Chapter 7

Appendices

References

Abbreviations

English summary

Nederlandse samenvatting

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Abbreviations

AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
APOE	Apolipoprotein E
APP	Amyloid-β precursor protein
APPtg	APP transgenic
ARM	Activated response microglia
ATM	Axonal tract-associated microglia
ATP	Adenosine triphosphate
BBB	Blood brain barrier
BCSFB	Blood cerebrospinal fluid barrier
BDNF	Brain derived neurotrophic factor
BLMB	Blood leptomeningeal barrier
BSA	Bovine serum albumin
C9orf72	Chromosome 9 open reading frame 72
CA	Cornu ammonis
CAA	Cerebral amyloid angiopathy
CAM	CNS-associated macrophage
CLDN5	Claudin-5
CNS	Central nervous system
COP	Commited oligodendrocyte precursor
CPM	Counts per million
CSF	Cerebrospinal fluid
CTR	Control
CWM	Control white matter
DAM	Disease-associated microglia
DE	Differential expression
DEG	Differentially expressed gene
DG	Dentate gyrus
EAE	Experimental autoimmune encephalomyelitis
EC	Enthorinal cortex
EGLN1	Egl-9 family hypoxia inducible factor 1
EOAD	Early-onset Alzheimer's disease

EWCE	Expression weighted cell type enrichment
FACS	Fluorescence-activated cell sorting
FANS	Fluorescence-activated nuclei sorting
FC	Frontal cortex
FLT1	Fms related receptor tyrosine kinase 1
FPKM	Fragments per kilobase of transcript per million
FTD	Frontotemporal dementia
MS	Multiple sclerosis
FTD-GRN	Progranulin-associated frontotemporal dementia
GABA	Gamma-aminobutyric acid
GDNF	Glial derived neurotrophic factor
GM	Grey matter
GRN	Progranulin
GWAS	Genome wide association studies
HIF1	Hypoxia inducible factor 1
HLA-DR	Major histocompatibility complex class II, DR alpha
HSP	Heat shock protein
HVG	Highly variable gene
IEG	Immediate-early gene
IGF	Insulin-like growth factor
IHC	Immunohistochemistry
ISF	Interstitial fluid
ISS	In situ sequencing
LOAD	Late-onset Alzheimer's disease
LPS	Lipopolysaccharide
LRP1	LDL receptor related protein 1
MAPT	Microtubule associated protein tau
MFO	Myelin forming oligodendrocyte
MOG	Myelin oligodendrocyte glycoprotein
MOGAD	MOG-associated disease
MOL	Mature oligodendrocyte
MSC	Mesenchymal stem cell
NAWM	Normal appearing white matter

NBB	Netherlands brain bank
NCL	Neuronal ceroid lipofuscinosis
NFO	Newly formed oligodendrocyte
NMOSD	Neuromyelitis optica spectrum disorder
NS	Normal serum
OC	Occipital cortex
OPC	Oligodendrocyte progenitor cell
OTC	Occipitotemporal cortex
PCA	Principal component analysis
PlGF	Placental growth factor
PLP1	Proteolipid protein 1
PSEN	Presenilin-1
PSENtg	PSEN transgenic
PTN	Pleiotrophin
RNAseq	RNA-sequencing
scRNAseq	Single-cell RNA-sequencing
SMC	Smooth muscle cell
snRNAseq	Single-nucleus RNA-sequencing
ST	Spatial transcriptomics
TAUtg	Tau transgenic
TBI	Traumatic brain injury
TC	Temporal cortex
UMAP	Uniform manifold approximation and projection
UMI	Unique molecular identifier
WAM	White matter-associated microglia
WGCNA	Weighted gene coexpression network analysis
WM	White matter
WML	White matter lesion

English summary

The brain is a complex organ composed of a wide variety of cell types with intricate cellular organization, that is far from being completely understood. The human brain comprises over 100 billion neurons with an even larger number of supporting cells (glia) and a vascular architecture that is comprised of multiple distinct cell types. Understanding heterogeneity of the cells in the healthy brain and how these are affected by diseases will help reveal new targets for future therapies and biomarkers to monitor the disease. In this thesis, we studied cellular heterogeneity of the brain in relation to neurodegenerative diseases, in particular multiple sclerosis (MS), Alzheimer's disease (AD) and frontotemporal dementia (FTD).

