing smaller effects, the sample size needs to be larger. The larger sample sets boost the power in order to be able to identify true disease associated alterations (Button, 2013). Suboptimal cohort designs with small sample sizes might lead to an overestimation (or underestimation) of biomarker performance (Ioannidis, 2008).

Sources of bias can be introduced in different phases of a proteomic analysis which might reduce the reliability of the results. These potential confounders include both preanalytical, analytical and biological variability. Biological factors are connected to the individual, such as age, sex, medication and diet. Additionally, there is a possibility of variability connected to the circadian rhythm or seasonal influences which might confound the interpretation (Caruso, 2020; Lim, 2018). Women

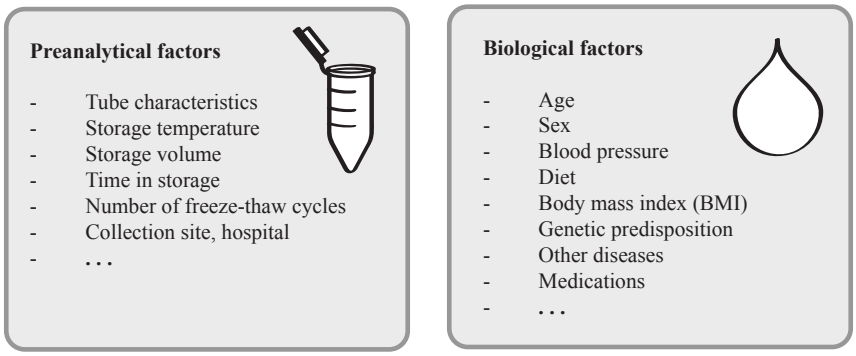

Figure 11: Different types of potential confounders can be introduced in different stages of a study.

have another level of variability connected to their menstrual cycle which may influence their protein levels (Birse, 2015; Bradley, 2018). Preanalytical factors are linked to sample handling and processing, such as collection tube characteristics, storage temperature, storage volume and the number of freeze-thaw cycles (Hampel, 2018a). It is important to reflect on potential confounders, but it is difficult to separate the different factors from each other without specific experiments addressing a particular set of preanalytical factors. Sample handling procedures prior to analysis is preferably standardized to avoid as many potential preanalytical confounders as possible. Some neurodegenerative subgroups are rare, which makes multicenter studies essential. Standardized operating procedures are an important tool to overcome these types of potential confounders. However, if a biomarker is too prone to be influenced by preanalytical factors, it might not have the robustness required for clinical practice. Nevertheless, identification of disease-relevant protein alterations has the potential to increase the knowledge of disease etiology and pathogenesis which may shed light on potential strategies for prevention as well as suitable therapeutics.

In addition to standardized protocols, well characterized and structured cohorts with associated clinical and demographic information is also of importance. This enables assessment of potential biological confounders, such as medication, genetic factors, presence of co-morbidities, and allows further subdivisions within diagnostic groups. Such stratifications might be necessary to uncover differences in underlying mechanisms or associations to the pathogenesis of the disease. All types of factors that might influence the protein profiles should preferably be collected and included in the analysis.

Aβ42 exemplifies a protein known to be influenced by several preanalytical factors (Hansson, 2018a). For example, the measured Aβ concentration is affected by the type of collection tube due to adsorption onto tube walls. This variability puts demands on the use of suitable tubes to reduce unspecific binding, and to standardize the collection tube to obtain comparable measurements between different centers (Bjerke, 2010; Fourier, 2015; Perret-Liaudet, 2012). However, the use of the ratio of Aβ42/Aβ40 reduces the interference of several preanalytical factors and is therefore more robust in this regard (Gervaise-Henry, 2017; Vanderstichele, 2016; Willemse, 2017).

In addition to the preanalytical and biological variables, the outcome of the analysis varies depending on several analytical factors. The sample composition can for example be altered by depletion, enrichment, and fractionation. Furthermore, different factors such as heating, enzyme digestion, dilutions and buffer components can affect the obtained results. Depletion, enrichment, fractionation, and enzyme digestion are more associated within mass spectrometry-based methods. For the antibody-based suspension bead arrays utilized to profile proteins in CSF within the presented investigations, no such procedures have been applied. However, the buffer composition is of importance, and a buffer supplemented with albumin and IgG is used during sample labeling with biotin to avoid bias related to the potentially varying levels of these high abundant proteins. This creates a more similar ratio of protein content and biotin across samples. Furthermore, the dilution factor affects the output and have been optimized to enable detection of many proteins. The selected dilution factor is not optimal for all proteins but enables a high-throughput and multiplexed setup. According to our standard operating procedure, all samples are heat treated at 56°C for 30 minutes before incubation with the suspension bead array. This has been observed to increase the measured signals for many antibodies generated within the Human Protein Atlas (Häggmark, 2013).

The prevalence of other assay-related confounders can be reduced if a proper randomization of the samples is performed. However, a complete randomization can lead to an unbalanced distribution and thereby introduce additional confounding factors (Burger, 2021). Randomization can be stratified to ensure balance of importance parameters. A proper stratified randomization enables reduction of potential batch effects through suitable normalization strategies (McShane, 2017). The constrained randomization can be based on several variables, such as diagnostic group, age, and sex. However, in a longitudinal study it is preferred to treat all samples belonging to the same individual as similar as possible, since the aim is to compare differences within the same subject (Burger, 2021).

In conclusion, there are many challenges connected to experimental design. Confounders have the potential to bias the interpretation of the generated data. Whereas the technical variability should be as small as possible, some degree of biological variability can be maintained in order to be able to generalize the results onto the population of interest (Altman, 2015). An appropriate experimental design is the foundation to obtaining meaningful and relevant results.

Workflow and reproducible data analysis

After the raw data has been generated, there is often a long process before meaningful interpretations can be obtained. The data workflow includes data wrangling (cleaning and standardization), quality control of the data, and the actual data analysis where results can be obtained. Lastly, the workflow includes interpretations and communication of the obtained conclusions.

The first step of the workflow is data wrangling, where the raw data is cleaned and transformed into a suitable format. This process may include merging multiple data sources into a single data set, and at the same time remove variables that are unnecessary or irrelevant for a certain study. The appropriate data structure depends on the project and the planned downstream analysis. Data wrangling also includes cleaning, for example identification of gaps and standardization of variables to enable efficient downstream analysis. An example of a common cleaning step when working with clinical data from several cites is to unify the sex identifiers. One hospital might use female/male, another F/M and a third 0/1 including a code key (for example 0 = female, 1 = male). However, other variables are not as straightforward to standardize. Different cohorts may for example use different definitions of mild cognitive impairment and might use different exclusion and inclusion criteria. This adds another dimension of complexity to the analysis, and it is important to handle these types of complicating factors properly. Furthermore, this part of the workflow can include an evaluation whether the data is within the expected range and to identify extreme outliers. Another thing to be aware of is when 0 is a possible value within a variable that also contains gaps. Sometimes "0" is automatically added into blank cells, which can result in a massive error in the data. It is a good idea to make sure that all zeros are intended and to add NA or similar when data is missing. It could also be relevant to make sure that suitable names (short, descriptive, and consistent) are used for the variables. Data wrangling can be time-consuming, but a comprehensive initial examination, cleaning and organizing of the data makes the rest of the workflow more efficient.

In an optimally designed project this part of the workflow would be done once. After this step, a complete overview of the included variables would be obtained. However, in an actual research setting this is often not the case. Data that was missing in the beginning of a project might become available later due to various reasons, for instance by tracking down data in patient journals or finalized analyses of additional markers, such as measurements of AD core markers at another site. The cohort data is often continuously updated and refined. Different strategies can be applied to

avoid exclusion of individuals due to gaps in important variables. Every individual could be valuable, especially if CSF is already available and ready for analysis. Therefore, individuals with gaps in their meta-data can be included in the experimental analysis when the missing data is expected to become available. Additionally, the data workflow can be structured to enable the incorporation of additional information in a later stage of a project, for example by re-running the initial part of data wrangling, quality control and subsequent downstream analysis.

A software environment that can track all steps in the analysis is preferable over manual updating in for example an excel sheet with a point-and-click user interface. There is a considerable risk of copy-paste errors when updating the information manually, and it is in practice impossible to properly document all the steps that have been performed. Instead, it is easier to follow the progression by using a programming-oriented environment where every step is outlined in code in a linear fashion. The choice of programming language depends on the project, but both R and Python are commonly used languages for data analysis within life science. The free, open source R (R Core Team, 2020) is a language with a massive set of packages for data formatting, statistical modelling, machine learning and visualizations. The language is surrounded by a large community and many statistics and machine learning papers are accompanied by an R package which enables immediate access to new statistical techniques and implementations.

By using R for the data analysis, the workflow becomes more reproducible and transparent. Another person should be able to look at the code and understand it, including yourself in a year or only in a month. However, this might be challenging if the code is not accompanied by proper documentations and descriptions. A strategy to aid the understanding of the code, as well as facilitating a reproducible data analysis, is to use dynamic documents where the output is directly generated from the underlying data and code. Dynamic documents can combine code, results including rendered figures, as well as contextual narrative that includes information about *why* the analysis was performed in a certain way. The explicit link between code, output, and the reasoning behind, can make it easier to understand a workflow. However, it is important to remember that these types of documents do not guarantee reproducible research, but it is an important step towards it (Xie, 2015). Already in 1984, Donald Knuth published the basis for these types of dynamic documents, the *literate programming* paradigm (Knuth, 1984).

"Let us change our traditional attitude to the construction of programs:
Instead of imagining that our main task is to instruct a computer what to do,
let us concentrate rather on explaining to human beings what
we want a computer to do."

- Donald Knuth 1984

This emphasizes the necessity to clearly explain code and thereby increase the transparency and comprehensibility. R Markdown is an example of a tool that includes both code and text in one file that can be used to analyze data as well as generate reports that are reproducible (Allaire, 2021; Xie, 2018; Xie, 2020). Information about the environment, comprising of information about operating system, R version, and loaded packages including versions, can easily be added to the generated report.

Another advantage with a structured, clear, and reproducible workflow is the ability to reuse and repurpose code. The research progress accelerates if you don't spend time writing things that you already have written elsewhere. It saves time. The DRY principle (Don't Repeat Yourself) was coined in 1999 by Andrew Hunt and David Thomas (Hunt, 1999) and highlights the advantages of reusing code as often as possible which makes the code easier to understand and maintain. The first person to benefit from this is yourself, since it becomes easier to reuse code written in a previous project or in another part of a project. An important part of the R language is the ease of constructing user-defined functions which can automate common tasks and allow for reuse of code. It is good practice to consider writing a function whenever something needs to be written more than twice. Functions can be created using *function()* or be obtained from the many packages available through The Comprehensive R Archive Network (CRAN).

Furthermore, it is easier to make sure that a data analysis workflow is reproducible and understandable if the project is self-contained and relative links are used (Xie, 2015). It is preferable if all the files connected to a certain project, including raw data, scripts, patient information, figures, and reports among other files, are well-organized into appropriate folders. It is vital to keep the original raw data files intact (read-only). All these things make it easier to share your analysis and make sure that it is understandable and transparent. Even if it is not possible to make the complete project publicly available, for example due to confidentiality issues regarding the clinical information connected to the included individuals, the use of self-contained projects enables collaborators to access and reproduce the analysis.

Lastly, another aspect to consider is where the code connected to a study is stored. It should at least be backed up in a second location in addition to the used computer, but it is even better if the code is stored in a space shared within the team. However, the best practice is to let the code live within a version control system, for example GitHub. This enables tracking of changes and simplifies code collaborations (Stoudt, 2021).

Quality control and normalizations

All types of assays have some intrinsic sources of variability. Analytical variation can be reduced but never completely removed. There are different tools and strategies that can be used to assess the quality of the data. Quality control is essential to detect bias and is an important step to ensure meaningful and reliable results. Different types of control measures can be included in the experimental design to enable assessment of the analytical factors that might affect assay performance. The quality control spans both sample and protein dimensions and include both common tasks as well as assay specific evaluations.

Intra-assay validation strategies can assess the variation of the data obtained from different wells located on the same assay plate. This part of the quality control can be used to to ensure that the data obtained from different wells containing the same sample are as similar as possible. Intra-assay variation can be assessed by calculating the coefficient of variation, defined as the standard deviation divided by the mean, between replicated samples in the same assay. This is usually done per protein and can be used as a filtering strategy of proteins when the technical variation is determined to be too high. Sometimes, every sample is analyzed several times, for example in triplicates, in the same assay. This enables the variation to be assessed for each and every sample. However, this is not efficient when analyzing hundreds of samples. The intra-assay variation can instead be assessed by analyzing dedicated replication samples, for example three replicates per 96-well microtiter plate. It is a tradeoff between analyzing as many individual samples as possible but still be able to accurately evaluate the quality of an assay. Depending on volume availability, the technical replicates can consist of one sample, or a pool of samples, from the same study. If the sample volume is scarce within the cohort, a sample collected from another individual that is similar to the study population can be used, since it is used to assess the overall intra-assay variability of an assay and not the quality of the samples (Tworoger, 2006). The technical replicates are preferably included in the experimental procedure as early as possible to ensure that the entire process is represented. Furthermore, inter-assay variability can be assessed to determine the reproducibility of an assay performed on different days. All samples, or a subset of them, are re-analyzed and the precision is measured. This quality control procedure ensures that the obtained data is consistent over time.

Other types of controls that can be included in the experiments include negative and positive controls. These types of controls can be included both on a protein level and a sample level. The signal background can be assessed by including negative controls in the form of blank wells. The

content of a blank well is preferably as similar as possible to the sample wells, with the only difference that the sample is omitted. In the case of a suspension bead array assay, the blank wells are included already in the initial step where the samples are labeled with biotin. A secondary use of blank wells, when working with microtiter plates, is to use them as a verification that the intended plate was included in the correct spot and in the right orientation. This should be carefully controlled in the laboratory, but it is useful to use the blanks to confirm that all plates were analyzed as planned. For suspension bead arrays, the controls on a protein level include bare beads that are used to control unspecific binding to the surface of the beads. Additionally, positive controls in the form of sample loading controls can be included. The loading control beads have an antibody immobilized which target a high-abundant protein in the measured samples, and make sure that all sample transfers have been performed correctly.

One problem with the identification of biologically relevant associations may be connected to sample sets with limited numbers of included individuals. However, what number of individuals that constitutes a "large sample set" is context dependent. The prevalence of a disease is one factor that may influence what is regarded as many samples, as well as similar studies in the same field. For example, fewer individuals can be regarded as many when studying FTD, compared to AD. Moreover, the utilized protein profiling technology further determines what constitutes a large sample set. The sufficient number of included individuals depends on the homogeneity of the sample set and the questions to be answered. All protein profiling platforms have an intrinsic limit to the number of samples that can be analyzed in the same analysis round. If more samples are to be analyzed, they need to be divided into different batches. However, non-biological differences are likely to occur between the different batches of data, and these differences ought to be minimized (Hong, 2016). Data normalization can be used to diminish unwanted variation while maintaining the quality of the data, as long as a proper randomization has been performed to make sure that the groups are balanced between batches. The selected normalization strategy needs to be adapted depending on the dimensions on the data batches. Hong et al. 2016 developed a strategy suitable for diminishing batch effects based on the dimensions and characteristics of data generated using the suspension bead array. This enables the analysis of larger sample sets that span several assay batches. Moreover, variation introduced due to different sources of systematic bias can also include various types of instrument drift. A deep understanding of the experimental procedures and technologies that were used to generate the data improves the ability to identify and diminish introduced variation.

Dimensionality reduction methods can be used to extract information from a multidimensional data set and visualize the distribution of the data. The aim with the technique is to increase the interpretability while preserving as much of the variability as possible. Due to its unsupervised nature, it can be used for exploration and quality control by visualizing potential patterns in the data. Unexpected patterns can be investigated further, for example if one batch cluster alone, while all other batches cluster together. The dimensionality reduction methods can be divided into two main types: linear and non-linear. Principal component analysis (PCA) is a linear dimensionality reduction method that has been widely used for many years. PCA finds new uncorrelated variables called principal components, based on the input variables. The first principal component describes the largest variance in the dataset, and the following principal components are ordered based on how much variance they describe. This enables representations of the data based on the first principal components which by definition will describe the most variance in the dataset. A scree plot can be used to visualize the degree of variance that the principal components describe, and can be used to determine how many principal components that are relevant to extract in order to retain a certain amount of variance (Cattell, 1966). A predefined percentage of explained variance can also be used to determine how many principal components to keep. This is a common but rather subjective strategy that depends on the circumstances and objectives. However, usually a plane is used to visualize the multidimensional space, constructed by the first two principal components. To describe the quality of this graphical representation of the dataset, each principal component can be complemented with information regarding how much of the variance in the dataset it captures, usually as a percentage of the total variance. In addition to linear dimensionality reduction techniques there are non-linear alternatives. In 2018, a new non-linear dimensionality reduction method was published, named uniform manifold approximation and projection (UMAP) (McInnes, 2018). It has been suggested that UMAP outperforms linear dimensionality reduction methods, as well as another non-linear method called t-distributed stochastic neighbor embedding (t-SNE) (Maaten, 2008) since UMAP has been found to better preserve the global structure of the data. One disadvantage with t-SNE is that only distances within the same cluster is meaningful. It is not certain that distances are correctly preserved in the low-dimensional space when points are far apart in the high-dimensional space (Trozzi, 2021). This means that it is hard to say whether two clusters located closer to each other are more similar compared to two clusters located further apart in the low-dimensional graphical representation of the data.

Different types of dimensionality reduction methods, both linear and non-linear, can be applied to investigate potential confounders in a dataset. Due to the different properties of the methods, it can be a good idea to explore the dataset using several approaches. These types of low-dimensional

graphical representations of a dataset can be used to ensure that the individuals are not clustered based on for example sex, delivery plates, experimental plates, or other parameters such as the site where the samples were collected (in the case of a multicenter study). Some types of non-biologically relevant variation can be handled using different normalization strategies, but sometimes a group of study subjects need to be filtered out. And in the worst case: the entire dataset can be failed due to the existence of large non-relevant variance that will prevent detection of disease-relevant differences.

Identifying potential sources of non-disease relevant variability and striving for the prevention or reduction of their effects will optimize biomarker studies. This will hopefully lead to an increased understanding of the studied diseases.

Data analysis and visualizations

In addition to a suitable experimental design, and subsequent proper quality control, it is vital to ensure a statistically sound data analysis that takes the underlying assumptions of a certain test into account. During this phase results are obtained, figures are generated, and interpretations can be made.

The generated data can be used to draw conclusions about the population of interest. The research question might be to investigate whether there are altered levels of a certain protein in a specific disease group compared to the control group, for example *Are the levels of amyloid beta altered in CSF from patients with AD compared to individuals that are cognitively intact?* Statistical hypothesis testing is a common approach to tackle this question. It starts by the formulation of a null hypothesis. In statistics, it is easier to disprove than to prove a hypothesis. Therefore, the focus is often on a null hypothesis: *There is no difference of amyloid beta levels in CSF between AD cases and controls.* The most common way to evaluate this statement is to compute a p-value. The p-value describes the probability of obtaining a value as least as extreme as the observed, given that the null hypothesis is correct. Since the p-value is connected to a *probability*, inference about the null hypothesis can be incorrect. The probability of rejecting the null hypothesis when it is true is called the type I error rate and is commonly set to 0.05. However, this is an arbitrary convention and other cut-offs can be justified depending on the circumstances. The second type of errors, type II error, is connected to failure to reject a false null hypothesis. The null hypothesis is rejected if the computed p-value is smaller than the accepted type I error rate. Different types of tests can be used to calculate p-values. The appropriate test depends on whether the data is normally distributed and the number of groups that will be compared. For example, a t-test can be used when two groups are to be compared if the data is known to follow a normal distribution. On the other hand, nonparametric methods can be used if the data is not normally distributed, such as Wilcoxon rank sum test also known as Mann-Whitney U test (when comparing two groups) and Kruskal-Wallis test (when comparing several groups).

There is an increased risk of false-positive findings when a large number of protein profiles are analyzed. This risk can be reduced by correction for multiple comparisons and there are several different strategies available (Noble, 2009), including Bonferroni correction and correction by the Benjamini-Hochberg procedure (Benjamini, 1995). The previous is a more conservative correction than the latter and simply multiplies all p-values with the number of comparisons. The suitable approach to handle multiple comparisons can be complex and context dependent. It depends on

the goal of the comparison and the cost of identifying a false positive compared to a false negative. If the goal is to be exploratory, to identify interesting patterns that will be further investigated in other settings, the p-values can be used as a ranking system: as a measure of the strength of evidence for rejecting the null hypothesis (Amrhein, 2017). In this case, a stringent correction for multiple comparisons becomes less critical. For example, it might be possible to be less stringent in a discovery cohort, if a more stringent approach is used for the validation cohort (Mattsson-Carlgren, 2020b).

Furthermore, it is important to remember that a statistically non-significant result does not *prove* that there are no differences between two groups. It does not prove the null hypothesis. Nor does a significant p-value *prove* that there is a difference. However, this is a common misconception and half of almost 800 examined articles nevertheless claim *no difference* or *no effect* due to a statistically non-significant result (Amrhein, 2019). There is a huge, but sometimes overlooked, difference between: *the protein levels were not found to be different* and *the protein levels were found to not be different*. Furthermore, two studies do not conflict, just because one obtained a statistically significant result, while the other did not. This might be due to a lack of power in the second study, for example by an insufficient sample size.

Absence of evidence is not evidence of absence.

- Douglas G Altman and J Martin Bland 1995

One way to overcome these problems is to make sure to include additional information describing the data from which a significant p-value was obtained. This includes appropriate figures which display the data points behind the numbers, for example by adding beeswarms to boxplots (Ho, 2019). Additionally, the credibility of the result will increase if the data is explored and reported in different ways where the p-value is just one part of a bigger picture. Additionally, the actual p-value should be presented instead of $p<0.05$ or just the phrase "*statistically significant*".

Unsupervised methods can be used to identify subgroups in the dataset. A number of unsupervised dimensionality reduction methods were discussed in the previous section *Study design: Quality control and normalizations*, including PCA, t-SNE and UMAP, as a mean to quality control the data. The same methods can be used to investigate potential disease-relevant subgroups. There is a large heterogeneity in different neurodegenerative disorders and the identification of disease-relevant

subgroups has the potential to increase our understanding of the diseases. Another unsupervised approach is to identify subgroups by different types of clustering methods. Two examples are K-means clustering, where a predefined number of clusters is pursued, and hierarchical clustering, where the number of clusters are not defined in advance. No response variables are used in the clustering, but if they are available, they can be used to visualize the distribution of for example cases and controls. It can be a difficult problem to determine how many clusters to choose, both in K-means clustering but also retrospectively in hierarchical clustering. Another aspect that needs to be handled is which measure of closeness to use. There are several linkage methods, including complete (largest dissimilarity), single (smallest dissimilarity), average (the average of the dissimilarities) and centroid (the dissimilarity between the centroids of two clusters). Additionally, there are different methods to calculate the distance, or the dissimilarity, between the observations. One classical distance measure is Euclidean distance, but correlation-based distances can also be used, such as Pearson correlation distance. All these things can influence the clustering result drastically. Unsupervised learning is useful for exploring the data and understanding the variation and structure within it. However, it can be hard to interpret it since there are no "truth" to compare the result with. Maybe a number of subgroups are identified within a specific disease, but it can be hard to evaluate if the clusters are meaningful or not (Hastie, 2017).

In supervised learning, a labelled dataset is used to train a model to accurately predict the classification (for example case or control) in a new dataset. Different supervised learning algorithms can be used, including random forest (Breiman, 2001) and the least absolute shrinkage and selection operator (LASSO) (Tibshirani, 1996). The learning algorithm needs to be able to generalize from the training data to correctly classify a new dataset. An overfitted model fits very well to the training data but perform poorly on the next dataset, the model is not able to accurately predict new data. To avoid overfitting, the tradeoff between bias and variance can be used. A model with high bias might be too simple and neglect to capture relevant features. A model with low bias on the other hand, can lead to overfitting where the model follows individual points too closely which is connected to a high variance in the algorithm. To capture all relevant features without overfitting the data, the optimal tradeoff between bias and variance is assessed.

Supervised machine learning consists of two distinct parts: model selection including optimization followed by a subsequent evaluation of the performance of the model. The data used for assessing the performance of the model needs to be separated from the rest of the data, an independent test set is required. The first part, model selection and optimization, include variable selection and tuning of hyperparameters. Cross-validation is a standard way to tune hyperparameters in a model

to avoid overfitting (Guyon, 2003). One strategy is *k*-fold cross validation where the training data is divided into *k* bins of equal size. For a five-fold cross validation, the data is split into five bins and five iterations are used for the tuning. In the first iteration, four bins are regarded as the training data and the fifth is the validation data. In the next iteration another bin is used as the validation data, and so on until each bin has been used as the validation set. The results from the different iterations are combined to form the selected model.

