Immunogenetics of Parkinson's disease: Translational studies from rodents to humans

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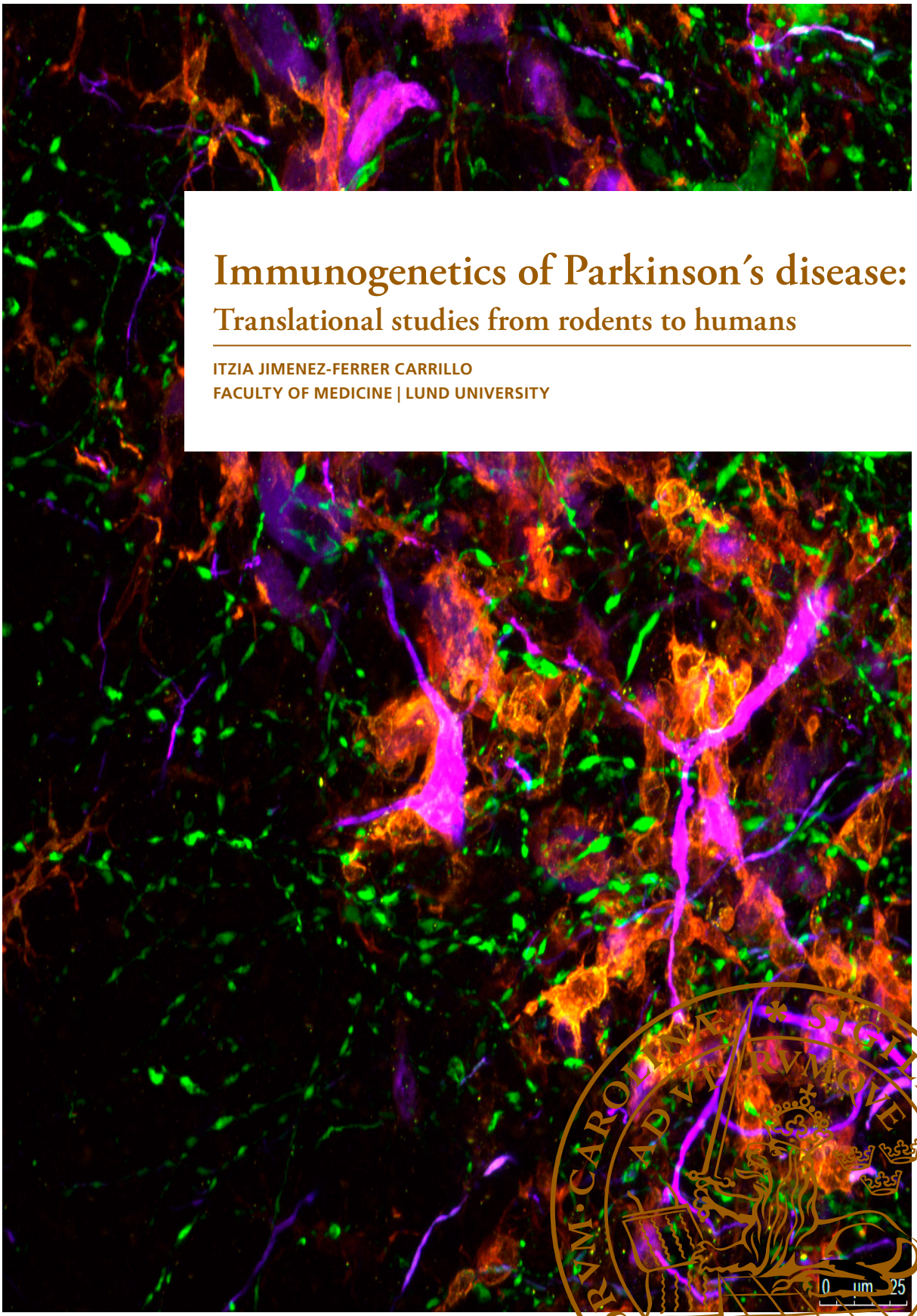

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Translational studies from rodents to humans

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LUND
UNIVERSITY

DOCTORAL DISSERTATION

With the approval of the Faculty of Medicine at Lund University this thesis will be defended on September 6,2019 at 13:15 in Segerfalksalen, Wallenberg Neurocentrum, Lund, Sweden.

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Abstract

Parkinson´s disease (PD) is a complex neurodegenerative disease, characterized by a progressive loss of dopaminergic neurons (DN) in the substantia nigra (SN) that innervate the striatum (ST) and pathological accumulation of alpha-synuclein (αsyn) protein in aggregates called Lewy bodies (LB) and Lewy neurites (LN). As a complex disease, PD presents a genetically heterogeneous origin. Mutations in single genes account for 5-10% of all the cases. The remaining 90-95% of the cases present a complex and multifactorial etiology, where there is an interplay between genetic and environmental factors that can synergize to initiate the selective degeneration of DN in the SN and the development of PD pathology. In this thesis, we aimed to contribute to the understating of the genetic architecture of PD, and its implications in the DN loss and inflammatory aspects of the disease. We first explored differences in dopaminergic susceptibility in two mouse strains that have a partial loss of Engrailed 1 (*en1*), a gene important for dopaminergic neuronal development and survival. Using linkage analysis, we identified 23 loci determining dopaminergic susceptibility. The next part of the thesis was focus on immune mechanisms in PD, for that we used a congenic rat strain to study whether allelic variants of *Mhc2ta*, could affect α-syn-induced pathology and dopaminergic neurodegeneration. Our results identified Mhc2ta as a facilitator and aggravator of PD-like αsyn pathology. The last part of this thesis, was focused on determining the frequency of known pathogenic variants causing PD, in a Swedish multi-center sample collection. Out of 7 studied pathogenic variants, we identified the *LRRK2* p.G2019S mutation and *SNCA* duplication to be present at a low frequency among Swedish patients, supporting the notion of the very complex genetic architecture of PD, and suggesting other factors underlying PD risk in this population. Overall, the results gathered in this thesis have given insight into the complex genetics underlying disease risk and identifying *MHC2TA* as a potential modulator of the immune response in PD.

Key words Parkinson´s disease; genetics; inflammation, α-synuclein, dopaminergic neurons, MHCII, Mhc2ta, Vra4, PFFs, microglia

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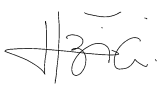
Signature  Date 2019-08-02

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Coverphoto The invisible guardians

The photography shows microglia cells dopaminergic neurons and phospho-alpha-synuclein in a rodent experimental brain.

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Unwelcome proteins
Tangling cobwebs in my brain
Don´t negate my life

(Haiku by Kendra Hough at the World Parkinson Congress 2019)

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II. **Jimenez-Ferrer I,** Jewett M, Tontanahal A, Romero-Ramos M, Swanberg M. *Allelic difference in Mhc2ta confers altered microglial activation and susceptibility to α-synuclein-induced dopaminergic neurodegeneration.* Neurobiology of Disease; 2017, 106:279-290

III. **Jimenez-Ferrer I,** Jewett M, Duenas-Rey A, Bäckstrom F, Boza-Serrano A, C.Luk K, Lee V, Deierborg T, Swanberg M. *The MHC class II transactivator modulates susceptibility to alpha-synuclein propagation and dopaminergic neurodegeneration in an in vivo rat model of Parkinson's disease*. Manuscript

IV. Puschmann A, **Jimenez-Ferrer I**, Lundbald-Anderson E, Mårtensson E, Hansson O, Odin P, Widner H, Brolin K, Mzezewa R, Kristense J, Soller M, Ygland Rödström E, Ross O, Toft M, Breedveld G, Bonifati V, Brodin L, Zettergren A, Sydow O, Linder J, Wirdefeldt K, Svenningsson P, Nissbrandt H, Carmine Beli A, Forsgren L, Swanberg M. *Low prevalence of known pathogenic mutations in dominant PD genes: A Swedish multicenter study* Accepted for publication in Parkinsonism and Related Disorders

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1. Jewett M, Dickson E, Brolin K, Negrini M, **Jimenez-Ferrer I**, Swanberg M. *Glutathione S-Transferase Alpha 4 Prevents Dopamine Neurodegeneration in a Rat Alpha-Synuclein Model of Parkinson's Disease*. Front Neurol. 2018 Apr 6; 9:222

2. Jewett M, **Jimenez-Ferrer I**, Swanberg M. *Astrocytic Expression of GSTA4 Is Associated to Dopaminergic Neuroprotection in a Rat 6-OHDA Model of Parkinson's disease.* Brain Sci. 2017 Jun 26;7(7):73

3. Puschmann A, Fiesel FC, Caulfield TR, Hudec R, Ando M, Truban D, Hou X, Ogaki K, Heckman MG, James ED, Swanberg M, **Jimenez-Ferrer I**, Hansson O, Opala G, Siuda J, Boczarska-Jedynak M, Friedman A, Koziorowski D, Rudzińska-Bar M, Aasly JO, Lynch T, Mellick GD, Mohan M, Silburn PA, Sanotsky Y, Vilariño-Güell C, Farrer MJ, Chen L, Dawson VL, Dawson TM, Wszolek ZK, Ross OA, Springer W. *Heterozygous PINK1 p.G411S increases risk of Parkinson's disease via a dominant-negative mechanism.* Brain. 2017 Jan;140(1):98-117.

4. Boza-Serrano, A., Ruiz, R., Sanchez-Varo, R., García-Revilla, J., Yang, Y., **Jimenez-Ferrer, I**., Paulus, A., Wennström, M., Vilalta, A., Allendorf, D., Davila, J. C., Stegmayr, J., Jiménez, S., Roca-Ceballos, M. A., Navarro-Garrido, V., Swanberg, M., Hsieh, C. L., Real, L. M., Englund, E., Linse, S. & 7 others, Ulf J. Nilsson, Brown, G. C., Gutierrez, A., Vitorica, J., Venero, J. L. & Tomas Deierborg, *Galectin-3, a novel endogenous TREM2 ligand, detrimentally regulates inflammatory response in Alzheimer's disease.* Acta Neuropathologica. 2019 Apr 20;

5. Medrano-Jiménez E*, **Jimenez-Ferrer I*,** Pedraza-Escalona M*, Álvarez-Arellano L, Cortés-Mendoza J, Herrera-Ruiz M, Jiménez-Ferrer E, Zamilpa A, Tortoriello J, Pedraza-Alva G, Pérez-Martínez L. *Malva parviflora extract ameliorates the deleterious effects of high fat diet on the cognitive deficit in a mouse model of Alzheimer´s disease by restoring microglial function via a PPARgamma dependent mechanism.* Journal of Neuroinflammation. 2019 Jul 10; 16:43

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6. Puschmann A, Fiesel FC, Caulfield TR, Hudec R, Ando M, Truban D, Hou X, Ogaki K, Heckman MG, James ED, Swanberg M, **Jimenez-Ferrer I**, Hansson O, Opala G, Siuda J, Boczarska-Jedynak M, Friedman A, Koziorowski D, Rudzinska-Bar M, Aasly JO, Lynch T, Mellick GD, Mohan M, Silburn PA, Sanotsky Y, Vilariño-Güell C, Farrer MJ, Chen L, Dawson VL, Dawson TM, Wszolek ZK, Ross OA, Springer W. *Reply: Heterozygous PINK1 p.G411S in rapid eye movement sleep behaviour disorder.* Brain. 2017 Jun;140(6):e33.

Review article

7. Bachiller S, **Jiménez-Ferrer I**, Paulus A, Yang Y, Swanberg M, Deierborg T, Boza-Serrano A. *Microglia in Neurological Diseases: A Road Map to Brain-Disease Dependent-Inflammatory Response.* Front Cell Neurosci. 2018 Dec 18; 12:488

Book chapter

8. **Jimenez-Ferrer I**, Swanberg M. *Immunogenetics of Parkinson´s Disease In*: Parkinson's disease: Pathogenesis and Clinical Aspects. Stoker TB, Greenland JC (Editors). Codon Publications, 2018 Dec 21

Summary

Parkinson´s disease (PD) is the second most common aged-related neurodegenerative disorder, affecting over 1-2% of worldwide population above the age of 65. PD is a complex neurodegenerative disease, characterized by a progressive loss of dopaminergic neurons (DN) in the substantia nigra (SN) that innervate the striatum (ST) and pathological accumulation of alpha-synuclein (αsyn) protein in aggregates called Lewy bodies (LB) and Lewy neurites (LN). As a complex disease, PD presents a genetically heterogeneous origin. Mutations in single genes account for 5-10% of all the cases. The remaining 90-95% of the cases present a complex and multifactorial etiology, where there is an interplay between genetic and environmental factors that can synergize to initiate the selective degeneration of DN in the SN and the development of PD pathology. So far, the most comprehensive meta-analysis of genome-wide association studies used large number of unrelated cases and compare with unrelated controls. These studies have identified at least 70 independent risk loci associated with PD that increase or decrease disease risk in a small yet potentially additive way. These variants include PD associated Human Leukocyte Antigen variants (HLA-variants), and carriage of multiple of such risk variants can double the risk of developing PD. The HLA region encodes the Major Histocompatibility Complex Class II (MHCII) molecules. These molecules have key roles in inflammatory processes and are up-regulated in microglial cells in PD patients. Inflammation in the central nervous system (CNS) has been widely studied and it is now regarded as a hallmark of PD. The fact that common genetic variant affecting expression levels of MHCII are associated with PD risk strongly supports a role of immune responses in PD susceptibility. The *MHCII* transactivator (*MHC2TA*) gene is the master regulator of *MHCII* expression. As previously demonstrated in a linkage analysis study that fine-mapped a rat, quantitative trait locus (QTL) regulating *MHCII* expression levels to a genomic interval within the *Mhc2ta* gene. As also shown in this study, a single nucleotide polymorphism (SNP) in the regulatory region of the human orthologue (*MHC2TA*) is also associated with increase susceptibility to complex disease with inflammatory components. Although inflammation and expression of MHCII are known to be involved in PD, the impact of physiological differential *Mhc2ta* expression on degeneration of dopaminergic neurons has not previously be been addressed before. In this thesis, we aimed to contribute to the understating of the genetic architecture of PD, and its implications in the DN loss and inflammatory aspects of the disease.

We first explored differences in dopaminergic susceptibility in two mouse strains that have a partial loss of Engrailed 1 (*en1*), a gene important for dopaminergic neuronal

development and survival. Using linkage analysis, we identified 23 loci determining dopaminergic susceptibility. Transcriptome analysis of dopaminergic neurons will be used to further investigate the role of these loci and to identify candidate genes.

The next part of the thesis was focus on immune mechanisms in PD, for that we used a congenic rat strain to study whether allelic variants of *Mhc2ta*, could affect α-syn-induced pathology and dopaminergic neurodegeneration. Our results show that the DA.VRA4 congenic rat strain present widespread αsyn pathology and dopaminergic neurodegeneration, in combination with increased hyperreactive microglia. Thus, we identified *Mhc2ta* as a facilitator and aggravator of PD-like αsyn pathology. Using a translational approach, we have started to study if *MHC2TA* variants and *HLA* variants are associated with PD in a Swedish patient-control cohort.

The last part of this thesis, was focused on determining the frequency of known pathogenic variants causing PD, in a Swedish multi-center sample collection. Out of 7 studied pathogenic variants, we identified the *LRRK2* p.G2019S mutation and *SNCA* duplication to be present at a low frequency among Swedish patients, supporting the notion of the very complex genetic architecture of PD, and suggesting other factors underlying PD risk in this population.

Overall, the results gathered in this thesis have given insight into the complex genetics underlying disease risk and identifying *MHC2TA* as a potential modulator of the immune response in PD.

Populärvetenskaplig sammanfattning

Parkinsons sjukdom är den näst vanligaste neurodegenerativa sjukdomen och drabbar 1-2% av befolkningen över 65 års ålder. Sjukdomen är komplex och karaktäriseras av en progressiv förlust av dopaminproducerande nervceller i en del av mellanhjärnan kallad substantia nigra. I de påvekade nervcellerna ses en ackumulering av aggregerade proteiner. Dessa aggregat innehåller alfa-synuklein och kallas Lewy kroppar.

Liksom andra komplexa sjukdomar har Parkinsons sjukdom en genetiskt heterogen bakgrund. Mutationer i specifika gener svarar för 5-10% av alla fall, medan resterande 90-95% av fallen orsakas av en kombination av genetiska faktorer och miljöfaktorer. Gener och miljö samverkar således för att initiera de sjukliga förändringarna i hjärnan, vilket resulterar i förlusten av nervceller.

Hittills har genetiska studier med ett stort antal obesläktade patienter och kontroller identifierat minst 90 områden i genomet med variationer som antingen ökar eller minskar risken för Parkinsons sjukdom. Trots att varje enskilt genetiskt område har en relativt liten påverkan på sjukdomsrisken kan kombinationen av flera riskvariationer dubblera risken för en individ att drabbas av Parkinsons sjukdom. I denna avhandling studeras hur genetiska variationer påverkar risken för Parkinsons sjukdom i sjukdomsmodeller och i patientkohorter, med särskilt fokus på inflammation.