Chapter 1 provides an overview of cell types in the brain and how these are affected by neurodegenerative diseases. Largely, cell types in the healthy brain can be divided in to neurons, oligodendrocytes, astrocytes, microglia and CNS-associated macrophages (CAMs), and distinct cell types that comprise the brain's vasculature, including endothelial cells, pericytes, smooth muscle cells and fibroblasts. Neurodegenerative diseases are characterized by neurodegeneration, however often the disease mechanisms initially affect other cell types, and neuronal damage occurs as a secondary effect. For example, in MS it is known that oligodendrocytes are severely affected, resulting in loss of myelin that covers and protects axons, making the axons prone to damage and degeneration. In MS and AD, a significant role for microglia has been implied by genetic studies and dysfunction of these cells may possibly underlie neurodegeneration in these diseases. In FTD, studies with mouse models point towards microglia activation underlying neurodegeneration. However, in this thesis we show that this is not the case in humans.

In **chapter 2**, we studied brain tissues from MS donors. MS is a neurodegenerative disease, characterized by demyelinated lesions in the CNS, ultimately resulting in damage to axons which leads to cognitive problems, blindness, impaired motor function or even paralysis. We performed RNAseq on three groups of tissues: 1) white matter from control donors (CWM); 2) normal-appearing white matter (NAWM) from MS donors (non-demyelinated); 3) white matter lesions (WML) from MS donors. Between NAWM and WML tissues, most differentially expressed genes associated with myelination and inflammation, likely caused by the extensive demyelination that is present in WMLs and infiltration of immune cells. When comparing NAWM with CWM tissues, inflammation and stress pathways were enriched that pointed towards microglia and CAMs, indicating that these cells show reactive changes independent of demyelination. To investigate how microglia/CAMs are affected in MS tissues, we performed scRNAseq on $CD45^{pos}CD11B^{pos}$ cells isolated from brain tissues from five MS donors. Of each donor, three brain regions were studied: 1) normal-appearing cortical tissue (NACT) which consisted of both grey and white matter; 2) NAWM; 3) WML. We identified eight subtypes of microglia/CAMs. One of these clusters showed enriched expression of immediate-early genes and heat shock proteins, and was particularly abundant in NAWM samples. Another cluster, that was enriched in WMLs, showed a typical activated/phagocytic microglia profile, that was similar to those described during developmental myelinogenesis. To confirm that this microglia phenotype arose in response to demyelination, we performed scRNAseq of microglia isolated from brains from mice fed with cuprizone. Indeed, we observed that a similar activated/phagocytic profile arose after demyelination, and disappeared in remyelinated mouse brains. These data indicate that microglia respond

to demyelination by adopting an activated/phagocytic profile, possibly as an attempt to stimulate (re)myelination. Thus, we showed that brain macrophages adopt distinct profiles in myelinated (NAWM) and demyelinated (WML) MS brain tissues, possibly representing an early stress response to MS pathology in NAWM and an activated/phagocytic phenotype accompanying demyelination.

Despite the valuable findings in **chapter 2**, there were also some disadvantages to the study design. Due to the dependance on fresh brain tissues for the cell isolation, we were able to include only five donors for scRNAseq. As an alternative approach, in **chapter 3** we set up a protocol to isolate nuclei from frozen brain tissues followed by snRNAseq. We validated, for microglia, that the nuclear transcriptome is a good proxy for the cellular transcriptome. Studying nuclei instead of cells allows to use archived, well characterized frozen brain tissues. These tissues can be obtained from brain banks and studied in large numbers at a time, avoiding confounding batch effects.