Next the prediction performance needs to be assessed and thereby also the separation capacity of the selected model. This part is done using the independent test data that was set aside in the beginning of the workflow. One way to assess the prediction performance is to use a receiver operating characteristics (ROC)-curve with corresponding area under the curve (AUC). An AUC of 1 corresponds to a perfect model, while 0.5 is a result from a non-informative classifier. The test data used for assessing the accuracy of the model should be as independent as possible, preferably from an independent cohort. However, when only one dataset is available, a fraction of the data can be dedicated for testing the model. The fraction used for training the data is usually larger (such as 4/5 or 2/3) than the fraction used for testing (1/5 or 1/3), but the exact number depend on application and total sample size. Enough samples are needed to successfully train the model, but a sufficient number of samples is also needed to test the model. The partitioning should be done before any analysis, to avoid leakage of information from the test to the training set. However, possible systematic bias in a cohort will still be present and might affect the results if the test set belong to the same cohort as the data used for model selection and optimization (Mattsson-Carlgren, 2020b).

To reduce the complexity of a model and to increase interpretability, it can be useful to use different feature selection techniques that identify informative variables in a dataset. Most feature selection methods can be divided into three major groups: filter-based, wrapper-based, and embedded methods. When using a filter-based method, the features are ranked based on a selected metric, for example p-values, and a selected cut-off is used to identify relevant variables. However, if a univariate scoring metric is used, potential multivariate patterns will not be considered. Specific combinations of variables might result in the best separation capacity, and this is not recognized by a method that scores the variables individually and independently. Additionally, the selected variables might be redundant, and it is possible that a smaller subset would achieve the same performance. A wrapper-based feature selection method attempts to identify a subset of informative variables by an iterative process. Different strategies can be applied, for example a greedy search strategy which can be divided into *forward selection* and *backward elimination*. In forward

selection variables are assessed and added one by one if they improve the model. The strategy is *greedy* since the strategy does not re-evaluate previous decisions of inclusion or exclusion of a variable in regard to new information obtained (Guyon, 2003). Embedded feature selection methods include algorithms that have built-in feature selection strategies. LASSO (Tibshirani, 1996) is an example of an embedded feature selection method which aims at reducing the complexity of the model and thereby increase the generalizability. LASSO performs L1 regularization where unimportant coefficients shrink to zero and are thereby excluded which simplifies the model. Combinations of several proteins have the possibility to reflect a complex disease more precisely than single biomarkers. Small individual differences between two groups can together result in a successful separation. A variable can even be useless by itself but contribute to the separation when combined with other variables. Machine learning algorithms can recognize hidden patterns in multidimensional data and are thereby useful for feature selection tasks.

In conclusion, many strategies can be applied to explore and learn from the data. Different techniques can be used to extract important patterns from which interpretations can be made and conclusions can be drawn. However, different types of validation procedures are necessary to assess the characteristics of the identified profiles. Other protein profiling technologies can be used to verify the observed protein profiles in the same cohort. Furthermore, biological verification includes the analysis of additional independent cohorts to confirm the observed patterns.

Part IV: Present investigations

The aim with the four studies included in this thesis is to identify and characterize potential disease-relevant proteins within neurodegenerative diseases. A better understanding of the protein alterations linked to neurodegenerative conditions will contribute to an increased knowledge of the disease development and identification of potential subgroups. CSF was selected as sample material due to its proximity to the brain and potential to reflect the molecular pathophysiology connected to these diseases. The study of proteins with potential disease relevance may ultimately enhance the ability to diagnose and treat these devastating conditions. However, since these diseases often are complex and multifactorial, this is a challenging mission that requires a united effort from many researchers, but the present investigations nevertheless represent a contribution towards this goal.

The antibody-based suspension bead array assay is an important pillar of these four studies. This procedure to analyze proteins in a multiplexed and high-throughput fashion was developed in 2008 using blood-derived samples (Schwenk, 2008). The suspension bead array, as previously discussed, allows hundreds of proteins to be analyzed in hundreds of samples in the same assay. This is enabled through direct labeling of whole samples with biotin, and antibodies coupled to color-coded beads. A streptavidin-conjugated fluorophore enables detection of binding events, bead by bead, using a Luminex instrument. The procedure has been further refined and applied in many publications since (Arner, 2015; Ayoglu, 2014; Bachmann, 2014; Bedri, 2019; Byström, 2014; Byström, 2018; Byström, 2017; Darmanis, 2013; Dodig-Crnković, 2020; Drobin, 2019; Drobin, 2020; Drobin, 2013; Häggmark, 2014; Häggmark, 2012; Hamsten, 2015; Hong, 2020; Iglesias, 2021; Lourido, 2017; Lourido, 2021; Månberg, 2021; Mikus, 2017; Mikus, 2021; Neiman, 2013; Neiman, 2011; Qundos, 2016; Qundos, 2013; Razzaq, 2021; Reuterswärd, 2018; Schwenk, 2010a; Schwenk, 2010b; Schwenk, 2011; Strandberg, 2020). Additionally, the procedure has been adjusted to measure proteins in other types of biofluids, including CSF (Andersson, 2019; Byström, 2014; Häggmark, 2013; Häggmark, 2016; Häggmark-Månberg, 2017; Lindblad, 2021; Paslawski, 2021; Pin, 2019; Remnestål, 2016; Remnestål, 2020), cervicovaginal secretions or lavage (Månberg, 2019; Röhl, 2021), dried blood spots (Dezfouli, 2020; Hamsten, 2015) and bronchoalveolar lavage (BAL) (Hamsten, 2016).

The set of proteins to be analyzed needs to be determined in the beginning of each study. Even though the suspension bead array format enables the study of hundreds of proteins in the same assay, the proteins need to be selected prior to the analysis. This is an important step that is essential for the entire study. We select proteins based on the specific research focus of the study using a foundation of knowledge about the disease. The list often includes proteins identified previously from other internal neurodegenerative protein profiling efforts, as well as interesting pathways or

mechanisms and is complemented with published candidates identified through literature search. When the protein list is compiled, the next step is to select high-quality and well-characterized antibodies to profile the proteins. This is another crucial component of the presented studies, and the vast majority of the selected antibodies were polyclonal rabbit antibodies from the Human Protein Atlas project (HPA, www.proteinatlas.org).

One important thing to highlight is that all four studies are cross-disciplinary collaborations and include contributions from several groups with different expertise. These types of united efforts are beneficial for studies investigating disease-related protein alterations. Medical doctors with the clinical point-of-view and well-defined cohorts are needed, as well as people with expertise in advanced technological instruments and data analysis strategies. The interpretation of the results will improve if people with several different backgrounds provide their perspective and contribute with their specific knowledge.

In **Paper I** we investigated proteins connected to the presence and progression of AD in four different cohorts. In **Paper II** we explored protein profiles in relation to the core AD CSF biomarkers in an asymptomatic elderly cohort to elucidate the pathological processes preceding AD development. **Paper III** focuses on dementia connected to PD, here we investigated the protein profiles in relation to different cognitive screening tests. In the last paper, **Paper IV**, we analyzed proteins in a genetic FTD cohort, to investigate the ability of a combined panel of proteins to reflect disease progression. The full versions of the present investigations are included in the appendix, but the following chapter will cover short descriptions of the studies.

Paper I:
Multi-cohort protein profiling reveals higher levels of six brain-enriched proteins in Alzheimer's disease patients

AD is the neurodegenerative disease with the most well-studied set of core CSF biomarkers: t-tau, p-tau and Aβ42. These biomarkers have a precise specificity and sensitivity and provide diagnostically relevant information already at early disease stages (Blennow, 2019). However, additional biomarkers representing other pathophysiological processes would be beneficial, especially additional biomarkers with altered levels before symptom onset. Identifying the patients that will develop Alzheimer's disease is crucial and will be even more important when disease modifying treatments are available.

In the initial phase, 216 proteins were analyzed in CSF from individuals from two separate cohorts from the AETIONOMY project, a European consortium focusing on PD and AD. Six proteins were observed at increased CSF levels in AD compared to controls in both cohorts, namely amphiphysin (AMPH), aquaporin 4 (AQP4), cAMP-regulated phosphoprotein 21 (ARPP21), growth-associated protein 43 (GAP43), neurofilament medium polypeptide (NEFM), and synuclein beta (SNCB). An interesting group to study in the context of AD is individuals with mild cognitive impairment (MCI). SNCB had significantly increased levels already in the MCI group compared to controls. However, MCI is a diverse group, and we decided to divide the group based on t-tau, p-tau and Aβ42 levels to reduce the heterogeneity and could thereafter identify increased levels of AMPH, AQP4, ARPP21, GAP43 and SNCB in the MCI subgroups with high t-tau or p-tau compared to controls (**Figure 1**). Next, we investigated these proteins potential association with the core AD biomarkers and found a strong correlation between both t-tau and p-tau and the same five proteins, while the correlations to NEFM were weak. Furthermore, the correlations to Aβ42 were weak or moderate for all six proteins. Biological verification in two additional Swedish cohorts was performed, and we identified concordant trends for all six proteins.

One of the cohorts included follow-up information about potential conversion to AD for the individuals in the MCI group. Ten of these individuals had converted to AD after sample collection, with conversion times ranging from less than 2 years up to 11 years post lumbar puncture. We identified a correlation between the protein levels of AMPH, AQP4, ARPP21, GAP43 and SNCB and the number of years left to conversion.

In conclusion, six proteins were observed with increased levels in CSF from patients with AD compared to controls. The patterns of AMPH, AQP4, ARPP21, GAP43, NEFM and SNCB was extensively replicated and might reflect early disease-related changes in the brain. Their potential role in AD pathogenesis will be continuously investigated.

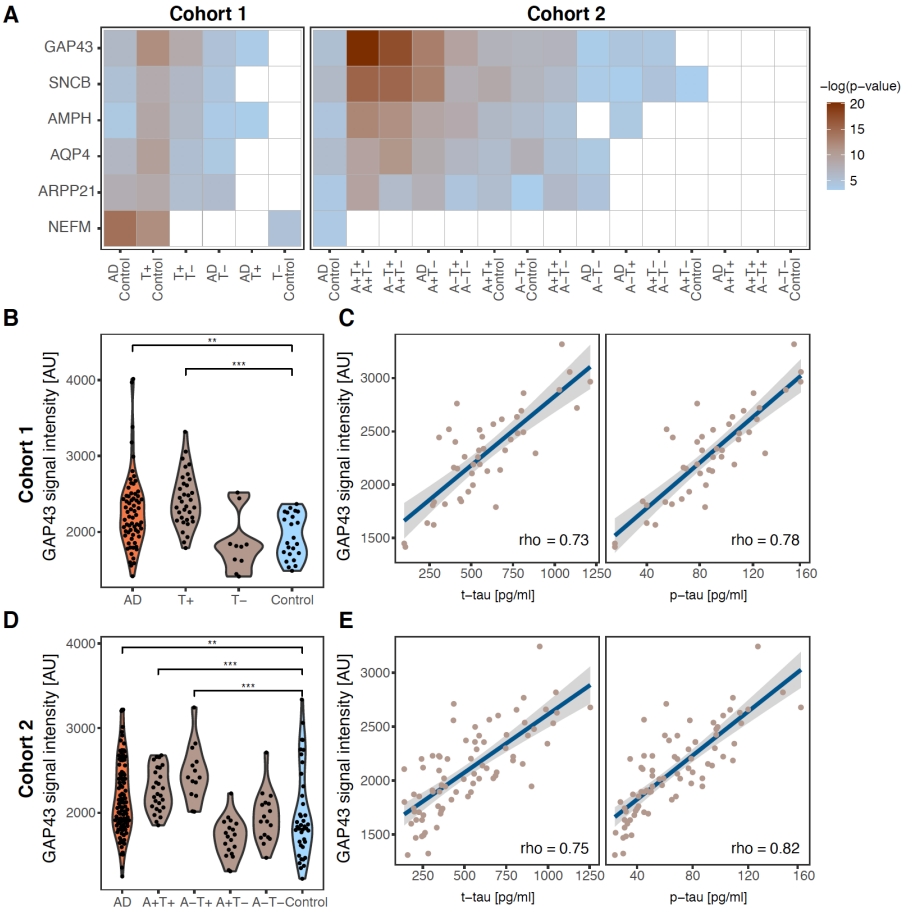

Figure 1: A. Heatmap per cohort visualizing the p-values obtained per protein for the comparison between AD, controls and various MCI subgroups. White color indicates a p-value above 0.05. B. Protein profiles of GAP43 in cohort 1. The MCI group is divided based on CSF concentrations of t-tau and p-tau. C. Correlations between GAP43 levels and respectively t-tau and p-tau, in the MCI individuals in cohort 1. D. Protein profiles of GAP43 in cohort 2. The MCI group is divided based on the levels of t-tau, p-tau, and Aβ42. E. Correlations between GAP43levels and respectively t-tau and p-tau, in the MCI individuals in cohort 2.

Paper II:
Association of CSF proteins with tau and amyloid β levels in asymptomatic 70-year-olds

A better understanding of the early pathophysiological processes preceding AD symptom onset is needed. In this study, we investigated the CSF protein profiles of 104 proteins, measured using the suspension bead arrays, in relation to the core AD CSF markers: t-tau, p-tau and Aβ42, to further characterize the molecular mechanisms behind AD. The included individuals were asymptomatic 70-year-olds recruited from the H70 Gothenburg Birth Cohort Studies (Kern, 2018; Rydberg Sterner, 2019).

Significant correlations were found between 63 of the studied proteins and the concentration of t-tau, p-tau or/and Aβ42. Both SNCB and GAP43 were among the proteins with the strongest correlation to t-tau and/or p-tau (SNCB, t-tau rho = 0.80; GAP43, p-tau rho = 0.78) and neuronal cell adhesion molecule (NRCAM) displayed the strongest correlation with Aβ42 out of the studied proteins (rho = 0.33), followed by neuronal pentraxin-1 (NPTX1, rho = 0.32).

To explore if the correlation between the studied proteins and the core AD markers changed in the preclinical stage of AD, the individuals were dichotomized in two different ways. First, they were divided based on their Aβ42/Aβ40 ratio, with a cut-off point determined by the bimodal distribution. Six proteins showed significantly increased levels in the amyloid-positive individuals: GAP43, SNCB, brain acid-soluble protein 1 (BASP1), rabphilin-3A (RPH3A), dimethylarginine dimethylaminohydrolase-1 (DDAH1) and aquaporin 4 (AQP4).

Additionally, 33 out of the 63 proteins with a correlation to t-tau showed a significantly different slope between the amyloid-positive and the amyloid-negative groups. One of these proteins was neural cell adhesion molecule L1-like protein (CHL1), with the largest association to t-tau levels in the amyloid negative group (**Figure 1**). When investigating the correlation to p-tau, 24 proteins had a significantly different slope between the two groups. No protein had a significantly different slope when comparing the correlations between the proteins and Aβ42 levels.

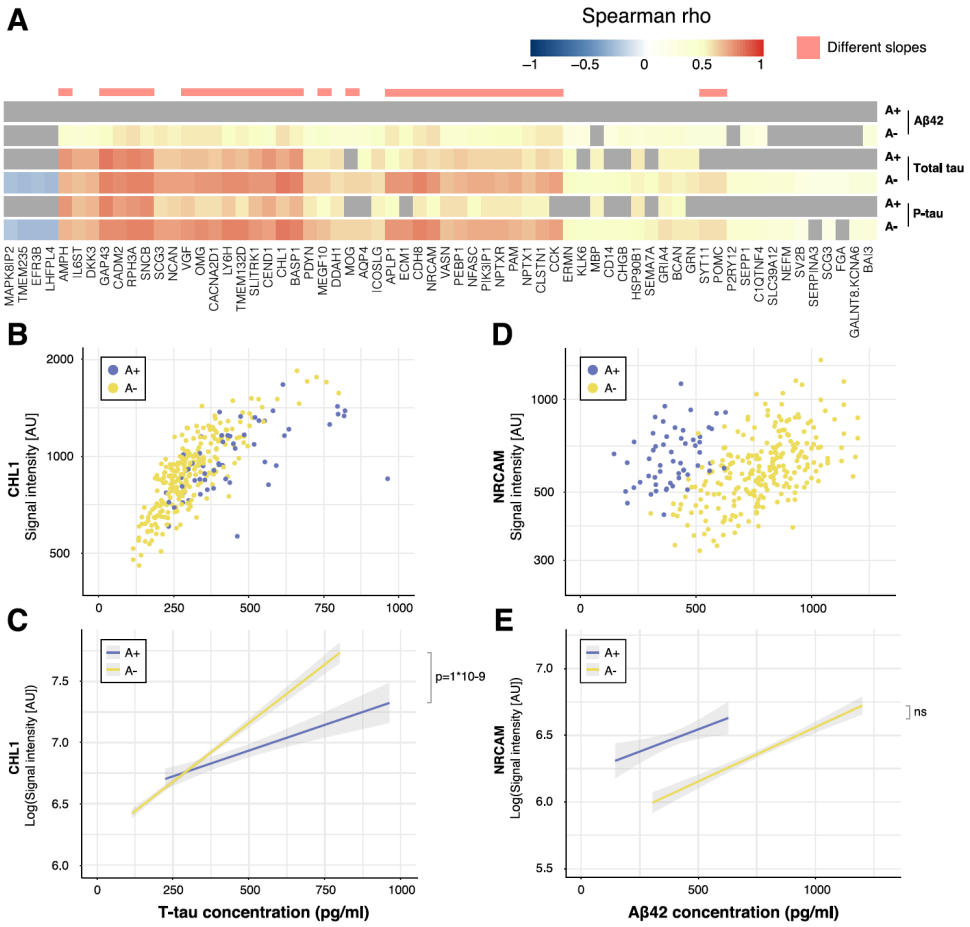

Figure 1: Associations with the core AD CSF biomarkers for individuals dichotomized based on CSF Aβ42/Aβ40 ratio. A) Heatmap visualizing the spearman correlation between each group and all three established biomarkers. Grey indicates a non-significant correlation. B) The association between CHL1 levels and t-tau concentration where both amyloid positive (A+) and amyloid negative (A-) individuals displayed a significant correlation. C) The association between CHL1 and t-tau revealed significantly different slopes between the amyloid status groups. D) The association between NRCAM levels and Aβ42 concentration. A significant correlation was observed within the amyloid negative individuals (A-) but not in amyloid positive individuals (A+). E) The association between NRCAM levels and Aβ42 concentration did not reveal significantly different slopes between the amyloid status groups.

Next, the individuals were divided based on their CDR score but no significant difference in protein levels could be observed between the two groups, neither for the AD core biomarkers nor the other studied proteins. Furthermore, we investigated the studied proteins in relation to other suggested markers: NfL, NRGN as well as *APOE* ε4 carrier status.

In conclusion, more than half of the studied carefully selected proteins correlated with at least one of the core AD biomarkers. Interestingly, when dividing the individuals into two groups based on their Aβ42/Aβ40 ratio, where amyloid-positive individuals potentially represent the preclinical stage of AD and amyloid-negative represents normal aging, 33 proteins displayed a significant difference in the slopes between the two groups when correlating the proteins with t-tau, p-tau and Aβ42 levels. Two groups could be identified from these 33 proteins: a group of transmembrane proteins and a group of proteins involved in or connected to synaptic vesicle transport. Further studies are needed to investigate if these proteins are involved in, or affected by, tau or amyloid pathology present in AD.

Paper III:
Cerebrospinal fluid levels of kininogen-1 indicate early cognitive impairment in Parkinson's disease

Cognitive impairment is a common component in PD (Aarsland, 2021; Emre, 2007) and protein markers to predict PD dementia are needed. To provide insights into the protein profiles associated with cognitive performance in PD, we analyzed CSF from idiopathic PD patients without overt dementia from the AETIONOMY project, a European multicenter consortium focusing on PD and AD. Levels of 216 proteins were analyzed using an antibody-based suspension bead array. The cognitive function of the patients was assessed with three different cognitive scales: Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), Montreal Cognitive Assessment (MoCA) and Mini-Mental State Examination (MMSE). The main focus was the RBANS total index scores, and the patients were divided into two groups based on the cut-off for average cognition in healthy individuals of the same age, i.e. a score of 90.

Fourteen proteins were identified with different levels between the group with low RBANS (<90) and the group with high RBANS (>90) based on Wilcoxon rank sum test. When adjusting for potential confounders, including age, sex, disease duration as well as lifestyle and clinical parameters, three proteins remained significant. These three proteins, a disintegrin and metalloproteinase with thrombospondin motifs 1 (ADAMTS1), kallikrein-6 (KLK6) and kininogen-1 (KNG1), were all observed at higher levels in the patients with low RBANS score (**Figure 1**).

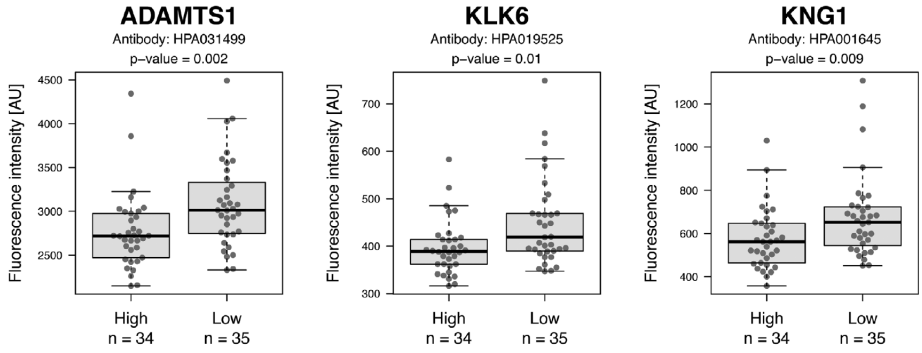

Figure 1. Protein profiles for ADAMTS1, KLK6 and KNG1 in CSF from PD patients with low and high RBANS total score, respectively.

After further evaluation, KNG1 was determined to be most stable and had the strongest association to the cognitive status, i.e. high or low RBANS. Furthermore, KNG1 was also observed to have an association with MoCA total score, but not with MMSE total score. An increasing body of evidence demonstrate that MoCA outperforms MMSE when it comes to identifying PD-related mild cognitive impairment or dementia (Dalrymple-Alford, 2010; Hoops, 2009; Mazancova, 2020; Skorvanek, 2018; Zadikoff, 2008), which might explain this pattern.

In conclusion, higher CSF levels of KNG1 were observed in PD patients with low RBANS scores. Further evaluation in larger cohorts is needed to assess its potential as a marker for cognitive decline in PD.

Paper IV:
A panel of CSF proteins separates genetic frontotemporal dementia from presymptomatic mutation carriers: a GENFI study

When investigating the early disease processes in FTD, it can be useful to study the genetic form of FTD due to the autosomal dominant inheritance pattern. The study of genetic FTD enables the stratification of the included individuals into three groups: individuals with symptomatic genetic FTD (affected mutation carriers, AMC), individuals with mutations in disease associated genes but without symptoms (presymptomatic mutation carriers, PMC), and one group without mutations in these genes (mutation non-carriers, NC). The individuals (n = 221) were recruited as a part of the GENetic Frontotemporal dementia Initiative (GENFI) study (Rohrer, 2015). Participants that were enrolled in GENFI had a 50% risk of FTD due to a pathogenic mutation in a first degree relative.

We analyzed the levels of 111 proteins in CSF samples from the included individuals, using the suspension bead array assay. The first objective was to identify a panel of proteins that could separate affected (AMC) from unaffected individuals, where unaffected included both PMC and NC. The second objective was to identify a panel that could separate PMC from NC. To identify such panels, we utilized two machine learning based algorithms: Random Forest and Least absolute shrinkage and selection operator (LASSO).

When comparing affected and unaffected individuals, we identified a panel of proteins that successfully separated the two groups. The panel included four proteins: neurofilament medium polypeptide (NEFM), aquaporin 4 (AQP4), neuronal pentraxin 2 (NPTX2) and neurosecretory protein VGF (VGF). The panel was further evaluated using PCA and hierarchical clustering, as well as the proteins univariate difference between the two groups (**Figure 1**).