I den första delen av denna avhandling studieras möss som förlorat en av två kopior av genen *engrailed-1*, vilket hos en av de studerade musstammarna orsakar en Parkinsonlik förlust av dopaminproducerande nervceller. Studien identifierade 23 delvis överlappande genetiska områden som påverkar känsligheten för att utveckla den Parkinsonlika celldöden och dessa studeras nu vidare för att identifiera de bakomliggande processerna.

Ett av de områden där humanstudier tidigare har identifierat riskvariationer för Parkinsons sjukdom kodar för MHCII molekyler. MHCII sitter på ytan av immunceller och har en nyckelroll i inflammatoriska processer. Tecken på inflammation i hjärnan är karaktäristiskt för Parkinsons sjukdom och inkluderar också ett ökat MHCII uttryck. Att genetiska variationer som påverkar MHCII ökar risken att drabbas av Parkinsons sjukdom är ett starkt stöd för att inflammation påverkar sjukdomsutveklingen. Den dominerande faktorn som reglerar uttrycket av MHCII på celler är proteinet MHC2TA och genetiska variationer i MHC2TA reglerar känsligheten för inflammation hos både människor och råttor.

I den andra delen av denna avhandling har två olika modeller i råtta använts för att studera hur genetiska variationer som styr *Mhc2ta* uttryck påverkar Parkinsonlik sjukdom. De två studierna visar att ett lägre uttryck av *Mhc2ta* ökar känsligheten för

nervcelldöd, förekomst av proteinaggregat och motoriska symptom, vilka samtliga är karaktäristiska för Parkinsons sjukdom. I kommande studier kommer variationer i MHC2TA och MHCII gener studeras i patientmaterial för att se om dessa påverkar risk och progression av Parkinsons sjukdom.

I den sista delen av denna avhandling har förekomsten av sjukdomsorsakande mutationer studerats hos mer än 10% av alla patienter med Parkinsons sjukdom i Sverige. Studien visar att dessa mutationer är mycket ovanliga och endast förklarar en bråkdel av den totala ärftligheten av Parkinsons sjukdom. Detta motiverar starkt fortsatt forskning, då identifieringen av genetiska riskfaktorer kan ge oss nya verktyg för att behandla denna allvarliga och i nuläget obotliga sjukdom.

Resumen

La enfermedad de Parkinson (EP) es el segundo trastorno neurodegenerativo relacionado con la edad más común, que afecta a más del 1-2% de la población mundial mayor de 65 años. La EP es una enfermedad neurodegenerativa compleja, caracterizada por la pérdida progresiva de neuronas dopaminérgicas (ND) en la sustancia negra (SN) que inervan el cuerpo estriado (ST) y la acumulación patológica de la proteína alfa-sinucleína (αsyn) en agregados llamados cuerpos de Lewy (LB) y neuritas de Lewy (LN). Como enfermedad compleja, la EP presenta un origen genéticamente heterogéneo. Se han identificado mutaciones en genes individuales que representan el 5-10% de todos los casos. El 90-95% restante de los casos presenta una etiología compleja y multifactorial, donde los factores genéticos y ambientales interactúan y pueden actuar de manera sinérgica para iniciar la degeneración selectiva de ND en el SN y el desarrollo de la patología de EP. Hasta ahora, el metanálisis más completo de los estudios de asociación de todo el genoma, ha utilizado un gran número de casos no relacionados entre ellos y se han comparado con controles no relacionados entre ellos. Estos estudios han identificado al menos 70 loci de riesgo independientes asociados con la EP que aumentan o disminuyen el riesgo de una manera pequeña pero que pueden actuar de manera aditiva. Estas variantes incluyen variantes del antígeno leucocitario humano (HLA), asociado a la EP y la presencia de múltiples de estas variantes pueden duplicar el de riesgo de desarrollar EP. En la región HLA, se encuentran codificadas las moléculas del complejo principal de histocompatibilidad de clase II (MHCII). Estas moléculas tienen funciones clave en procesos inflamatorios y se ha reportado estar expresadas en células microgliales en pacientes con EP. La inflamación en el sistema nervioso central (SNC) ha sido ampliamente estudiada y ahora se reconoce como una caracteristica de la EP. El hecho de que existan variantes genéticas comunes que regulan los niveles de expresión de MHCII, y que estos esten asociados con el riesgo de EP, respalda firmemente el papel del sistema immune en la susceptibilidad o durante el desarrollo de la EP. El gen transactivador de MHCII (MHC2TA) es el regulador maestro de la expresión de MHCII. Previamente, se ha demostrado, en estudios de mapeo genetico, que diferencias en los niveles de expression de MHCII están regulados por un interval genómico dentro del gen Mhc2ta en ratas. Como se ha demonstrado también en este studio, un polimorfismo de un solo nucleótido (SNP) en la región reguladora del ortólogo gen en humano (MHC2TA) se ha asociado con mayor susceptibilidad a enfermedades complejas con un componente inflamatorio. Aunque se sabe que la inflamación y la expresión de MHCII están involucradas en la EP, el impacto de la expresión fisiológica diferencial de Mhc2ta sobre la degeneración de las neuronas

dopaminérgicas no se ha abordado previamente. En esta tesis, nuestro objetivo fue contribuir a la comprensión de la arquitectura genética de la EP, y sus implicaciones en la pérdida de DN y los aspectos inflamatorios de la enfermedad.

Abbreviations and nomenclature

APC	Antigen presenting cells
Arg1	Arginase 1, anti-inflammatory citokine
BSA	Bovine serum albumin
Cd11b	(Mac1) complement receptor type 3
CD4	Cluster of differentiation 4
CD74	Cluster of differentiation 74
CD8	Cluster of differentiation 8
CE	Coefficient of error
CIITA	Class II major histocompatibility complex Transactivator
cM	Centimorgan
CNS	Central nervous system
CNVs	Copy number variations
COMT	Catechol-O-methyltransferase
CR3/43	HLA DR + DP + DQ, MHCII
CRC	Clinical Research Center
CSF	Cerebrospinal fluid
DA	Dark agouti
DAB	3,3′-Diaminobenzidine
DBS	Deep brain stimulation
ddPCR	Droplet digital PCR
DN	Dopaminergic neurons
DNA	Deoxyribonucleic acid
DQA2	Human HLA specific allele
DRA	Human HLA specific allele
DRB5	Human HLA specific allele
EBM11	Monoclonal antibody to human macrophages
EM	Expectation-maximization
En1	Engrailed-1
En2	Engrailed-2
F1	First filial generation
F2	Second filial generation
G	Unit for centrifugation steps
GABA	Gamma-aminobutyric acid
GBA	Glucocerebrosidase
GFP	Green fluorescent protein
gnomAD	Genome aggregation database
LB	Lewy bodies
LD	Linkage disequilibrium
LN	Lewy neurites
LOD	Odds favouring linkage
LPS	Lipopolysaccharide
LRRK2	Leucine-rich repeat kinase 2
Mac1	(Cd11b) complement receptor type 3
MAO	Monoamine oxidase
MHC2TA	Class II major histocompatibility complex Transactivator
MHCII	Major Histocompatibility Complex Class II
MLPA	Multiplex ligation-dependent probe amplification
Mm	Millimetre
MPBC	Multipark´s biobank sample collection
NACl	Sodium chloride
NSAID	Nonsteroidal anti-inflammatory drug
OD	Optical density
OR	Odds ratio
PARK2	Parkin E3 ubiquitin protein
PARK7	Parkinsonism associated deglycase
PD	Parkinson´s Disease
PFFs	Preform fribrils
PINK1	PTEN-induced kinase 1
PVA-DABCO	Polyvinil alcohol mounting medium with DABCO
PVG	Piebald Virol Glaxo
qPCR	Quantitative polymerase chain reaction
qRT-PCR	Real-time reverse transcription-PCR
QTL	Quantitative trait locus
rAAV	Recombinant adeno-associated virus
RBD	Sleep behavior disorder
RLS	Restless legs syndrome
RNA	Ribonucleic acid
RNases	Ribonucleases
RNasezap	RNase decontamination solution
ROIs	Regions of interest
SD	Sprague Dawley
SN	Substantia nigra

GWAS	Genome-wide association study	**SNCA**	Synculein alpha
H2-Q2	Mouse histocompatibilty 2, Q region locus 2	**SNP**	Single nucleotide polymorphisms
H2-Q7	Mouse histocompatibility 2, Q region locus 7	**SPSS**	Statistical software
HLA	Human Leukocyte Antigen	**SRS**	Systematic random samples
HRM	High-resolution melt	**Ssf**	Section-sampling fraction
IFN	Interferon	**ST**	Striatum
IFNγ	Interferon gamma , cytokine involve in actiavation of macrophages	**TGF-β1**	Transforming growth factor beta 1 cytokine
IL-10	Interleukin 10 , anti-inflammatory cytokine	**TGF-β2**	Transforming growtg factor beta 2 cytokine
IL-12	Interleukin 12, stimulate growth and function of T cells	**TH**	Tyrosine hydroxylase
IL-1β	Interleukin 1 beta pro-inflammatory cytokine	**TLR2**	Toll-like receptor 2
IL-2	Interleukin 2 regulates funcion of T cells	**TNF-α**	Tumor necrosis factor alpha, pro-inflammatory cytokine
IL-4	Interleukin 4 stimulates B cell and T cel proliferation	**µL**	Microliter
IL-5	Interleukin 5 stimulates B cell growth	**µM**	Micrometer
IL-6	Interleukin 6	**VPS-35**	Vacuolar protein sorting-associated protein 35
IL-8	Interleukin 8	**WPRE**	Woodchuck hepatitis virus posttranscriptionl regulatory element
LacZ	lac operon	**αsyn**	Alpha-synuclein
		pαsyn	phosphorylated alpha-synuclein

Nomenclature

Gene names are written in italics, capital letter when referred to the human gene (e.g. *SNCA, LRRK2, MHC2TA*), non capital letter when referring at the mouse version of the gene (e.g. *en1*), and first letter capitalized when referring to the gene in rats (e.g. *Mhc2ta*). Proteins are identified with the commonly used names (e.g. MHCII, αsyn)

Preface

What make us different?

Despite the vast phenotypic spectrum humans display, approximately 99.9% of our genetic makeup is shared by us all. Notwithstanding this compelling fact, we still carry within us a unique narrative written in a 4-letter alphabet: a plethora of subtle differences that define who we are and, in some way, make each and everyone of us unique. Therefore, whether we are very similar or very different, it is a matter of perspective.

Thus our uniqueness is caused by the presence of many particular variants or many slight differences in various genes that will have an effect on a wide range of manifestations both in health and disease. Not only do we differ in obvious physical traits (height, eye color, etc.), but also in our propensity and resilience to disease. Depending on the amount of variants affecting that, these traits can be monogenic (when one variant is responsible for the physical trait), or complex traits (where multiple variants exert an effect on the trait). Many common chronic diseases with adult onset appear to be caused by multiple genes, usually interacting with environmental factors. In the last decade an increase in the development of high-throughput genomic technologies, and the use of transgenic and congenic animal models, have shed light on how genetic diversity impacts disease risk and progression.

Recently, there has been a boom of companies offering services of sequencing and ancestry; opening the landscape to early detection of people at risk of developing such complex diseases. For that to happen, the identification of the genetic risk factors is needed. Such testing will be particularly important for high-risk individuals in disease where prevention by lifestyle changes is possible to reduce the risk of certain diseases, or to potentiate the effect of certain drugs.

In this thesis, we employed transgenic and congenic animals to navigate the nuances of genetic risk factors in PD. We focused specifically on factors whose impact is mostly exerted through immune mechanisms. Although PD is a well-known disease, firstly described more than 200 years ago, it is still considered incurable. It is a textbook example of a disease with a complex genetic architecture, in which the presence of many genetic variants and environmental factors come into play. Although still many disease-modifying risk factors are yet to be identified, here we present a comprehensive approach on how to find them and evaluate their functional consequences from a translational perspective.

Introduction

Parkinson´s Disease

Parkinson´s disease (PD) is a complex and heterogeneous neurodegenerative disease that affects around 1-1.5% of the population older than 65 years, rising to 3.5% at age 85-89 years (de Lau et al., 2006; Pringsheim et al., 2014). Being the second most common neurodegenerative aged-related brain disorder, it is estimated to affect 7 to 10 million people worldwide (Aarsland et al., 2011). Unfortunately, until today, there are no treatments that delay or modify PD progression.

Symptoms and therapeutic strategies

PD patients suffer from a combination of progressive non-motor and motor symptoms that increasingly impair daily function and quality of life. Non-motor symptoms which often start prior to diagnosis (prodromal stage of PD) are also frequently reported. Including cognitive impairment, dementia, restless legs syndrome (RLS), fatigue, hyposmia, constipation, and sleep behaviour disorder (RBD) (Geurtsen et al., 2014; Papagno et al., 2018; Trojano et al., 2018). The cardinal symptoms are the motor including tremor rigidity, slowness and balance problem. Importantly, these symptoms are often identified relatively late in the pathological process when approximately 50% of dopaminergic neurons have been lost in the substantia nigra (SN) (Ross et al., 2004).

Classical interventions have been designed to treat motor dysfunction in PD, including dopamine precursors, such as L-DOPA and dopamine agonists (amantadine, apomorphine, bromocriptine, caergoline, lisuride, pergolide, pramipexole, ropinrole, rotgotine). Although the use of dopamine precursors and dopamine agonists have shown alleviation of motor symptoms, the prolonged use of dopamine replacement therapies show no effect in alleviating motor fluctuations and dyskinesia (Caraceni et al., 1991) in later stages of the disease (e.g. the receptors, reuptake and circuitries are dysfunctional even when dopamine is replaced).

Other symptomatic treatments for PD that have been effective in treating tremor and dyskinesia for some patients include deep brain stimulation (DBS), monoamine oxidase (MAO) inhibitors (selegiline, rasagiline), and catechol-O-methyltrasnferase (COMT) inhibitors (entacapone, tolcapone) (Cacabelos, 2017).

Current clinical therapies are started when clinical diagnosis has occurred. Diagnosis of PD requires the presence of multiple motor features deficits (Postuma et al., 2015). Given that subtle or single motor abnormalities occur prior to diagnosis and alongside

early non-motor symptoms, according to the latest criteria by the International Parkinson and Movement Disorders Society, it is recommended to refer to this phase as prediagnostic PD phase (Schrag et al., 2015). Disease progression comprises three clear stages; **(i)** preclinical PD, characterized by the presence of neurodegenerative synucleinopathy without clinical symptoms, and defined by disease biomarkers when available; **(ii)** prodromal PD, when early symptoms and signs are present before PD diagnosis; **(iii)** clinical PD, in which diagnosis of PD has been made based on the presence of classical motor signs.

The identification of these pre-diagnostic PD stages have prompted the search for novel therapeutic approach that can stop or modify the course of the disease prior to the appearance of the symptoms.

Heterogeneity of patients

PD patients are a heterogeneous population, displaying a wide range of motor and non-motor symptoms, with a high inter-individual variation in disease onset and progression (Foltynie et al., 2002). This inter-individual variation supports the emerging picture of PD as a clinical syndrome with different subtypes (Erro et al., 2013; Foltynie et al., 2002) (Figure 1).

Assuming that homogenous groups of patients are more likely to share pathological and genetic features, it has been proposed that grouping subtypes of PD (e.g. mild pure motor, mild mixed motor-non-motor, sever non-motor dominant and sever motor dominant) in clusters may be relevant for elucidation of the underlying pathophysiology with crucial consequences for our understanding of disease progression, prognosis and treatment strategies (Erro et al., 2013; Mu et al., 2017).

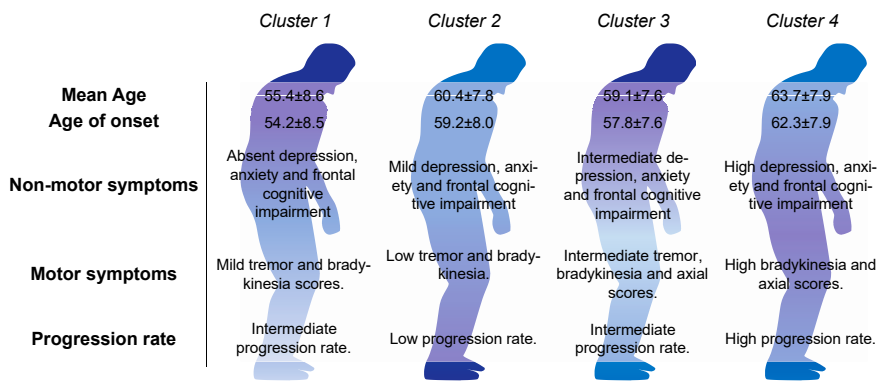


Figure 1 | Example of heterogeneity in PD patients. Decribed clusters of PD patients using clinical data (Erro et al., 2013). Further categorisation using biomarkers, genetics and imaging is in development.