As a first large-scale application of snRNAseq in the lab, in **chapter 4** we studied brain tissues from CTR and late onset AD (LOAD) donors. AD is the most common type of dementia, and is neuropathologically characterized by accumulation of extracellular amyloid-β and intracellular tau. From CTR and LOAD donors, two brain regions were studied, where we specifically selected the LOAD cohort such that the occipital cortex (OC) only contained amyloid-β pathology and the occipitotemporal cortex (OTC) both amyloid-β and tau pathology. We studied the snRNAseq profiles of seven distinct cell types, and only in microglia disease-associated changes were observed. 12 subtypes of microglia were identified, which largely could be grouped into six categories: 'homeostatic', 'AD1', 'AD2', 'pro-inflammatory', 'stress' and 'proliferation'. The abundance of AD1 microglia correlated significantly with amyloid-β load in the tissues, and these nuclei had an activated/phagocytic gene expression profile similar to microglia in amyloid mouse models. The abundance of AD2 microglia correlated significantly with tau load, and these nuclei had a gene expression profile that suggested that these cells were more neurotrophic. These data indicate that microglia adopt distinct transcriptomic profiles in response to amyloid-β and tau pathology in LOAD, which offers new targets for microglia-state-specific therapeutic strategies.

In **chapter 5** we studied brain tissues from FTD-GRN donors. FTD is the second most common form of dementia, and neurodegeneration is typically present in the frontal and temporal lobes, whilst the posterior part of the brain (such as the occipital cortex) is seemingly unaffected. GRN is a growth factor that has been implicated in angiogenesis, wound healing, inflammation, brain development and, more recently, lysosomal function, a property mainly attributed to microglia. Heterozygous mutations in the *GRN* gene cause haploinsufficiency of the resulting protein and account for approximately 30% of genetic FTD cases. Homozygous deletion of *GRN* in humans causes a different disease, namely neuronal ceroid lipofuscinosis. Regardless, *Grn*$^{-/-}$ mice are frequently studied as a model for FTD-GRN, where it was shown that microglia adopt reactive profiles, and this co-occurs with neurodegeneration. To investigate microglia profiles in FTD-GRN, we performed snRNAseq on frontal, temporal and occipital cortices from FTD-GRN and CTR donors. We did not observe profound microglia activation in FTD-GRN, neither in the RNAseq data nor *in situ* using IHC. Instead, we observed that astrocytes, endothelial cells, pericytes and fibroblasts were severely affected. Astrocytes adopted multiple profiles in FTD-GRN tissues,

characterized by enriched expression of genes associated with astrogliosis, BBB dysfunction, interferon signaling, oxidative stress and neuronal support. In endothelial cells, a depleted expression of homeostatic marker genes, such as *CLDN5*, *MFSD2A*, and *VEGF* was observed, as well as enriched expression of complement signaling and genes associated with BBB dysfunction. Additionally, we observed increased T-cell infiltration in FTD-GRN brain tissues *in situ*, possibly resulting from a dysfunctional BBB. Pericytes lost expression of homeostatic marker genes, and communication with endothelial cells. Fibroblasts were more abundant in FTD-GRN brain tissues, indicating that the tissue has become fibrotic. Taken together, we showed that not microglia, but the brain vasculature is severely affected in FTD-GRN brain tissues. In *Grn*$^{-/-}$ mice, we observed that similar vascular changes occurred in 2-months old mice, whereas microgliosis and neurodegeneration occurred after 12 months of age. This indicates that the neurovascular findings in FTD-GRN underlie neurodegeneration, and are an essential feature of FTD-GRN pathophysiology. Hence, neurovascular changes should be considered as a novel and prime target for therapeutic interventions of FTD-GRN.

Chapter 6 summarizes the contribution of the findings reported in this thesis to the understanding of the pathological mechanisms of neurodegenerative diseases, and discusses potential pitfalls and future perspectives. In this thesis we used postmortem human brain tissues to investigate how different cells are affected by the underlying neurodegenerative pathology. Studying postmortem human brain tissues has several potential pitfalls, including limited tissue availability, studying end-stage disease and inability to modulate the disease process. On the other hand, alternative approaches, such as mouse models, often do not fully recapitulate the human disease, and mouse and human brains are significantly different. Additionally, a wide variety of technical factors may affect experimental outcomes and comparison of reported differentially expressed genes between studies revealed low overlap. Hence, one should be aware of confounding factors when interpreting scientific data.