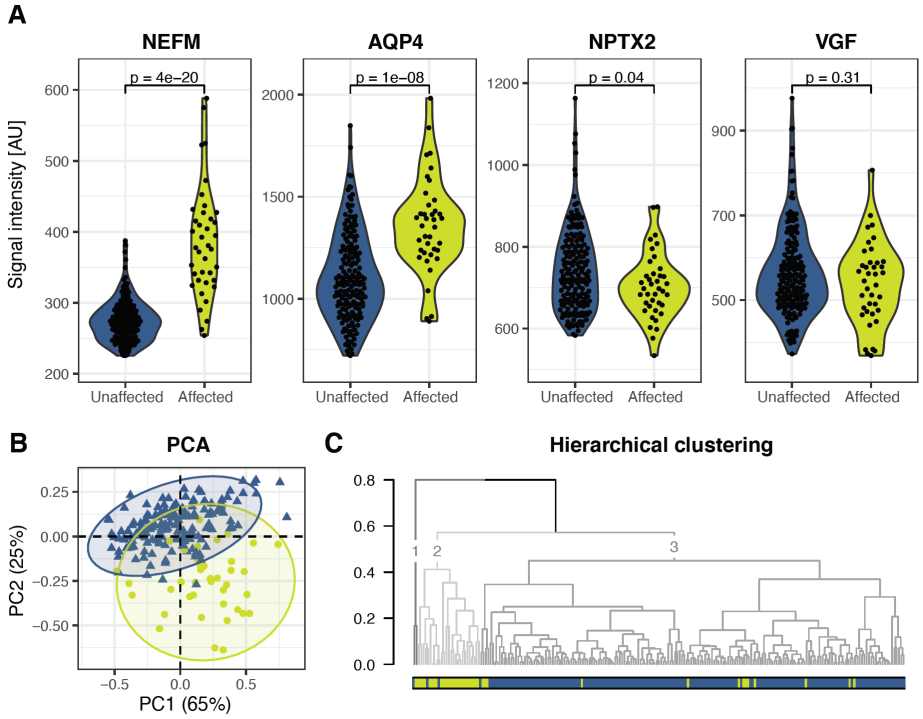

Figure 1: Affected vs unaffected. Yellow and circles = affected individuals (n = 39), blue and triangles = unaffected individuals (n =174). Four proteins (NEFM, AQP4, NPTX2 and VGF) selected by both Random forest and LASSO when comparing affected and unaffected individuals. A) Violin plots for the four proteins with p-values from Wilcoxon rank sum test. B) A PCA plot based on the four selected proteins. C) A hierarchical clustering based on principal component 1 and 2.

The next step was to investigate a panel with the potential to separate PMC from NC. Since both these groups consist of individuals without symptoms, the differences on a protein level are presumably smaller compared to the group of individuals that presented symptoms (AMC). We decided to focus the analysis on PMC with an expected future or present TDP-43 pathology and with less than ten years left until expected symptom onset, based on the mean age at disease onset per genetic group (Moore, 2020).

We identified five proteins, namely TAR DNA binding protein 43 (TARDBP), kininogen 1 (KNG1), heparin binding EGF like growth factor (HBEGF), myelin basic protein (MBP), and

calsyntenin-1 (CLSTN1), in addition to progranulin (GRN), that contributed to the separation between PMC and NC. However, the models had a lower prediction capacity than the model optimized for separating affected from unaffected individuals and additional studies and cohorts are needed to validate and further characterize these profiles.

In conclusion, the multivariate approach was fruitful and by using a machine learning based feature selection, additional patterns can be identified that improve the separation capacity. We successfully identified a panel that separated affected from unaffected individuals (consisting of NEFM, AQP4, NPTX2 and VGF) and we identified promising proteins for a panel separating PMC and NC, but this panel needs further work. Continued evaluation will elucidate the potential association of these proteins to FTD pathogenesis.

Concluding remarks

I have learnt a lot the last four years. But I have also experienced that the study of proteins in different diseases is more difficult and complex than I would ever have imagined. The time spent studying proteins has however strengthened my belief that proteins have a lot of things to tell us about the state of our bodies. As long as we are able to accurately decipher the protein profiles.

Due to its proximity to the brain, CSF is an attractive body fluid to work with when it comes to neurodegenerative disorders. I think that this is the body fluid with the highest potential to identify and characterize disease-relevant proteins for these diseases. However, when a potential biomarker has been well-validated in several CSF cohorts using different protein profiling technologies, it is advantageous if a plasma-based assay for the protein in question can be developed to increase availability and utility. Nevertheless, some proteins will be hard to translate into blood-tests, for example due to their presence in additional cell types outside the brain.

I would argue that the major bottleneck for further breakthroughs is the availability of large well-characterized and standardized CSF cohorts. There are several advanced protein profiling technologies available that enable a high sample throughput. The capacity of the suspension bead array technology that we have utilized in all four papers included in the present investigation, has been challenged these last 1.5 years due to the COVID-19 pandemic. Last year we developed a serological test based on several SARS-CoV-2 proteins (Hober, 2021). For this setup, the viral antigens were immobilized onto the surface of the beads, in order to detect the presence of antibodies to SARS-CoV-2 in plasma and serum. So far, we have analyzed more than 160,000 samples and several papers have been published based on data from these suspension bead arrays. Even if those numbers are beyond reach in a CSF-based neurodegenerative diseases setting, they still highlight the possibility and feasibility of investigating large sample sets when using the suspension bead array setup.

I would like to highlight eight proteins from the present investigations: NEFM, GAP43, SNCB, AMPH, NPTX2, VGF, AQP4, and KNG1. Several proteins, combined into a panel, have an increased potential to accurately reflect the state of heterogenous neurodegenerative diseases. The protein levels often overlap between different sample groups, and by analyzing a panel of proteins an enhanced discriminatory ability can be obtained. NEFM is a subunit of neurofilaments and was observed at increased levels in AD (**Paper I**) as well as in FTD (**Paper IV**) compared to controls.

Further studies are needed to investigate the potential added value of measuring NEFM in addition to the neuroaxonal damage marker NfL. Several of these eight proteins are associated with synapses: GAP43, SNCB, AMPH, NPTX2, and VGF. Higher levels of GAP43, SNCB, and AMPH were observed in AD compared to controls (**Paper I**) and correlate with tau levels in both AD (**Paper I**) and in asymptomatic elderly (**Paper II**). NPTX2 and VGF are also associated with synaptic function, but their levels were decreased in FTD in **Paper IV**. This pattern with some proteins that are present at increased levels and other proteins at decreased levels is advantageous. AQP4 is an astrocytic protein and higher levels were observed both in AD (**Paper I**) and FTD (**Paper IV**) compared to controls. Furthermore, higher levels of AQP4 were identified in asymptomatic but amyloid-positive individuals compared to amyloid-negative individuals in **Paper II**. Finally, increased levels of KNG1 were observed in PD patients without overt dementia but with low RBANS and MoCA total score (**Paper III**).

These eight proteins need, as always, further characterization and validation in additional cohorts to further elucidate their potential association to the pathogenesis in neurodegenerative diseases. Furthermore, the patterns need validation using other protein profiling technologies. The suspension bead array patterns of GAP43 and NEFM concord with an in-house developed PRM assay (Andersson, 2019), a commercial ELISA was used to confirm the suspension bead array profile of KNG1 (**Paper III**), and we have developed bead-based sandwich assays which provided support for on-target binding for NEFM (Remnestål, 2020), SNCB, AQP4, and AMPH (**Paper I**). We will continue to investigate these eight proteins in several cohorts. Currently, we are evaluating their pattern in additional AD and FTD cohorts, but also in an ALS cohort, to enable comparisons between different diseases.

Proteins reflecting additional pathological processes, including synaptic dysfunction as well as neuroinflammation, will likely in the future be added to the current set of clinically used biomarkers: Aβ, tau and NfL. Furthermore, disease-relevant proteins that do not reach the biomarker status might still help us to disentangle the underlying mechanisms and provide us with a deepened understanding of the wide range of pathological processes present in neurodegenerative diseases. To achieve this, we need appropriate study designs with large and well-characterized cohorts, advanced protein profiling technologies, and suitable data analysis strategies. This thesis covers selected aspects of these topics related to the pursuit of CSF biomarkers for neurodegenerative diseases.

Acknowledgements

First of all, I would like to acknowledge all the people who have donated samples to the included studies. Without you, our research would not be possible. I would also like to acknowledge the financial support from the Swedish FTD Initiative (The Schörling Foundation), EU/EFPIA Innovative Medicines Initiative Joint Undertaking (AETIONOMY), Alzheimerfonden, Demensfonden, Åhlén foundation, and the KTH Center for Applied Precision Medicine (Erling-Persson Family Foundation).

Peter, du är den bästa handledare man kan tänka sig. Tack för att du alltid tror på mig och backar mig. Du är otroligt duktig på att skapa en bra stämning som gör att man känner sig trygg, sedd och respekterad. Det är inte alltid lätt med forskning. Men när man kommer till dig när man är som mest nere och stressad, så har du en otrolig förmåga att vända situationen och se till att man lämnar ditt rum med ny energi och ett nytt perspektiv som gör att man kan ta itu med problemen. Eller så har du fått mig att inse att jag inte hade ett problem från första början. Din förmåga att se den stora bilden, men samtidigt ha koll på alla projekt på detaljnivå är imponerande!

Anna. Du är en fantastik handledare, person, och vän! Vi har haft så mycket kul tillsammans, inte minst på våra resor! Jag uppskattar alla dina smarta kommentarer under åren, du har verkligen hjälp mig att utvecklas på många plan. Tack för din entusiasm och all den energi och glädje du sprider!

Julia och **Jennie**, mina bästa kumpaner! Tack för att jag fått dela den här resan tillsammans med er, utan er hade det inte varit detsamma. Julia, tack för ditt lugn och ständiga uppmuntran! Jennie, tänk att det är sex år sen vi började i gruppen som sommarjobbare! Tack för all peppning under åren, ”*You can do it!”,* och påminnelser om att man faktiskt får planera om när saker och ting tar längre tid än vad jag tror. Du har en otrolig förmåga att se folk!

Jochen, thank you for giving me the opportunity to stay in the group as a research engineer after my master thesis project! I have learnt a lot and have enjoyed our discussions throughout the years! You always ask complicated questions and challenges me which I appreciate. And thank you for always sharing things you come across that might be of interest to me.

Bästa skrivbordsraden, **Cecilia H** och **August**, mitt R-team! Tack för all inspiration och alla förslag på hur vi kan utveckla vår kodning och testa nya saker. Våra coffee and code sessions är fantastiska, hoppas det kan bli fler sådana framöver! August, kan jag hoppas på att du kommer ta med mer hembakt bröd till jobbet när vi börjar med frukostarna igen? Cecilia, nu blir det mer brädspel och kanske till och med lite klättring?!

Eni vad skulle vi göra utan dig? Du är stommen i labbet och har så mycket kunskap som är ovärderlig! Jag hoppas att jag lärt mig 10% av vad du kan när det kommer till labbande, då kan jag känna mig nöjd. När jag är tillbaka mer på kontoret är det definitivt dags för fler sushi-luncher! **Cecilia M**, när jag började i gruppen pratades det om dig som någon slags rockstjärna. Du var mammaledig och alla pratade om dig och väntade på att du skulle komma tillbaka! Det känns passande att vi sedan dess har gjort så mycket kul tillsammans, inklusive gått på konsert! **Elisa**, I love your smile and your brain, you are a wonderful person and researcher! **Tea**, tack för allt kul vi haft genom åren, både på och utanför jobbet!

Jamil och **Lovisa**, det har varit så kul att få följa er sen ni gjorde ert kandidatexamensarbete hos oss! Ni har blivit en viktig del i gruppen och har stenkoll på allt i labbet! **Sara** and **Shaghayegh**, I'm so happy that you joined the group! Too bad we didn't have more office time together, I hope this will change from now on. I wish you all the best with your continued PhD studies! **Matilda**. Varmare person får man ju leta efter. Dessutom har du har väldigt bra koll på allt i labbet! Man ser direkt vad i labbet som hör till Matilda, och man önskar att det var ens egna experiment. **Annika**, vi har följt varandra i 10 år nu! Tack för allt kul vi haft tillsammans genom åren! Och tack för att du delar med dig av din sandwich-expertis! **Ronald** and **Claudia** thanks for being great captains over the facilities! **Leo**, det ska bli spännande att se vad du jobbat på senaste året! **Maryam** and **Paul**, welcome to the PAPP-crew! **Maria Jesus** thank you for always keeping your door open when I need help or someone to discuss things with! **Mahya**, thank you for including me in several interesting projects. Thank you for all the laughs and our frequent happy dances in the lab! **Arash**, tack för att just du var min handledare under mitt examensarbete! Jag uppskattar alla råd jag fick av dig när jag skulle börja min doktorandresa där för fyra år sedan.

David K, **Andreas, Christian, Casper, Diana, Fredrik,** and **Emmie** and all past and current members of alfa 2, thank you for making SciLifeLab such a nice place to work at! **Max**, tänk att du hjälpte mig att plugga till min sista kurs på civilingenjörsprogrammet, och nu har du funnits där igen med bullar under avhandlingsskrivandet! Tack för alla diskussioner genom åren och all den energi du sprider!

Lovisa, så skönt att kunna bolla alla funderingar inför disputationen med dig. Jag tror på oss! **Philippa R**, tack för att jag fick börja min doktorandresa tillsammans med just dig och malaria-projektet!

I would also like to recognize some of the people that I have had the opportunity to work with from other universities. **Linn**, jag är så glad över att jag har fått samarbeta med dig i våra FTD projekt! Jag vill även tacka **Carro** och **Abbe** för alla värdefulla diskussioner! Tack **Per** för att du inkluderade oss i AETIONOMY projekten! **Ioanna**, det har varit ett nöje att samarbeta med dig. Hoppas vi kan jobba tillsammans på fler projekt framöver! **Kaj**, **Henrik**, **Martin** och **Kim**, tack för alla värdefulla kommentarer och ert ständiga engagemang! Jag vill också tacka **Ulf** och **Johan** för malaria-projektet! **Frideborg**, **Alexandra**, **Gabriella, Vilde** och **Kristina** tack för att jag fått jobba med er på flera spännande HIV projekt!

PAPP: Nilsson lab and Schwenk lab. I first got to meet PAPP in 2014 and I knew from the start that I wanted to be a part of this fantastic group of people. In 2015 I started as a summer worker, and you lived up to my expectations! In 2016 I got the privilege to do my master thesis project with you. I was very happy when Jochen contacted me during the summer to ask me if I wanted to work in the facility as a research engineer! The time in the facility gave me a lot of experience working in the lab and I learnt a lot. But I knew that I wanted to do a PhD and Peter gave me this opportunity in 2017. A lot of great people have left the group since I started (including **Arash**, **Cecilia ET**, **David**, **Elin**, **Kimi**, **Laura**, **Lucía**, **Mahya**, **Maja**, **Maria**, **Mun-Gwan**, **Philippa R**, **Philippa P**, **Ragna, Sanna**, **Ulrika** and many summer workers and master thesis students), and a lot of other great people have joined. But one thing that never change is the trust, respect, curiosity, and our drive to always do better research. We always share and care. We share our knowledge and ideas, and we care for each other and make sure to cover for each other when we need help. I believe that this attitude takes us further and enables us to do great research. Lastly, and maybe most important, we have a lot of fun together! The last 1.5 years have been strange with home offices etc., but I hope that I will be able to meet you in person more often from now on!

Tack **Sophia** och **Stefan** för era värdefulla kommentarer under mitt 2-årsseminarium. **Johan**, ett extra stort tack till dig som tog dig tid att granska den här avhandlingen. I am very grateful to all people that took time to read selected parts of the thesis and have provided me with helpful comments and suggestion!

Jag vill också passa på och tacka de människor som funnits där för mig utanför jobbet. Ett stort tack till mina fina vänner som har fått mig att skratta, låtit mig gråta och peppat mig in i det sista! Nu finns det snart mycket mer tid till allt kul vi brukar hitta på! Till sist vill jag tacka min stora familj för många nyfikna frågor genom åren! **Pappa** och **Mamma**, tack för att ni alltid ställer alltid upp för mig och alltid är lika uppmuntrande över vad jag än tar mig för. **Brorsan**, nu har jag tid för fler upptåg! **Joakim**, tänk att jag fått dela halva mitt liv med dig. Du hjälper mig att fokusera på det viktiga, tack för att du alltid finns där för mig. Nu väntar nya äventyr!

References

Aarsland, D., Andersen, K., Larsen, J. P., Lolk, A., Nielsen, H., & Kragh-Sørensen, P. (2001). Risk of dementia in Parkinson's disease: a community-based, prospective study. *Neurology, 56*(6), 730-736. doi:10.1212/wnl.56.6.730

Aarsland, D., Batzu, L., Halliday, G. M., Geurtsen, G. J., Ballard, C., Ray Chaudhuri, K., & Weintraub, D. (2021). Parkinson disease-associated cognitive impairment. *Nat Rev Dis Primers, 7*(1), 47. doi:10.1038/s41572-021-00280-3

Adigal, S. S., Rizvi, A., Rayaroth, N. V., John, R. V., Barik, A., Bhandari, S., George, S. D., Lukose, J., Kartha, V. B., & Chidangil, S. (2021). Human tear fluid analysis for clinical applications: progress and prospects. *Expert Rev Mol Diagn, 21*(8), 767-787. doi:10.1080/14737159.2021.1941879

Aebersold, R., & Mann, M. (2003). Mass spectrometry-based proteomics. *Nature, 422*(6928), 198-207. doi:10.1038/nature01511

Aisen, P. S., Cummings, J., Jack, C. R., Jr., Morris, J. C., Sperling, R., Frölich, L., Jones, R. W., Dowsett, S. A., Matthews, B. R., Raskin, J., Scheltens, P., & Dubois, B. (2017). On the path to 2025: understanding the Alzheimer's disease continuum. *Alzheimers Res Ther, 9*(1), 60. doi:10.1186/s13195-017-0283-5

Alagaratnam, J., von Widekind, S., De Francesco, D., Underwood, J., Edison, P., Winston, A., Zetterberg, H., & Fidler, S. (2021). Correlation between CSF and blood neurofilament light chain protein: a systematic review and meta-analysis. *BMJ Neurol Open, 3*(1), e000143. doi:10.1136/bmjno-2021-000143

Albert, M. S., DeKosky, S. T., Dickson, D., Dubois, B., Feldman, H. H., Fox, N. C., Gamst, A., Holtzman, D. M., Jagust, W. J., Petersen, R. C., Snyder, P. J., Carrillo, M. C., Thies, B., & Phelps, C. H. (2011). The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement, 7*(3), 270-279. doi:10.1016/j.jalz.2011.03.008

Aldo, P., Marusov, G., Svancara, D., David, J., & Mor, G. (2016). Simple Plex(™) : A Novel Multi-Analyte, Automated Microfluidic Immunoassay Platform for the Detection of Human and Mouse Cytokines and Chemokines. *Am J Reprod Immunol, 75*(6), 678-693. doi:10.1111/aji.12512

Algenäs, C., Agaton, C., Fagerberg, L., Asplund, A., Björling, L., Björling, E., Kampf, C., Lundberg, E., Nilsson, P., Persson, A., Wester, K., Pontén, F., Wernérus, H., Uhlén, M., Ottosson Takanen, J., & Hober, S. (2014). Antibody performance in western blot applications is context-dependent. *Biotechnol J, 9*(3), 435-445. doi:10.1002/biot.201300341

Allaire, J., Xie, Y., McPherson, J., Luraschi, J., Ushey, K., Atkins, A., Wickham, H., Cheng, J., Chang, W., & Iannone, R. (2021). rmarkdown: Dynamic Documents for R. Retrieved from https://CRAN.R-project.org/package=rmarkdown.

Altman, D. G., & Bland, J. M. (1995). Absence of evidence is not evidence of absence. *Bmj, 311*(7003), 485. doi:10.1136/bmj.311.7003.485

Altman, N., & Krzywinski, M. (2015). Points of significance: Sources of variation. *Nat Methods, 12*(1), 5-6. doi:10.1038/nmeth.3224

Amrhein, V., Greenland, S., & McShane, B. (2019). Scientists rise up against statistical significance. *Nature, 567*(7748), 305-307. doi:https://doi.org/10.1038/d41586-019-00857-9

Amrhein, V., Korner-Nievergelt, F., & Roth, T. (2017). The earth is flat ($p > 0.05$): significance thresholds and the crisis of unreplicable research. *PeerJ, 5*, e3544. doi:10.7717/peerj.3544

Anderson, L. (2014). Six decades searching for meaning in the proteome. *J Proteomics, 107*, 24-30. doi:10.1016/j.jprot.2014.03.005

Anderson, N. L., & Anderson, N. G. (2002). The human plasma proteome: history, character, and diagnostic prospects. *Mol Cell Proteomics, 1*(11), 845-867. doi:10.1074/mcp.r200007-mcp200

Andersson, A., Remnestål, J., Nellgard, B., Vunk, H., Kotol, D., Edfors, F., Uhlen, M., Schwenk, J. M., Ilag, L. L., Zetterberg, H., Blennow, K., Månberg, A., Nilsson, P., & Fredolini, C. (2019). Development of parallel reaction monitoring assays for cerebrospinal fluid proteins associated with Alzheimer's disease. *Clin Chim Acta, 494*, 79-93. doi:10.1016/j.cca.2019.03.243

Armstrong, M. J., Litvan, I., Lang, A. E., Bak, T. H., Bhatia, K. P., Borroni, B., Boxer, A. L., Dickson, D. W., Grossman, M., Hallett, M., Josephs, K. A., Kertesz, A., Lee, S. E., Miller, B. L., Reich, S. G., Riley, D. E., Tolosa, E., Tröster, A. I., Vidailhet, M., & Weiner, W. J. (2013). Criteria for the diagnosis of corticobasal degeneration. *Neurology, 80*(5), 496-503. doi:10.1212/WNL.0b013e31827f0fd1

Arner, P., Henjes, F., Schwenk, J. M., Darmanis, S., Dahlman, I., Ireskjö, B. M., Naredi, P., Agustsson, T., Lundholm, K., Nilsson, P., & Rydén, M. (2015). Circulating carnosine dipeptidase 1 associates with weight loss and poor prognosis in gastrointestinal cancer. *PLoS One, 10*(4), e0123566. doi:10.1371/journal.pone.0123566

Ashton, N. J., Janelidze, S., Al Khleifat, A., Leuzy, A., van der Ende, E. L., Karikari, T. K., Benedet, A. L., Pascoal, T. A., Lleó, A., Parnetti, L., Galimberti, D., Bonanni, L., Pilotto, A., Padovani, A., Lycke, J., Novakova, L., Axelsson, M., Velayudhan, L., Rabinovici, G. D., Miller, B., Pariante, C., Nikkheslat, N., Resnick, S. M., Thambisetty, M., Schöll, M., Fernández-Eulate, G., Gil-Bea, F. J., López de Munain, A., Al-Chalabi, A., Rosa-Neto, P., Strydom, A., Svenningsson, P., Stomrud, E., Santillo, A., Aarsland, D., van Swieten, J. C., Palmqvist, S., Zetterberg, H., Blennow, K., Hye, A., & Hansson, O. (2021a). A multicentre validation study of the diagnostic value of plasma neurofilament light. *Nat Commun, 12*(1), 3400. doi:10.1038/s41467-021-23620-z

Ashton, N. J., Pascoal, T. A., Karikari, T. K., Benedet, A. L., Lantero-Rodriguez, J., Brinkmalm, G., Snellman, A., Schöll, M., Troakes, C., Hye, A., Gauthier, S., Vanmechelen, E., Zetterberg, H., Rosa-Neto, P., & Blennow, K. (2021b). Plasma p-tau231: a new biomarker for incipient Alzheimer's disease pathology. *Acta Neuropathol, 141*(5), 709-724. doi:10.1007/s00401-021-02275-6

Asken, B. M., Elahi, F. M., La Joie, R., Strom, A., Staffaroni, A. M., Lindbergh, C. A., Apple, A. C., You, M., Weiner-Light, S., Brathaban, N., Fernandes, N., Karydas, A., Wang, P., Rojas, J. C., Boxer, A. L., Miller, B. L., Rabinovici, G. D., Kramer, J. H., & Casaletto, K. B. (2020). Plasma Glial Fibrillary Acidic Protein Levels Differ Along the Spectra of Amyloid Burden and Clinical Disease Stage. *J Alzheimers Dis, 78*(1), 265-276. doi:10.3233/jad-200755

Assarsson, E., Lundberg, M., Holmquist, G., Björkesten, J., Thorsen, S. B., Ekman, D., Eriksson, A., Rennel Dickens, E., Ohlsson, S., Edfeldt, G., Andersson, A. C., Lindstedt, P., Stenvang, J., Gullberg, M., & Fredriksson, S. (2014). Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. *PLoS One, 9*(4), e95192. doi:10.1371/journal.pone.0095192

Ayoglu, B., Chaouch, A., Lochmuller, H., Politano, L., Bertini, E., Spitali, P., Hiller, M., Niks, E. H., Gualandi, F., Ponten, F., Bushby, K., Aartsma-Rus, A., Schwartz, E., Le Priol, Y., Straub, V., Uhlen, M., Cirak, S., t Hoen, P. A., Muntoni, F., Ferlini, A., Schwenk, J. M., Nilsson, P., & Al-Khalili Szigyarto, C. (2014). Affinity proteomics within rare diseases: a BIO-NMD study for blood biomarkers of muscular dystrophies. *EMBO Mol Med, 6*(7), 918-936. doi:10.15252/emmm.201303724

Bachmann, J., Burte, F., Pramana, S., Conte, I., Brown, B. J., Orimadegun, A. E., Ajetunmobi, W. A., Afolabi, N. K., Akinkunmi, F., Omokhodion, S., Akinbami, F. O., Shokunbi, W. A., Kampf, C.,

Pawitan, Y., Uhlen, M., Sodeinde, O., Schwenk, J. M., Wahlgren, M., Fernandez-Reyes, D., & Nilsson, P. (2014). Affinity proteomics reveals elevated muscle proteins in plasma of children with cerebral malaria. *PLoS Pathog, 10*(4), e1004038. doi:10.1371/journal.ppat.1004038

Barschke, P., Oeckl, P., Steinacker, P., Al Shweiki, M. R., Weishaupt, J. H., Landwehrmeyer, G. B., Anderl-Straub, S., Weydt, P., Diehl-Schmid, J., Danek, A., Kornhuber, J., Schroeter, M. L., Prudlo, J., Jahn, H., Fassbender, K., Lauer, M., van der Ende, E. L., van Swieten, J. C., Volk, A. E., Ludolph, A. C., & Otto, M. (2020). Different CSF protein profiles in amyotrophic lateral sclerosis and frontotemporal dementia with C9orf72 hexanucleotide repeat expansion. *J Neurol Neurosurg Psychiatry, 91*(5), 503-511. doi:10.1136/jnnp-2019-322476

Beach, T. G., Monsell, S. E., Phillips, L. E., & Kukull, W. (2012). Accuracy of the clinical diagnosis of Alzheimer disease at National Institute on Aging Alzheimer Disease Centers, 2005-2010. *J Neuropathol Exp Neurol, 71*(4), 266-273. doi:10.1097/NEN.0b013e31824b211b

Bedri, S. K., Nilsson, O. B., Fink, K., Månberg, A., Hamsten, C., Ayoglu, B., Manouchehrinia, A., Nilsson, P., Olsson, T., Hillert, J., Grönlund, H., & Glaser, A. (2019). Plasma protein profiling reveals candidate biomarkers for multiple sclerosis treatment. *PLoS One, 14*(5), e0217208. doi:10.1371/journal.pone.0217208

Bendes, A., Dale, M., Mattsson, C., Dodig-Crnković, T., Iglesias, M. J., Schwenk, J. M., & Fredolini, C. (2021). Bead-Based Assays for Validating Proteomic Profiles in Body Fluids. *Methods Mol Biol, 2344*, 65-78. doi:10.1007/978-1-0716-1562-1_5

Benjamini, Y., & Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society. Series B (Methodological), 57*, 289-300.