Evidence of pathophysiological mechanisms in PD patients

Neuropathologically PD is characterized by reduction of striatal dopamine and loss of nigral dopaminergic neurons (DN), concentrated in the SN (Bjorklund et al., 2007). Overall, the degree of striatal dopaminergic atrophy occurs prior to the nigral neurodegeneration (Kordower et al., 2013), suggesting that the degenerative process begins in the striatal axons, and then protracts retrogradely to the respective dopaminergic neurons: indeed, motor symptoms often appear when approximately 80% of striatal dopamine and 50% of cells are lost (Burke et al., 2013). In addition to dopaminergic neurodegeneration, another key hallmark is the pathological intracellular accumulation of alpha-synuclein protein (αsyn) within neuronal somas, known as Lewy bodies (LB), and along their axons, known as Lewy neurites (LN) (Spillantini et al., 1997). It has been reported that non-dopaminergic neurons such as GABAergic, cholinergic, and serotonergic neurons, are also affected to a certain extent in PD. However, how and when these specific neuronal phenotypes contribute to the range of PD symptoms is not completely determined. Several pathological processes including; mitochondrial DNA damage, respiratory chain dysfunction, oxidative stress, and αsyn inclusions, along with inflammation have also been reported in human PD (Devi et al., 2008).

In the context of inflammation the underlying pathological processes are not fully understood, although a growing body of evidence supports its role in PD. The first evidence of an involvement of inflammation in PD was derived from the first clinical and pathological description of the disease in 1817, which was reprinted in the special issue on the 200-year anniversary of the essay (Parkinson, 2002). More direct evidence of inflammation in the brain came from post-mortem analysis of the brains of PD patients, in which an increase in the number of Major Histocompatibility Class two (MHCII) positive cells were observed in the SN (McGeer, Itagaki, Boyes, et al., 1988). Furthermore, activated microglial markers such as CR3/43 and EBM11 have been shown to be higher in the SN of PD patients, and these correlate with neuronal degeneration in SN and putamen (Imamura et al., 2003). The presence of MHCII positive microglia also correlates with increased αsyn deposition (Croisier et al., 2005), thereby further strengthening the link between inflammation and PD. However, whether this microglial activation is a response to the ongoing neurodegeneration or an inducer of the disease remains to be defined.

Apart from microglia activation, pro-inflammatory cytokines such as increased levels of IL-1β and IL-6 have been found in blood and cerebrospinal fluid (CSF) of PD patients (Blum-Degen et al., 1995). Furthermore, TGF- β1 and TGF- β2, two pro-inflammatory cytokines, levels were elevated in post-mortem CSF of patients with PD, in comparison with age and gender-matched controls (Vawter et al., 1996). Lymphocyte cells have been observed in SN after post-mortem analyses of PD patients in CNS (Croisier et al., 2005; McGeer, Itagaki, Akiyama, et al., 1988; McGeer, Itagaki, Boyes, et al., 1988). Interestingly, in the periphery, there was a reduction in the CD4+ CD8+ T-cells ratio, and a decreased level of regulatory T-cells. (Baba et al., 2005; Stevens et al., 2012). Recently, reactive T-cells against αsyn peptides were reported to be found in PD

patients. This response was mediated by CD4+ or CD8+ T cells and was specially robust with a defined subset of *MHC* alleles (Sulzer et al., 2017). These data demonstrate that αsyn epitopes can induce T cell mediated immune responses in PD patients. So it is clear that MHCII is involved in the presentation of αsyn to T cells.

Etiology of Parkinson´s disease

The cause of PD is still unknown, a complex interaction between environmental and genetic factors has been proposed. Although a number of genetic mutations that can cause PD have been identified, these are associated with familiar forms of PD and account for less that 10% of the cases (Hernandez et al., 2016). Thus, indicating that the genetic architecture of PD is complex and the interplay with environmental factors is not completely understood.

Monogenic forms or familial forms of PD

The first studies into the role of genetic factors in PD focused on the identification of rare mutations causing familial forms of the disease. Mutations that have been causatively linked to PD map to the genes encoding αsyn (*SNCA*) (Polymeropoulos et al., 1997; Singleton et al., 2003), leucine-rich repeat kinase 2 (*LRRK2*) (Zimprich et al., 2004), parkin (*PARK2*), PTEN-induced putative kinase 1 (*PINK1*) (Kitada et al., 1998; Valente et al., 2004), vacuolar protein sorting-associated protein 35 (*VPS-35*) (Zimprich et al., 2011), *DJ1* (*PARK7*) (Bonifati et al., 2003), and glucocerebrosidase (*GBA*) (Figure 2). However, only less than 10% of the cases can be attributed to these specifics genetic mutations. The identification of these genes also has led to the identification of key mechanisms and their corresponding molecular players in PD etiology. This can be illustrated by *SNCA*, which is both neuropathologically and genetically linked to PD as LB and LN containing αsyn are present in both familial and idiopathic PD.

Idiopathic forms or non familial forms

90% of PD cases present a complex and multifactorial etiology, where common genetic variants and environmental factors could synergize to initiate the selective degeneration of dopaminergic neurons in the SN and the development of PD pathology (Hamza et al., 2010).

In the quest to understand the etiology of idiopathic PD, efforts have been made to identify genetic variants associated with a disease risk. So far, the most comprehensive meta-analysis of genome-wide association studies (GWAS) has identified 41 genetic risk loci for PD, each representing common genetic variants conferring an increased risk of developing PD (Chang et al., 2017; Nalls et al., 2014) (Figure 2).

At the moment of writing this thesis, a recent collaborative study between research groups and the commercial genetics company 23andMe published in biorxiv, reported 70 independent common genetic risk loci for PD.

Almost doubling the number of known PD risk loci. Interestingly these variants explained 26-36% of heritable risk of PD in individuals of European ancestry (https://www.biorxiv.org/content/10.1101/388165v2). It is important to bear in mind, that at this point the single nucleotide polymorphisms (SNPs) reported to be associated with PD are unlikely to be the actual functional variant but rather are in linkage disequilibrium (LD) with a polymorphism that affects risk. Thus, the true causative gene or genes remains elusive.

This data highlights the relevance of common genetic variants in risk pathology, and the need for identifying the genetic component of the disease that remain unidentified.

Therefore, high-quality epidemiological studies with large sample sizes in combination with exome and whole genome sequencing technologies and objective measurements of phenotypes and exposures are needed to identify causal novel risk factors.

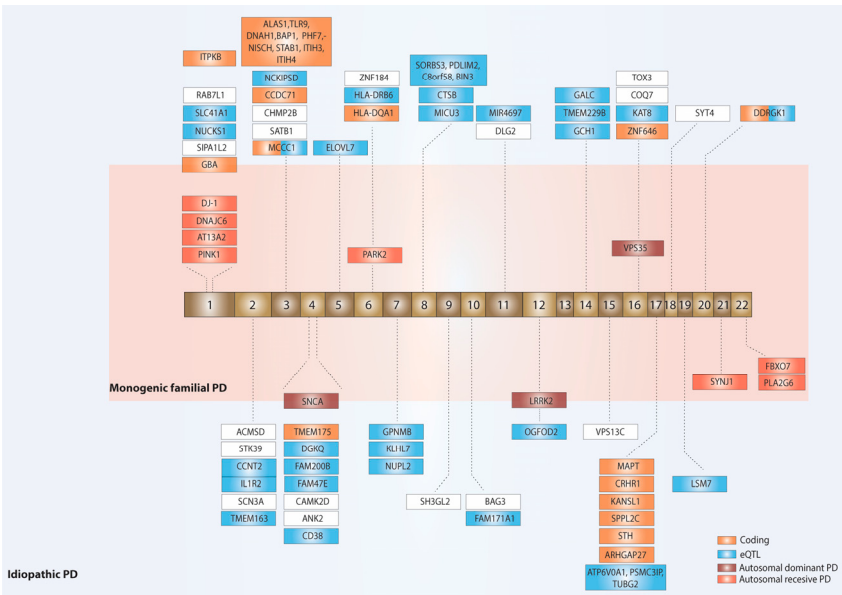


Figure 2 | **Insights into the genetics of Parkinson´s Disease (PD)** .41 PD-risk loci identified in meta-analysis GWAS (Chang et al., 2017). Candidate genes are annotated for each region that has been significantly associated with PD . For some of the regions more there are more than once candidate gene.

Immunogenetics

Immunogenetics specifically studies the relationship between genetics and the immune system, that is, how genetic variants contribute to inter-individual variation in immune response and risk of disease.

We have reviewed and reported that many of the genetic variants linked to monogenic PD also play a critical role in modulating inflammatory responses (Jimenez-Ferrer et al., 2018). *LRRK2* and *SNCA* genes, two of the most studied genes in monogenic PD, have immunogenetics components. Mutations in *LRRK2* account for 1-2% of all PD cases (Paisan-Ruiz et al., 2004; Zimprich et al., 2004) but the prevalence vary substantially depending on the studied population. *LRRK2* encodes a large protein with multiple functions and has a moderate homology to the receptor-interacting protein kinases, a family of kinases with a role in cellular signal transduction events in immune cells (Cook et al., 2017). Mutations and copy number variations (CNVs) in the *SNCA* gene (Polymeropoulos et al., 1997; Spillantini et al., 1997) are linked to dominantly inherited monogenic PD (Singleton et al., 2003).

Furthermore, common variations in the non-coding region of *SNCA* gene, including a SNP that affect gene expression levels are associated to idiopathic PD risk (Campelo et al., 2017). In addition, LB and LN containing αsyn accumulations are present in both familial and idiopathic PD. Recently; it was shown that αsyn is a ligand for toll-like receptor 2 (TLR2) (Kim et al., 2013). This receptor is present on T-lymphocytes, B-lymphocytes, monocytes and macrophages, cells that are part of the adaptive immune system. Moreover, it has been reported that both helper and cytotoxic T lymphocytes can be activated upon recognition of αsyn epitopes presented on MHC molecules (Sulzer et al., 2017). This data further strengthen the notion that αsyn can thus elicit both innate and adaptive immune responses.

Moreover, from the last meta-analysis of GWAS, two risk loci lie within the human leukocyte antigen (*HLA*) region. The *HLA* is one of the most polymorphic regions in the human genome. It encodes both MHCI and MHCII molecules that present antigens to CD8+ and CD4+ T-lymphocytes, respectively. Different *HLA* alleles encode MHC molecules with different antigen-binding capacity and affinity, with certain alleles being associated with complex disorders, including autoimmune diabetes and rheumatoid arthritis (Miyadera et al., 2015). Several studies have found association between SNPs and alleles in the *HLA* class II region and PD (Figure 3). Interestingly, from a GWAS, an association between late-onset PD and a non-coding variant in *HLA* has been reported (Hamza et al., 2010). This variant has been reported to be a cis-acting expression quantitative trait loci (eQTL) that correlates significantly with expression levels of specific *HLA* alleles; HLA-DRA, DRB5, and DQA2 (Montgomery et al., 2010; Stranger et al., 2007). This provides functional insight into the observed increased expression of MHCII molecules in PD brains and how allelic variability can affect the interaction between antigen-presenting cells and lymphocytes.

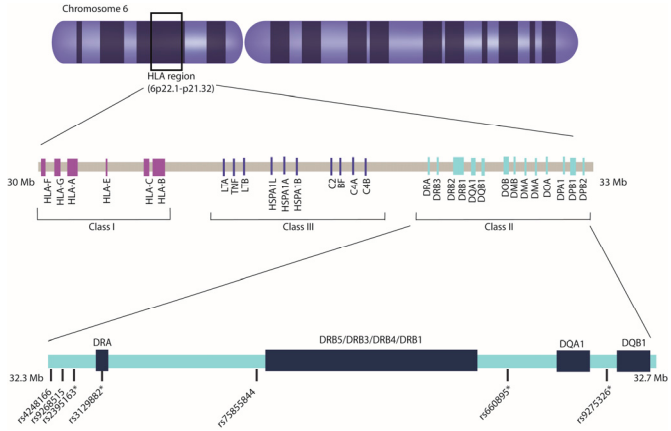


Figure 3 | **Single nucleotide polymorphism (SNPs) in the human leukocyte antinge (HLA) locus associated with increased risk for Parkinson´s disease (PD).** Map of HLA class I, II and III regions indicating alleles and SNPs associated with PD. An asterisk (') denotes that the SNP is acting as an expression quantitative trait locus (eQTL).(Adapted from (Wissemann et al., 2013)

Environmental risk factors

An increasing amount of environmental factors have been found to modify PD risk, with aging being the most prominent, since there is a substantial increase in the incidence after 65 years (de Lau & Breteler, 2006; Pringsheim et al., 2014).

Environmental factors associated with an increased PD risk includes pesticide exposure with the odds ratios (OR) ranging from 1.28 to 2.56 have been reported in different meta-analyses (Noyce et al., 2012; Van Maele-Fabry et al., 2012), farming and well-water consumption, rural living, and head injury which can cause long-lasting brain inflammation and aggregation of αsyn, contributing thus to an increased risk of PD. For these factors an OR range from 1.04 to 1.91 (Martino et al., 2017; Noyce et al., 2012).

In contrast, other factors are associated negatively with the onset of PD. The most studied includes, cigarette smoking (Hernan et al., 2002; Noyce et al., 2012; Sugita et al., 2001) and coffee consumption (Hernan et al., 2002; Noyce et al., 2012). Pooled results in meta-analyses have shown a reduced risk of PD with an OR of 0.64 to 0.59. Interestingly, an inverse association with PD onset has also been seen for the use of the Swedish oral moist tobacco (snus) and chew tobacco (Yang et al., 2017). However, the biological mechanism behind these two correlations has not been determined yet. Other factors have also been reported to decrease PD risk, although they have not been studied extensively. Among these is the regular use of non-steroidal anti-inflammatory drug, with ibuprofen being significantly associated with a reduced risk for PD (Rees et al., 2011)

The gastrointestinal tract has an extensive immune and neuronal network and is in direct contact with the external environment. According to the Braak hypothesis, the αsyn Lewy pathology initially appears during the pre-motor stage of PD, during which the enteric nervous system is affected by αsyn. It has been proposed that PD pathology actually starts in the gut and propagates through the vagus nerve. (Braak et al., 2003). Indeed, there is also evidence of the impact of gut microbiota on environmental factors associated with PD, such as smoking. The relationship between smoking and gut microbiota remains to be determined. However, there is evidence that smoking improve gut barrier function in humans (Prytz et al., 1989). Importantly, smoking also seems to have an effect on gut microbiome composition (Benjamin et al., 2012; Biedermann et al., 2014).

However, whether these simultaneous changes are causally related to each other and eventually to PD, is yet to be elucidated. Moreover, a possible reverse effect of gut microbiota on smoking propensity and its relevance for PD is an interesting field for future studies.

Gene-environment interactions

The incomplete penetrance of monogenic forms of PD and the complex genetic architecture of idiopathic PD suggest the presence of a synergistic interaction between genetics, environment, and aging to cause disease, since not everyone with a genetic mutation linked to PD will develop the disease. Similarly, not everyone exposed to a pesticide will develop PD.

Studies have shown evidence of the link between inflammation, PD genetic risk, and environment. The SNP located in *HLA-DRA* (rs3129882) associated with increased MHCII molecule expression has been reported to significantly increase the risk of PD, in synergy with environmental exposure to pyrethroid (Kannarkat et al., 2015). Greater understanding of the causes behind the interactive effect of genes and environment can lead to treatments that will slow, stop, or even prevent disease.

Genetic delineation of unknown risk factors

Both genetic variants and environmental factors contribute to the multifactorial origins of phenotypic variation and disease risk. Identifying these genetic variants is fundamental to determine/define new risk factors, diagnostic markers, and possible drug targets. In order to delineate these variants, different methods and analyses can be employed.

Linkage analysis studies

Linkage analysis is a well-established statistical method for mapping heritable traits to their chromosome locations. Each chromosome location is characterized by specific genetic markers, and individuals that are related will share the same marker allele. Thus, if a region of the genome influences a certain trait, markers lying in the same region are physically linked to this influential locus and will be inherited together, known as to be in linkage disequilibrium (LD) with the phenotype. Linkage analysis can be performed in large families with individuals who exhibit a heritable trait of interest, and members of the families that have been genotyped for markers to identify disease-causing genetic regions, or in controlled crosses of experimental species that present a differential phenotype for certain traits (Cantor, 1995).