Molecular biological techniques are advancing rapidly and in particular scRNAseq and snRNAseq have greatly enabled our ability to study cellular heterogeneity of the brain. The downside of sc/snRNAseq is loss of spatial information and potentially biased cell/nucleus isolation. With the advent of spatial transcriptomics techniques, where RNA expression is quantified *in situ*, transcriptomic analyses can be performed without prior isolation of cells or nuclei whilst retaining spatial information. This opens up many new avenues, in particular when the technique can be applied on a subcellular level. Potential examples of the application of this technique are mapping of cellular subtypes identified with sc/snRNAseq onto the tissue in relation to pathology (e.g. AD1 microglia with amyloid-β plaques), or studying cell-cell interactions. Lastly, open questions remain how induction of cellular subtypes identified with sc/snRNAseq is regulated, and whether these subtypes translate into functional subtypes. Multimodal studies, where transcriptomics is combined with epigenomics or proteomics may help answer these questions.

Nederlandse samenvatting

De hersenen zijn een complex orgaan dat bestaat uit een grote verscheidenheid aan celtypen met een ingewikkelde cellulaire organisatie, die nog lang niet volledig wordt begrepen. Het menselijk brein bestaat uit meer dan 100 miljard neuronen met een nog groter aantal ondersteunende cellen (glia) en een vasculaire architectuur die bestaat uit meerdere verschillende celtypen. Inzicht in de heterogeniteit van de cellen in de gezonde hersenen en hoe deze worden beïnvloed door ziekten, zal helpen bij het identificeren van nieuwe aanknopingspunten voor toekomstige therapieën en bio-markers om de ziekte te volgen. In dit proefschrift hebben we de cellulaire heterogeniteit van de hersenen bestudeerd in relatie tot neurodegeneratieve ziekten, in het bijzonder multiple sclerose (MS), de ziekte van Alzheimer (AD) en fronto-temporale dementie (FTD).

Hoofdstuk 1 geeft een overzicht van de verschillende celtypen in de hersenen en hoe deze worden beïnvloed door neurodegeneratieve ziekten. Grotendeels kunnen cellen in de gezonde hersenen worden onderverdeeld in neuronen, oligodendrocyten, astrocyten, microglia en CNS-geassocieerde macrofagen (CAM), en verschillende celtypen die het vaatstelsel van de hersenen vormen, waaronder endotheelcellen, pericyten, glad spierweefsel en fibroblasten. Neurodegeneratieve ziekten worden gekenmerkt door neurodegeneratie, maar vaak richten de ziektemechanismen zich in eerste instantie op andere celtypes, en is neuronale schade een secundair effect. Bij MS is het bijvoorbeeld bekend dat oligodendrocyten ernstig worden aangetast, wat leidt tot verlies van myeline dat axonen beschermd, waardoor de axonen vatbaar zijn voor beschadiging en degeneratie. Bij MS en AD is een significante rol voor microglia geïmpliceerd door genetische onderzoeken. Een verstoorde functie van deze cellen kan mogelijk ten grondslag liggen aan neurodegeneratie bij deze ziekten. In FTD wijzen studies met muismodellen in de richting van microglia-activering die ten grondslag ligt aan neurodegeneratie. In dit proefschrift laten we echter zien dat dit niet het geval is bij mensen.