Benussi, A., Karikari, T. K., Ashton, N., Gazzina, S., Premi, E., Benussi, L., Ghidoni, R., Rodriguez, J. L., Emeršič, A., Simrén, J., Binetti, G., Fostinelli, S., Giunta, M., Gasparotti, R., Zetterberg, H., Blennow, K., & Borroni, B. (2020). Diagnostic and prognostic value of serum NfL and p-Tau(181) in frontotemporal lobar degeneration. *J Neurol Neurosurg Psychiatry, 91*(9), 960-967. doi:10.1136/jnnp-2020-323487

Berg, D., Postuma, R. B., Adler, C. H., Bloem, B. R., Chan, P., Dubois, B., Gasser, T., Goetz, C. G., Halliday, G., Joseph, L., Lang, A. E., Liepelt-Scarfone, I., Litvan, I., Marek, K., Obeso, J., Oertel, W., Olanow, C. W., Poewe, W., Stern, M., & Deuschl, G. (2015). MDS research criteria for prodromal Parkinson's disease. *Mov Disord, 30*(12), 1600-1611. doi:10.1002/mds.26431

Berglund, L., Andrade, J., Odeberg, J., & Uhlén, M. (2008). The epitope space of the human proteome. *Protein Sci, 17*(4), 606-613. doi:10.1110/ps.073347208

Biomarkers Definitions Working Group. (2001). Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther, 69*(3), 89-95. doi:10.1067/mcp.2001.113989

Birse, K., Arnold, K. B., Novak, R. M., McCorrister, S., Shaw, S., Westmacott, G. R., Ball, T. B., Lauffenburger, D. A., & Burgener, A. (2015). Molecular Signatures of Immune Activation and Epithelial Barrier Remodeling Are Enhanced during the Luteal Phase of the Menstrual Cycle: Implications for HIV Susceptibility. *J Virol, 89*(17), 8793-8805. doi:10.1128/jvi.00756-15

Bischof, G. N., Dodich, A., Boccardi, M., van Eimeren, T., Festari, C., Barthel, H., Hansson, O., Nordberg, A., Ossenkoppele, R., Sabri, O., Giovanni, B. F. G., Garibotto, V., & Drzezga, A. (2021). Clinical validity of second-generation tau PET tracers as biomarkers for Alzheimer's disease in the context of a structured 5-phase development framework. *Eur J Nucl Med Mol Imaging, 48*(7), 2110-2120. doi:10.1007/s00259-020-05156-4

Bjerke, M., Portelius, E., Minthon, L., Wallin, A., Anckarsäter, H., Anckarsäter, R., Andreasen, N., Zetterberg, H., Andreasson, U., & Blennow, K. (2010). Confounding factors influencing amyloid Beta concentration in cerebrospinal fluid. *Int J Alzheimers Dis, 2010*. doi:10.4061/2010/986310

Björling, E., & Uhlén, M. (2008). Antibodypedia, a portal for sharing antibody and antigen validation data. *Mol Cell Proteomics, 7*(10), 2028-2037. doi:10.1074/mcp.M800264-MCP200

Blennow, K. (2021). Phenotyping Alzheimer's disease with blood tests. *Science, 373*(6555), 626-628. doi:10.1126/science.abi5208

Blennow, K., Hampel, H., Weiner, M., & Zetterberg, H. (2010). Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. *Nat Rev Neurol, 6*(3), 131-144. doi:10.1038/nrneurol.2010.4

Blennow, K., & Zetterberg, H. (2012). Sjukdomar i centrala nervsystemet. In P. Nilsson-Ehle, M. B. Söderlund, & E. Theodorsson (Eds.), *Klinisk kemi i praktisk medicin* (9 ed., pp. 553-577). Lund: Studentlitteratur.

Blennow, K., & Zetterberg, H. (2018). Biomarkers for Alzheimer's disease: current status and prospects for the future. *J Intern Med, 284*(6), 643-663. doi:10.1111/joim.12816

Blennow, K., & Zetterberg, H. (2019). Fluid biomarker-based molecular phenotyping of Alzheimer's disease patients in research and clinical settings. *Prog Mol Biol Transl Sci, 168*, 3-23. doi:10.1016/bs.pmbts.2019.07.006

Boiten, W. A., van Steenoven, I., Xiao, M., Worley, P. F., Lemstra, A. W., & Teunissen, C. E. (2020). Pathologically Decreased CSF Levels of Synaptic Marker NPTX2 in DLB Are Correlated with Levels of Alpha-Synuclein and VGF. *Cells, 10*(1). doi:10.3390/cells10010038

Boroumand, M., Olianas, A., Cabras, T., Manconi, B., Fanni, D., Faa, G., Desiderio, C., Messana, I., & Castagnola, M. (2021). Saliva, a bodily fluid with recognized and potential diagnostic applications. *J Sep Sci*. doi:10.1002/jssc.202100384

Borràs, E., & Sabidó, E. (2017). What is targeted proteomics? A concise revision of targeted acquisition and targeted data analysis in mass spectrometry. *Proteomics, 17*(17-18). doi:10.1002/pmic.201700180

Boulo, S., Kuhlmann, J., Andreasson, U., Brix, B., Venkataraman, I., Herbst, V., Rutz, S., Manuilova, E., Vandijck, M., Dekeyser, F., Bjerke, M., Pannee, J., Charoud-Got, J., Auclair, G., Mazoua, S., Pinski, G., Trapmann, S., Schimmel, H., Emons, H., Quaglia, M., Portelius, E., Korecka, M., Shaw, L. M., Lame, M., Chambers, E., Vanderstichele, H., Stoops, E., Leinenbach, A., Bittner, T., Jenkins, R. G., Kostanjevecki, V., Lewczuk, P., Gobom, J., Zetterberg, H., Zegers, I., & Blennow, K. (2020). First amyloid β1-42 certified reference material for re-calibrating commercial immunoassays. *Alzheimers Dement, 16*(11), 1493-1503. doi:10.1002/alz.12145

Bradley, F., Birse, K., Hasselrot, K., Noël-Romas, L., Introini, A., Wefer, H., Seifert, M., Engstrand, L., Tjernlund, A., Broliden, K., & Burgener, A. D. (2018). The vaginal microbiome amplifies sex hormone-associated cyclic changes in cervicovaginal inflammation and epithelial barrier disruption. *Am J Reprod Immunol, 80*(1), e12863. doi:10.1111/aji.12863

Brat, D. J. (2018). Normal Brain Histopathology. In A. Perry & D. J. Brat (Eds.), *Practical Surgical Neuropathology: A Diagnostic Approach* (2 ed., pp. 19-37): Elsevier.

Breiman, L. (2001). Random Forests. *Machine Learning, 45*(1), 5-32. doi:10.1023/A:1010933404324

Bridel, C., van Wieringen, W. N., Zetterberg, H., Tijms, B. M., Teunissen, C. E., Alvarez-Cermeno, J. C., Andreasson, U., Axelsson, M., Backstrom, D. C., Bartos, A., Bjerke, M., Blennow, K., Boxer, A., Brundin, L., Burman, J., Christensen, T., Fialova, L., Forsgren, L., Frederiksen, J. L., Gisslen, M., Gray, E., Gunnarsson, M., Hall, S., Hansson, O., Herbert, M. K., Jakobsson, J., Jessen-Krut, J., Janelidze, S., Johannsson, G., Jonsson, M., Kappos, L., Khademi, M., Khalil, M., Kuhle, J., Landen, M., Leinonen, V., Logroscino, G., Lu, C. H., Lycke, J., Magdalinou, N. K., Malaspina, A., Mattsson, N., Meeter, L. H., Mehta, S. R., Modvig, S., Olsson, T., Paterson, R. W., Perez-Santiago, J., Piehl, F., Pijnenburg, Y. A. L., Pyykko, O. T., Ragnarsson, O., Rojas, J. C., Romme Christensen, J., Sandberg, L., Scherling, C. S., Schott, J. M., Sellebjerg, F. T., Simone, I. L., Skillback, T., Stilund, M., Sundstrom, P., Svenningsson, A., Tortelli, R., Tortorella, C., Trentini, A., Troiano, M., Turner, M. R., van Swieten, J. C., Vagberg, M., Verbeek, M. M., Villar, L. M., Visser, P. J., Wallin, A., Weiss, A., Wikkelso, C., & Wild, E. J.

(2019). Diagnostic Value of Cerebrospinal Fluid Neurofilament Light Protein in Neurology: A Systematic Review and Meta-analysis. *JAMA Neurol*. doi:10.1001/jamaneurol.2019.1534

Burger, B., Vaudel, M., & Barsnes, H. (2021). Importance of Block Randomization When Designing Proteomics Experiments. *J Proteome Res, 20*(1), 122-128. doi:10.1021/acs.jproteome.0c00536

Button, K. S., Ioannidis, J. P., Mokrysz, C., Nosek, B. A., Flint, J., Robinson, E. S., & Munafò, M. R. (2013). Power failure: why small sample size undermines the reliability of neuroscience. *Nat Rev Neurosci, 14*(5), 365-376. doi:10.1038/nrn3475

Byström, S., Ayoglu, B., Häggmark, A., Mitsios, N., Hong, M. G., Drobin, K., Forsström, B., Fredolini, C., Khademi, M., Amor, S., Uhlen, M., Olsson, T., Mulder, J., Nilsson, P., & Schwenk, J. M. (2014). Affinity proteomic profiling of plasma, cerebrospinal fluid, and brain tissue within multiple sclerosis. *J Proteome Res, 13*(11), 4607-4619. doi:10.1021/pr500609e

Byström, S., Eklund, M., Hong, M. G., Fredolini, C., Eriksson, M., Czene, K., Hall, P., Schwenk, J. M., & Gabrielson, M. (2018). Affinity proteomic profiling of plasma for proteins associated to area-based mammographic breast density. *Breast Cancer Res, 20*(1), 14. doi:10.1186/s13058-018-0940-z

Byström, S., Fredolini, C., Edqvist, P. H., Nyaiesh, E. N., Drobin, K., Uhlen, M., Bergqvist, M., Ponten, F., & Schwenk, J. M. (2017). Affinity Proteomics Exploration of Melanoma Identifies Proteins in Serum with Associations to T-Stage and Recurrence. *Transl Oncol, 10*(3), 385-395. doi:10.1016/j.tranon.2017.03.002

Cairns, D. A. (2011). Statistical issues in quality control of proteomic analyses: good experimental design and planning. *Proteomics, 11*(6), 1037-1048. doi:10.1002/pmic.201000579

Camporesi, E., Nilsson, J., Brinkmalm, A., Becker, B., Ashton, N. J., Blennow, K., & Zetterberg, H. (2020). Fluid Biomarkers for Synaptic Dysfunction and Loss. *Biomark Insights, 15*, 1177271920950319. doi:10.1177/1177271920950319

Caruso, B., Bovo, C., & Guidi, G. C. (2020). Causes of Preanalytical Interferences on Laboratory Immunoassays - A Critical Review. *Ejifcc, 31*(1), 70-84.

Cattell, R. B. (1966). The Scree Test For The Number Of Factors. *Multivariate Behav Res, 1*(2), 245-276. doi:10.1207/s15327906mbr0102_10

Cecchin, D., Garibotto, V., Law, I., & Goffin, K. (2021). PET Imaging in Neurodegeneration and Neuro-oncology: Variants and Pitfalls. *Semin Nucl Med, 51*(5), 408-418. doi:10.1053/j.semnuclmed.2021.03.003

Chatterjee, P., Pedrini, S., Stoops, E., Goozee, K., Villemagne, V. L., Asih, P. R., Verberk, I. M. W., Dave, P., Taddei, K., Sohrabi, H. R., Zetterberg, H., Blennow, K., Teunissen, C. E., Vanderstichele, H. M., & Martins, R. N. (2021). Plasma glial fibrillary acidic protein is elevated in cognitively normal older adults at risk of Alzheimer's disease. *Transl Psychiatry, 11*(1), 27. doi:10.1038/s41398-020-01137-1

Cicognola, C., Janelidze, S., Hertze, J., Zetterberg, H., Blennow, K., Mattsson-Carlgren, N., & Hansson, O. (2021). Plasma glial fibrillary acidic protein detects Alzheimer pathology and predicts future conversion to Alzheimer dementia in patients with mild cognitive impairment. *Alzheimers Res Ther, 13*(1), 68. doi:10.1186/s13195-021-00804-9

Clarke, M. T. M., Brinkmalm, A., Foiani, M. S., Woollacott, I. O. C., Heller, C., Heslegrave, A., Keshavan, A., Fox, N. C., Schott, J. M., Warren, J. D., Blennow, K., Zetterberg, H., & Rohrer, J. D. (2019). CSF synaptic protein concentrations are raised in those with atypical Alzheimer's disease but not frontotemporal dementia. *Alzheimers Res Ther, 11*(1), 105. doi:10.1186/s13195-019-0564-2

Colom-Cadena, M., Spires-Jones, T., Zetterberg, H., Blennow, K., Caggiano, A., DeKosky, S. T., Fillit, H., Harrison, J. E., Schneider, L. S., Scheltens, P., de Haan, W., Grundman, M., van Dyck, C. H., Izzo, N. J., & Catalano, S. M. (2020). The clinical promise of biomarkers of synapse damage

or loss in Alzheimer's disease. *Alzheimers Res Ther, 12*(1), 21. doi:10.1186/s13195-020-00588-4

Dalrymple-Alford, J. C., MacAskill, M. R., Nakas, C. T., Livingston, L., Graham, C., Crucian, G. P., Melzer, T. R., Kirwan, J., Keenan, R., Wells, S., Porter, R. J., Watts, R., & Anderson, T. J. (2010). The MoCA: well-suited screen for cognitive impairment in Parkinson disease. *Neurology, 75*(19), 1717-1725. doi:10.1212/WNL.0b013e3181fc29c9

Darmanis, S., Cui, T., Drobin, K., Li, S. C., Öberg, K., Nilsson, P., Schwenk, J. M., & Giandomenico, V. (2013). Identification of candidate serum proteins for classifying well-differentiated small intestinal neuroendocrine tumors. *PLoS One, 8*(11), e81712. doi:10.1371/journal.pone.0081712

Dezfouli, M., Bergström, S., Skattum, L., Abolhassani, H., Neiman, M., Torabi-Rahvar, M., Franco Jarava, C., Martin-Nalda, A., Ferrer Balaguer, J. M., Slade, C. A., Roos, A., Fernandez Pereira, L. M., López-Trascasa, M., Gonzalez-Granado, L. I., Allende-Martinez, L. M., Mizuno, Y., Yoshida, Y., Friman, V., Lundgren, Å., Aghamohammadi, A., Rezaei, N., Hernández-Gonzalez, M., von Döbeln, U., Truedsson, L., Hara, T., Nonoyama, S., Schwenk, J. M., Nilsson, P., & Hammarström, L. (2020). Newborn Screening for Presymptomatic Diagnosis of Complement and Phagocyte Deficiencies. *Front Immunol, 11*, 455. doi:10.3389/fimmu.2020.00455

Dhiman, K., Blennow, K., Zetterberg, H., Martins, R. N., & Gupta, V. B. (2019). Cerebrospinal fluid biomarkers for understanding multiple aspects of Alzheimer's disease pathogenesis. *Cell Mol Life Sci, 76*(10), 1833-1863. doi:10.1007/s00018-019-03040-5

Disanto, G., Barro, C., Benkert, P., Naegelin, Y., Schädelin, S., Giardiello, A., Zecca, C., Blennow, K., Zetterberg, H., Leppert, D., Kappos, L., Gobbi, C., & Kuhle, J. (2017). Serum Neurofilament light: A biomarker of neuronal damage in multiple sclerosis. *Ann Neurol, 81*(6), 857-870. doi:10.1002/ana.24954

Dodig-Crnković, T., Hong, M. G., Thomas, C. E., Häussler, R. S., Bendes, A., Dale, M., Edfors, F., Forsström, B., Magnusson, P. K. E., Schuppe-Koistinen, I., Odeberg, J., Fagerberg, L., Gummesson, A., Bergström, G., Uhlén, M., & Schwenk, J. M. (2020). Facets of individual-specific health signatures determined from longitudinal plasma proteome profiling. *EBioMedicine, 57*, 102854. doi:10.1016/j.ebiom.2020.102854

Dong, J., & Ueda, H. (2017). ELISA-type assays of trace biomarkers using microfluidic methods. *Wiley Interdiscip Rev Nanomed Nanobiotechnol, 9*(5). doi:10.1002/wnan.1457

Drobin, K., Assadi, G., Hong, M. G., Andersson, E., Fredolini, C., Forsström, B., Reznichenko, A., Akhter, T., Ek, W. E., Bonfiglio, F., Hansen, M. B., Sandberg, K., Greco, D., Repsilber, D., Schwenk, J. M., D'Amato, M., & Halfvarson, J. (2019). Targeted Analysis of Serum Proteins Encoded at Known Inflammatory Bowel Disease Risk Loci. *Inflamm Bowel Dis, 25*(2), 306-316. doi:10.1093/ibd/izy326

Drobin, K., Marczyk, M., Halle, M., Danielsson, D., Papiez, A., Sangsuwan, T., Bendes, A., Hong, M. G., Qundos, U., Harms-Ringdahl, M., Wersäll, P., Polanska, J., Schwenk, J. M., & Haghdoost, S. (2020). Molecular Profiling for Predictors of Radiosensitivity in Patients with Breast or Head-and-Neck Cancer. *Cancers (Basel), 12*(3). doi:10.3390/cancers12030753

Drobin, K., Nilsson, P., & Schwenk, J. M. (2013). Highly multiplexed antibody suspension bead arrays for plasma protein profiling. *Methods Mol Biol, 1023*, 137-145. doi:10.1007/978-1-4614-7209-4_8

Dubois, B., Feldman, H. H., Jacova, C., Cummings, J. L., Dekosky, S. T., Barberger-Gateau, P., Delacourte, A., Frisoni, G., Fox, N. C., Galasko, D., Gauthier, S., Hampel, H., Jicha, G. A., Meguro, K., O'Brien, J., Pasquier, F., Robert, P., Rossor, M., Salloway, S., Sarazin, M., de Souza, L. C., Stern, Y., Visser, P. J., & Scheltens, P. (2010). Revising the definition of Alzheimer's disease: a new lexicon. *Lancet Neurol, 9*(11), 1118-1127. doi:10.1016/s1474-4422(10)70223-4

Dubois, B., Feldman, H. H., Jacova, C., Dekosky, S. T., Barberger-Gateau, P., Cummings, J., Delacourte, A., Galasko, D., Gauthier, S., Jicha, G., Meguro, K., O'Brien, J., Pasquier, F., Robert, P., Rossor, M., Salloway, S., Stern, Y., Visser, P. J., & Scheltens, P. (2007). Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. *Lancet Neurol, 6*(8), 734-746. doi:10.1016/s1474-4422(07)70178-3

Dubois, B., Feldman, H. H., Jacova, C., Hampel, H., Molinuevo, J. L., Blennow, K., DeKosky, S. T., Gauthier, S., Selkoe, D., Bateman, R., Cappa, S., Crutch, S., Engelborghs, S., Frisoni, G. B., Fox, N. C., Galasko, D., Habert, M. O., Jicha, G. A., Nordberg, A., Pasquier, F., Rabinovici, G., Robert, P., Rowe, C., Salloway, S., Sarazin, M., Epelbaum, S., de Souza, L. C., Vellas, B., Visser, P. J., Schneider, L., Stern, Y., Scheltens, P., & Cummings, J. L. (2014). Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. *Lancet Neurol, 13*(6), 614-629. doi:10.1016/s1474-4422(14)70090-0

Dubois, B., Hampel, H., Feldman, H. H., Scheltens, P., Aisen, P., Andrieu, S., Bakardjian, H., Benali, H., Bertram, L., Blennow, K., Broich, K., Cavedo, E., Crutch, S., Dartigues, J. F., Duyckaerts, C., Epelbaum, S., Frisoni, G. B., Gauthier, S., Genthon, R., Gouw, A. A., Habert, M. O., Holtzman, D. M., Kivipelto, M., Lista, S., Molinuevo, J. L., O'Bryant, S. E., Rabinovici, G. D., Rowe, C., Salloway, S., Schneider, L. S., Sperling, R., Teichmann, M., Carrillo, M. C., Cummings, J., & Jack, C. R., Jr. (2016). Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria. *Alzheimers Dement, 12*(3), 292-323. doi:10.1016/j.jalz.2016.02.002

Dubois, B., Villain, N., Frisoni, G. B., Rabinovici, G. D., Sabbagh, M., Cappa, S., Bejanin, A., Bombois, S., Epelbaum, S., Teichmann, M., Habert, M. O., Nordberg, A., Blennow, K., Galasko, D., Stern, Y., Rowe, C. C., Salloway, S., Schneider, L. S., Cummings, J. L., & Feldman, H. H. (2021). Clinical diagnosis of Alzheimer's disease: recommendations of the International Working Group. *Lancet Neurol, 20*(6), 484-496. doi:10.1016/s1474-4422(21)00066-1

Duits, F. H., Martinez-Lage, P., Paquet, C., Engelborghs, S., Lleó, A., Hausner, L., Molinuevo, J. L., Stomrud, E., Farotti, L., Ramakers, I., Tsolaki, M., Skarsgård, C., Åstrand, R., Wallin, A., Vyhnalek, M., Holmber-Clausen, M., Forlenza, O. V., Ghezzi, L., Ingelsson, M., Hoff, E. I., Roks, G., de Mendonça, A., Papma, J. M., Izagirre, A., Taga, M., Struyfs, H., Alcolea, D. A., Frölich, L., Balasa, M., Minthon, L., Twisk, J. W. R., Persson, S., Zetterberg, H., van der Flier, W. M., Teunissen, C. E., Scheltens, P., & Blennow, K. (2016). Performance and complications of lumbar puncture in memory clinics: Results of the multicenter lumbar puncture feasibility study. *Alzheimers Dement, 12*(2), 154-163. doi:10.1016/j.jalz.2015.08.003

Elahi, F. M., Casaletto, K. B., La Joie, R., Walters, S. M., Harvey, D., Wolf, A., Edwards, L., Rivera-Contreras, W., Karydas, A., Cobigo, Y., Rosen, H. J., DeCarli, C., Miller, B. L., Rabinovici, G. D., & Kramer, J. H. (2020). Plasma biomarkers of astrocytic and neuronal dysfunction in early- and late-onset Alzheimer's disease. *Alzheimers Dement, 16*(4), 681-695. doi:10.1016/j.jalz.2019.09.004

Emre, M., Aarsland, D., Brown, R., Burn, D. J., Duyckaerts, C., Mizuno, Y., Broe, G. A., Cummings, J., Dickson, D. W., Gauthier, S., Goldman, J., Goetz, C., Korczyn, A., Lees, A., Levy, R., Litvan, I., McKeith, I., Olanow, W., Poewe, W., Quinn, N., Sampaio, C., Tolosa, E., & Dubois, B. (2007). Clinical diagnostic criteria for dementia associated with Parkinson's disease. *Mov Disord, 22*(12), 1689-1707; quiz 1837. doi:10.1002/mds.21507

Engelborghs, S., Niemantsverdriet, E., Struyfs, H., Blennow, K., Brouns, R., Comabella, M., Dujmovic, I., van der Flier, W., Frölich, L., Galimberti, D., Gnanapavan, S., Hemmer, B., Hoff, E., Hort, J., Iacobaeus, E., Ingelsson, M., Jan de Jong, F., Jonsson, M., Khalil, M., Kuhle, J., Lleó, A., de Mendonça, A., Molinuevo, J. L., Nagels, G., Paquet, C., Parnetti, L., Roks, G., Rosa-Neto, P., Scheltens, P., Skårsgard, C., Stomrud, E., Tumani, H., Visser, P. J., Wallin, A., Winblad, B., Zetterberg, H., Duits, F., & Teunissen, C. E. (2017). Consensus guidelines for lumbar puncture

in patients with neurological diseases. *Alzheimers Dement (Amst), 8*, 111-126. doi:10.1016/j.dadm.2017.04.007

Engvall, E., & Perlmann, P. (1971). Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. *Immunochemistry, 8*(9), 871-874. doi:10.1016/0019-2791(71)90454-x

FDA-NIH Biomarker Working Group. (2016). *BEST (Biomarkers, EndpointS, and other Tools) Resource [Internet, last updated 2021]*. Silver Spring (MD), Bethesda (MD): Food and Drug Administration (US), National Institutes of Health (US).