A quantitative trait is a measurable phenotype emerging from genetic and environmental factors (Grisel, 2000) The quantitative trait is therefore expected to display a normal distribution in a population (however, non-normal phenotypes can also be analysed such as disease/no diseases or score). A genetic region identified to be linked to a measurable phenotype is termed quantitative trait locus (QTL). QTL represents the likelihood that a genotype in LD with the marker is influencing the phenotype; this probability is termed the logarithm of odds (LOD) score (Klein et al., 2012). Linkage analysis has been used to study complex and quantitative traits.

The term complex derives from multiple genetic and environmental factors determining disease risk. Thus, linkage analysis of complex traits provides statistical evidence of the involvement of such multiple risk variants in disease risk. For instance, a LOD score of 2 indicates 100 times higher chance of having a QTL in the target population at the specific location, than expected at random.

QTL analysis detects natural variability. Derived from two inbred parent populations, F1 animals are genetically identical and heterozygous at every locus throughout the genome. In the F2 population, a random distribution of genetic variability inherent in the parent strains is generated as a result of chromosomal recombination. QTL regions identified through an F2 are often broad (e.g. 20 centiMorgans (cM)) and can contain hundred of genes. The generation of congenic strains represents an extension of QTL analysis that is commonly employed to confirm and fine-map a QTL region. This strategy involves transferring a QTL interval from one inbred strain (i.e. donor strain) into another inbred strain (i.e. recipient strain) using marker-assisted breeding over ten

generations of backcrossing. After ten generations, heterozygotes for the donor region are intercrossed to generate congenic strains. The resulting congenic strains are homozygous at the QTL locus and for the recipient strain in the background genome.

DA.VRA4 congenic model to study immune allelic variants

A congenic strain is generated by transferring genomic segments from a donor strain to a recipient strain genome by repeated backcrossing and selection. Marker-assisted selection can be used to reduce the number of backcross generations. By selecting founders in each generation with the least background, a 0.1% pure congenic can be obtained by 5 generations (Wakeland et al., 1997).

The congenic strains thus allow us to study, in isolation, the effect of a QTL and dissect its role by functional studies, since congenic animals are genetically identical animals. However, it is important to note that due to the loss of the random assortment of parental alleles, the effect of interaction between alleles cannot by addressed in these strains.

In the context of this thesis, the DA.VRA4 congenic rat strain was used to perform functional studies of different major histocompatibility complex class II transactivator (*Mhc2ta*) alleles, encoded within the VRA4 locus.

The VRA4 locus was identified as regulating MHCII expression levels in the CNS after mechanical nerve injury, by linkage analysis in a cross between DA and (PVG^{AV1}) (Lidman et al., 2003). Initially the PVG^{AV1} strain, which expresses the same MHC haplotype as DA (Lundberg et al., 2001), was backcrossed multiple times to DA to create the DA.VRA4 congenic strain, carrying PVG alleles in the VRA4 region on a DA strain background.

Furthermore, the VRA4 locus was fine-mapped, and *Mhc2ta* identified as the candidate gene responsible for differential expression of MHCII (Swanberg et al., 2005). In addition to that, translational studies demonstrated that a SNP in the regulatory region of the orthologous human version of the *MHC2TA* gene is associated with differential expression of both *MHC2TA* and *MHCII* genes in peripheral blood cells and with increased susceptibility to rheumatoid arthritis, multiple sclerosis and myocardial infarction (Swanberg et al., 2005).

MHC2TA gene encodes the protein CIITA, and represent the major regulator of *MHCII* expression (Figure 4).

There is increasing evidence of the concomitant inflammatory process in PD. The fact that common genetic variants affecting expression levels of MHCII are associated with PD risk, strongly supports a role of immune response in disease susceptibility. MHCII molecules are expressed by antigen-presenting cells in the brain parenchyma (microglia, astrocytes and infiltrating macrophages). These antigen-presenting cells are responsible for antigen presentation to CD4+ T-lymphocytes, bridging the innate and adaptive immune system. The observation that genetic variability of *MHC2TA* (CIITA*)*, both in rats and humans, results in differential *MHCII* expression associated with

susceptibility to complex diseases with inflammatory components, prompted us to further look into its potential role in determining PD risk.

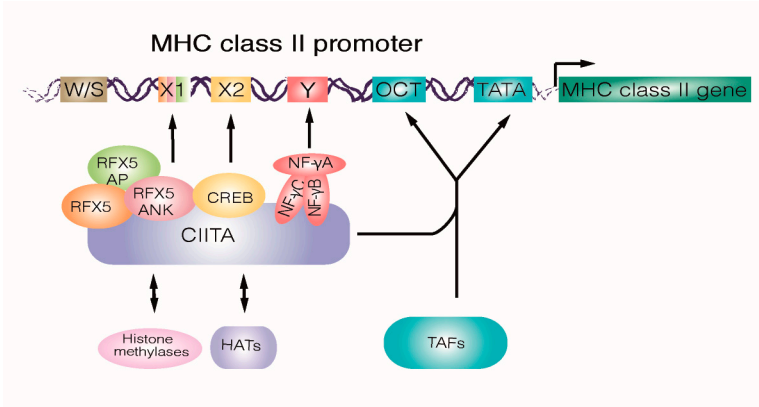


Figure 4 Scheme of MHCII expression regulation by CIITA (MHC2TA) .The SXY module located upstream of the MHC class II genes transcription initiation site. CIITA binding elements RFX: regulatory factor X (RFX5,RFX5AP,RFX5ANK), CREB: cyclic AMP responsive element-binding protein. NF-γ: nuclear transcription factor Y. CIITA coordinates the recuirment of chromatin modification and remodelling fators (HATs and histone methylases) and factors that are involved in transcription initiation (TAFs).

Genetic association studies

Complex diseases present a more nuanced genetic architecture compared to monogenic disorders, in which the presence or absence of disease alleles usually predicts the presence or absence of disease completely. For genetically complex disease, risk alleles are less deterministic and more probabilistic; the presence of a high-risk allele may only mildly increase the chance of disease, and these variants will have weak frequency. To track these genetic causative variants in complex disease, GWAS are performed. GWAS are based on association tests of common SNP variants frequencies between patients and controls. Variations with higher frequency in the patients groups are called associated with increased risk of disease. The usual conclusion of GWAS studies is that the polymorphism being tested either affects risk of disease directly, or is a marker for genetic variant in LD that affects risk of disease.

The sequencing technology has facilitated the discovery of common polymorphisms in genes, outside genes, or in intronic regions. Since the human genome project, the number of SNPs has been increasing. The number of SNPs in public databases is now over 1,000,000 (Sachidanandam et al., 2001)

Modelling of Parkinson´s disease pathology

Animal models are pivotal for studying the molecular underpinning of PD and its related symptoms, and have significantly advanced our understanding of PD.

The en1$^{+/-}$ transgenic mouse model

Engrailed-1 (*en1*), is a developmental gene of the homeobox family, essential for the development of mesencephalic DN (Le Pen et al., 2008; Sonnier et al., 2007) and axon guidance (Fuchs et al., 2012). *en1* plays an essential role in the survival and maintenance of these cells, together with *en2* (Alvarez-Fischer et al., 2011), a paralogue to *en1*, which exhibits overlapping molecular functions but distinct spatiotemporal expression (Hanks et al., 1995). Additionally, polymorphisms in the human orthologue of this gene (*EN1*) have been (weakly) associated with PD in humans (Fuchs et al., 2009; Haubenberger et al., 2011).

Several studies from partial or total loss of *en1* have revealed that the outcome varies depending on the mouse strain. The total loss of *en1* is perinatal lethal in 129/Sv, C57Bl/6x129/Sv, and SwissOF1 strains, but C57Bl/6- *en1*$^{-/-}$ are born alive (Bilovocky et al., 2003; Wurst et al., 1994).

In the context of PD, these phenotypic differences between SwissOF1 and C57Bl/6 strain *en1*-hemizygous strains are relevant for PD. While SwissOF1-*en1*$^{+/-}$ mice display dystrophic nigrostriatal terminals, progressive dopaminergic neurodegeneration, motor deficits, and depressive-like behaviour (Le Pen et al., 2008; Nordstroma et al., 2015; Sonnier et al., 2007), C57Bl/6-*en1*$^{+/-}$ mice appear normal and require additionally complete *en2* deletion for developing degeneration of DN (Sgado et al., 2006). This data proves that genetic factors outside the *en1* locus are responsible for the strain-dependent differential dopaminergic neurodegeneration induced by *en1*$^{+/-}$, and that these two strains can be used for identifying loci linked to DN loss, and axonal pathology, using a linkage analysis strategy, which has been employed in Paper I.

αsyn overexpression rat models

Point mutations in the *SNCA* gene, encoding for αsyn, and multiplications of the *SNCA* locus have been identified in monogenic forms of PD (Polymeropoulos et al., 1997; Singleton et al., 2003). The relevance of αsyn to sporadic PD is strongly supported by the presence of pathogenic aggregates of αsyn in neurons of patients (Spillantini et al., 1997). This has motivated the development of animal models based on αsyn overexpression. Moreover, several types of viral vectors have been used to deliver human αsyn transgene into the SN, by stereotactic injection; primarily lentiviruses and adeno-associated vectors (rAAV) (Decressac et al., 2012; Kirik et al., 2002; Lo Bianco et al., 2002).

The use of rAAV has been shown to be efficient in inducing overexpression of human wild-type αsyn in the nigral DN, accompanied by cellular and axonal pathologies in Sprague Dawley (SD) rats (Kirik et al., 2002). In the rAAV model, the expression of the transgene is dependent on different factors: the AAV serotype used (AAV2, 5, and 6), the number of viable vector particles injected, and critically, the efficiency of gene expression. In this regard, improvements have been made over the years, which include, increasing the expression efficiency using a neuron-specific synpasin-1 promoter, and a woodchuck hepatitis virus element (WPRE) (Decressac et al., 2012).

In SD rats, the rAAV6-αsyn model results in progressive loss of TH-positive neurons, ranging between 60 and 80% reduction in TH-positive cell numbers in the SN, accompanied by reduction of striatal TH+ innervation and axonal pathology (Decressac et al., 2012). The axonal changes include αsyn containing dystrophic axons, which are remarkably similar to those observed in brains from PD patients (Braak et al., 2000). The presence of axonal pathology is visible 3 weeks after injection, prior to DN loss. Furthermore, rAAV6-αsyn overexpression has been shown to induce motor impairment (Decressac et al., 2012), and has been broadly reproduced in different rat models (Aldrin-Kirk et al., 2014; Jewett et al., 2018).

In the context of inflammation, the rAAV-mediated overexpression of human wild-type αsyn has been shown to induce an early and persistent inflammatory reaction in SD rats (Sanchez-Guajardo et al., 2010). The injection of rAAV6 to overexpress human αsyn strategy has been employed in Paper II.

αsyn seeding and propagation *in vivo* models

The αsyn overexpression models give us insight into pathogenic mechanisms of αsyn; however, the expression levels that results from the rAAV6 transgene delivery is far from levels associated with idiopathic PD and also far beyond the levels in cases with *SNCA* duplications or triplications, where a duplication of *SNCA* give rise to a idiopathic-like PD and triplications of *SNCA* give rise to early and sever PD. Besides, these models lack the formation of LB-like and Lewy neurites-like structures that, as mentioned in previous sections, are mainly composed by pathogenic aggregated forms of αsyn. In order to understand the mechanisms underlying aggregation, misfolding and propagation of αsyn, *in vivo* models based on preform fibrils (PFFs) have been developed.

The preform fibrils (PFFs) propagation model was first developed *in vitro* by exposing primary neuronal cultures to PFFs αsyn. PFFs are generated from recombinant αsyn monomers and subsequently sonicated into smaller fibrils (50 nm). (Volpicelli-Daley et al., 2011) and administer to primary neuronal culture, inducing aggregation of αsyn (Luk et al., 2009; Volpicelli-Daley et al., 2014).

In vivo, it has been shown that the local administration of sonicated PFFs αsyn acting as seeds, starting a pathogenic cascade, whereby endogenous αsyn levels are triggered to misfold and form aggregates. In this regard, human αsyn PFFs have been injected in the striatum of transgenic mice expressing a mutant form of human αsyn (A53) (Luk,

Kehm, Zhang, et al., 2012) and in rats (Abdelmotilib et al., 2017). This model has been also reproduced using mouse αsyn PFFs in mice (Harms et al., 2017; Luk, Kehm, Carroll, et al., 2012), in rats (Duffy et al., 2018; Paumier et al., 2015) and most recently in non-human primates (Shimozawa et al., 2017).

The PFFs model recapitulates the emergence of LB-like and LN-like pathology in regions that are connected in the brain. For example, following striatal PFFs injection, regions innervating the striatum were shown to display signs of phosphorylated αsyn (pαsyn), which has been shown to be a pathogenic form (Duffy et al., 2018; Luk, Kehm, Carroll, et al., 2012; Luk, Kehm, Zhang, et al., 2012; Paumier et al., 2015), hence suggesting that axonal terminals can internalize the PFFs, and retrogradely transport and seed endogenous αsyn. Interestingly, *in vivo* administration of PFFs in αsyn-/- mice does not result in formation of αsyn aggregates or dopaminergic degeneration (Luk, Kehm, Zhang, et al., 2012), highlighting the requirement of normal endogenous αsyn as substrate. Nevertheless, this model has shown to be able do develop LN-like and LB-like aggregates, recapitulating events of the pathological cascade in PD.

The development of the pathological cascade and significant neurodegenerative changes in the presence of PFFs acting as pathogenic seeds, can take up to 6 months (Duffy et al., 2018; Paumier et al., 2015). Furthermore, it has been shown that the use of human αsyn PFFs in mice induced αsyn pathology to a lesser extent compared to mouse αsyn PFFs in the mice (Rey et al., 2016), thus suggesting a partial species barrier related to seeding and pathology triggering.

These observations led to the development of a new model using SD rats that combine the overexpression of human αsyn transgene and administration of human PFFs. This model recapitulates many of the pathophysiological hallmarks observed in human PD, such as aggregates of pαsyn, microgliosis, DN loss in SN, and motor impairment, in a short time span (Thakur et al., 2017). The use of human αsyn PFFs as seeds of αsyn pathology has been used in combination with human αsyn overexpression in DA.VRA4 congenic rats in Paper III.

Importantly, the *en1*$^{+/-}$ transgenic model together with the αsyn overexpression and αsyn propagation in DA.VRA4 congenic rats models, used in this thesis, develop spontaneous dopaminergic neuronal loss and striatal axonal swellings, inclusions of aggregated αsyn and αsyn-mediated neuronal loss and behaviour impairments thereby replicating the defining pathological hallmarks of PD, and driving insightful advances into understanding the pathogenic mechanisms underpinning PD.

Aims of the thesis

The overriding aim of this thesis was to bring us closer to elucidating the genetic factors that regulate the susceptibility to Parkinson´s disease (PD), through immune mechanisms.

In line with this goal, a series of studies were conducted to further our understanding of the effect of genetic variants on PD pathology, with special focus on the immune system in experimental models and humans. The main aims can be summarized as follows:

1. To delineate novel genetic susceptibility loci linked to dopaminergic neurodegeneration, by performing linkage analysis in engrailed-1 (*en1)* hemizygous mice.

2. To assess the effect of common genetic variants of the Class II Major Histocompatibility Complex Transactivator (*Mhc2ta)* on alpha-synuclein (αsyn) overexpression-induced dopaminergic neurodegeneration and microglial activation patterns in two congenic rat strains.

3. To determine the effect of allelic differences of *Mhc2ta* on αsyn aggregation and propagation in the αsyn-seeding combined model.

4. To evaluate the prevalence of monogenic forms of PD in the Swedish PD patient population, by performing mutation screening in *LRRK2* and *SNCA* genes.

Experimental considerations

A brief description of the methods utilized in the papers constituting this thesis is compiled in the following section. More details can be found in the corresponding paper.

All experimental procedures performed were conducted in accordance with guidelines set by the Ethical Committee for the use of laboratory animals in the Lund-Malmö region Sweden.

Parkinson´s disease models

In this thesis, three approaches were used to model three critical aspects of PD.