In **hoofdstuk 2** hebben we hersenweefsel van MS-donoren bestudeerd. MS is een neurodegeneratieve ziekte, gekenmerkt door gedemyeliniseerde laesies in het centrale zenuwstelsel. Uiteindelijk leidt deze demyelinisatie tot schade aan axonen met cognitieve problemen, blindheid, verminderde motorische functie of zelfs verlamming als gevolg. We hebben met behulp van RNAseq de gen activiteit van drie groepen weefsels bepaald: 1) witte stof van controle-donoren (CWM); 2) normaal lijkende witte stof (NAWM) van MS-donoren (niet-gedemyeliniseerd); 3) witte stof laesies (WML) van MS-donoren. De meeste differentieel tot expressie gebrachte genen tussen NAWM en WML waren geassocieerd met myelinisatie en ontsteking, waarschijnlijk veroorzaakt door de uitgebreide demyelinisatie die aanwezig is in WMLs en de infiltratie van immuun cellen. Bij het vergelijken van de genexpressie profielen van NAWM met CWM-weefsels, waren ontstekings- en stressroutes verrijkt die wezen op betrokkenheid van microglia en CAMs, wat aangeeft dat deze cellen reactieve veranderingen vertonen, onafhankelijk van demyelinisatie. Om te onderzoeken hoe microglia/CAMs worden beïnvloed in MS-weefsels, hebben we het genexpressie niveau in individuele cellen bepaald door middel van single cell RNA sequencing (scRNAseq) van $CD45^{pos}CD11B^{pos}$-cellen die we hebben geïsoleerd uit hersenweefsel van vijf MS-donoren. Van elke donor werden drie hersengebieden bestudeerd: 1) normaal lijkend corticaal weefsel (NACT) dat bestond uit zowel grijze als witte stof; 2) NAWM; 3) WML. We identificeerden acht subtypes van microglia en CAMs. Een van deze subtypes vertoonde een verrijkte

expressie van 'immediate-early' genen en genen voor 'heat shock'-eiwitten, en was vooral aanwezig in NAWM-weefsels. Een ander cluster dat was verrijkt in WMLs, vertoonde een typisch geactiveerd/fagocytisch microglia profiel, dat vergelijkbaar was met het profiel dat is beschreven bij myelinisatie tijdens de ontwikkeling. Om te bevestigen dat dit microglia-fenotype ontstond als reactie op demyelinisatie, voerden we scRNAseq uit van microglia geïsoleerd uit de hersenen van muizen aan wiens dieet cuprizone was toegevoegd, een stof die leidt tot verlies van myeline. Inderdaad zagen we dat een vergelijkbaar geactiveerd/fagocytisch profiel ontstond na demyelinisatie en dit profiel verdween in geremyeliniseerde muizenhersenen. Deze bevindingen geven aan dat microglia reageren op demyelinisatie door een geactiveerd/fagocytisch profiel aan te nemen, mogelijk als een poging om myeline herstel te stimuleren. In deze studie hebben we aangetoond dat hersenmacrofagen verschillende profielen aannemen in gemyeliniseerde (NAWM) en gedemyeliniseerde (WML) MS-hersenweefsels, wat mogelijk duidt op een vroege stressrespons op MS-pathologie in NAWM en een geactiveerd/fagocytisch fenotype na demyelinisatie.

Ondanks de waardevolle bevindingen in **hoofdstuk 2** waren er ook enkele nadelen aan de onderzoeksopzet. Vanwege de afhankelijkheid van vers hersenweefsel voor de cel isolatie, waren we in staat om slechts vijf donoren voor scRNAseq te includeren. Als alternatieve benadering hebben we in **hoofdstuk 3** een protocol geïmplementeerd om kernen te isoleren uit bevroren hersenweefsel, gevolgd door single nucleus RNA sequencing (snRNAseq). We hebben voor microglia gevalideerd dat het nucleaire transcriptoom een goede benadering is van het cellulaire transcriptoom. Door kernen in plaats van cellen te bestuderen, kunnen gearchiveerde, goed gekarakteriseerde bevroren hersenweefsels worden gebruikt. Deze weefsels kunnen worden verkregen uit hersenbanken en in grote aantallen tegelijk worden bestudeerd, waardoor batch-effecten worden vermeden.