Feneberg, E., Steinacker, P., Lehnert, S., Schneider, A., Walther, P., Thal, D. R., Linsenmeier, M., Ludolph, A. C., & Otto, M. (2014). Limited role of free TDP-43 as a diagnostic tool in neurodegenerative diseases. *Amyotroph Lateral Scler Frontotemporal Degener, 15*(5-6), 351-356. doi:10.3109/21678421.2014.905606

Fenn, J. B., Mann, M., Meng, C. K., Wong, S. F., & Whitehouse, C. M. (1989). Electrospray ionization for mass spectrometry of large biomolecules. *Science, 246*(4926), 64-71. doi:10.1126/science.2675315

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res, 12*(3), 189-198. doi:10.1016/0022-3956(75)90026-6

Fourier, A., Portelius, E., Zetterberg, H., Blennow, K., Quadrio, I., & Perret-Liaudet, A. (2015). Pre-analytical and analytical factors influencing Alzheimer's disease cerebrospinal fluid biomarker variability. *Clin Chim Acta, 449*, 9-15. doi:10.1016/j.cca.2015.05.024

Fredriksson, S., Gullberg, M., Jarvius, J., Olsson, C., Pietras, K., Gústafsdóttir, S. M., Ostman, A., & Landegren, U. (2002). Protein detection using proximity-dependent DNA ligation assays. *Nat Biotechnol, 20*(5), 473-477. doi:10.1038/nbt0502-473

Freischmidt, A., Wieland, T., Richter, B., Ruf, W., Schaeffer, V., Müller, K., Marroquin, N., Nordin, F., Hübers, A., Weydt, P., Pinto, S., Press, R., Millecamps, S., Molko, N., Bernard, E., Desnuelle, C., Soriani, M. H., Dorst, J., Graf, E., Nordström, U., Feiler, M. S., Putz, S., Boeckers, T. M., Meyer, T., Winkler, A. S., Winkelman, J., de Carvalho, M., Thal, D. R., Otto, M., Brännström, T., Volk, A. E., Kursula, P., Danzer, K. M., Lichtner, P., Dikic, I., Meitinger, T., Ludolph, A. C., Strom, T. M., Andersen, P. M., & Weishaupt, J. H. (2015). Haploinsufficiency of TBK1 causes familial ALS and fronto-temporal dementia. *Nat Neurosci, 18*(5), 631-636. doi:10.1038/nn.4000

Gaetani, L., Blennow, K., Calabresi, P., Di Filippo, M., Parnetti, L., & Zetterberg, H. (2019). Neurofilament light chain as a biomarker in neurological disorders. *J Neurol Neurosurg Psychiatry*. doi:10.1136/jnnp-2018-320106

Galasko, D., Xiao, M., Xu, D., Smirnov, D., Salmon, D. P., Dewit, N., Vanbrabant, J., Jacobs, D., Vanderstichele, H., Vanmechelen, E., & Worley, P. (2019). Synaptic biomarkers in CSF aid in diagnosis, correlate with cognition and predict progression in MCI and Alzheimer's disease. *Alzheimers Dement (N Y), 5*, 871-882. doi:10.1016/j.trci.2019.11.002

Gervaise-Henry, C., Watfa, G., Albuisson, E., Kolodziej, A., Dousset, B., Olivier, J. L., Jonveaux, T. R., & Malaplate-Armand, C. (2017). Cerebrospinal Fluid Aβ42/Aβ40 as a Means to Limiting Tube- and Storage-Dependent Pre-Analytical Variability in Clinical Setting. *J Alzheimers Dis, 57*(2), 437-445. doi:10.3233/jad-160865

Gold, L., Ayers, D., Bertino, J., Bock, C., Bock, A., Brody, E. N., Carter, J., Dalby, A. B., Eaton, B. E., Fitzwater, T., Flather, D., Forbes, A., Foreman, T., Fowler, C., Gawande, B., Goss, M., Gunn, M., Gupta, S., Halladay, D., Heil, J., Heilig, J., Hicke, B., Husar, G., Janjic, N., Jarvis, T., Jennings, S., Katilius, E., Keeney, T. R., Kim, N., Koch, T. H., Kraemer, S., Kroiss, L., Le, N., Levine, D., Lindsey, W., Lollo, B., Mayfield, W., Mehan, M., Mehler, R., Nelson, S. K., Nelson, M., Nieuwlandt, D., Nikrad, M., Ochsner, U., Ostroff, R. M., Otis, M., Parker, T., Pietrasiewicz, S., Resnicow, D. I., Rohloff, J., Sanders, G., Sattin, S., Schneider, D., Singer, B., Stanton, M.,

Sterkel, A., Stewart, A., Stratford, S., Vaught, J. D., Vrkljan, M., Walker, J. J., Watrobka, M., Waugh, S., Weiss, A., Wilcox, S. K., Wolfson, A., Wolk, S. K., Zhang, C., & Zichi, D. (2010). Aptamer-based multiplexed proteomic technology for biomarker discovery. *PLoS One, 5*(12), e15004. doi:10.1371/journal.pone.0015004

Gómez de San José, N., Massa, F., Halbgebauer, S., Oeckl, P., Steinacker, P., & Otto, M. (2021). Neuronal pentraxins as biomarkers of synaptic activity: from physiological functions to pathological changes in neurodegeneration. *J Neural Transm (Vienna)*. doi:10.1007/s00702-021-02411-2

Gorno-Tempini, M. L., Hillis, A. E., Weintraub, S., Kertesz, A., Mendez, M., Cappa, S. F., Ogar, J. M., Rohrer, J. D., Black, S., Boeve, B. F., Manes, F., Dronkers, N. F., Vandenberghe, R., Rascovsky, K., Patterson, K., Miller, B. L., Knopman, D. S., Hodges, J. R., Mesulam, M. M., & Grossman, M. (2011). Classification of primary progressive aphasia and its variants. *Neurology, 76*(11), 1006-1014. doi:10.1212/WNL.0b013e31821103e6

Greaves, C. V., & Rohrer, J. D. (2019). An update on genetic frontotemporal dementia. *J Neurol, 266*(8), 2075-2086. doi:10.1007/s00415-019-09363-4

Guyon, I., & Elisseeff, A. (2003). An introduction to variable and feature selection. *Journal of Machine Learning Research, 3*, 1157-1182.

Häggmark, A., Byström, S., Ayoglu, B., Qundos, U., Uhlen, M., Khademi, M., Olsson, T., Schwenk, J. M., & Nilsson, P. (2013). Antibody-based profiling of cerebrospinal fluid within multiple sclerosis. *Proteomics, 13*(15), 2256-2267. doi:10.1002/pmic.201200580

Häggmark, A., Mikus, M., Mohsenchian, A., Hong, M. G., Forsström, B., Gajewska, B., Baranczyk-Kuzma, A., Uhlen, M., Schwenk, J. M., Kuzma-Kozakiewicz, M., & Nilsson, P. (2014). Plasma profiling reveals three proteins associated to amyotrophic lateral sclerosis. *Ann Clin Transl Neurol, 1*(8), 544-553. doi:10.1002/acn3.83

Häggmark, A., Neiman, M., Drobin, K., Zwahlen, M., Uhlen, M., Nilsson, P., & Schwenk, J. M. (2012). Classification of protein profiles from antibody microarrays using heat and detergent treatment. *N Biotechnol, 29*(5), 564-570. doi:10.1016/j.nbt.2011.10.005

Häggmark, A., Schwenk, J. M., & Nilsson, P. (2016). Neuroproteomic profiling of human body fluids. *Proteomics Clin Appl, 10*(4), 485-502. doi:10.1002/prca.201500065

Häggmark-Månberg, A., Nilsson, P., & Schwenk, J. M. (2017). Neuroproteomic Profiling of Cerebrospinal Fluid (CSF) by Multiplexed Affinity Arrays. *Methods Mol Biol, 1598*, 247-254. doi:10.1007/978-1-4939-6952-4_11

Hampel, H., O'Bryant, S. E., Molinuevo, J. L., Zetterberg, H., Masters, C. L., Lista, S., Kiddle, S. J., Batrla, R., & Blennow, K. (2018a). Blood-based biomarkers for Alzheimer disease: mapping the road to the clinic. *Nat Rev Neurol, 14*(11), 639-652. doi:10.1038/s41582-018-0079-7

Hampel, H., Vergallo, A., Aguilar, L. F., Benda, N., Broich, K., Cuello, A. C., Cummings, J., Dubois, B., Federoff, H. J., Fiandaca, M., Genthon, R., Haberkamp, M., Karran, E., Mapstone, M., Perry, G., Schneider, L. S., Welikovitch, L. A., Woodcock, J., Baldacci, F., & Lista, S. (2018b). Precision pharmacology for Alzheimer's disease. *Pharmacol Res, 130*, 331-365. doi:10.1016/j.phrs.2018.02.014

Hamsten, C., Skattum, L., Truedsson, L., von Dobeln, U., Uhlen, M., Schwenk, J. M., Hammarstrom, L., Nilsson, P., & Neiman, M. (2015). Heat differentiated complement factor profiling. *J Proteomics, 126*, 155-162. doi:10.1016/j.jprot.2015.05.027

Hamsten, C., Wiklundh, E., Gronlund, H., Schwenk, J. M., Uhlen, M., Eklund, A., Nilsson, P., Grunewald, J., & Häggmark-Månberg, A. (2016). Elevated levels of FN1 and CCL2 in bronchoalveolar lavage fluid from sarcoidosis patients. *Respir Res, 17*(1), 69. doi:10.1186/s12931-016-0381-0

Hanes, J., Kovac, A., Kvartsberg, H., Kontsekova, E., Fialova, L., Katina, S., Kovacech, B., Stevens, E., Hort, J., Vyhnalek, M., Boonkamp, L., Novak, M., Zetterberg, H., Hansson, O., Scheltens,

P., Blennow, K., Teunissen, C. E., & Zilka, N. (2020). Evaluation of a novel immunoassay to detect p-tau Thr217 in the CSF to distinguish Alzheimer disease from other dementias. *Neurology, 95*(22), e3026-e3035. doi:10.1212/wnl.0000000000010814

Hansson, O. (2021). Biomarkers for neurodegenerative diseases. *Nat Med, 27*(6), 954-963. doi:10.1038/s41591-021-01382-x

Hansson, O., Lehmann, S., Otto, M., Zetterberg, H., & Lewczuk, P. (2019). Advantages and disadvantages of the use of the CSF Amyloid β (Aβ) 42/40 ratio in the diagnosis of Alzheimer's Disease. *Alzheimers Res Ther, 11*(1), 34. doi:10.1186/s13195-019-0485-0

Hansson, O., Mikulskis, A., Fagan, A. M., Teunissen, C., Zetterberg, H., Vanderstichele, H., Molinuevo, J. L., Shaw, L. M., Vandijck, M., Verbeek, M. M., Savage, M., Mattsson, N., Lewczuk, P., Batrla, R., Rutz, S., Dean, R. A., & Blennow, K. (2018a). The impact of preanalytical variables on measuring cerebrospinal fluid biomarkers for Alzheimer's disease diagnosis: A review. *Alzheimers Dement, 14*(10), 1313-1333. doi:10.1016/j.jalz.2018.05.008

Hansson, O., Seibyl, J., Stomrud, E., Zetterberg, H., Trojanowski, J. Q., Bittner, T., Lifke, V., Corradini, V., Eichenlaub, U., Batrla, R., Buck, K., Zink, K., Rabe, C., Blennow, K., & Shaw, L. M. (2018b). CSF biomarkers of Alzheimer's disease concord with amyloid-β PET and predict clinical progression: A study of fully automated immunoassays in BioFINDER and ADNI cohorts. *Alzheimers Dement, 14*(11), 1470-1481. doi:10.1016/j.jalz.2018.01.010

Hansson, O., Zetterberg, H., Buchhave, P., Londos, E., Blennow, K., & Minthon, L. (2006). Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. *Lancet Neurol, 5*(3), 228-234. doi:10.1016/s1474-4422(06)70355-6

Harada, C. N., Natelson Love, M. C., & Triebel, K. L. (2013). Normal cognitive aging. *Clin Geriatr Med, 29*(4), 737-752. doi:10.1016/j.cger.2013.07.002

Hastie, T., Tibshirani, R., & Friedman, J. (2017). *The Elements of Statistical Learning: Data Mining, Inference, and Prediction* (2 ed.): Springer.

Haussler, R. S., Bendes, A., Iglesias, M., Sanchez-Rivera, L., Dodig-Crnkovic, T., Byström, S., Fredolini, C., Birgersson, E., Dale, M., Edfors, F., Fagerberg, L., Rockberg, J., Tegel, H., Uhlen, M., Qundos, U., & Schwenk, J. M. (2019). Systematic Development of Sandwich Immunoassays for the Plasma Secretome. *Proteomics, 19*(15), e1900008. doi:10.1002/pmic.201900008

Heller, C., Foiani, M. S., Moore, K., Convery, R., Bocchetta, M., Neason, M., Cash, D. M., Thomas, D., Greaves, C. V., Woollacott, I. O., Shafei, R., Van Swieten, J. C., Moreno, F., Sanchez-Valle, R., Borroni, B., Laforce, R., Jr., Masellis, M., Tartaglia, M. C., Graff, C., Galimberti, D., Rowe, J. B., Finger, E., Synofzik, M., Vandenberghe, R., de Mendonca, A., Tagliavini, F., Santana, I., Ducharme, S., Butler, C. R., Gerhard, A., Levin, J., Danek, A., Frisoni, G., Sorbi, S., Otto, M., Heslegrave, A. J., Zetterberg, H., & Rohrer, J. D. (2020). Plasma glial fibrillary acidic protein is raised in progranulin-associated frontotemporal dementia. *J Neurol Neurosurg Psychiatry, 91*(3), 263-270. doi:10.1136/jnnp-2019-321954

Hjelm, B., Forsstrom, B., Igel, U., Johannesson, H., Stadler, C., Lundberg, E., Ponten, F., Sjoberg, A., Rockberg, J., Schwenk, J. M., Nilsson, P., Johansson, C., & Uhlen, M. (2011). Generation of monospecific antibodies based on affinity capture of polyclonal antibodies. *Protein Sci, 20*(11), 1824-1835. doi:10.1002/pro.716

Hjelm, B., Forsström, B., Löfblom, J., Rockberg, J., & Uhlén, M. (2012). Parallel immunizations of rabbits using the same antigen yield antibodies with similar, but not identical, epitopes. *PLoS One, 7*(12), e45817. doi:10.1371/journal.pone.0045817

Ho, J., Tumkaya, T., Aryal, S., Choi, H., & Claridge-Chang, A. (2019). Moving beyond P values: data analysis with estimation graphics. *Nat Methods, 16*(7), 565-566. doi:10.1038/s41592-019-0470-3

Hober, S., Hellström, C., Olofsson, J., Andersson, E., Bergström, S., Jernbom Falk, A., Bayati, S., Mravinacova, S., Sjöberg, R., Yousef, J., Skoglund, L., Kanje, S., Berling, A., Svensson, A. S., Jensen, G., Enstedt, H., Afshari, D., Xu, L. L., Zwahlen, M., von Feilitzen, K., Hanke, L., Murrell, B., McInerney, G., Karlsson Hedestam, G. B., Lendel, C., Roth, R. G., Skoog, I., Svenungsson, E., Olsson, T., Fogdell-Hahn, A., Lindroth, Y., Lundgren, M., Maleki, K. T., Lagerqvist, N., Klingström, J., Da Silva Rodrigues, R., Muschiol, S., Bogdanovic, G., Arroyo Mühr, L. S., Eklund, C., Lagheden, C., Dillner, J., Sivertsson, Å., Havervall, S., Thålin, C., Tegel, H., Pin, E., Månberg, A., Hedhammar, M., & Nilsson, P. (2021). Systematic evaluation of SARS-CoV-2 antigens enables a highly specific and sensitive multiplex serological COVID-19 assay. *Clin Transl Immunology, 10*(7), e1312. doi:10.1002/cti2.1312

Hober, S., & Uhlén, M. (2008). Human protein atlas and the use of microarray technologies. *Curr Opin Biotechnol, 19*(1), 30-35. doi:10.1016/j.copbio.2007.11.006

Hoffman, P. N., Cleveland, D. W., Griffin, J. W., Landes, P. W., Cowan, N. J., & Price, D. L. (1987). Neurofilament gene expression: a major determinant of axonal caliber. *Proc Natl Acad Sci U S A, 84*(10), 3472-3476. doi:10.1073/pnas.84.10.3472

Höglinger, G. U., Respondek, G., Stamelou, M., Kurz, C., Josephs, K. A., Lang, A. E., Mollenhauer, B., Müller, U., Nilsson, C., Whitwell, J. L., Arzberger, T., Englund, E., Gelpi, E., Giese, A., Irwin, D. J., Meissner, W. G., Pantelyat, A., Rajput, A., van Swieten, J. C., Troakes, C., Antonini, A., Bhatia, K. P., Bordelon, Y., Compta, Y., Corvol, J. C., Colosimo, C., Dickson, D. W., Dodel, R., Ferguson, L., Grossman, M., Kassubek, J., Krismer, F., Levin, J., Lorenzl, S., Morris, H. R., Nestor, P., Oertel, W. H., Poewe, W., Rabinovici, G., Rowe, J. B., Schellenberg, G. D., Seppi, K., van Eimeren, T., Wenning, G. K., Boxer, A. L., Golbe, L. I., & Litvan, I. (2017). Clinical diagnosis of progressive supranuclear palsy: The movement disorder society criteria. *Mov Disord, 32*(6), 853-864. doi:10.1002/mds.26987

Holtzman, D. M., Carrillo, M. C., Hendrix, J. A., Bain, L. J., Catafau, A. M., Gault, L. M., Goedert, M., Mandelkow, E., Mandelkow, E. M., Miller, D. S., Ostrowitzki, S., Polydoro, M., Smith, S., Wittmann, M., & Hutton, M. (2016). Tau: From research to clinical development. *Alzheimers Dement, 12*(10), 1033-1039. doi:10.1016/j.jalz.2016.03.018

Hong, M. G., Dodig-Crnković, T., Chen, X., Drobin, K., Lee, W., Wang, Y., Edfors, F., Kotol, D., Thomas, C. E., Sjöberg, R., Odeberg, J., Hamsten, A., Silveira, A., Hall, P., Nilsson, P., Pawitan, Y., Uhlén, M., Pedersen, N. L., Hägg, S., Magnusson, P. K., & Schwenk, J. M. (2020). Profiles of histidine-rich glycoprotein associate with age and risk of all-cause mortality. *Life Sci Alliance, 3*(10). doi:10.26508/lsa.202000817

Hong, M. G., Lee, W., Nilsson, P., Pawitan, Y., & Schwenk, J. M. (2016). Multidimensional Normalization to Minimize Plate Effects of Suspension Bead Array Data. *J Proteome Res, 15*(10), 3473-3480. doi:10.1021/acs.jproteome.5b01131

Honig, L. S., Vellas, B., Woodward, M., Boada, M., Bullock, R., Borrie, M., Hager, K., Andreasen, N., Scarpini, E., Liu-Seifert, H., Case, M., Dean, R. A., Hake, A., Sundell, K., Poole Hoffmann, V., Carlson, C., Khanna, R., Mintun, M., DeMattos, R., Selzler, K. J., & Siemers, E. (2018). Trial of Solanezumab for Mild Dementia Due to Alzheimer's Disease. *N Engl J Med, 378*(4), 321-330. doi:10.1056/NEJMoa1705971

Hoops, S., Nazem, S., Siderowf, A. D., Duda, J. E., Xie, S. X., Stern, M. B., & Weintraub, D. (2009). Validity of the MoCA and MMSE in the detection of MCI and dementia in Parkinson disease. *Neurology, 73*(21), 1738-1745. doi:10.1212/WNL.0b013e3181c34b47

Huang, L., Shao, D., Wang, Y., Cui, X., Li, Y., Chen, Q., & Cui, J. (2021). Human body-fluid proteome: quantitative profiling and computational prediction. *Brief Bioinform, 22*(1), 315-333. doi:10.1093/bib/bbz160

Hunt, A., & Thomas, D. (1999). *The Pragmatic Programmer* (1st ed.): Addison-Wesley Professional.