1. To study the dopaminergic cell loss present in PD, we have used the *en1*$^{+/-}$ transgenic mouse model.
2. To determine the αsyn-induced effects on microglial activation, dopaminergic neurodegeneration and motor deficits observed in PD, we have used the well-studied rAAV6-αsyn overexpression model in a new paradigm using a congenic rat strain.
3. To assess the progressive spread of αsyn observed in PD, we have used a recently developed seeding model, in which the combination of a nigral rAAV6-αsyn injection precedes the striatal injection of αsyn PFFs seeds.

en1$^{+/-}$ transgenic animals

Breeding scheme

In order to genetically map the strain-specific susceptibility to dopaminergic neurodegeneration induced by *en1* hemizygosity in paper I, F2 animals with *en1*$^{+/-}$ and *en1*$^{+/+}$ genotype were generated on a SwissOF1xC57Bl/6 background. These strains were chosen due to the fact that SwissOF1 mice display PD-like pathology with preferential loss of dopaminergic neurons (DN) in substantia nigra pars compacta (SN) when *en1* hemizygous, but C57Bl/6 mice do not display DN degeneration. To generate the F2 generation, C57Bl/6 *en1*$^{+/+}$ males were crossed with SwissOF1 *en1*$^{+/-}$ females to generate the F1 generation. Subsequently, from F1, *en1*$^{+/+}$ males were intercrossed with *en1*$^{+/-}$ females to produce the F2 generation (Figure 5). In total, 129 F2-*en1*$^{+/-}$ and 57 F2-*en1*$^{+/+}$ males were included and sacrificed at 17 weeks of age. At this point, the loss of DN is reduced significantly in the SwissOF1, and it has been shown that there is no additional loss between 24 and 48 weeks (Sonnier et al., 2007).

Using a speed congenic approach (Wakeland et al., 1997), the transgene *en1*$^{+/-}$ was transferred from SwissOF1 *en1*$^{+/-}$ to the C57Bl/6 background. The speed congenic approach consisted of repeated backcrossing to C57Bl/6 *en1*$^{+/+}$ females, starting with an F2 *en1*$^{+/-}$ male. In each generation, *en1*$^{+/-}$ males were subjected to single nucleotide polymorphism (SNP) analysis (Illumina Golden Gate assay) and the *en1*$^{+/-}$ with the highest number of C57Bl/6 alleles was kept for backcrossing to produce the next generation.

This marker-assisted selection allowed us to generate the F2 generation in less than half the number of generations and time required by the classic protocol, which is based only on the presence of the desired gene, and the gene donor is backcrossed serially to the recipient strain. In contrast, the speed congenic, which is based on the presence of the desired gene and the absence of contaminating donor genes from other parts of the genome. The C57Bl/6-N4 generation had an average of <3% SwissOF1 alleles outside the en1 locus.

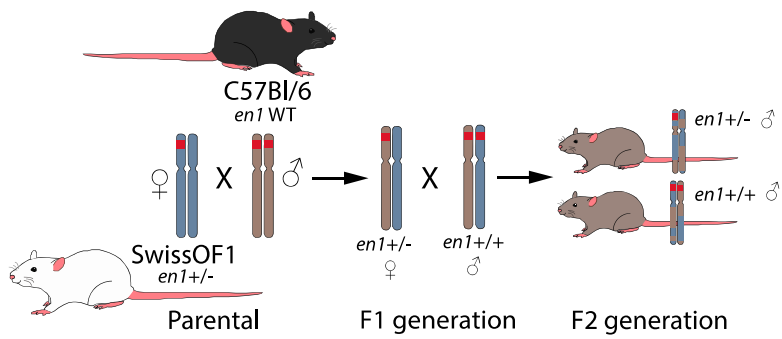


Figure 5 | Breeding scheme for generating F2 population of *en1*$^{+/+}$ and *en1*$^{+/-}$ mice on a SwissOF1 background. Parental C57Bl/6-*en1*$^{+/+}$ males (wild type *en1* gene) and SwissOF1-*en1*$^{+/-}$ females were crossed to generate the F1 generation, from which en1+/+ males and en1+/- females were intercrossed to generated the F2 generation (en1+/- and en1+/+ male mice). A total of 129 F2-en1+/- and 57 F2-en1+/+ males were generated and sacrificed at 17 weeks of age.

Genotyping

To identify single allele knockout of en1, PCR was performed with primers for *LacZ*, which was used to knockout one copy of en1. We also performed qPCR using the SsoAdvanced$_{tm}$ SYBR Green Supermix (Bio-Rad) for genotyping of *LacZ*. Primers used can be found in Table 1.

Vra4 Congenic animals

From a previous QTL analysis study, the Vra4 locus was identified and linked to susceptibility to inflammatory diseases (Harnesk et al., 2008; Lidman et al., 2003; Swanberg et al., 2005). Further genetic fine-mapping of this region revealed variations in the promoter region of *Mhc2ta* (Swanberg et al., 2005), and two congenic rat strains were generated to study the effect of these variations in inflammatory complex diseases (Harnesk et al., 2008).

In this thesis, we have made use of these congenic rat strains in the αsyn-induced pathology and propagation double-hit seeding models. Founders for DA and DA.VRA4 strains were originated and kindly provided by Professor Piehl at Karolinska Institute, Stockholm, Sweden. The animals used in these studies were from our breeding, and used to establish breeding colonies at Clinical Research Center (CRC) in Malmö.

The DA.VRA4 congenic rats were initially bred as previously described (Harnesk et al., 2008). The original VRA4 alleles donors were PVG males. Repeated backcrossing to the DA recipient strain was performed to create a congenic strain, which after 10 generations will have theoretically <0.1% of the donor genome outside the VRA4 locus (Figure 6).

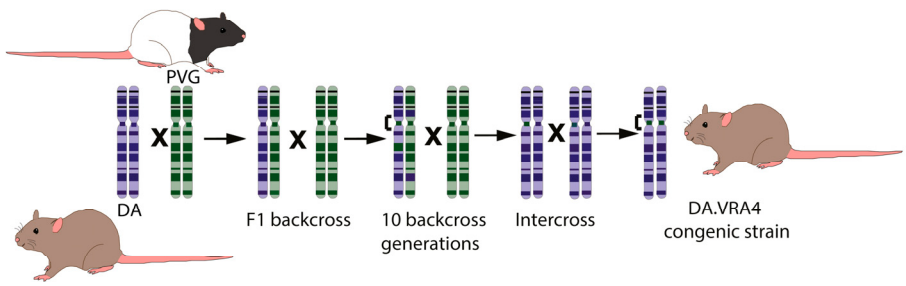


Figure 6 | Breeding scheme for generating DA.VRA4 congenic strain.
Parental PVG and DA were intercrossed to give origin to the F1 and backcrossed 10 times with selection on the VRA4 region (open bracket); an intercross generated homozygous DA.VRA4 congenic strain, with >99.9% DA genome outside the PVG fragment.

Genotyping

Genomic DNA was extracted from tail tips, and High-resolution melt (HRM) analysis was conducted for genotyping using two markers, one inside the congenic region and the other in the non-congenic region. DNA from DA and PVG parental rats were included for each marker as a control. Primers used can be found in Table 1.

Tabel 1 | Primers used for genoypting
Primers were synthezied at Eurofins and were used at 10 μM concentration.

Region	Primer name	Forward Sequence (5′-3′)	Reverse sequence (5′-3′)	Paper
en1-LacZ	*LacZ*	TGTATGAACGGTCTGGTCTTTG	AACAGGTATTCGCTGGTCACTT′	I
Vra4 locus	*D10Rat95*	TTACACCTCTCCAACACTGCC	TCATAGGAGGGAATACGGACA	II
Outside Vra4 locus	*D10Rat47*	TTTTTCCCCTTCTTTCTGACTG	TCACAATTCTCCACGTGAGG	II

Viral Vector based model

In order to express human αsyn in our congenic animal model, we employed unilateral stereotactic injections of adeno-associated viral vector serotype 6 (rAAV6) into the SN of DA and DA.VRA4 animals. The construct consisted of full-length human wild type αsyn. The expression was driven by the synapsin-1 promoter and enhanced using a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) (Decressac et al., 2012).

The immunogenicity of the rAAV vectors highlights the relevance of using a non-immunogenic transgene as a control, to distinguish the effects of the transgene from the immunogenicity of the vector. In our model the effect of αsyn expression was compared to an AAV6-GFP (+WPRE) control vector (Figure 7A). The viral vector concentration for each batch was assessed with qRT-PCR, where expression of WPRE sequence was quantified to determine the titer of the virus (both rAAV6 vectors were obtained from AAV Vector Lab, Multipark platform, Lund, Sweden). Several points need to be considered when using rAAV6 vectors, to ensure reproducibility of the results. For instance, storing the virus at the right temperature, perform titration pilot experiments to test the efficacy of the virus, etc. It is important to bear in mind that the transgene copy number injected is an estimated value, and we do not know the amount of genome copies taken up by each cell. Genome copies and dilutions of the vectors used in paper II and paper III can be found in Table 2.

Table 2 | rAAV titers used in the experiments.

	Paper I	Paper II
rAAV6-GFP	9.6E+14 GC/ml	1.02E+10 GC/ml
rAAV6-αsyn	3.6E+14 GC/ml	1.02E+10 GC/ml

Preformed fibrils seeding model

Aggregation and misfolding of αsyn play a central role in the pathogenesis of PD. In rodents PD-like αsyn pathology can be induced by overexpression of wild type or mutated forms of αsyn through injection of viral vectors, or, alternatively, through intracerebral administration of preformed αsyn fibrils (PFFs).

The PFFs model is based on the discovery that short αsyn fibrils, injected into the brain, can act as seeds for the formation of αsyn aggregates using endogenous αsyn as substrate. (Luk, Kehm, Carroll, et al., 2012; Luk, Kehm, Zhang, et al., 2012). The restricted diffusion of the injected PFFs from the site of injection is a limitation of this

approach, particularly when the PFFs are injected into the striatum, which makes it difficult to induce pathology in a substantial fraction of SN.

In paper III, we have combined the rAAV6-mediate overexpression of human αsyn and addition of exogenous of PPFs. Striatal delivery of αsyn-seeds, 5 µg (2.5 µl per site), composed of human αsyn preformed fibrils (PFFs) were unilaterally injected in the striatum of 12 weeks of age DA and DA.VRA4, 2 weeks after rAAV6-human-αsyn vector (αsyn-substrate) delivery in SN. PFFs were kindly generated and provided by Assistant Professor Kelvin C. Luk and Professor Virginia Lee at Pennsylvania University. Prior to injections, the PFFs were thawed and sonicated at room temperature by probe sonication to produce PFFs seeds (Thakur et al., 2017; Volpicelli-Daley et al., 2014). To visualize the PFFs seeds after sonication, electron microscopy was performed at the Lund University Bioimaging Centre (Figure 7B).

This model combines two inducers of αsyn pathology: an increase in the expression levels, and the generation of toxic forms, thereby making this model suitable for determining αsyn anatomic spread, seeding effects and conformational changes and αsyn-induced dopaminergic pathology.

The surgical procedure can induce a rather strong inflammatory response, rendering the use of an appropriate control is essential. In this study, we have used two control groups to consider the effects of αyn-substrate, αsyn-seeds, and the surgical procedure itself. The αyn+PFFs vs GFP+PFFs-control group comparison, allowed us to distinguish the effect caused by the presence of αyn-seeds without substrate and gave us information regarding unspecific rAAV6-immunogenicity and GFP-toxicity. In the other hand, the αyn+PFFs vs αyn+BSA-control group comparison, provided information regarding the αsyn substrate alone and the response to the second surgery injection (Figure 7C).

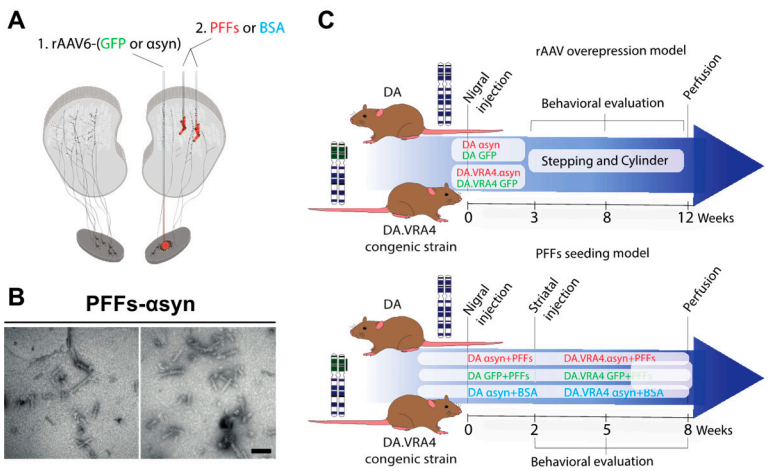


Figure 7 | Experimental design and injection scheme

(A). Injection scheme for rAAV-mediated-αsyn neurodegeneration (1.) and PFFs-αsyn-seeding model (1. and 2.) **(B)**. Representative TransEM images PFFs before (left) and after (right) sonication and suspension in DPBS (scale bar 100 µM). **(C)**. Experimental design, study timeline and control groups in Paper II (top) and Paper III (bottom).

Stereotactic delivery of rAAV6 and PFFs

Animal surgery

All surgical procedures were performed under 1-2% isoflurane anaesthesia in a 2:1 Oxygen/Nitrogen oxide mixture and the depth of anaesthesia was monitored during all steps of the surgery. Animals were placed and fixed in a stereotaxic frame (Kopf Instruments) (Figure 8). The eyes of animals were covered with eye ointment (Viscotears) to prevent eye dryness, and the head was disinfected with 70% ethanol. Marcain was used as local anaesthetic, and a small midline incision was made by a sterilized scalpel. Thereafter, the skull was exposed gently and cleared of blood and fluids. Once the skull was clean it was thinned using a dental drill to make a burr hole at the determined anterior-posterior and medial-lateral midbrain coordinates given in Table 3. The rAAVs (2 µl) or PFFs (2.5 µl per site) solution was injected using a 10µL Hamilton syringe fitted with a glass capillary (outer diameter of 250 µM). The capillary was left in place for 2 min after injection to allow for diffusion before being retracted carefully. After closing the wound, we provided post-operative analgesia by subcutaneous injection of 0.15 ml Metacam. It should be noted that Metacam is a nonsteroidal anti-inflammatory drug (NSAID), and we cannot rule out the immediate anti-inflammatory effects of one single administration, however, the effects studied in this thesis are based upon stable transgene expression, which is reached approximately two weeks after injection, and persist long-term. In addition, the use of Metacam will be compared to that of groups used as control. All animals were then placed in clean cages on a heated pad for recovery and monitored for the next 48 hours.

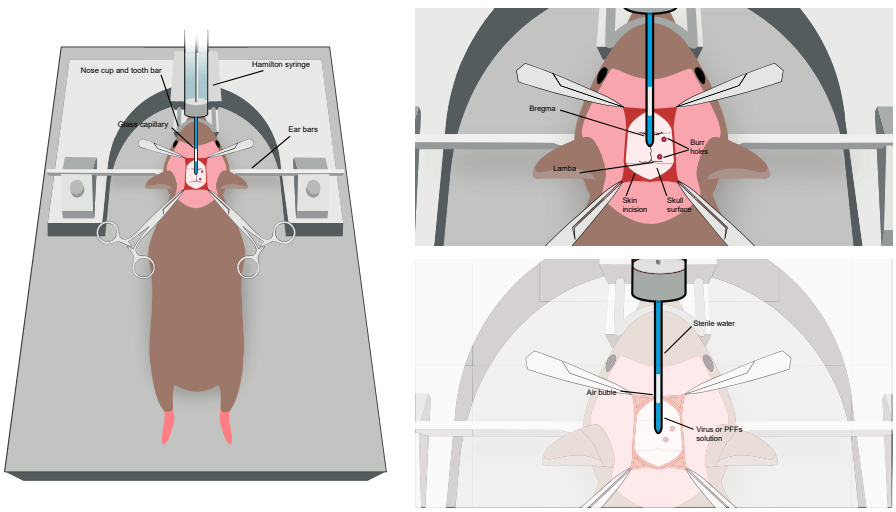


Figure 8 | **Scheme of stereotactic injection setup for rAAV and PFFs delivery**

Tabel 3. Coordinates in mm relative to Bregma and dural surface (Paxinos et al., 1980)
Tabeltext is better placed above the table.

Coordinates	Paper II	Paper III	
	SN	SN	ST
Anteroposterior	-5.3	-5.3	0.1 and -0.4
Mediolateral	-1.7	-1.7	-3.0 and -3.0
Dorsoventral	-7.2	-7.2	-5.0 and -4.5

Behavioral analysis

Both non-motor and motor features characterize PD. Striatal dopamine depletion has been identified as the major cause of the motor symptoms. In animals, different behavioural test are used to assess the presence of motor impairment. In this thesis, we have determined the presence of motor impairment using two behavioural tests performed at 3, 8 and 12 weeks after vector injection (paper II), and 2, 5 and 8 weeks after vector injection (paper III). The same experimenter, blinded to the group-identity, performed each test in the same testing room, and at the same interval during the day (9-12 AM) throughout the study.

Stepping test

This test allowed us to address forelimb akinesia, which is the loss or impairment of voluntary movement. We used the adjusting steps protocol described in (Olsson et al., 1995).