Als een eerste grootschalige toepassing van snRNAseq in het laboratorium, hebben we in **hoofdstuk 4** hersenweefsel van CTR- en late-onset AD (LOAD)-donoren bestudeerd. AD is de meest voorkomende vorm van dementie en wordt neuropathologisch gekenmerkt door accumulatie van extracellulair amyloïde-β en intracellulair tau. Van CTR- en LOAD-donoren werden twee hersenregio's bestudeerd, waarbij we het LOAD-cohort dusdanig selecteerden dat de occipitale cortex (OC) alleen amyloïde-β-pathologie bevatte en de occipitotemporale cortex (OTC) zowel amyloïde-β- als tau-pathologie. We bestudeerden de snRNAseq-profielen van zeven verschillende celtypen en alleen in microglia werden ziekte gerelateerde veranderingen waargenomen. Er werden 12 subtypes van microglia geïdentificeerd, die grotendeels konden worden onderverdeeld in zes categorieën: 'homeostatisch', 'AD1', 'AD2', 'pro-inflammatoir', 'stress' en 'proliferatie'. De hoeveelheid van AD1-microglia correleerde significant met de hoeveelheid amyloïde-β in de weefsels, en deze kernen hadden een geactiveerd/fagocytisch genexpressieprofiel, vergelijkbaar met microglia in amyloïde muismodellen. De hoeveelheid AD2-microglia correleerde significant met de hoeveelheid tau en deze kernen hadden een genexpressieprofiel dat suggereerde dat deze microglia ondersteunend waren voor de neuronen. Deze gegevens suggereren dat microglia verschillende genexpressie profielen aannemen als reactie op amyloïde-β en tau-pathologie in LOAD, wat nieuwe aanknopingspunten geeft voor microglia-subtype specifieke therapeutische strategieën.

In **hoofdstuk 5** hebben we hersenweefsel van FTD-GRN donoren bestudeerd. FTD is de op een na (na AD) meest voorkomende vorm van dementie, en neurodegeneratie is typisch

aanwezig in de frontale en temporale kwabben, terwijl het achterste deel van de hersenen (zoals de occipitale kwab) gespaard blijft. GRN is een groeifactor die is betrokken bij angiogenese, wondgenezing, ontsteking, hersenontwikkeling en meer recentelijk lysosomale functies, een rol die voornamelijk wordt toegeschreven aan microglia. Heterozygote mutaties van het *GRN*-gen veroorzaken haplo-insufficiëntie van het resulterende eiwit en ongeveer 30% van de genetische FTD-gevallen hebben een *GRN* mutatie. Homozygote deletie van *GRN* bij mensen veroorzaakt een andere ziekte, neuronale ceroide lipofuscinose. Desondanks worden *Grn*$^{-/-}$ muizen vaak bestudeerd als een model voor FTD-GRN, en hierin werd aangetoond dat microglia reactieve profielen aannemen wat tegelijk gebeurt met neurodegeneratie. Om microglia-profielen in FTD-GRN te onderzoeken hebben we snRNAseq uitgevoerd op frontale, temporale en occipitale kwabben van FTD-GRN- en CTR-donoren. Hierin hebben we geen microglia-activering waargenomen in FTD-GRN, noch in de RNAseq-gegevens noch *in situ* met behulp van IHC. In plaats daarvan zagen we dat astrocyten, endotheelcellen, pericyten en fibroblasten extreme veranderingen hadden ondergaan. Astrocyten namen meerdere profielen aan in FTD-GRN-weefsels, gekenmerkt door verrijkte expressie van genen geassocieerd met astrogliose, bloed-hersen-barrière (BHB-)disfunctie, interferon-signalering, oxidatieve stress en neuronale ondersteuning. In endotheelcellen werd een verminderde expressie van homeostatische markergenen, zoals *CLDN5*, *MFSD2A* en *VEGF*, waargenomen, evenals een verrijkte expressie van complementsignalering en genen geassocieerd met BHB-disfunctie. Bovendien zagen we verhoogde T-celinfiltratie in FTD-GRN-hersenweefsels *in situ*, mogelijk als gevolg van een disfunctionele BHB. Pericyten verloren expressie van homeostatische markergenen en communicatie met endotheelcellen. Fibroblasten waren meer aanwezig in FTD-GRN-hersenweefsels, wat aangeeft dat het weefsel mogelijk fibrotischer is geworden. Samenvattend toonden we aan dat niet microglia, maar de hersenvasculatuur ernstig was aangetast in FTD-GRN-hersenweefsel. Bij *Grn*$^{-/-}$ muizen zagen we dat vergelijkbare vasculaire veranderingen optraden bij muizen van 2 maanden oud, terwijl microgliose en neurodegeneratie optraden bij een leeftijd van 12 maanden. Dit geeft aan dat de neurovasculaire bevindingen in FTD-GRN mogelijk ten grondslag liggen aan neurodegeneratie en een essentieel kenmerk zijn van FTD-GRN pathofysiologie. Daarom moeten neurovasculaire veranderingen worden beschouwd als een nieuw en belangrijk doelwit voor therapeutische interventies van FTD-GRN.