Iglesias, M. J., Schwenk, J. M., & Odeberg, J. (2021). Affinity Proteomics Assays for Cardiovascular and Atherosclerotic Disease Biomarkers. *Methods Mol Biol, 2344*, 163-179. doi:10.1007/978-1-0716-1562-1_12

Ioannidis, J. P. (2008). Why most discovered true associations are inflated. *Epidemiology, 19*(5), 640-648. doi:10.1097/EDE.0b013e31818131e7

Irwin, D. J. (2016). Tauopathies as clinicopathological entities. *Parkinsonism Relat Disord, 22 Suppl 1*(0 1), S29-33. doi:10.1016/j.parkreldis.2015.09.020

Jack, C. R., Jr., Bennett, D. A., Blennow, K., Carrillo, M. C., Dunn, B., Haeberlein, S. B., Holtzman, D. M., Jagust, W., Jessen, F., Karlawish, J., Liu, E., Molinuevo, J. L., Montine, T., Phelps, C., Rankin, K. P., Rowe, C. C., Scheltens, P., Siemers, E., Snyder, H. M., & Sperling, R. (2018). NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimers Dement, 14*(4), 535-562. doi:10.1016/j.jalz.2018.02.018

Jack, C. R., Jr., Bennett, D. A., Blennow, K., Carrillo, M. C., Feldman, H. H., Frisoni, G. B., Hampel, H., Jagust, W. J., Johnson, K. A., Knopman, D. S., Petersen, R. C., Scheltens, P., Sperling, R. A., & Dubois, B. (2016). A/T/N: An unbiased descriptive classification scheme for Alzheimer disease biomarkers. *Neurology, 87*(5), 539-547. doi:10.1212/wnl.0000000000002923

Jäkel, S., & Dimou, L. (2017). Glial Cells and Their Function in the Adult Brain: A Journey through the History of Their Ablation. *Front Cell Neurosci, 11*, 24. doi:10.3389/fncel.2017.00024

Janelidze, S., Mattsson, N., Palmqvist, S., Smith, R., Beach, T. G., Serrano, G. E., Chai, X., Proctor, N. K., Eichenlaub, U., Zetterberg, H., Blennow, K., Reiman, E. M., Stomrud, E., Dage, J. L., & Hansson, O. (2020a). Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. *Nat Med, 26*(3), 379-386. doi:10.1038/s41591-020-0755-1

Janelidze, S., Stomrud, E., Smith, R., Palmqvist, S., Mattsson, N., Airey, D. C., Proctor, N. K., Chai, X., Shcherbinin, S., Sims, J. R., Triana-Baltzer, G., Theunis, C., Slemmon, R., Mercken, M., Kolb, H., Dage, J. L., & Hansson, O. (2020b). Cerebrospinal fluid p-tau217 performs better than p-tau181 as a biomarker of Alzheimer's disease. *Nat Commun, 11*(1), 1683. doi:10.1038/s41467-020-15436-0

Johanson, C. E., Duncan, J. A., 3rd, Klinge, P. M., Brinker, T., Stopa, E. G., & Silverberg, G. D. (2008). Multiplicity of cerebrospinal fluid functions: New challenges in health and disease. *Cerebrospinal Fluid Res, 5*, 10. doi:10.1186/1743-8454-5-10

Kalia, L. V., & Lang, A. E. (2015). Parkinson's disease. *Lancet, 386*(9996), 896-912. doi:10.1016/s0140-6736(14)61393-3

Karas, M., & Hillenkamp, F. (1988). Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. *Anal Chem, 60*(20), 2299-2301. doi:10.1021/ac00171a028

Karikari, T. K., Emeršič, A., Vrillon, A., Lantero-Rodriguez, J., Ashton, N. J., Kramberger, M. G., Dumurgier, J., Hourregue, C., Čučnik, S., Brinkmalm, G., Rot, U., Zetterberg, H., Paquet, C., & Blennow, K. (2021). Head-to-head comparison of clinical performance of CSF phospho-tau T181 and T217 biomarkers for Alzheimer's disease diagnosis. *Alzheimers Dement, 17*(5), 755-767. doi:10.1002/alz.12236

Karikari, T. K., Pascoal, T. A., Ashton, N. J., Janelidze, S., Benedet, A. L., Rodriguez, J. L., Chamoun, M., Savard, M., Kang, M. S., Therriault, J., Schöll, M., Massarweh, G., Soucy, J. P., Höglund, K., Brinkmalm, G., Mattsson, N., Palmqvist, S., Gauthier, S., Stomrud, E., Zetterberg, H., Hansson, O., Rosa-Neto, P., & Blennow, K. (2020). Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. *Lancet Neurol, 19*(5), 422-433. doi:10.1016/s1474-4422(20)30071-5

Karlsson, M., Zhang, C., Méar, L., Zhong, W., Digre, A., Katona, B., Sjöstedt, E., Butler, L., Odeberg, J., Dusart, P., Edfors, F., Oksvold, P., von Feilitzen, K., Zwahlen, M., Arif, M., Altay, O., Li,

X., Ozcan, M., Mardonoglu, A., Fagerberg, L., Mulder, J., Luo, Y., Ponten, F., Uhlén, M., & Lindskog, C. (2021). A single-cell type transcriptomics map of human tissues. *Sci Adv, 7*(31). doi:10.1126/sciadv.abh2169

Karran, E., & Hardy, J. (2014). Antiamyloid therapy for Alzheimer's disease--are we on the right road? *N Engl J Med, 370*(4), 377-378. doi:10.1056/NEJMe1313943

Kern, S., Syrjanen, J. A., Blennow, K., Zetterberg, H., Skoog, I., Waern, M., Hagen, C. E., van Harten, A. C., Knopman, D. S., Jack, C. R., Jr., Petersen, R. C., & Mielke, M. M. (2019). Association of Cerebrospinal Fluid Neurofilament Light Protein With Risk of Mild Cognitive Impairment Among Individuals Without Cognitive Impairment. *JAMA Neurol, 76*(2), 187-193. doi:10.1001/jamaneurol.2018.3459

Kern, S., Zetterberg, H., Kern, J., Zettergren, A., Waern, M., Hoglund, K., Andreasson, U., Wetterberg, H., Borjesson-Hanson, A., Blennow, K., & Skoog, I. (2018). Prevalence of preclinical Alzheimer disease: Comparison of current classification systems. *Neurology, 90*(19), e1682-e1691. doi:10.1212/wnl.0000000000005476

Khalil, M., Teunissen, C. E., Otto, M., Piehl, F., Sormani, M. P., Gattringer, T., Barro, C., Kappos, L., Comabella, M., Fazekas, F., Petzold, A., Blennow, K., Zetterberg, H., & Kuhle, J. (2018). Neurofilaments as biomarkers in neurological disorders. *Nat Rev Neurol, 14*(10), 577-589. doi:10.1038/s41582-018-0058-z

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., & Långström, B. (2004). Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. *Ann Neurol, 55*(3), 306-319. doi:10.1002/ana.20009

Knuth, D. E. (1984). Literate programming. *The Computer Journal, 27*(2), 97-111.

Köhler, G., & Milstein, C. (1975). Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature, 256*(5517), 495-497. doi:10.1038/256495a0

Kuhle, J., Barro, C., Andreasson, U., Derfuss, T., Lindberg, R., Sandelius, Å., Liman, V., Norgren, N., Blennow, K., & Zetterberg, H. (2016). Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electrochemiluminescence immunoassay and Simoa. *Clin Chem Lab Med, 54*(10), 1655-1661. doi:10.1515/cclm-2015-1195

Kumar, A., Singh, A., & Ekavali. (2015). A review on Alzheimer's disease pathophysiology and its management: an update. *Pharmacol Rep, 67*(2), 195-203. doi:10.1016/j.pharep.2014.09.004

Landegren, U., & Hammond, M. (2021). Cancer diagnostics based on plasma protein biomarkers: hard times but great expectations. *Mol Oncol, 15*(6), 1715-1726. doi:10.1002/1878-0261.12809

Lantero Rodriguez, J., Karikari, T. K., Suárez-Calvet, M., Troakes, C., King, A., Emersic, A., Aarsland, D., Hye, A., Zetterberg, H., Blennow, K., & Ashton, N. J. (2020). Plasma p-tau181 accurately predicts Alzheimer's disease pathology at least 8 years prior to post-mortem and improves the clinical characterisation of cognitive decline. *Acta Neuropathol, 140*(3), 267-278. doi:10.1007/s00401-020-02195-x

Lewczuk, P., Ermann, N., Andreasson, U., Schultheis, C., Podhorna, J., Spitzer, P., Maler, J. M., Kornhuber, J., Blennow, K., & Zetterberg, H. (2018). Plasma neurofilament light as a potential biomarker of neurodegeneration in Alzheimer's disease. *Alzheimers Res Ther, 10*(1), 71. doi:10.1186/s13195-018-0404-9

Libiger, O., Shaw, L. M., Watson, M. H., Nairn, A. C., Umaña, K. L., Biarnes, M. C., Canet-Avilés, R. M., Jack, C. R., Jr., Breton, Y. A., Cortes, L., Chelsky, D., Spellman, D. S., Baker, S. A., Raghavan, N., & Potter, W. Z. (2021). Longitudinal CSF proteomics identifies NPTX2 as a prognostic biomarker of Alzheimer's disease. *Alzheimers Dement*. doi:10.1002/alz.12353

Lim, A. S. P., Gaiteri, C., Yu, L., Sohail, S., Swardfager, W., Tasaki, S., Schneider, J. A., Paquet, C., Stuss, D. T., Masellis, M., Black, S. E., Hugon, J., Buchman, A. S., Barnes, L. L., Bennett, D. A., & De Jager, P. L. (2018). Seasonal plasticity of cognition and related biological measures in adults with and without Alzheimer disease: Analysis of multiple cohorts. *PLoS Med, 15*(9), e1002647. doi:10.1371/journal.pmed.1002647

Lim, B., Sando, S. B., Grøntvedt, G. R., Bråthen, G., & Diamandis, E. P. (2020). Cerebrospinal fluid neuronal pentraxin receptor as a biomarker of long-term progression of Alzheimer's disease: a 24-month follow-up study. *Neurobiol Aging, 93*, 97.e91-97.e97. doi:10.1016/j.neurobiolaging.2020.03.013

Lindblad, C., Pin, E., Just, D., Al Nimer, F., Nilsson, P., Bellander, B. M., Svensson, M., Piehl, F., & Thelin, E. P. (2021). Fluid proteomics of CSF and serum reveal important neuroinflammatory proteins in blood-brain barrier disruption and outcome prediction following severe traumatic brain injury: a prospective, observational study. *Crit Care, 25*(1), 103. doi:10.1186/s13054-021-03503-x

Lindskog, M., Rockberg, J., Uhlén, M., & Sterky, F. (2005). Selection of protein epitopes for antibody production. *Biotechniques, 38*(5), 723-727. doi:10.2144/05385st02

Lipman, N. S., Jackson, L. R., Trudel, L. J., & Weis-Garcia, F. (2005). Monoclonal versus polyclonal antibodies: distinguishing characteristics, applications, and information resources. *Ilar j, 46*(3), 258-268. doi:10.1093/ilar.46.3.258

Lourido, L., Ayoglu, B., Fernandez-Tajes, J., Oreiro, N., Henjes, F., Hellstrom, C., Schwenk, J. M., Ruiz-Romero, C., Nilsson, P., & Blanco, F. J. (2017). Discovery of circulating proteins associated to knee radiographic osteoarthritis. *Sci Rep, 7*(1), 137. doi:10.1038/s41598-017-00195-8

Lourido, L., Paz-González, R., Ruiz-Romero, C., Nilsson, P., & Blanco, F. J. (2021). Serum Proteomic Profiling in Rheumatoid Arthritis by Antibody Suspension Bead Arrays. *Methods Mol Biol, 2259*, 143-151. doi:10.1007/978-1-0716-1178-4_8

Maaten, L. v. d., & Hinton, G. (2008). Visualizing Data using t-SNE. *Journal of Machine Learning Research, 9*, 2579-2605.

Maes, E., Cho, W. C., & Baggerman, G. (2015). Translating clinical proteomics: the importance of study design. *Expert Rev Proteomics, 12*(3), 217-219. doi:10.1586/14789450.2015.1041512

Månberg, A., Bradley, F., Qundos, U., Guthrie, B. L., Birse, K., Noël-Romas, L., Lindskog, C., Bosire, R., Kiarie, J., Farquhar, C., Burgener, A. D., Nilsson, P., & Broliden, K. (2019). A High-throughput Bead-based Affinity Assay Enables Analysis of Genital Protein Signatures in Women At Risk of HIV Infection. *Mol Cell Proteomics, 18*(3), 461-476. doi:10.1074/mcp.RA118.000757

Månberg, A., Skene, N., Sanders, F., Trusohamn, M., Remnestål, J., Szczepińska, A., Aksoylu, I. S., Lönnerberg, P., Ebarasi, L., Wouters, S., Lehmann, M., Olofsson, J., von Gohren Antequera, I., Domaniku, A., De Schaepdryver, M., De Vocht, J., Poesen, K., Uhlén, M., Anink, J., Mijnsbergen, C., Vergunst-Bosch, H., Hübers, A., Kläppe, U., Rodriguez-Vieitez, E., Gilthorpe, J. D., Hedlund, E., Harris, R. A., Aronica, E., Van Damme, P., Ludolph, A., Veldink, J., Ingre, C., Nilsson, P., & Lewandowski, S. A. (2021). Altered perivascular fibroblast activity precedes ALS disease onset. *Nat Med, 27*(4), 640-646. doi:10.1038/s41591-021-01295-9

Mandelkow, E. M., & Mandelkow, E. (2012). Biochemistry and cell biology of tau protein in neurofibrillary degeneration. *Cold Spring Harb Perspect Med, 2*(7), a006247. doi:10.1101/cshperspect.a006247

Marimuthu, A., O'Meally, R. N., Chaerkady, R., Subbannayya, Y., Nanjappa, V., Kumar, P., Kelkar, D. S., Pinto, S. M., Sharma, R., Renuse, S., Goel, R., Christopher, R., Delanghe, B., Cole, R. N., Harsha, H. C., & Pandey, A. (2011). A comprehensive map of the human urinary proteome. *J Proteome Res, 10*(6), 2734-2743. doi:10.1021/pr2003038

Masliah, E., Mallory, M., Alford, M., DeTeresa, R., Hansen, L. A., McKeel, D. W., Jr., & Morris, J. C. (2001). Altered expression of synaptic proteins occurs early during progression of Alzheimer's disease. *Neurology, 56*(1), 127-129. doi:10.1212/wnl.56.1.127

Masters, C. L., Bateman, R., Blennow, K., Rowe, C. C., Sperling, R. A., & Cummings, J. L. (2015). Alzheimer's disease. *Nat Rev Dis Primers, 1*, 15056. doi:10.1038/nrdp.2015.56

Mattsson, N., Carrillo, M. C., Dean, R. A., Devous, M. D., Sr., Nikolcheva, T., Pesini, P., Salter, H., Potter, W. Z., Sperling, R. S., Bateman, R. J., Bain, L. J., & Liu, E. (2015). Revolutionizing Alzheimer's disease and clinical trials through biomarkers. *Alzheimers Dement (Amst), 1*(4), 412-419. doi:10.1016/j.dadm.2015.09.001

Mattsson, N., Lönneborg, A., Boccardi, M., Blennow, K., & Hansson, O. (2017). Clinical validity of cerebrospinal fluid Aβ42, tau, and phospho-tau as biomarkers for Alzheimer's disease in the context of a structured 5-phase development framework. *Neurobiol Aging, 52*, 196-213. doi:10.1016/j.neurobiolaging.2016.02.034

Mattsson, N., Zetterberg, H., Janelidze, S., Insel, P. S., Andreasson, U., Stomrud, E., Palmqvist, S., Baker, D., Tan Hehir, C. A., Jeromin, A., Hanlon, D., Song, L., Shaw, L. M., Trojanowski, J. Q., Weiner, M. W., Hansson, O., & Blennow, K. (2016). Plasma tau in Alzheimer disease. *Neurology, 87*(17), 1827-1835. doi:10.1212/wnl.0000000000003246

Mattsson-Carlgren, N., Janelidze, S., Palmqvist, S., Cullen, N., Svenningsson, A. L., Strandberg, O., Mengel, D., Walsh, D. M., Stomrud, E., Dage, J. L., & Hansson, O. (2020a). Longitudinal plasma p-tau217 is increased in early stages of Alzheimer's disease. *Brain, 143*(11), 3234-3241. doi:10.1093/brain/awaa286

Mattsson-Carlgren, N., Palmqvist, S., Blennow, K., & Hansson, O. (2020b). Increasing the reproducibility of fluid biomarker studies in neurodegenerative studies. *Nat Commun, 11*(1), 6252. doi:10.1038/s41467-020-19957-6

Mazancova, A. F., Růžička, E., Jech, R., & Bezdicek, O. (2020). Test the Best: Classification Accuracies of Four Cognitive Rating Scales for Parkinson's Disease Mild Cognitive Impairment. *Arch Clin Neuropsychol*. doi:10.1093/arclin/acaa039

McCarron, M., McCallion, P., Reilly, E., Dunne, P., Carroll, R., & Mulryan, N. (2017). A prospective 20-year longitudinal follow-up of dementia in persons with Down syndrome. *J Intellect Disabil Res, 61*(9), 843-852. doi:10.1111/jir.12390

McGrowder, D. A., Miller, F., Vaz, K., Nwokocha, C., Wilson-Clarke, C., Anderson-Cross, M., Brown, J., Anderson-Jackson, L., Williams, L., Latore, L., Thompson, R., & Alexander-Lindo, R. (2021). Cerebrospinal Fluid Biomarkers of Alzheimer's Disease: Current Evidence and Future Perspectives. *Brain Sci, 11*(2). doi:10.3390/brainsci11020215

McInnes, L., Healy, J., & Melville, J. (2018). UMAP: Uniform Manifold Approximation and Projection for Dimension Reduction.

McKeith, I. G., Boeve, B. F., Dickson, D. W., Halliday, G., Taylor, J. P., Weintraub, D., Aarsland, D., Galvin, J., Attems, J., Ballard, C. G., Bayston, A., Beach, T. G., Blanc, F., Bohnen, N., Bonanni, L., Bras, J., Brundin, P., Burn, D., Chen-Plotkin, A., Duda, J. E., El-Agnaf, O., Feldman, H., Ferman, T. J., Ffytche, D., Fujishiro, H., Galasko, D., Goldman, J. G., Gomperts, S. N., Graff-Radford, N. R., Honig, L. S., Iranzo, A., Kantarci, K., Kaufer, D., Kukull, W., Lee, V. M. Y., Leverenz, J. B., Lewis, S., Lippa, C., Lunde, A., Masellis, M., Masliah, E., McLean, P., Mollenhauer, B., Montine, T. J., Moreno, E., Mori, E., Murray, M., O'Brien, J. T., Orimo, S., Postuma, R. B., Ramaswamy, S., Ross, O. A., Salmon, D. P., Singleton, A., Taylor, A., Thomas, A., Tiraboschi, P., Toledo, J. B., Trojanowski, J. Q., Tsuang, D., Walker, Z., Yamada, M., & Kosaka, K. (2017). Diagnosis and management of dementia with Lewy bodies: Fourth consensus report of the DLB Consortium. *Neurology, 89*(1), 88-100. doi:10.1212/wnl.0000000000004058

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., & Stadlan, E. M. (1984). Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology, 34*(7), 939-944. doi:10.1212/wnl.34.7.939

McKhann, G. M., Knopman, D. S., Chertkow, H., Hyman, B. T., Jack, C. R., Jr., Kawas, C. H., Klunk, W. E., Koroshetz, W. J., Manly, J. J., Mayeux, R., Mohs, R. C., Morris, J. C., Rossor, M. N., Scheltens, P., Carrillo, M. C., Thies, B., Weintraub, S., & Phelps, C. H. (2011). The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement, 7*(3), 263-269. doi:10.1016/j.jalz.2011.03.005

McShane, L. M. (2017). In Pursuit of Greater Reproducibility and Credibility of Early Clinical Biomarker Research. *Clin Transl Sci, 10*(2), 58-60. doi:10.1111/cts.12449

Medeiros, R., & LaFerla, F. M. (2013). Astrocytes: conductors of the Alzheimer disease neuroinflammatory symphony. *Exp Neurol, 239*, 133-138. doi:10.1016/j.expneurol.2012.10.007

Mikus, M., Drobin, K., Gry, M., Bachmann, J., Lindberg, J., Yimer, G., Aklillu, E., Makonnen, E., Aderaye, G., Roach, J., Fier, I., Kampf, C., Gopfert, J., Perazzo, H., Poynard, T., Stephens, C., Andrade, R. J., Lucena, M. I., Arber, N., Uhlen, M., Watkins, P. B., Schwenk, J. M., Nilsson, P., & Schuppe-Koistinen, I. (2017). Elevated levels of circulating CDH5 and FABP1 in association with human drug-induced liver injury. *Liver Int, 37*(1), 132-140. doi:10.1111/liv.13174

Mikus, M., Järnbert-Pettersson, H., Johansson, C., Nilsson, P., Scheynius, A., & Alm, J. (2021). Protein profiles in plasma: Development from infancy to 5 years of age. *Proteomics Clin Appl, 15*(4), e2000038. doi:10.1002/prca.202000038

Moore, K. M., Nicholas, J., Grossman, M., McMillan, C. T., Irwin, D. J., Massimo, L., Van Deerlin, V. M., Warren, J. D., Fox, N. C., Rossor, M. N., Mead, S., Bocchetta, M., Boeve, B. F., Knopman, D. S., Graff-Radford, N. R., Forsberg, L. K., Rademakers, R., Wszolek, Z. K., van Swieten, J. C., Jiskoot, L. C., Meeter, L. H., Dopper, E. G., Papma, J. M., Snowden, J. S., Saxon, J., Jones, M., Pickering-Brown, S., Le Ber, I., Camuzat, A., Brice, A., Caroppo, P., Ghidoni, R., Pievani, M., Benussi, L., Binetti, G., Dickerson, B. C., Lucente, D., Krivensky, S., Graff, C., Öijerstedt, L., Fallström, M., Thonberg, H., Ghoshal, N., Morris, J. C., Borroni, B., Benussi, A., Padovani, A., Galimberti, D., Scarpini, E., Fumagalli, G. G., Mackenzie, I. R., Hsiung, G. R., Sengdy, P., Boxer, A. L., Rosen, H., Taylor, J. B., Synofzik, M., Wilke, C., Sulzer, P., Hodges, J. R., Halliday, G., Kwok, J., Sanchez-Valle, R., Lladó, A., Borrego-Ecija, S., Santana, I., Almeida, M. R., Tábuas-Pereira, M., Moreno, F., Barandiaran, M., Indakoetxea, B., Levin, J., Danek, A., Rowe, J. B., Cope, T. E., Otto, M., Anderl-Straub, S., de Mendonça, A., Maruta, C., Masellis, M., Black, S. E., Couratier, P., Lautrette, G., Huey, E. D., Sorbi, S., Nacmias, B., Laforce, R., Jr., Tremblay, M. L., Vandenberghe, R., Damme, P. V., Rogalski, E. J., Weintraub, S., Gerhard, A., Onyike, C. U., Ducharme, S., Papageorgiou, S. G., Ng, A. S. L., Brodtmann, A., Finger, E., Guerreiro, R., Bras, J., & Rohrer, J. D. (2020). Age at symptom onset and death and disease duration in genetic frontotemporal dementia: an international retrospective cohort study. *Lancet Neurol, 19*(2), 145-156. doi:10.1016/s1474-4422(19)30394-1

Mullard, A. (2021). Controversial Alzheimer's drug approval could affect other diseases. *Nature, 595*(7866), 162-163. doi:10.1038/d41586-021-01763-9

Nadler, W. M., Waidelich, D., Kerner, A., Hanke, S., Berg, R., Trumpp, A., & Rösli, C. (2017). MALDI versus ESI: The Impact of the Ion Source on Peptide Identification. *J Proteome Res, 16*(3), 1207-1215. doi:10.1021/acs.jproteome.6b00805

Nasreddine, Z. S., Phillips, N. A., Bédirian, V., Charbonneau, S., Whitehead, V., Collin, I., Cummings, J. L., & Chertkow, H. (2005). The Montreal Cognitive Assessment, MoCA: a brief screening

tool for mild cognitive impairment. *J Am Geriatr Soc, 53*(4), 695-699. doi:10.1111/j.1532-5415.2005.53221.x

Neiman, M., Fredolini, C., Johansson, H., Lehtio, J., Nygren, P. A., Uhlen, M., Nilsson, P., & Schwenk, J. M. (2013). Selectivity analysis of single binder assays used in plasma protein profiling. *Proteomics, 13*(23-24), 3406-3410. doi:10.1002/pmic.201300030

Neiman, M., Hedberg, J. J., Donnes, P. R., Schuppe-Koistinen, I., Hanschke, S., Schindler, R., Uhlen, M., Schwenk, J. M., & Nilsson, P. (2011). Plasma profiling reveals human fibulin-1 as candidate marker for renal impairment. *J Proteome Res, 10*(11), 4925-4934. doi:10.1021/pr200286c

Niemantsverdriet, E., Struyfs, H., Duits, F., Teunissen, C. E., & Engelborghs, S. (2015). Techniques, Contraindications, and Complications of CSF Collection Procedures. In F. Deisenhammer, F. Sellebjerg, C. E. Teunissen, & H. Tumani (Eds.), *Cerebrospinal Fluid in Clinical Neurology* (pp. 35-57). Cham: Springer International Publishing.