Steps for each forelimb were counted and averaged across trials. For that, a distance of 90 cm is marked on a smooth, clean surface, cleared from distracting objects. The experimenter held the animal with one hand fixing the hind limbs and slightly raising the hind part above the surface. With the other hand, the investigator lifted and locked in the contralateral forepaw and kept it out of the visual field of the animal. At the initiation of the experiment, the animals were habituated and pre-trained in the test for 3 days. Afterwards, the performance is scored in triplicates over three consecutive days. The animal is moved slowly sideways, first in the forehand, and then in the backhand direction. The number of adjusting steps was counted for both paws in the backhand and forehand directions movement, resulting in 4 parameters recorded at each time.

The results are presented as the average number of adjusting steps made by the forehand contralateral to the injections.

Cylinder test

To evaluate locomotor asymmetry during natural exploratory behaviour, we performed the cylinder test (Schallert et al., 2000). In this test, as the animal moves within an open-top, clear glass cylinder, the forelimb activity is recorded while rearing against the wall. The forelimb use was defined by the placement of the whole palm on the wall. The animals were placed in a glass cylinder, in front of two mirrors positioned at a 90° angle behind to allow for assessment of the full cylinder surface. Spontaneous

use of the forepaws was video-recorded and analysed *post hoc*. The first 20 touches made were counted. The data from this test is presented as the average number of left paw touches as a percentage of the total, (intact animals average around 50%).

Tissue processing

Perfusion and brain dissection

en1$^{+/-}$ Transgenic Mice

All animals were sedated by intraperitoneal injection of sodium pentobarbital (40mg/μL), and subsequently perfused through the ascending aorta with ice-cold saline (0.9% NaCl) for 3 minutes. After isolating the brain, the brain was placed in a mice brain matrix, to use one hemisphere for histology and the other for RNA/protein quantifications. The cerebellum was sliced off and post-fixed in PFA (4%, pH 7.4) for 20 minutes, and then transferred to saline for subsequent *LacZ* staining. The right hemispheres were placed on an ice-cold plate, and the striatum and midbrain were carefully dissected and snap-frozen. The remaining brain was placed in PFA (4%, pH 7.4) and post-fixed overnight and subsequently cryoprotected in 30% sucrose (PBS, with 0.01% sodium azide). The brain hemispheres were sectioned on a freezing sledge microtome (MICROM, HM450, ThermoScientific TM, US) and coronal sections were collected in a 1:12 series at a thickness of 35 μM.

DA.VRA4 Congenic rats

All animals were sedated by intraperitoneal injection of sodium pentobarbital (40mg/μL), and subsequently perfused through the ascending aorta with ice-cold saline (0.9% NaCl) and freshly prepared 4% paraformaldehyde (PFA, pH 7.4). Brains were then removed and post-fixed in 5mL 4% PFA overnight. A cryoprotecting sucrose solution (30% in PBS and 0.01 % sodium azide) was then used for storing the brains until sectioning. The brains were sectioned on a freezing sledge microtome (MICROM, HM450, ThermoScientific TM, US) and coronal sections were collected in a 1:12 series at a thickness of 35 μM. For gene expression experiments, heat-proof tools were cleaned with ethanol and rinsed thoroughly prior to being baked at 180°C for several hours to inactivate RNases. Animals were deeply anaesthetized intraperitoneal with pentobarbital and subsequently perfused through the ascending aorta with ice-cold saline (0.9% NaCl); the brain was isolated and placed in a rat brain slice matrix where midbrain and striatum were cut out with fine razor blades cleaned with RNaseZap to preserve the RNA. Both right and left hemispheres were dissected, resulting in 4 pieces of approximately 30 mg each per brain. Each piece was placed in lysis matrix sterile tubes and snap-frozen and stored at -80°C for subsequent RNA extraction.

Histological analysis

Immunochemistry

To determine the presence of proteins, we performed immunohistochemical stainings on free-floating sections using primary and secondary biotinylated antibodies listed in Table 4. We used a standard peroxidase-based method. It is important to remember that an immunohistochemical stain depends on the integrity of an antibody-antigen interaction, which can be affected by the fixation method used; the amount, age and pH of the PFA and the time the tissue is left in the PFA. All these variables can be mitigated to a great extent by antigen retrieval method. For that, sections were incubated in Tris/EDTA (pH 9.0) at 80°C for 45 min. All sections were quenched, and then blocked with the appropriate serum solution, prior to overnight incubation with the primary antibody on a shaker at room temperature. The next day, sections were washed three times in PBS to remove any unspecific binding.

Diaminobenzidine (DAB) method

For visualisation of the secondary antibody using the 3´diaminobenzidine (DAB) method, the secondary antibody incubation were performed in serum for 1 hour at room temperature, and the sections were subsequently washed three times for 10 minutes in PBS. Thereafter, we developed the section using 3´diaminobenzidine (DAB) and peroxide (3%). After mounting the sections on gelatine coated slides, we air-dried the tissue overnight before dehydrating the slides in a series of ascending concentration of ethanol, xylene. The slides were then coverslipped with DPX mounting medium (Sigma).

Immunofluorescence

To analyze two or more proteins in the same cell or tissue, double immunofluorescence stainings were performed using fluorescently tagged secondary antibodies. After the secondary antibody incubation, all steps were performed in the dark to prevent photobleaching of the conjugated secondary antibody. The fluorescent stained sections were mounted onto gelatine-covered slides and secured with coverslips using PVA-DABCO (Sigma) and visualised using the Confocal Microscopy Multipark platform.

Table 4 | **Antibodies used in the experiments**

Antigen	Company	Host	Dilution	Paper
αsyn (211)	Santa Cruz (sc-12767)	Mouse	1:1,000	I, II
GFP	Abcam (ab13970)	Chicken	1:1,000	I, II
pαsyn (pS129)	Abcam (EP1536Y)	Rabbit	1:2,000	II
TH	Millipore (AB152)	Rabbit	1:1,000	I, II
Iba1	Abcam (ab139590)	Chicken	1:1,000	II
MHCII	Abcam (ab23990)	Mouse	1:500	I, II
Mac1 (CD11b)	Bio-Rad (MCA275GA)	Mouse	1:500	I
Biotinylated anti-Rabbit	Vector Laboratories	Horse	1:200	II
Biotinylated anti-Mouse	Vector Laboratories	Goat	1:200	III
Alexa 647 anti-mouse	Abcam (ab150119)	Goat	1:200	III
Alexa 488 anti-rabbit	Abcam (ab150081)	Goat	1:200	III
Alexa 594 anti-chicken	Abcam (ab150176)	Goat	1:200	III

Dopaminergic neurodegeneration analysis

To determine the extent of dopaminergic neurodegeneration in all the different models used in this thesis, we applied three different methods.

Midbrain stereology analysis

In general, determining the number of cells in our regions of interest (Striatum and SN) is complicated, due to the fact that cutting these brain regions into sections results in cutting the cells in the tissue. Thus, the number of cell fragments in the sections differs from the original number of cells in the tissue and as a consequence, estimates of cell numbers are based solely on counts of cell fragments in sections. The optical fractionator method uses thick sections and estimates the total number of cells sampled in a systematic randomly sampling set of unbiased virtual counting space covering the entire region of interest, with uniform distance between virtual counting spaces in X,Y, and Z directions. In paper I, II and III dopaminergic neuron loss in the SN was determined by unbiased stereological estimations of the TH+ cells in SN, using the optical fractionator principle (West et al., 1990). According to this principle, an unbiased estimate number of a specific cellular population can be obtained if random sample series are systematically taken, and a section-sampling fraction (ssf) is counted. To do so, an observer blinded to the experimental group of the animal performed the counts every determine ssf (ssf= 3 paper I, ssf=6 paper II and III) covering the full extent of the SN yielding 8-10 sections per animal. Tracing regions of interest (ROIs) was done using a 5X/0.11 lens, and counting was performed with 100X/1.30 lens magnification and immersion oil. A series of counting frames were systematically and randomly distributed over a grid spanning the SN. The total population estimate was calculated using optical fractionator estimates (interval, step area, area of counting frame).

A maximal Gunderson coefficient error (CE) of 0.08 was accepted for cells to be counted. It has been shown that the rAAV injection in SN can cause down-regulation of TH previous to degeneration (Albert et al., 2019) which affects all the groups to a certain extent.

Striatal densitometry analysis

The extent of striatal degeneration was measured by optical densitometry. Dorsal striatum receives dopamine input from the SN, while ventral striatum is innervated by dopamine-producing cells in the ventral tegmental area (VTA) (Macdonald et al., 2011) . In PD, degeneration of SN is substantially greater than that in VTA. Furthermore, in our rat models we deliver rAAV6 vectors in the SN. Thus, we focused on the dorsal denervation of TH+ fibres. For this the entire striatum was divided into two equal halves along the dorsoventral axis. The dorsal striatal TH+ fibre optical density was determined using the ImageJ software at four coronal levels (1.60, 0.48, -0.26 and -0.40 mm relative to Bregma).

The images were obtained using a Light microscope at 2x magnification and analysed using the optical density values obtained from the Rodbard calibration curve, after being transformed into grey-measuring and subtracting background staining optical density (OD) of the corpus callosum for each animal to corrected for nonspecific background staining.

The calibration allows the program to assign a known OD mean grey value to a ROI under our experimental conditions. The data are presented as percentage of contralateral side to determine the loss of dopaminergic axonal fibres

Axonal swellings

To complement the study of striatal atrophy, the amount of axonal degeneration was determined by analysing axonal swellings, a feature of an on going degenerative process of the nerve terminal. We took an ImageJ-based semi-automatized approach. Four sections from each animal at Bregma distance 0.72–0.92 mm in mice and 1.60, 0.48, -0.26 and -0.40 in rats were analysed. High-resolution Z-stack 25x magnification images were taken using the same microscope, camera and software as for optical densitometry. Two pictures were taken of each section representing the dorsal and ventral part of the caudate-putamen of striatum for every section. The ImageJ software was used to identify the swellings and calculate the total number of swellings and their size by setting an exclusion threshold for particles < 3 μm^2. A genotype-blind operator performed image acquisition and processing.

Alpha-synuclein-induced pathology

Scoring of αsyn-induced pathology

For histological mapping, immunoreactive pαsyn+ inclusions/cells and neurites were mapped at previously described (Luk et al., 2012) rostral-caudal levels ([4.68,3.00] [1.44,0.60] [-0.60, -1.08] [-3.60, -4.44] [-5.52, -6.12] [-7.80, -8.64] [-9.48, -10.32] mm). The presence of pser-129-positive accumulations was assessed in a blinded manner by two independent researchers screening every section at 20x. To assess the accumulation of αsyn pathology (pser129-positivity) in animals, we used a modified scale (Table 5) from one described previously (Rey et al., 2016) for each section at the levels mentioned above. Data is presented at group level and shown as average values from each animal score.

Table 5 | αsyn pathology scoring scale

Score	Pathology degree	Description
0	No pathology	No aggregates
1	Sparse	Very few neurites, max 1 nuclear deposits, scattered dot-like inclusions
2	Mild	Varicose and filiform neurites, with or without presence of nuclear deposits
3	Moderate	Many nuclear and perinuclear, large areas with dot-like inclusions
4	Dense	Many nuclear, perinuclear, filiform neurites and Lewy neurite-like inclusions
5	Severe	Many Lewy bodies-like and Lewy neurites-like and dot-like inclusions
6	Severe	Many Lewy bodies like and Lewy-neurites-like inclusions

Assessment of inflammatory state of microglia and peripheral immune response

To determine the expression at RNA and protein levels of specific local and peripheral inflammatory and immune response markers in our congenic model, we performed gene expression, protein, and morphological analyses.

Gene expression analyses

RNA isolation and quantitative PCR

The striatal and midbrain tissue were collected promptly after brain isolation. The dissection was performed with sterilized tools on ice to minimize RNA degradation, and all collected samples were snap-frozen in dry ice. The tissue was treated with 1 ml of Trizol (Qiagen) and homogenized using lysis matrix tubes and MP fastprep homogenizer. Following that, total RNA was extracted from around 30 mg brain tissue (ST and SN) from DA and DA.VRA4 animals using the miRNeasy kit (Qiagen, USA). Samples of total RNA (100 ng) were reverse-transcribed using SuperScript III First-Strand Synthesis System. Quantitative real-time PCR was conducted using SYBR Green (Applied Biosystems) with specific primers listed in Table 6. Using GENORM analysis, *β-actin* and *GAPDH* were used as reference genes, and relative expression levels were determined according to the ΔΔCt method. Data are presented as fold change compared to the control group or contralateral side.

Table 6 | Gene expression analysis primers
Primers were designed using Primer3 software and synthezied at Eurofins. All primers were used at 10 μM concentration.

Gene	Forward primer (5´-3´)	Reverse primer (5´-3´)
GAPDH	CAACTCCCTCAAGATTGTCAGCA	GGCATGGACTGTGGTCATG
β-actin	AAGTCCCTCACCCTCCCAAAAG	AAGCAATGCTGTCACCTTCCC
Arg-1	GTGGCGTTGACCTTGTCTTG	GCCTGGTTCTGTTCGGTTTG
CD86	GCTCTCAGTGATCGCCAAC	TCTTTGTAGGTTTCGGGTATC
CD74	GTGATGCACCTGCTTACGAAGT	CTCCGGGAAGCTCCCCT
Mhc2ta	CATACTCTCTGTGTGCCACCATGG	AGTTCGATCTCTTCCTCCCCA
MHCII	TTCCCCCGACCAAAATGGAG	GAGCCTCAAAGCTGGCAAAC
Iba1	GAGGAATGGGTAGAAAGGGG	AGGAAGTGCTTGTTGATCCC

Serological analysis

Serum collection

Blood was collected by cardiac puncture at the terminal stage of the study from deeply sedated animals. A wide-bore needle was used for blood sample collection. The collected blood was kept at room temperature to form a clot (~30 minutes). Subsequently, the collection tubes were centrifuged at 2500 G for 15 minutes, and serum (supernatant) was collected. The serum was stored at -80°C until analysis.

Multiplex measurement of proinflammatory cytokines in serum

Meso Scale Discovery (MSD) plates were used to evaluate the cytokine levels (Pro-inflammatory panels for IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, TNF-α) in serum samples. To measure the cytokines, we used 25 µl sample/well diluted in PBS1:2. The plate was loaded with 50 µl per well of controls and unknown samples, incubated for 2h at room temperature with shaking, and washed with PBS. For detection, antibody was added per well and incubated for 2h with shaking at 800 rpm at room temperature, and finally, washed three times with PBS. The plate was developed using the 4x reading buffer diluted one time with distilled water and read using the QuickPlex Q120 reader from Mesoscale.

Stereological estimation and morphological characterization of microglia

Microglia activation is a complex process accompanied by morphological and gene expression changes. The morphology of microglia is one of its more dynamic characteristics. (Davis et al., 1994). Furthermore, different types of microglia have been reported in human brain (Torres-Platas et al., 2014). Thus, brain cells are morphologically similar across species, suggesting highly conserved functions. According to their morphology microglial cells can be categorized into four broadly distinct subtypes (Sanchez-Guajardo et al., 2010); Surveilling microglia (Type A), cells with no visible cytoplasm, a round dense nucleus, and with long thin processes with little branching; hyper-ramified (Type B), cells with a visible thin cytoplasm surrounding a dense nucleus; processes are very long and thin, with many branches of less define edges; hypertrophic (Type C), cells with elongated and irregular body, enlarged and less defined nucleus, and with shorter re-defined processes of varying thinness and little branching; amoeboid (Type D), has big cell body merging with the processes, the nucleus occupies most of the cell body and is not always distinguishable; processes are few thick and short, a drawback of this characterization using morphometric parameters is that, this type of microglia is indistinguishable from peripheral macrophages.

To characterize further the state of the microglia in the DA and DA.VRA4 animals we obtained stereological estimates of four distinct cell profiles. The counts were performed every six sections and the counting frame size was (56 x 56) µm for SN and

(53 x 53) μm in ST. Within the counting frame, the optical fractionator estimated the total number of Mac1+ (CD11b) cells , using the systematic random samples (SRS) set of unbiased counting spaces, covering the selected counting frame in the region of interest. The SRS layout was standardized to 211x211. The threshold for the coefficient of error for each animal was set at 0.06.

Immunohistochemical analysis and quantification of MHCII+ cells

To determine the response mediated by MHCII quantification of MHCII+ cells were made in 3-4 equally distant sections of the SN and striatum using a bright-field Leica DM1600B microscope at a magnification of 10x. MHCII is expressed by professional antigen presentation cells (APCs), whereas in the brain MHCII is mainly expressed by microglia and astrocytes. MHCII+ cells close to the site of injection or needle track were discarded from the analysis to avoid quantification of MHCII cells in response to mechanical injury associated with injection, and not to the presence of the transgene.