Hoofdstuk 6 vat de bijdrage van de in dit proefschrift beschreven bevindingen aan het begrijpen van de pathologische mechanismen van neurodegeneratieve ziekten samen, bespreekt mogelijke tekortkomingen en schetst toekomstperspectieven. In dit proefschrift hebben we postmortem menselijk hersenweefsel gebruikt om te onderzoeken hoe verschillende cellen worden beïnvloed door de onderliggende neurodegeneratieve pathologie. Het bestuderen van postmortaal menselijk hersenweefsel heeft verschillende valkuilen, waaronder beperkte weefselbeschikbaarheid, het bestuderen van ziekte in het eindstadium en het onvermogen om het ziekteproces experimenteel te beïnvloeden. Aan de andere kant recapituleren alternatieve benaderingen, zoals muismodellen, vaak niet volledig de ziekte, en de hersenen van muizen en mensen zijn significant verschillend. Bovendien kan een breed scala aan technische factoren de experimentele resultaten beïnvloeden en uit vergelijking van gerapporteerde differentieel tot expressie gebrachte genen tussen onderzoeken bleek er een lage reproduceerbaarheid te zijn. Daarom moet men zich bewust zijn van deze beperkingen bij het interpreteren van wetenschappelijke data.

Moleculaire biologietechnieken ontwikkelen zich snel en met name scRNAseq en snRNAseq hebben het bestuderen van cellulaire heterogeniteit van de hersenen mogelijk gemaakt. Het nadeel van sc/snRNAseq is het verlies van ruimtelijke informatie en mogelijk selectie bias door de gebruikte procedures voor cel/kern isolatie. Met de komst van 'spatial transcriptomics' technieken, waarbij RNA-expressie *in situ* wordt bestudeerd, kunnen genexpressie analyses worden uitgevoerd zonder voorafgaande isolatie van cellen of kernen, waarbij ruimtelijke informatie behouden blijft. Dit opent veel nieuwe wegen, vooral wanneer de techniek op sub cellulair niveau kan worden toegepast. Voorbeelden van toepassing van deze techniek zijn het in kaart brengen van cellulaire subtypes geïdentificeerd met sc/snRNAseq op het weefsel in relatie tot pathologie (bijv. lokalisatie van AD1 microglia met amyloïde-β plaques), of het bestuderen van cel-cel interacties. Ten slotte blijven er open vragen over hoe inductie van cellulaire subtypen geïdentificeerd met sc/snRNAseq wordt gereguleerd en of deze subtypen zich vertalen in functionele subtypen. Multimodale studies, waarbij genexpressie analyses worden gecombineerd met epigenetica of eiwit analyses, kunnen helpen bij het beantwoorden van deze vragen.

Publications and papers in progress

First authorship (this thesis)

Gerrits, E.*, Heng, Y.*, Boddeke, H.W.G.M., Eggen, B.J.L. (2020). Transcriptional profiling of microglia; current state of the art and future perspectives. *Glia*, 68(4), 740-755. https://doi.org/10.1002/glia.23767. (**Chapter 3**) *shared first authorship

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Second authorship

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Other

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