Nilsson, J., Gobom, J., Sjödin, S., Brinkmalm, G., Ashton, N. J., Svensson, J., Johansson, P., Portelius, E., Zetterberg, H., Blennow, K., & Brinkmalm, A. (2021). Cerebrospinal fluid biomarker panel for synaptic dysfunction in Alzheimer's disease. *Alzheimers Dement (Amst), 13*(1), e12179. doi:10.1002/dad2.12179

Nilsson, P., Paavilainen, L., Larsson, K., Odling, J., Sundberg, M., Andersson, A. C., Kampf, C., Persson, A., Al-Khalili Szigyarto, C., Ottosson, J., Björling, E., Hober, S., Wernérus, H., Wester, K., Pontén, F., & Uhlen, M. (2005). Towards a human proteome atlas: high-throughput generation of mono-specific antibodies for tissue profiling. *Proteomics, 5*(17), 4327-4337. doi:10.1002/pmic.200500072

Noble, W. S. (2009). How does multiple testing correction work? *Nat Biotechnol, 27*(12), 1135-1137. doi:10.1038/nbt1209-1135

Norgren, N., Rosengren, L., & Stigbrand, T. (2003). Elevated neurofilament levels in neurological diseases. *Brain Res, 987*(1), 25-31. doi:10.1016/s0006-8993(03)03219-0

O'Brien, J. T., & Herholz, K. (2015). Amyloid imaging for dementia in clinical practice. *BMC Med, 13*, 163. doi:10.1186/s12916-015-0404-6

Oeckl, P., Halbgebauer, S., Anderl-Straub, S., Steinacker, P., Huss, A. M., Neugebauer, H., von Arnim, C. A. F., Diehl-Schmid, J., Grimmer, T., Kornhuber, J., Lewczuk, P., Danek, A., Ludolph, A. C., & Otto, M. (2019). Glial Fibrillary Acidic Protein in Serum is Increased in Alzheimer's Disease and Correlates with Cognitive Impairment. *J Alzheimers Dis, 67*(2), 481-488. doi:10.3233/jad-180325

Olsson, B., Lautner, R., Andreasson, U., Öhrfelt, A., Portelius, E., Bjerke, M., Hölttä, M., Rosén, C., Olsson, C., Strobel, G., Wu, E., Dakin, K., Petzold, M., Blennow, K., & Zetterberg, H. (2016). CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. *Lancet Neurol, 15*(7), 673-684. doi:10.1016/s1474-4422(16)00070-3

Palmqvist, S., Janelidze, S., Quiroz, Y. T., Zetterberg, H., Lopera, F., Stomrud, E., Su, Y., Chen, Y., Serrano, G. E., Leuzy, A., Mattsson-Carlgren, N., Strandberg, O., Smith, R., Villegas, A., Sepulveda-Falla, D., Chai, X., Proctor, N. K., Beach, T. G., Blennow, K., Dage, J. L., Reiman, E. M., & Hansson, O. (2020). Discriminative Accuracy of Plasma Phospho-tau217 for Alzheimer Disease vs Other Neurodegenerative Disorders. *Jama, 324*(8), 772-781. doi:10.1001/jama.2020.12134

Palmqvist, S., Mattsson, N., & Hansson, O. (2016). Cerebrospinal fluid analysis detects cerebral amyloid-β accumulation earlier than positron emission tomography. *Brain, 139*(Pt 4), 1226-1236. doi:10.1093/brain/aww015

Pannee, J., Törnqvist, U., Westerlund, A., Ingelsson, M., Lannfelt, L., Brinkmalm, G., Persson, R., Gobom, J., Svensson, J., Johansson, P., Zetterberg, H., Blennow, K., & Portelius, E. (2014). The amyloid-β degradation pattern in plasma--a possible tool for clinical trials in Alzheimer's disease. *Neurosci Lett, 573*, 7-12. doi:10.1016/j.neulet.2014.04.041

Paslawski, W., Bergström, S., Zhang, X., Remnestål, J., He, Y., Boxer, A., Månberg, A., Nilsson, P., & Svenningsson, P. (2021). Cerebrospinal Fluid Proteins Altered in Corticobasal Degeneration. *Mov Disord*. doi:10.1002/mds.28543

Pereira, J. B., Janelidze, S., Smith, R., Mattsson-Carlgren, N., Palmqvist, S., Teunissen, C. E., Zetterberg, H., Stomrud, E., Ashton, N. J., Blennow, K., & Hansson, O. (2021). Plasma GFAP is an early marker of amyloid-β but not tau pathology in Alzheimer's disease. *Brain*. doi:10.1093/brain/awab223

Perlmutter, J. S. (2021). FDA's green light, science's red light. *Science, 372*(6549), 1371. Retrieved from https://doi.org/10.1126/science.abk0575

Perret-Liaudet, A., Pelpel, M., Tholance, Y., Dumont, B., Vanderstichele, H., Zorzi, W., Elmoualij, B., Schraen, S., Moreaud, O., Gabelle, A., Thouvenot, E., Thomas-Anterion, C., Touchon, J., Krolak-Salmon, P., Kovacs, G. G., Coudreuse, A., Quadrio, I., & Lehmann, S. (2012). Risk of Alzheimer's disease biological misdiagnosis linked to cerebrospinal collection tubes. *J Alzheimers Dis, 31*(1), 13-20. doi:10.3233/jad-2012-120361

Picotti, P., Bodenmiller, B., & Aebersold, R. (2013). Proteomics meets the scientific method. *Nat Methods, 10*(1), 24-27. doi:10.1038/nmeth.2291

Pin, E., Sjöberg, R., Andersson, E., Hellstrom, C., Olofsson, J., Jernbom Falk, A., Bergström, S., Remnestal, J., Just, D., Nilsson, P., & Månberg, A. (2019). Array-Based Profiling of Proteins and Autoantibody Repertoires in CSF. *Methods Mol Biol, 2044*, 303-318. doi:10.1007/978-1-4939-9706-0_19

Postuma, R. B., Berg, D., Stern, M., Poewe, W., Olanow, C. W., Oertel, W., Obeso, J., Marek, K., Litvan, I., Lang, A. E., Halliday, G., Goetz, C. G., Gasser, T., Dubois, B., Chan, P., Bloem, B. R., Adler, C. H., & Deuschl, G. (2015). MDS clinical diagnostic criteria for Parkinson's disease. *Mov Disord, 30*(12), 1591-1601. doi:10.1002/mds.26424

Preische, O., Schultz, S. A., Apel, A., Kuhle, J., Kaeser, S. A., Barro, C., Gräber, S., Kuder-Buletta, E., LaFougere, C., Laske, C., Vöglein, J., Levin, J., Masters, C. L., Martins, R., Schofield, P. R., Rossor, M. N., Graff-Radford, N. R., Salloway, S., Ghetti, B., Ringman, J. M., Noble, J. M., Chhatwal, J., Goate, A. M., Benzinger, T. L. S., Morris, J. C., Bateman, R. J., Wang, G., Fagan, A. M., McDade, E. M., Gordon, B. A., & Jucker, M. (2019). Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer's disease. *Nat Med, 25*(2), 277-283. doi:10.1038/s41591-018-0304-3

Quiroz, Y. T., Zetterberg, H., Reiman, E. M., Chen, Y., Su, Y., Fox-Fuller, J. T., Garcia, G., Villegas, A., Sepulveda-Falla, D., Villada, M., Arboleda-Velasquez, J. F., Guzmán-Vélez, E., Vila-Castelar, C., Gordon, B. A., Schultz, S. A., Protas, H. D., Ghisays, V., Giraldo, M., Tirado, V., Baena, A., Munoz, C., Rios-Romenets, S., Tariot, P. N., Blennow, K., & Lopera, F. (2020). Plasma neurofilament light chain in the presenilin 1 E280A autosomal dominant Alzheimer's disease kindred: a cross-sectional and longitudinal cohort study. *Lancet Neurol, 19*(6), 513-521. doi:10.1016/s1474-4422(20)30137-x

Qundos, U., Drobin, K., Mattsson, C., Hong, M. G., Sjoberg, R., Forsstrom, B., Solomon, D., Uhlen, M., Nilsson, P., Michaelsson, K., & Schwenk, J. M. (2016). Affinity proteomics discovers decreased levels of AMFR in plasma from Osteoporosis patients. *Proteomics Clin Appl, 10*(6), 681-690. doi:10.1002/prca.201400167

Qundos, U., Hong, M. G., Tybring, G., Divers, M., Odeberg, J., Uhlen, M., Nilsson, P., & Schwenk, J. M. (2013). Profiling post-centrifugation delay of serum and plasma with antibody bead arrays. *J Proteomics, 95*, 46-54. doi:10.1016/j.jprot.2013.04.020

R Core Team. (2020). R: A Language and Environment for Statistical Computing. *R Foundation for Statistical Computing*. Retrieved from https://www.R-project.org/

Rabinovici, G. D., Gatsonis, C., Apgar, C., Chaudhary, K., Gareen, I., Hanna, L., Hendrix, J., Hillner, B. E., Olson, C., Lesman-Segev, O. H., Romanoff, J., Siegel, B. A., Whitmer, R. A., & Carrillo,

M. C. (2019). Association of Amyloid Positron Emission Tomography With Subsequent Change in Clinical Management Among Medicare Beneficiaries With Mild Cognitive Impairment or Dementia. *Jama, 321*(13), 1286-1294. doi:10.1001/jama.2019.2000

Randolph, C., Tierney, M. C., Mohr, E., & Chase, T. N. (1998). The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS): preliminary clinical validity. *J Clin Exp Neuropsychol, 20*(3), 310-319. doi:10.1076/jcen.20.3.310.823

Rascovsky, K., Hodges, J. R., Knopman, D., Mendez, M. F., Kramer, J. H., Neuhaus, J., van Swieten, J. C., Seelaar, H., Dopper, E. G., Onyike, C. U., Hillis, A. E., Josephs, K. A., Boeve, B. F., Kertesz, A., Seeley, W. W., Rankin, K. P., Johnson, J. K., Gorno-Tempini, M. L., Rosen, H., Prioleau-Latham, C. E., Lee, A., Kipps, C. M., Lillo, P., Piguet, O., Rohrer, J. D., Rossor, M. N., Warren, J. D., Fox, N. C., Galasko, D., Salmon, D. P., Black, S. E., Mesulam, M., Weintraub, S., Dickerson, B. C., Diehl-Schmid, J., Pasquier, F., Deramecourt, V., Lebert, F., Pijnenburg, Y., Chow, T. W., Manes, F., Grafman, J., Cappa, S. F., Freedman, M., Grossman, M., & Miller, B. L. (2011). Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. *Brain, 134*(Pt 9), 2456-2477. doi:10.1093/brain/awr179

Razzaq, M., Iglesias, M. J., Ibrahim-Kosta, M., Goumidi, L., Soukarieh, O., Proust, C., Roux, M., Suchon, P., Boland, A., Daiain, D., Olaso, R., Havervall, S., Thalin, C., Butler, L., Deleuze, J. F., Odeberg, J., Morange, P. E., & Trégouët, D. A. (2021). An artificial neural network approach integrating plasma proteomics and genetic data identifies PLXNA4 as a new susceptibility locus for pulmonary embolism. *Sci Rep, 11*(1), 14015. doi:10.1038/s41598-021-93390-7

Remnestål, J., Just, D., Mitsios, N., Fredolini, C., Mulder, J., Schwenk, J. M., Uhlen, M., Kultima, K., Ingelsson, M., Kilander, L., Lannfelt, L., Svenningsson, P., Nellgard, B., Zetterberg, H., Blennow, K., Nilsson, P., & Häggmark-Månberg, A. (2016). CSF profiling of the human brain enriched proteome reveals associations of neuromodulin and neurogranin to Alzheimer's disease. *Proteomics Clin Appl, 10*(12), 1242-1253. doi:10.1002/prca.201500150

Remnestål, J., Öijerstedt, L., Ullgren, A., Olofsson, J., Bergström, S., Kultima, K., Ingelsson, M., Kilander, L., Uhlén, M., Månberg, A., Graff, C., & Nilsson, P. (2020). Altered levels of CSF proteins in patients with FTD, presymptomatic mutation carriers and non-carriers. *Transl Neurodegener, 9*(1), 27. doi:10.1186/s40035-020-00198-y

Reuterswärd, P., Bergström, S., Orikiiriza, J., Lindquist, E., Bergström, S., Andersson Svahn, H., Ayoglu, B., Uhlén, M., Wahlgren, M., Normark, J., Ribacke, U., & Nilsson, P. (2018). Levels of human proteins in plasma associated with acute paediatric malaria. *Malar J, 17*(1), 426. doi:10.1186/s12936-018-2576-y

Rissin, D. M., Kan, C. W., Campbell, T. G., Howes, S. C., Fournier, D. R., Song, L., Piech, T., Patel, P. P., Chang, L., Rivnak, A. J., Ferrell, E. P., Randall, J. D., Provuncher, G. K., Walt, D. R., & Duffy, D. C. (2010). Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. *Nat Biotechnol, 28*(6), 595-599. doi:10.1038/nbt.1641

Röhl, M., Tjernlund, A., Lajoie, J., Edfeldt, G., Bradley, F., Bergström, S., Kaldhusdal, V., Åhlberg, A., Månberg, A., Omollo, K., Boily-Larouche, G., Asghar, M., Kwon, D. S., Oyugi, J., Kimani, J., Nilsson, P., Fowke, K. R., & Broliden, K. (2021). HIV-Exposed Seronegative Sex Workers Express Low T-Cell Activation and an Intact Ectocervical Tissue Microenvironment. *Vaccines (Basel), 9*(3). doi:10.3390/vaccines9030217

Rohrer, J. D., Nicholas, J. M., Cash, D. M., van Swieten, J., Dopper, E., Jiskoot, L., van Minkelen, R., Rombouts, S. A., Cardoso, M. J., Clegg, S., Espak, M., Mead, S., Thomas, D. L., De Vita, E., Masellis, M., Black, S. E., Freedman, M., Keren, R., MacIntosh, B. J., Rogaeva, E., Tang-Wai, D., Tartaglia, M. C., Laforce, R., Jr., Tagliavini, F., Tiraboschi, P., Redaelli, V., Prioni, S., Grisoli, M., Borroni, B., Padovani, A., Galimberti, D., Scarpini, E., Arighi, A., Fumagalli, G., Rowe, J. B., Coyle-Gilchrist, I., Graff, C., Fallström, M., Jelic, V., Ståhlbom, A. K., Andersson,

C., Thonberg, H., Lilius, L., Frisoni, G. B., Pievani, M., Bocchetta, M., Benussi, L., Ghidoni, R., Finger, E., Sorbi, S., Nacmias, B., Lombardi, G., Polito, C., Warren, J. D., Ourselin, S., Fox, N. C., Rossor, M. N., & Binetti, G. (2015). Presymptomatic cognitive and neuroanatomical changes in genetic frontotemporal dementia in the Genetic Frontotemporal dementia Initiative (GENFI) study: a cross-sectional analysis. *Lancet Neurol, 14*(3), 253-262. doi:10.1016/s1474-4422(14)70324-2

Rojas, J. C., Wang, P., Staffaroni, A. M., Heller, C., Cobigo, Y., Wolf, A., Goh, S. M., Ljubenkov, P. A., Heuer, H. W., Fong, J. C., Taylor, J. B., Veras, E., Song, L., Jeromin, A., Hanlon, D., Yu, L., Khinikar, A., Sivasankaran, R., Kieloch, A., Valentin, M. A., Karydas, A. M., Mitic, L. L., Pearlman, R., Kornak, J., Kramer, J. H., Miller, B. L., Kantarci, K., Knopman, D. S., Graff-Radford, N., Petrucelli, L., Rademakers, R., Irwin, D. J., Grossman, M., Ramos, E. M., Coppola, G., Mendez, M. F., Bordelon, Y., Dickerson, B. C., Ghoshal, N., Huey, E. D., Mackenzie, I. R., Appleby, B. S., Domoto-Reilly, K., Hsiung, G. R., Toga, A. W., Weintraub, S., Kaufer, D. I., Kerwin, D., Litvan, I., Onyike, C. U., Pantelyat, A., Roberson, E. D., Tartaglia, M. C., Foroud, T., Chen, W., Czerkowicz, J., Graham, D. L., van Swieten, J. C., Borroni, B., Sanchez-Valle, R., Moreno, F., Laforce, R., Graff, C., Synofzik, M., Galimberti, D., Rowe, J. B., Masellis, M., Finger, E., Vandenberghe, R., de Mendonça, A., Tagliavini, F., Santana, I., Ducharme, S., Butler, C. R., Gerhard, A., Levin, J., Danek, A., Otto, M., Sorbi, S., Cash, D. M., Convery, R. S., Bocchetta, M., Foiani, M., Greaves, C. V., Peakman, G., Russell, L., Swift, I., Todd, E., Rohrer, J. D., Boeve, B. F., Rosen, H. J., & Boxer, A. L. (2021). Plasma Neurofilament Light for Prediction of Disease Progression in Familial Frontotemporal Lobar Degeneration. *Neurology, 96*(18), e2296-e2312. doi:10.1212/wnl.0000000000011848

Rydberg Sterner, T., Ahlner, F., Blennow, K., Dahlin-Ivanoff, S., Falk, H., Havstam Johansson, L., Hoff, M., Holm, M., Horder, H., Jacobsson, T., Johansson, B., Johansson, L., Kern, J., Kern, S., Machado, A., Mellqvist Fassberg, M., Nilsson, J., Ribbe, M., Rothenberg, E., Ryden, L., Sadeghi, A., Sacuiu, S., Samuelsson, J., Sigstrom, R., Skoog, J., Thorvaldsson, V., Waern, M., Westman, E., Wetterberg, H., Zetterberg, H., Zetterberg, M., Zettergren, A., Ostling, S., & Skoog, I. (2019). The Gothenburg H70 Birth cohort study 2014-16: design, methods and study population. *Eur J Epidemiol, 34*(2), 191-209. doi:10.1007/s10654-018-0459-8

Salloway, S., Sperling, R., Fox, N. C., Blennow, K., Klunk, W., Raskind, M., Sabbagh, M., Honig, L. S., Porsteinsson, A. P., Ferris, S., Reichert, M., Ketter, N., Nejadnik, B., Guenzler, V., Miloslavsky, M., Wang, D., Lu, Y., Lull, J., Tudor, I. C., Liu, E., Grundman, M., Yuen, E., Black, R., & Brashear, H. R. (2014). Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer's disease. *N Engl J Med, 370*(4), 322-333. doi:10.1056/NEJMoa1304839

Sanchez-Valle, R., Heslegrave, A., Foiani, M. S., Bosch, B., Antonell, A., Balasa, M., Llado, A., Zetterberg, H., & Fox, N. C. (2018). Serum neurofilament light levels correlate with severity measures and neurodegeneration markers in autosomal dominant Alzheimer's disease. *Alzheimers Res Ther, 10*(1), 113. doi:10.1186/s13195-018-0439-y

Sancho, L., Contreras, M., & Allen, N. J. (2021). Glia as sculptors of synaptic plasticity. *Neurosci Res, 167*, 17-29. doi:10.1016/j.neures.2020.11.005

Schindler, S. E., Bollinger, J. G., Ovod, V., Mawuenyega, K. G., Li, Y., Gordon, B. A., Holtzman, D. M., Morris, J. C., Benzinger, T. L. S., Xiong, C., Fagan, A. M., & Bateman, R. J. (2019). High-precision plasma beta-amyloid 42/40 predicts current and future brain amyloidosis. *Neurology*. doi:10.1212/wnl.0000000000008081

Schindler, S. E., Gray, J. D., Gordon, B. A., Xiong, C., Batrla-Utermann, R., Quan, M., Wahl, S., Benzinger, T. L. S., Holtzman, D. M., Morris, J. C., & Fagan, A. M. (2018). Cerebrospinal fluid biomarkers measured by Elecsys assays compared to amyloid imaging. *Alzheimers Dement, 14*(11), 1460-1469. doi:10.1016/j.jalz.2018.01.013

Schmidt, M. E., Chiao, P., Klein, G., Matthews, D., Thurfjell, L., Cole, P. E., Margolin, R., Landau, S., Foster, N. L., Mason, N. S., De Santi, S., Suhy, J., Koeppe, R. A., & Jagust, W. (2015). The influence of biological and technical factors on quantitative analysis of amyloid PET: Points to consider and recommendations for controlling variability in longitudinal data. *Alzheimers Dement, 11*(9), 1050-1068. doi:10.1016/j.jalz.2014.09.004

Schutzer, S. E., Liu, T., Natelson, B. H., Angel, T. E., Schepmoes, A. A., Purvine, S. O., Hixson, K. K., Lipton, M. S., Camp, D. G., Coyle, P. K., Smith, R. D., & Bergquist, J. (2010). Establishing the proteome of normal human cerebrospinal fluid. *PLoS One, 5*(6), e10980. doi:10.1371/journal.pone.0010980

Schwartzentruber, J., Cooper, S., Liu, J. Z., Barrio-Hernandez, I., Bello, E., Kumasaka, N., Young, A. M. H., Franklin, R. J. M., Johnson, T., Estrada, K., Gaffney, D. J., Beltrao, P., & Bassett, A. (2021). Genome-wide meta-analysis, fine-mapping and integrative prioritization implicate new Alzheimer's disease risk genes. *Nat Genet, 53*(3), 392-402. doi:10.1038/s41588-020-00776-w

Schwenk, J. M., Gry, M., Rimini, R., Uhlen, M., & Nilsson, P. (2008). Antibody suspension bead arrays within serum proteomics. *J Proteome Res, 7*(8), 3168-3179. doi:10.1021/pr700890b

Schwenk, J. M., Igel, U., Kato, B. S., Nicholson, G., Karpe, F., Uhlen, M., & Nilsson, P. (2010a). Comparative protein profiling of serum and plasma using an antibody suspension bead array approach. *Proteomics, 10*(3), 532-540. doi:10.1002/pmic.200900657

Schwenk, J. M., Igel, U., Neiman, M., Langen, H., Becker, C., Bjartell, A., Ponten, F., Wiklund, F., Gronberg, H., Nilsson, P., & Uhlen, M. (2010b). Toward next generation plasma profiling via heat-induced epitope retrieval and array-based assays. *Mol Cell Proteomics, 9*(11), 2497-2507. doi:10.1074/mcp.M110.001560

Schwenk, J. M., & Nilsson, P. (2011). Antibody suspension bead arrays. *Methods Mol Biol, 723*, 29-36. doi:10.1007/978-1-61779-043-0_3

Sevigny, J., Suhy, J., Chiao, P., Chen, T., Klein, G., Purcell, D., Oh, J., Verma, A., Sampat, M., & Barakos, J. (2016). Amyloid PET Screening for Enrichment of Early-Stage Alzheimer Disease Clinical Trials: Experience in a Phase 1b Clinical Trial. *Alzheimer Dis Assoc Disord, 30*(1), 1-7. doi:10.1097/wad.0000000000000144

Sjoberg, R., Mattsson, C., Andersson, E., Hellstrom, C., Uhlen, M., Schwenk, J. M., Ayoglu, B., & Nilsson, P. (2016). Exploration of high-density protein microarrays for antibody validation and autoimmunity profiling. *N Biotechnol, 33*(5 Pt A), 582-592. doi:10.1016/j.nbt.2015.09.002

Sjoberg, R., Sundberg, M., Gundberg, A., Sivertsson, A., Schwenk, J. M., Uhlen, M., & Nilsson, P. (2012). Validation of affinity reagents using antigen microarrays. *N Biotechnol, 29*(5), 555-563. doi:10.1016/j.nbt.2011.11.009

Sjöstedt, E., Zhong, W., Fagerberg, L., Karlsson, M., Mitsios, N., Adori, C., Oksvold, P., Edfors, F., Limiszewska, A., Hikmet, F., Huang, J., Du, Y., Lin, L., Dong, Z., Yang, L., Liu, X., Jiang, H., Xu, X., Wang, J., Yang, H., Bolund, L., Mardinoglu, A., Zhang, C., von Feilitzen, K., Lindskog, C., Pontén, F., Luo, Y., Hökfelt, T., Uhlén, M., & Mulder, J. (2020). An atlas of the protein-coding genes in the human, pig, and mouse brain. *Science, 367*(6482). doi:10.1126/science.aay5947

Skogs, M., Stadler, C., Schutten, R., Hjelmare, M., Gnann, C., Björk, L., Poser, I., Hyman, A., Uhlén, M., & Lundberg, E. (2017). Antibody Validation in Bioimaging Applications Based on Endogenous Expression of Tagged Proteins. *J Proteome Res, 16*(1), 147-155. doi:10.1021/acs.jproteome.6b00821

Skorvanek, M., Goldman, J. G., Jahanshahi, M., Marras, C., Rektorova, I., Schmand, B., van Duijn, E., Goetz, C. G., Weintraub, D., Stebbins, G. T., & Martinez-Martin, P. (2018). Global scales for cognitive screening in Parkinson's disease: Critique and recommendations. *Mov Disord, 33*(2), 208-218. doi:10.1002/mds.27233

Smith, L. M., & Kelleher, N. L. (2013). Proteoform: a single term describing protein complexity. *Nat Methods, 10*(3), 186-187. doi:10.1038/nmeth.2369