Linkage analysis

In order to explain the genetic basis of differential susceptibility to dopaminergic cell death, a linkage analysis was implemented for mapping quantitative trait loci (QTL) linked to PD-like neurodegenerative changes. QTL analysis allowed us to link TH+ nigral degeneration, axonal swelling load and axonal swelling size to specific regions of chromosomes in our generated F2 *en1*$^{+/-}$ model and the parental strains using parental strain-specific SNPs as genetic markers.

Genome-wide SNP assay

To perform the genome-wide linkage analysis, a panel of 377 SNPs was used as molecular markers. The SNP genotyping was performed at the SNP&SEQ technology platform at Uppsala University. Parental SwissOF1, C57Bl/6 and the F2 generation were genotyped. SwissOF1 and C57Bl/6 strains were used to find parental-specific alleles. To fulfil the requirement of parental-specific SNP, it needs to vary between C57Bl/6 and SwissOF1 parental but not within the population of outbred SwissOF1 used for intercrossing. Following these criteria, 126 from the 377 SNPs were parental-specific. As we only had 20cM of genomic coverage on this linkage analysis, the resolution of the QTL was too low to detect the genes affected by the causative polymorphisms.

Single QTL analysis

To identify genetic regions linked to neurodegeneration, data were analyzed using R/QTL, and *Scanone* function was used for single QTL analysis. For expectation-maximization (EM) and Haley-Knott methods, the genotype probabilities were calculated with 0.5cM distance and a genotyping error rate set at 0.001. For multiple imputations, the genotype was simulated with 1000 simulation replicates, step length of 0.5cM and error probability of 0.001. Significance thresholds for the logarithm of the odds favouring linkage (LOD) score were obtained by a permutation test, with 1000 permutations using Haley-Knot-regression. The single QTL method is based on the assumption that there is only one QTL, and the scan is performed at one locus at a time. This method is less powerful for detecting regions linked to multigenic traits.

Multiple QTL analysis

To determine QTLs with additive or epistatic effects, we performed multiple-QTL analysis. This method increases the power to detect QTLs linked to multigenic traits, but it requires a larger sample size. The multiple QTL models were fitted starting with the locus with the highest logarithm of the odds (LOD) score in the single-QTL model. The models were iteratively built by scanning for interactive and additive loci using the *addqtl* function with Haley-Knot regression. *Fitqtl* was used to fit the models. Loci and interactions with p-value, $p < 0.05$ in the drop-one-term ANOVA were kept in the model and used in scanning for additional loci. Genotype probabilities were calculated with a step length of 0.1 cM and error probability of 0.001. To estimate the positions of QTLs in the model, we calculated the approximate 95% Bayes credible intervals (Manichaikul et al., 2006). Significance thresholds for the multiple QTL models were estimated by permuting randomly-selected positions 5000 times and taking the LOD score at the 95^{th} percentile. This estimation was done for the full model LOD scores as well as for individual QTL LOD scores in the model. The full model significance threshold is thus a measure of significance of the full model, while the QTL LOD score significance threshold gives an estimation of the contribution of a specific QTL to the respective model.

Translational studies

In paper IV, we assessed a Swedish cross-sectional PD case cohort to determine the prevalence of seven point mutations and one CNV in two disease-causing genes, for which roles in immune system have been suggested previously. Mutations in LRRK2 account for 1-2% of all PD cases (Paisan-Ruiz et al., 2004; Zimprich et al., 2004) but the prevalence vary substantially depending on the population studied. LRRK2 is highly expressed in particular immune cells and has been biochemically linked to the intertwined pathways regulating inflammation, mitochondrial function, and

autophagy/lysosomal function. Mutations in the SNCA gene (Polymeropoulos et al., 1997; Spillantini et al., 1997) and copy number variations (CNVs) are linked to dominantly inherited monogenic PD (Singleton et al., 2003). αsyn is a ligand for toll-like receptor 2 (TLR2) (Kim et al., 2013). TLR2 is also present on T-lymphocytes, B-lymphocytes, monocytes and macrophages, cells that are part of the adaptive immune system. Moreover, it has been reported that αsyn epitopes can be presented on MHC molecules and be recognized by and activate both helper- and cytotoxic T-lymphocytes (Sulzer et al., 2017). αsyn can thus elicit both innate and adaptive immune responses.

Swedish Parkinson Genetics Network

All major clinical research centres in Sweden with access to DNA from PD patients were invited to participate. 2,206 PD patients from 7 cohorts in 4 geographical areas were included (Figure 9) ; this number accounts for ~10% of the country's estimated number of PD patients. All study participants had provided written informed consent to their participation. Ethical approval was obtained from the regional ethical review boards responsible for the contributing research centres.

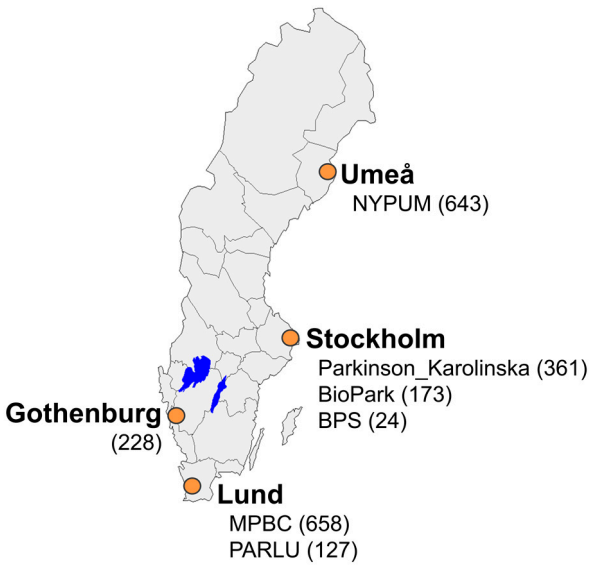


Figure 9| The Swedish Parkinson Genetics Network.
Map showing the locations and names of the seven contributing independent research studies. Number of DNA samples from PD patients analyzed in this study are shown in brackets.

TaqMan SNP Genotyping Assays

Seven point mutations in *SNCA* and *LRRK2* were analysed using commercial TaqMan SNP Genotyping Assays. The use of commercial assays that have been previously tested, ensures the discrimination between alleles, and it has been optimized, thus it is a reproducible method. To avoid the detection of false positive, it is important to include a positive control that allows a clear distinction of the two alleles for the detection of very rare mutations, it is not trivial to find a positive control sample. Therefore, it is important to confirm the suspected positive samples by another method.

Table 5 |TaqMan assay used
Nomenclature refers to transcript version NM_000345.3 (SNCA) and NM_198578.3 (LRRK2). Positive control samples were available for SNCA c.157G>A, for LRRK2 c.4309A>C and LRRK2 c.6055G>A.

Gene and SNP number	Substitution
SNCA (rs104893878)	c.88G>C p.(Ala30Pro)
SNCA (rs104893877)	c.157G>A p.(Ala53Thr)
LRRK2 (rs74163686)	c.4309A>C p.(Asn1437His)
LRRK2 (rs34995376)	c.4332G>A p.(Arg1441His)
LRRK2 (rs35801418)	c.5096A>G p.(Tyr1699Cys)
LRRK2 (rs34637584)	c.6055G>A p.(Gly2019Ser)
LRRK2 (rs35870237)	c.6059T>C p.(Ile2020Thr)

Twenty-seven samples that were considered positive or tentatively positive in the TaqMan allelic discrimination assays were analysed by Sanger sequencing.

Copy number variation (CNV)

Analysis of SNCA CNV was performed by two different methods.

Digital droplet PCR

The majority (1,566) of samples were analysed at the Clinical Genetics Department in Lund using digital droplet PCR. Using predesigned PrimePCR ddPCR Copy Number Variation Assays (Bio-Rad Laboratories).

TaqMan CNV analysis

A minority of samples (685 additional samples, plus 24 that were positive or possibly positive in digital PCR), were tested using TaqMan CNV analysis, using real-time polymerase chain reaction and unquenching of fluorescent probes for *SNCA* (TaqMan Copy Number Assay ID: Hs03506784_cn), and the ribonuclease P RNA component H1 gene *RPPH1* (TaqMan copy number reference assay no. 4403326) as reference gene. It is important to run each samples in quadruplicates, to provide confidence values for each copy number call, and to identify any possible technical failure during the reaction, and to consider the use of a positive control to identify abnormal read coverage. The CNV runs were done on an Applied Biosystem real-time PCR system and analysed using CopyCaller software.

Clinical data

Basic clinical data were extracted from the research databases included in the *Swedish Parkinson Genetic Network*, to characterize the composition of the case series and to describe the phenotype of the mutation carriers. Further, clinical information was partially complemented with data from the Swedish Parkinson Register. For the allele frequencies of known pathogenic mutations in dominant PD genes, we performed search in the literature and accessed The Genome Aggregation Database (gnomAD).

Statistical analysis

In paper I, the assumption of normality was tested using the Kolmogorov-Smirnov test. Non-parametric data were subsequently subjected to a, Kruskal-Wallis with Dunn´s multiple comparisons. All linkage analysis tests were performed in R.

In paper II and III all quantitative data were analysed using GraphPad. A significance level of $\alpha = 0.05$ was chosen for all analyses. Data are expressed as the mean ± standard error of the mean (SEM). Parametric data were subjected to an unpaired Student´s T-test two-tailed was used to analyse any direction of the effect. One-way ANOVA or Two-way ANOVA followed by post hoc analysis using either Bonferroni or Tukey´s multiple comparison test were performed in parametric data when adequate. Correlation analyses were performed using the Pearson correlation coefficient (r) using a 95% confidence interval

In paper IV, only descriptive data is presented.

Results

Paper I

Identification of multiple QTLs linked to neuropathology in the *engrailed-1* heterozygous mouse model of Parkinson´s disease

Transgenic *en1*$^{+/-}$ hemizygous model of PD

It has been shown that *en1* hemizygosity (*en1*$^{+/-}$) leads to loss of DNs in the midbrain and motor impairment in SwissOF1 animals (Sonnier et al., 2007), replicating features reminiscent of PD. However, with the same single-allele knockout C57Bl/6 mice display a normal phenotype (Sgado et al., 2006). Since the two strains respond differently to the same genetic manipulation of the *en1* gene, genetic factors outside the *en1*-region regulate the response to hemizygosity. To study genetic variants outside the *en1* locus that underlie this differential susceptibility to neurodegeneration, we intercrossed SwissOF1-*en1*$^{+/-}$ and C57Bl/6 mice and confirmed that C57bl/6 mice are resistant to *en1* hemizygosity. The resulting F2 generation (C57Bl/6*SwissOF1-*en1*$^{+/-}$) was then used for whole-genome linkage analysis to map susceptibility alleles/loci for dopaminergic neurodegeneration.

We confirmed that en1 hemizygosity resulted in loss of DNs in SwissOF1-*en1*$^{+/-}$ animals but not in C57Bl/6-*en1*$^{+/-}$ at 17 weeks of age (Figure 10A; F-G). The loss of 24% of DNs in SwissOF1-*en1*$^{+/-}$ compared to WT animals is in line with previous studies, where the *en1*-hemizygous mice replicates many pathological features reminiscent of the disease, including progressive dopaminergic degeneration, motor impairment and depressive-like behaviour (Nordstroma et al., 2015). In addition, our results show that wild-type C57Bl/6 mice have 29% fewer dopaminergic neurons in the SNpc compared to wild-type SwissOF1 mice ($p<0.0001$) (Figure 10A,F and G). We then analysed the amount of dystrophic dopaminergic axon terminals in the striatum of *en1*$^{+/-}$ animals. Interestingly, both strains displayed comparable loads of axonal swellings (Figure 10H-J), indicating axonal pathology in both strains. However, the protection of dopaminergic cell somas was observed only in C57Bl/6-*en1*$^{+/-}$ mice.

Interestingly, F2-*en1*$^{+/-}$ mice had fewer but larger swellings compared to SwissOF1-*en1*$^{+/-}$ and C56Bl/6-*en1*$^{+/-}$ (Figure 10B, C and H-J).

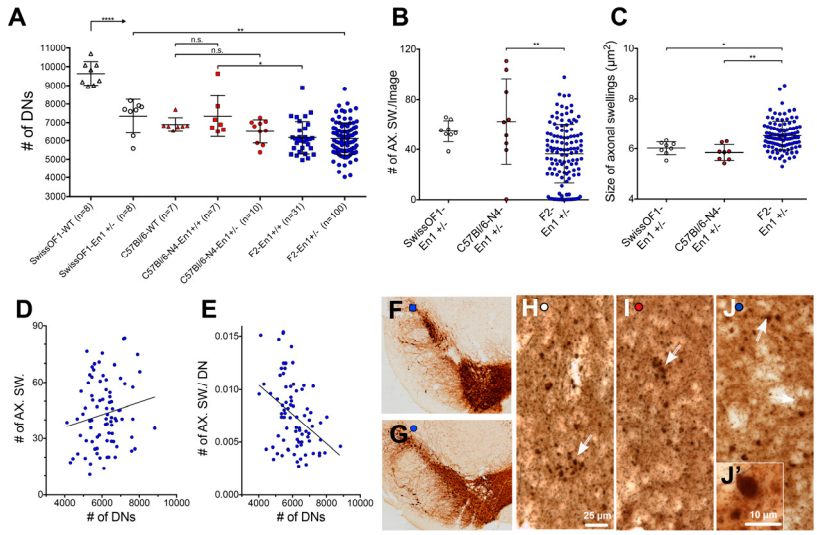


Figure 10 | Quantification of extent of dopaminergic neurodegeration of the differeC57Bl/6-*en1*$^{+/-}$, SwissOF1-*en1*$^{+/-}$ mice and intercrosses

(A) Stereological quantification of DNs in SNpc at 17 weeks of age. Individual data points and the mean ± S.D. are shown.**(B)** Number of axonal swellings in parental SwissOF1-*en1*$^{+/-}$, C57Bl/6-*en1*$^{+/-}$ and F2-*en1*$^{+/-}$. **(C)** Size of axonal swellings in the parental strains and F2 generation. **(D)** Correlation of axonal swellings number vs number of DNs (n.s.) . **(E)** correlation of number of swellings per DN vs number of remaining DN (r=-0.37, p<0.001).. Representative images used for stereological quantifications **(F)** F2-*en1*$^{+/+}$ and **(G)** F2-*en1*$^{+/-}$.Photomicrogrhaps of representative axonal swellings in **(H)** SwissOF1-*en1*$^{+/-}$. , **(I)** C57BL/6-*en1*$^{+/-}$, and **(J)** F2-*en1*$^{+/-}$

TH+ dystrophic axonal spheroids (axonal swellings) have been shown to contain abnormal autophagic vacuoles (Nordstroma et al., 2015) and to be an early neuropathological change in PD (Burke & O'Malley, 2013).

In F2-*en1*$^{+/-}$ mice, the number of axonal swellings did not show correlation to the DN number in SN (Figure 10D). This can be due to the fact axonal swellings appear before nigral neurons degeneration, and have been lost along with the respective soma of degenerate DN. Thus, we calculated load of axonal swellings, i.e. relative number of axonal swellings divided by nigral DN remaining number estimates, as phenotype.

In F2-*en1*$^{+/-}$ mice, the load of axonal swellings correlated with the number of DN when taking previous neuronal loss into account (Figure 10E).

QTLs linked to loss of dopaminergic neurons and axonal swellings

To identify loci linked to dopaminergic neurodegeneration in *en1*$^{+/-}$ animals, linkage analysis was performed using R-QTL. For that F2 *en1*$^{+/-}$ animals were whole-genome SNP genotyped, and the phenotypic variance of DNs in SN, the load of striatal axonal swellings, and size of axonal swellings were assessed. After that, the linkage of alleles across the genome to these quantitative traits was performed.

QTLs linked to nigral dopaminergic neurons loss

The phenotypic spread observed in the number of total TH+ neurons in SNpc (Figure 10A) suggested a complex genetic regulation of the trait. Single QTL analysis, which assumes a single QTL for the phenotype, revealed no significant peaks across the genome. Thus, multiple QTL analysis was performed. With this approach, eight loci were identified (Figure 11B), and the model included interactions between them that explained 74% of the phenotypic variance (logarithm of odds (LOD) 28, p02.4E-9) (Table 6). SwissOF1 and C57Bl/6 parental alleles in eight distinct and interacting QTLs regulate this phenotype. Not only C57Bl/6 alleles were linked to higher numbers of remaining DNs, but multiple QTL analysis reveal the effect on a mixed genetic background, where SwissOF1 alleles also showed to be protective. If all C57Bl/6 alleles were protective, the maximum and minimum values in F2 would equal those of the WT strains.