Sperling, R. A., Aisen, P. S., Beckett, L. A., Bennett, D. A., Craft, S., Fagan, A. M., Iwatsubo, T., Jack, C. R., Jr., Kaye, J., Montine, T. J., Park, D. C., Reiman, E. M., Rowe, C. C., Siemers, E., Stern, Y., Yaffe, K., Carrillo, M. C., Thies, B., Morrison-Bogorad, M., Wagster, M. V., & Phelps, C. H. (2011). Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement, 7*(3), 280-292. doi:10.1016/j.jalz.2011.03.003

Sperling, R. A., Rentz, D. M., Johnson, K. A., Karlawish, J., Donohue, M., Salmon, D. P., & Aisen, P. (2014). The A4 study: stopping AD before symptoms begin? *Sci Transl Med, 6*(228), 228fs213. doi:10.1126/scitranslmed.3007941

Stadler, C., Hjelmare, M., Neumann, B., Jonasson, K., Pepperkok, R., Uhlén, M., & Lundberg, E. (2012). Systematic validation of antibody binding and protein subcellular localization using siRNA and confocal microscopy. *J Proteomics, 75*(7), 2236-2251. doi:10.1016/j.jprot.2012.01.030

Stoudt, S., Vásquez, V. N., & Martinez, C. C. (2021). Principles for data analysis workflows. *PLoS Comput Biol, 17*(3), e1008770. doi:10.1371/journal.pcbi.1008770

Strandberg, K., Ayoglu, B., Roos, A., Reza, M., Niks, E., Signorelli, M., Fasterius, E., Pontén, F., Lochmüller, H., Domingos, J., Ala, P., Muntoni, F., Aartsma-Rus, A., Spitali, P., Nilsson, P., & Szigyarto, C. A. (2020). Blood-derived biomarkers correlate with clinical progression in Duchenne muscular dystrophy. *J Neuromuscul Dis, 7*(3), 231-246. doi:10.3233/jnd-190454

Strong, M. J., Abrahams, S., Goldstein, L. H., Woolley, S., McLaughlin, P., Snowden, J., Mioshi, E., Roberts-South, A., Benatar, M., HortobáGyi, T., Rosenfeld, J., Silani, V., Ince, P. G., & Turner, M. R. (2017). Amyotrophic lateral sclerosis - frontotemporal spectrum disorder (ALS-FTSD): Revised diagnostic criteria. *Amyotroph Lateral Scler Frontotemporal Degener, 18*(3-4), 153-174. doi:10.1080/21678421.2016.1267768

Strozyk, D., Blennow, K., White, L. R., & Launer, L. J. (2003). CSF Abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study. *Neurology, 60*(4), 652-656. doi:10.1212/01.wnl.0000046581.81650.d0

Suárez-Calvet, M., Karikari, T. K., Ashton, N. J., Lantero Rodríguez, J., Milà-Alomà, M., Gispert, J. D., Salvadó, G., Minguillon, C., Fauria, K., Shekari, M., Grau-Rivera, O., Arenaza-Urquijo, E. M., Sala-Vila, A., Sánchez-Benavides, G., González-de-Echávarri, J. M., Kollmorgen, G., Stoops, E., Vanmechelen, E., Zetterberg, H., Blennow, K., & Molinuevo, J. L. (2020). Novel tau biomarkers phosphorylated at T181, T217 or T231 rise in the initial stages of the preclinical Alzheimer's continuum when only subtle changes in Aβ pathology are detected. *EMBO Mol Med, 12*(12), e12921. doi:10.15252/emmm.202012921

Svenningsson, P., Westman, E., Ballard, C., & Aarsland, D. (2012). Cognitive impairment in patients with Parkinson's disease: diagnosis, biomarkers, and treatment. *Lancet Neurol, 11*(8), 697-707. doi:10.1016/s1474-4422(12)70152-7

Swift, I. J., Sogorb-Esteve, A., Heller, C., Synofzik, M., Otto, M., Graff, C., Galimberti, D., Todd, E., Heslegrave, A. J., van der Ende, E. L., Van Swieten, J. C., Zetterberg, H., & Rohrer, J. D. (2021). Fluid biomarkers in frontotemporal dementia: past, present and future. *J Neurol Neurosurg Psychiatry, 92*(2), 204-215. doi:10.1136/jnnp-2020-323520

Taoufik, E., Kouroupi, G., Zygogianni, O., & Matsas, R. (2018). Synaptic dysfunction in neurodegenerative and neurodevelopmental diseases: an overview of induced pluripotent stem-cell-based disease models. *Open Biol, 8*(9). doi:10.1098/rsob.180138

Tatebe, H., Kasai, T., Ohmichi, T., Kishi, Y., Kakeya, T., Waragai, M., Kondo, M., Allsop, D., & Tokuda, T. (2017). Quantification of plasma phosphorylated tau to use as a biomarker for brain

Alzheimer pathology: pilot case-control studies including patients with Alzheimer's disease and down syndrome. *Mol Neurodegener, 12*(1), 63. doi:10.1186/s13024-017-0206-8

Taussig, M. J., Fonseca, C., & Trimmer, J. S. (2018). Antibody validation: a view from the mountains. *N Biotechnol, 45*, 1-8. doi:10.1016/j.nbt.2018.08.002

The Food and Drug Administration (FDA). (2021). FDA Grants Accelerated Approval for Alzheimer's Drug [Press release]. Retrieved from https://www.fda.gov/news-events/press-announcements/fda-grants-accelerated-approval-alzheimers-drug

Thijssen, E. H., La Joie, R., Strom, A., Fonseca, C., Iaccarino, L., Wolf, A., Spina, S., Allen, I. E., Cobigo, Y., Heuer, H., VandeVrede, L., Proctor, N. K., Lago, A. L., Baker, S., Sivasankaran, R., Kieloch, A., Kinhikar, A., Yu, L., Valentin, M. A., Jeromin, A., Zetterberg, H., Hansson, O., Mattsson-Carlgren, N., Graham, D., Blennow, K., Kramer, J. H., Grinberg, L. T., Seeley, W. W., Rosen, H., Boeve, B. F., Miller, B. L., Teunissen, C. E., Rabinovici, G. D., Rojas, J. C., Dage, J. L., & Boxer, A. L. (2021). Plasma phosphorylated tau 217 and phosphorylated tau 181 as biomarkers in Alzheimer's disease and frontotemporal lobar degeneration: a retrospective diagnostic performance study. *Lancet Neurol, 20*(9), 739-752. doi:10.1016/s1474-4422(21)00214-3

Thijssen, E. H., La Joie, R., Wolf, A., Strom, A., Wang, P., Iaccarino, L., Bourakova, V., Cobigo, Y., Heuer, H., Spina, S., VandeVrede, L., Chai, X., Proctor, N. K., Airey, D. C., Shcherbinin, S., Duggan Evans, C., Sims, J. R., Zetterberg, H., Blennow, K., Karydas, A. M., Teunissen, C. E., Kramer, J. H., Grinberg, L. T., Seeley, W. W., Rosen, H., Boeve, B. F., Miller, B. L., Rabinovici, G. D., Dage, J. L., Rojas, J. C., & Boxer, A. L. (2020). Diagnostic value of plasma phosphorylated tau181 in Alzheimer's disease and frontotemporal lobar degeneration. *Nat Med, 26*(3), 387-397. doi:10.1038/s41591-020-0762-2

Thompson, E. J. (1995). Cerebrospinal fluid. *J Neurol Neurosurg Psychiatry, 59*(4), 349-357. doi:10.1136/jnnp.59.4.349

Thul, P. J., Akesson, L., Wiking, M., Mahdessian, D., Geladaki, A., Ait Blal, H., Alm, T., Asplund, A., Bjork, L., Breckels, L. M., Backstrom, A., Danielsson, F., Fagerberg, L., Fall, J., Gatto, L., Gnann, C., Hober, S., Hjelmare, M., Johansson, F., Lee, S., Lindskog, C., Mulder, J., Mulvey, C. M., Nilsson, P., Oksvold, P., Rockberg, J., Schutten, R., Schwenk, J. M., Sivertsson, A., Sjostedt, E., Skogs, M., Stadler, C., Sullivan, D. P., Tegel, H., Winsnes, C., Zhang, C., Zwahlen, M., Mardinoglu, A., Ponten, F., von Feilitzen, K., Lilley, K. S., Uhlen, M., & Lundberg, E. (2017). A subcellular map of the human proteome. *Science, 356*(6340). doi:10.1126/science.aal3321

Tibshirani, R. (1996). Regression shrinkage and selection via the lasso. *Journal of the Royal Statistical Society (Series B), 58*, 267-288.

Torres, V. I., Vallejo, D., & Inestrosa, N. C. (2017). Emerging Synaptic Molecules as Candidates in the Etiology of Neurological Disorders. *Neural Plast, 2017*, 8081758. doi:10.1155/2017/8081758

Trozzi, F., Wang, X., & Tao, P. (2021). UMAP as a Dimensionality Reduction Tool for Molecular Dynamics Simulations of Biomacromolecules: A Comparison Study. *J Phys Chem B, 125*(19), 5022-5034. doi:10.1021/acs.jpcb.1c02081

Tworoger, S. S., & Hankinson, S. E. (2006). Use of biomarkers in epidemiologic studies: minimizing the influence of measurement error in the study design and analysis. *Cancer Causes Control, 17*(7), 889-899. doi:10.1007/s10552-006-0035-5

Uhlén, M., Bandrowski, A., Carr, S., Edwards, A., Ellenberg, J., Lundberg, E., Rimm, D. L., Rodriguez, H., Hiltke, T., Snyder, M., & Yamamoto, T. (2016). A proposal for validation of antibodies. *Nat Methods, 13*(10), 823-827. doi:10.1038/nmeth.3995

Uhlén, M., Björling, E., Agaton, C., Szigyarto, C. A., Amini, B., Andersen, E., Andersson, A. C., Angelidou, P., Asplund, A., Asplund, C., Berglund, L., Bergström, K., Brumer, H., Cerjan, D., Ekström, M., Elobeid, A., Eriksson, C., Fagerberg, L., Falk, R., Fall, J., Forsberg, M.,

Björklund, M. G., Gumbel, K., Halimi, A., Hallin, I., Hamsten, C., Hansson, M., Hedhammar, M., Hercules, G., Kampf, C., Larsson, K., Lindskog, M., Lodewyckx, W., Lund, J., Lundeberg, J., Magnusson, K., Malm, E., Nilsson, P., Odling, J., Oksvold, P., Olsson, I., Oster, E., Ottosson, J., Paavilainen, L., Persson, A., Rimini, R., Rockberg, J., Runeson, M., Sivertsson, A., Sköllermo, A., Steen, J., Stenvall, M., Sterky, F., Strömberg, S., Sundberg, M., Tegel, H., Tourle, S., Wahlund, E., Waldén, A., Wan, J., Wernérus, H., Westberg, J., Wester, K., Wrethagen, U., Xu, L. L., Hober, S., & Pontén, F. (2005). A human protein atlas for normal and cancer tissues based on antibody proteomics. *Mol Cell Proteomics, 4*(12), 1920-1932. doi:10.1074/mcp.M500279-MCP200

Uhlén, M., Fagerberg, L., Hallstrom, B. M., Lindskog, C., Oksvold, P., Mardinoglu, A., Sivertsson, A., Kampf, C., Sjostedt, E., Asplund, A., Olsson, I., Edlund, K., Lundberg, E., Navani, S., Szigyarto, C. A., Odeberg, J., Djureinovic, D., Takanen, J. O., Hober, S., Alm, T., Edqvist, P. H., Berling, H., Tegel, H., Mulder, J., Rockberg, J., Nilsson, P., Schwenk, J. M., Hamsten, M., von Feilitzen, K., Forsberg, M., Persson, L., Johansson, F., Zwahlen, M., von Heijne, G., Nielsen, J., & Ponten, F. (2015). Tissue-based map of the human proteome. *Science, 347*(6220), 1260419. doi:10.1126/science.1260419

Uhlén, M., Karlsson, M. J., Hober, A., Svensson, A. S., Scheffel, J., Kotol, D., Zhong, W., Tebani, A., Strandberg, L., Edfors, F., Sjöstedt, E., Mulder, J., Mardinoglu, A., Berling, A., Ekblad, S., Dannemeyer, M., Kanje, S., Rockberg, J., Lundqvist, M., Malm, M., Volk, A. L., Nilsson, P., Månberg, A., Dodig-Crnkovic, T., Pin, E., Zwahlen, M., Oksvold, P., von Feilitzen, K., Häussler, R. S., Hong, M. G., Lindskog, C., Ponten, F., Katona, B., Vuu, J., Lindström, E., Nielsen, J., Robinson, J., Ayoglu, B., Mahdessian, D., Sullivan, D., Thul, P., Danielsson, F., Stadler, C., Lundberg, E., Bergström, G., Gummesson, A., Voldborg, B. G., Tegel, H., Hober, S., Forsström, B., Schwenk, J. M., Fagerberg, L., & Sivertsson, Å. (2019a). The human secretome. *Sci Signal, 12*(609). doi:10.1126/scisignal.aaz0274

Uhlén, M., Karlsson, M. J., Zhong, W., Tebani, A., Pou, C., Mikes, J., Lakshmikanth, T., Forsström, B., Edfors, F., Odeberg, J., Mardinoglu, A., Zhang, C., von Feilitzen, K., Mulder, J., Sjöstedt, E., Hober, A., Oksvold, P., Zwahlen, M., Ponten, F., Lindskog, C., Sivertsson, Å., Fagerberg, L., & Brodin, P. (2019b). A genome-wide transcriptomic analysis of protein-coding genes in human blood cells. *Science, 366*(6472). doi:10.1126/science.aax9198

Uhlén, M., Oksvold, P., Fagerberg, L., Lundberg, E., Jonasson, K., Forsberg, M., Zwahlen, M., Kampf, C., Wester, K., Hober, S., Wernerus, H., Björling, L., & Ponten, F. (2010). Towards a knowledge-based Human Protein Atlas. *Nat Biotechnol, 28*(12), 1248-1250. doi:10.1038/nbt1210-1248

Uhlén, M., Zhang, C., Lee, S., Sjöstedt, E., Fagerberg, L., Bidkhori, G., Benfeitas, R., Arif, M., Liu, Z., Edfors, F., Sanli, K., von Feilitzen, K., Oksvold, P., Lundberg, E., Hober, S., Nilsson, P., Mattsson, J., Schwenk, J. M., Brunnstrom, H., Glimelius, B., Sjoblom, T., Edqvist, P. H., Djureinovic, D., Micke, P., Lindskog, C., Mardinoglu, A., & Ponten, F. (2017). A pathology atlas of the human cancer transcriptome. *Science, 357*(6352). doi:10.1126/science.aan2507

Van Cauwenberghe, C., Van Broeckhoven, C., & Sleegers, K. (2016). The genetic landscape of Alzheimer disease: clinical implications and perspectives. *Genet Med, 18*(5), 421-430. doi:10.1038/gim.2015.117

van der Ende, E. L., Meeter, L. H., Poos, J. M., Panman, J. L., Jiskoot, L. C., Dopper, E. G. P., Papma, J. M., de Jong, F. J., Verberk, I. M. W., Teunissen, C., Rizopoulos, D., Heller, C., Convery, R. S., Moore, K. M., Bocchetta, M., Neason, M., Cash, D. M., Borroni, B., Galimberti, D., Sanchez-Valle, R., Laforce, R., Jr., Moreno, F., Synofzik, M., Graff, C., Masellis, M., Carmela Tartaglia, M., Rowe, J. B., Vandenberghe, R., Finger, E., Tagliavini, F., de Mendonca, A., Santana, I., Butler, C., Ducharme, S., Gerhard, A., Danek, A., Levin, J., Otto, M., Frisoni, G. B., Cappa, S., Pijnenburg, Y. A. L., Rohrer, J. D., & van Swieten, J. C. (2019a). Serum

neurofilament light chain in genetic frontotemporal dementia: a longitudinal, multicentre cohort study. *Lancet Neurol, 18*(12), 1103-1111. doi:10.1016/s1474-4422(19)30354-0

van der Ende, E. L., Meeter, L. H., Stingl, C., van Rooij, J. G. J., Stoop, M. P., Nijholt, D. A. T., Sanchez-Valle, R., Graff, C., Öijerstedt, L., Grossman, M., McMillan, C., Pijnenburg, Y. A. L., Laforce, R., Jr., Binetti, G., Benussi, L., Ghidoni, R., Luider, T. M., Seelaar, H., & van Swieten, J. C. (2019b). Novel CSF biomarkers in genetic frontotemporal dementia identified by proteomics. *Ann Clin Transl Neurol, 6*(4), 698-707. doi:10.1002/acn3.745

van der Ende, E. L., Xiao, M., Xu, D., Poos, J. M., Panman, J. L., Jiskoot, L. C., Meeter, L. H., Dopper, E. G., Papma, J. M., Heller, C., Convery, R., Moore, K., Bocchetta, M., Neason, M., Peakman, G., Cash, D. M., Teunissen, C. E., Graff, C., Synofzik, M., Moreno, F., Finger, E., Sánchez-Valle, R., Vandenberghe, R., Laforce, R., Jr., Masellis, M., Tartaglia, M. C., Rowe, J. B., Butler, C. R., Ducharme, S., Gerhard, A., Danek, A., Levin, J., Pijnenburg, Y. A., Otto, M., Borroni, B., Tagliavini, F., de Mendonca, A., Santana, I., Galimberti, D., Seelaar, H., Rohrer, J. D., Worley, P. F., & van Swieten, J. C. (2020). Neuronal pentraxin 2: a synapse-derived CSF biomarker in genetic frontotemporal dementia. *J Neurol Neurosurg Psychiatry, 91*(6), 612-621. doi:10.1136/jnnp-2019-322493

Van Regenmortel, M. H. (2009). What is a B-cell epitope? *Methods Mol Biol, 524*, 3-20. doi:10.1007/978-1-59745-450-6_1

van Steenoven, I., Koel-Simmelink, M. J. A., Vergouw, L. J. M., Tijms, B. M., Piersma, S. R., Pham, T. V., Bridel, C., Ferri, G. L., Cocco, C., Noli, B., Worley, P. F., Xiao, M. F., Xu, D., Oeckl, P., Otto, M., van der Flier, W. M., de Jong, F. J., Jimenez, C. R., Lemstra, A. W., & Teunissen, C. E. (2020). Identification of novel cerebrospinal fluid biomarker candidates for dementia with Lewy bodies: a proteomic approach. *Mol Neurodegener, 15*(1), 36. doi:10.1186/s13024-020-00388-2

Vanderstichele, H. M., Janelidze, S., Demeyer, L., Coart, E., Stoops, E., Herbst, V., Mauroo, K., Brix, B., & Hansson, O. (2016). Optimized Standard Operating Procedures for the Analysis of Cerebrospinal Fluid Aβ42 and the Ratios of Aβ Isoforms Using Low Protein Binding Tubes. *J Alzheimers Dis, 53*(3), 1121-1132. doi:10.3233/jad-160286

Verberk, I. M. W., Thijssen, E., Koelewijn, J., Mauroo, K., Vanbrabant, J., de Wilde, A., Zwan, M. D., Verfaillie, S. C. J., Ossenkoppele, R., Barkhof, F., van Berckel, B. N. M., Scheltens, P., van der Flier, W. M., Stoops, E., Vanderstichele, H. M., & Teunissen, C. E. (2020). Combination of plasma amyloid beta((1-42/1-40)) and glial fibrillary acidic protein strongly associates with cerebral amyloid pathology. *Alzheimers Res Ther, 12*(1), 118. doi:10.1186/s13195-020-00682-7

Villemagne, V. L., Fodero-Tavoletti, M. T., Masters, C. L., & Rowe, C. C. (2015). Tau imaging: early progress and future directions. *Lancet Neurol, 14*(1), 114-124. doi:10.1016/s1474-4422(14)70252-2

Vlassenko, A. G., McCue, L., Jasielec, M. S., Su, Y., Gordon, B. A., Xiong, C., Holtzman, D. M., Benzinger, T. L., Morris, J. C., & Fagan, A. M. (2016). Imaging and cerebrospinal fluid biomarkers in early preclinical alzheimer disease. *Ann Neurol, 80*(3), 379-387. doi:10.1002/ana.24719

Wang, M. J., Yi, S., Han, J. Y., Park, S. Y., Jang, J. W., Chun, I. K., Giau, V. V., Bagyinszky, E., Lim, K. T., Kang, S. M., An, S. S., Park, Y. H., Youn, Y. C., & Kim, S. (2016). Analysis of Cerebrospinal Fluid and [11C]PIB PET Biomarkers for Alzheimer's Disease with Updated Protocols. *J Alzheimers Dis, 52*(4), 1403-1413. doi:10.3233/jad-160143

Weston, P. S. J., Poole, T., O'Connor, A., Heslegrave, A., Ryan, N. S., Liang, Y., Druyeh, R., Mead, S., Blennow, K., Schott, J. M., Frost, C., Zetterberg, H., & Fox, N. C. (2019). Longitudinal measurement of serum neurofilament light in presymptomatic familial Alzheimer's disease. *Alzheimers Res Ther, 11*(1), 19. doi:10.1186/s13195-019-0472-5

Weston, P. S. J., Poole, T., Ryan, N. S., Nair, A., Liang, Y., Macpherson, K., Druyeh, R., Malone, I. B., Ahsan, R. L., Pemberton, H., Klimova, J., Mead, S., Blennow, K., Rossor, M. N., Schott, J. M., Zetterberg, H., & Fox, N. C. (2017). Serum neurofilament light in familial Alzheimer disease: A marker of early neurodegeneration. *Neurology, 89*(21), 2167-2175. doi:10.1212/wnl.0000000000004667

Willemse, E., van Uffelen, K., Brix, B., Engelborghs, S., Vanderstichele, H., & Teunissen, C. (2017). How to handle adsorption of cerebrospinal fluid amyloid β (1-42) in laboratory practice? Identifying problematic handlings and resolving the issue by use of the Aβ(42)/Aβ(40) ratio. *Alzheimers Dement, 13*(8), 885-892. doi:10.1016/j.jalz.2017.01.010

Xie, Y. (2015). *Dynamic Documents with R and knitr* (2nd ed.). Boca Raton, Florida: Chapman and Hall/CRC.

Xie, Y., Allaire, J. J., & Grolemund, G. (2018). *R Markdown: The Definitive Guide*: Chapman and Hall/CRC.

Xie, Y., Dervieux, C., & Riederer, E. (2020). *R Markdown Cookbook*: Chapman and Hall/CRC.

Zadikoff, C., Fox, S. H., Tang-Wai, D. F., Thomsen, T., de Bie, R. M., Wadia, P., Miyasaki, J., Duff-Canning, S., Lang, A. E., & Marras, C. (2008). A comparison of the mini mental state exam to the Montreal cognitive assessment in identifying cognitive deficits in Parkinson's disease. *Mov Disord, 23*(2), 297-299. doi:10.1002/mds.21837

Zetterberg, H. (2017). Review: Tau in biofluids - relation to pathology, imaging and clinical features. *Neuropathol Appl Neurobiol, 43*(3), 194-199. doi:10.1111/nan.12378

Zetterberg, H. (2019a). Blood-based biomarkers for Alzheimer's disease-An update. *J Neurosci Methods, 319*, 2-6. doi:10.1016/j.jneumeth.2018.10.025

Zetterberg, H., & Blennow, K. (2021). Moving fluid biomarkers for Alzheimer's disease from research tools to routine clinical diagnostics. *Mol Neurodegener, 16*(1), 10. doi:10.1186/s13024-021-00430-x

Zetterberg, H., Mörtberg, E., Song, L., Chang, L., Provuncher, G. K., Patel, P. P., Ferrell, E., Fournier, D. R., Kan, C. W., Campbell, T. G., Meyer, R., Rivnak, A. J., Pink, B. A., Minnehan, K. A., Piech, T., Rissin, D. M., Duffy, D. C., Rubertsson, S., Wilson, D. H., & Blennow, K. (2011). Hypoxia due to cardiac arrest induces a time-dependent increase in serum amyloid β levels in humans. *PLoS One, 6*(12), e28263. doi:10.1371/journal.pone.0028263

Zetterberg, H., van Swieten, J. C., Boxer, A. L., & Rohrer, J. D. (2019b). Review: Fluid biomarkers for frontotemporal dementias. *Neuropathol Appl Neurobiol, 45*(1), 81-87. doi:10.1111/nan.12530

Zetterberg, H., Wilson, D., Andreasson, U., Minthon, L., Blennow, K., Randall, J., & Hansson, O. (2013). Plasma tau levels in Alzheimer's disease. *Alzheimers Res Ther, 5*(2), 9. doi:10.1186/alzrt163