QTLs linked to extent of axonal pathology

Since axonal swellings are present in TH+ neurons and appear prior to degeneration of DN somas, the load of axonal swellings related to the number of DN neurons, was used as phenotype for linkage analysis. Although, C57Bl/6-en1+/- mice did not lose any DN, they displayed axonal swellings. Thus, nigral DN are not unaffected by *en1* hemizygosity, but C57Bl/6 animals are geared to contend with axonal degeneration it so that cell death is prevented. Single QTL analysis yielded one significant peak, Enli, located on chromosome 15 (Figure 11C). Subsequent multiple QTL analysis confirmed Enli and identified another six QTLs linked to load of axonal swellings in the striatum, with interactions between five of the loci (Figure 11C). Interestingly, mice homozygous for SwissOF1 alleles at En1i displayed the lowest load of axonal swellings. C57Bl/6 alleles at this locus are thus linked to the presence of more axonal swellings on remaining DN.

QTLs linked to striatal axonal swelling size

F2-*en1*$^{+/-}$ mice had a lower mean value of axonal swellings load, however, the size of axonal swellings in the F2 were larger compared to any of the parental *en1*$^{+/-}$ strains. Single QTL analysis did not reveal any significant locus linked to the size of DN axonal swellings in the striatal of F2-*en1*$^{+/-}$ mice (Figure 11D). However, multiple QTL scan identified eight QTLs and interactions between six of these (Figure 11D).

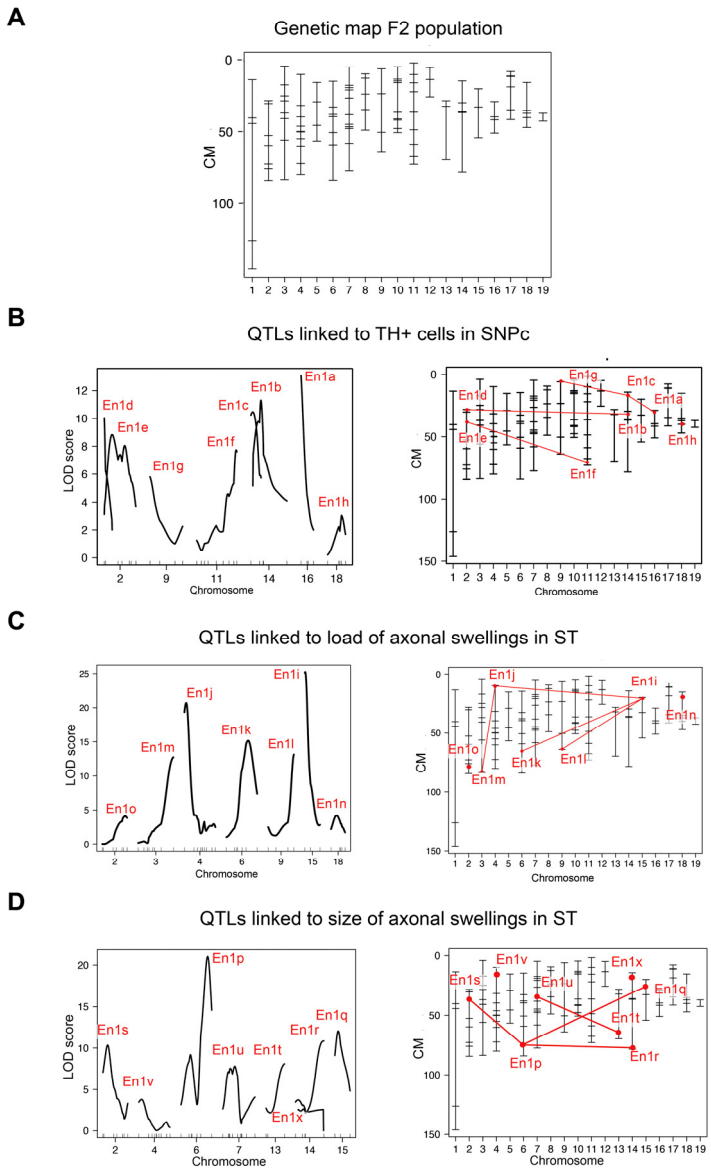


Figure 11 | QTLs and interactions discovered after single and multiple QTL analysis . (A) Genetic map of F2 population showing physical location of informative SNPs markers. (B-D) *left*.Multiple QTL scans reveal **(B)** 8 QTLs named en1a-h linked to TH+ cells in SNPc. **(C)** 7 QTLS linked to load of axonal swellings and **(D)** 8 QTLS linked to size of axonal swellings *right*. Chromosomal locations of QTLs in **(B, C and D)**, red lines between interacting loci.

In paper I, we identified 23 partly overlapping and interacting QTL linked to the number of DNs in SNpc and load and size of striatal axonal swellings (Table 7). With these data, we have been able to describe the effects of the *en1* hemizygozity same transgene in two mouse strains that are susceptible (SwissOF1) vs resistant (C57Bl/6) to PD-like pathology.

Table 7 |LOD-score, position and interactions of QTLs in the three respective models.
Full model statistics include p-value and LOD scores for the full QTL model with significance LOD threshold in parentheses. * Individual QTL below the respective genome-wide significance LOD threshold (Number of DNs in SNpc=7.1 , Number of axonal swellings per DN=4.8 , Average size of axonal swellings=8.7).

Phenotype	QTL	LOD score	Chr	Bayesian credible interval (cM)	Nearest marker	Interactions	Full model statistics	Variance explained
Number of DNs in SNpc	**En1a**	13.1	16	28.9-30.4	rs4180773	**En1c**	p=2.4E-09 LOD=28.3 (12.2)	74%
	En1b	11.3	14	27.2-34.9	rs3695574	**En1d**		
	En1c	10.5	14	14.3-22.4	rs3695383	**En1a. En1g**		
	En1d	10.0	2	28.2-29.0	rs13476490	**En1b**		
	En1e	8.8	2	36.0-67.8	rs3658729	**En1f**		
	En1f	7.7	11	69.5-72.9	rs13481230	**En1e**		
	En1g*	5.8	9	5.9-12.0	rs13480107	**En1c**		
	En1h*	3.0	18	31.7-46.5	rs6320743			
Number of axonal swellings per DN	**En1i**	25.2	15	19.9-22.3	rs3674266	**En1k, En1j, En1l**	p=1.7E-12 LOD=32.4 (10.1)	80%
	En1j	20.7	4	10.8-16.2	rs3653593	**En1i, En1m**		
	En1k	15.1	6	58.1-68.4	rs3152403	**En1i**		
	En1l	13.2	9	61.2-63.4	rs3694903	**En1i**		
	En1m	12.7	3	76.8-83.1	rs3724562	**En1j**		
	En1n*	4.3	18	20.1-36.0	rs3669543			
	En1o*	4.1	2	68.3-83.8	rs6376291			
Average size of axonal swellings	**En1p**	21.0	6	72.1-77.6	rs6387265	**En1q, En1r, En1s**	p=7.0E-11 LOD=30.2 (14.1)	74%
	En1q	12.1	15	23.9-30.9	rs3699312	**En1p**		
	En1r	10.8	14	69.2-78.4	rs3698545	**En1p**		
	En1s	10.3	2	34.7-42.7	rs13476507	**En1p**		
	En1t*	8.1	13	61.2-69.7	rs6316705	**En1u**		
	En1u*	7.8	7	15.7-36.2	rs3696018	**En1t**		
	En1v*	3.8	4	9.8-22.8	rs3653593			
	En1x*	3.7	14	14.2-29.2	rs3695383			

Paper II-III

Allelic difference in *Mhc2ta* confers altered microglial activation and susceptibility to α-synuclein-induced propagation and dopaminergic neurodegeneration

Vra4 congenic model to study immune effects on α-synuclein-induced spreading and neurodegeneration

The *Mhc2ta* is the master regulator of *MHCII* expression (Figure 4); the difference in expression levels of *Mhc2ta* and *MHCII* has been identified as a genetically regulated phenotype (Lidman et al., 2003; Lundberg et al., 2001; Piehl et al., 1999). From a QTL in an F2 intercross between two strains with differential *MHCII* expression levels, DA and PVGav1 strains, the VRA4 locus was identified as regulating MHCII expression levels in the CNS in the ventral root avulsion (VRA) model, in which ventral motor nerve rootlets are torn, resulting in extensive death of motoneurons, inflammation and little infiltration of immune cells (Lidman et al., 2003). Later, VRA4 alleles from PVG (low MHCII-expressing) rats were transferred to the DA (high MHCII-expressing) background (Harnesk et al., 2008) and the DA.VRA4 congenic strain was generated. Furthermore, using these two congenic rat strains (DA and DA.VRA4), the differential expression levels were finely mapped to allelic variants in the promoter region of *Mhc2ta*. (Harnesk et al., 2008; Swanberg et al., 2009; Swanberg et al., 2005). Moreover, allelic differences in *MHC2TA* affecting gene expression in susceptibility to complex inflammatory disease have been reported in animal models and humans. (Swanberg et al., 2005)

Here, we have used two known models of experimental PD in a novel paradigm that allows us to study human-orthologous genetic differences in *Mhc2ta* and *MHCII* expression levels: rAAV-αsyn-induced neurodegeneration (Decressac et al., 2012)(Figure 7) and αsyn PFFS seeding model (Thakur et al., 2017) (Figure 7).

Our two-congenic rat strains, DA and DA.VRA4, allow us to isolate the effect of these physiological relevant allelic variants of *Mhc2ta* since the two rat strains are identical, but differ in the *Mhc2ta* locus where DA.VRA4 animals present PVG alleles in the region containing the *Mhc2ta* gene, thus expressing lower levels of *Mhc2ta*.

The unilateral injection of rAAV6-αsyn in SNpc induced a stable αsyn transgene expression in neuronal cells throughout the nigrostriatal system in DA and DA.VRA4 strains in both experimental PD-models (Paper II and III) (Figure 12A). Moreover, axonal swellings, characteristic of axonopathy, were observed in the striatum of animals overexpressing αsyn (Figure 12B).

Our results thus show a stable transgene expression and presence of axonal swellings in the DA and DA.VRA4 strains.

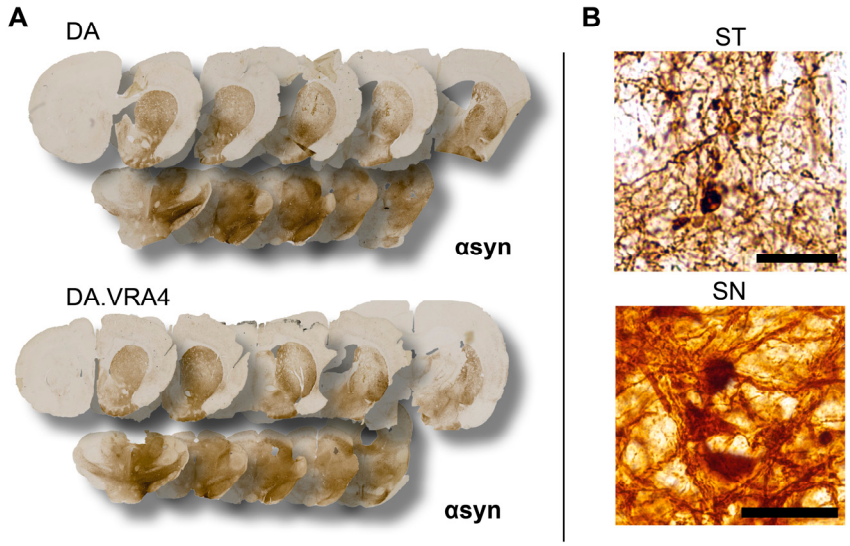


Figure 12 | Representative images of vector based asyn expression throughout the brain. (A) Low-magnification representation of αsyn overexpression in the midbrain and the terminal projection targest in the striatum of DA and DA.VRA4. **(B)** Higher magnification of αsyn overexpression showing pathological swellings and dystrophic axonal structures in the striatum (ST) and expression in the substantia nigra (SN) (scale bar =50 um) .

Allelic variants of *Mhc2ta* regulate differential antigen presentation capacity in response to αsyn

MHCII plays a major role in antigen presentation to T cells. We, therefore, examined the effect of *Mhc2ta* allelic variation in the antigen presentation capacity of immune cells present in the brain (microglia and macrophages). We first confirmed the lower *Mhc2ta* expression levels in ST and SN of DA.VRA4 at 12 weeks post rAAV6-αsyn injection (Figure 13A). The reduced expression in *Mhc2ta* expression levels resulted in lower MHCII expression levels in ST (2-fold vs DA, Figure 13A) and SN (3-fold compared to DA animals, Figure 13A), relative to Iba1. Also, lower levels of the cluster of differentiation 74 (CD74) in SN (2-fold vs DA, Figure 13A), a protein involved in the formation and transport of MHCII to the membrane, were observed in DA.VRA4 animals compared to DA. At a cellular level, lower MHCII immunoreactivity was observed in DA.VRA4 striatal cells compared to DA in response to αsyn-induced pathology (Figure 13B).

Therefore, we confirmed that *Mhc2ta* allelic variants affect the antigen presentation capacity in DA.VRA4 animals since there is a reduced expression level of critical components of the antigen presentation process (Paper II).

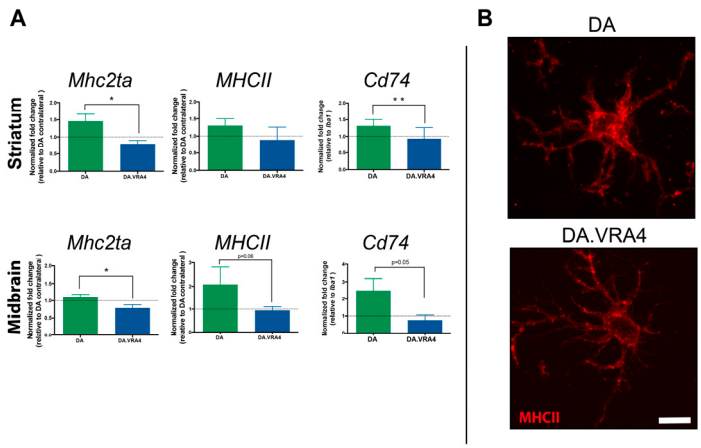


Figure 13 | Effects of *Mhc2ta* allelic variants in antigen presenting gene expression profiling. **(A)** Real-time qRT-PCR comparing the effects of VRA4-allelic variants on total expression of *Mhc2ta*, *MHCII* and *CD74* expression after 8-weeks of asyn overexpression related to the expression levels of *Iba1* in the striatum and midbrain of DA and DA.VRA4 animals. Ipsilateral *Mhc2ta* expression levels are significantly lower in the striatum of DA.VRA4 animals and midbrain relative to DA. MHCII expression was lower in DA.VRA4 rats compared to DA. CD74 levels presented a significant reduction in DA.VRA4 animals. Gene expression was log2 transformed using Iba1 or GAPDH expression levels as reference and data shown are normalized to contralateral DA levels. Fold change (mean ± S.E.M. n=8= shown). Unpaired t-test two-tailed was performed between groups, *p<0.05, ** p<0.01, *** p<0.001. **(B)** Representative images of MHCII immunoreactive cells in the striatum of DA and DA.VRA4 after rAAV-asyn and PFF injections.

αsyn induces widespread and sustained MHCII expression in association with accumulation of pαsyn

Antigen-presenting MHCII molecules are up-regulated on microglia in the brains of PD patients (McGeer, Itagaki, Boyes, et al., 1988). Given that *Mhc2ta* allelic variants have an impact in the antigen presentation capacity of DA.VRA4 animals, we mapped the anatomical distribution of MHCII+ cells in DA and DA.VRA4 animals in the αsyn-seeding model. MHCII+ cells were detected through the brain, including the cerebellum, motor cortex, insular cortex, amygdala, thalamus and olfactory tubercle (Figure 14A). Besides, the MHCII+ cell distribution matched the spatial distribution of pαsyn immunoreactivity, which is the most abundant synuclein modification in Lewy Bodies and a pathological hallmark of αsyn disease (Paper III).

To gain an in-depth understanding of whether reduced antigen presentation capacity affects αsyn-induced response in DA.VRA4 animals, a semi-quantitative analysis of MHCII+ cells was performed.

An increase in fold change (ipsilateral vs contralateral side) of MHCII+ cells was observed in ST of DA.VRA4 animals, 1.5-fold compared to DA in response to αsyn (Figure 14B-G). In the SN, there was a similar trend, albeit significance (Paper II).

We also analysed the co-localization of MHCII+ cells and pαsyn, using confocal microscopy. In several instances; MHCII co-localized with pαsyn (Figure 14H), indicating a role of MHCII+ cells in clearing and presenting pαsyn to the immune system. Interestingly, we noticed a lower Iba1 immunoreactivity in MHCII+ cells co-localizing with pαsyn this was associated with a hypertrophic cell body and an amoeboid morphology (Figure 14I), further supporting that MHCII+ cells are involved in the clearing of αsyn (Paper III).

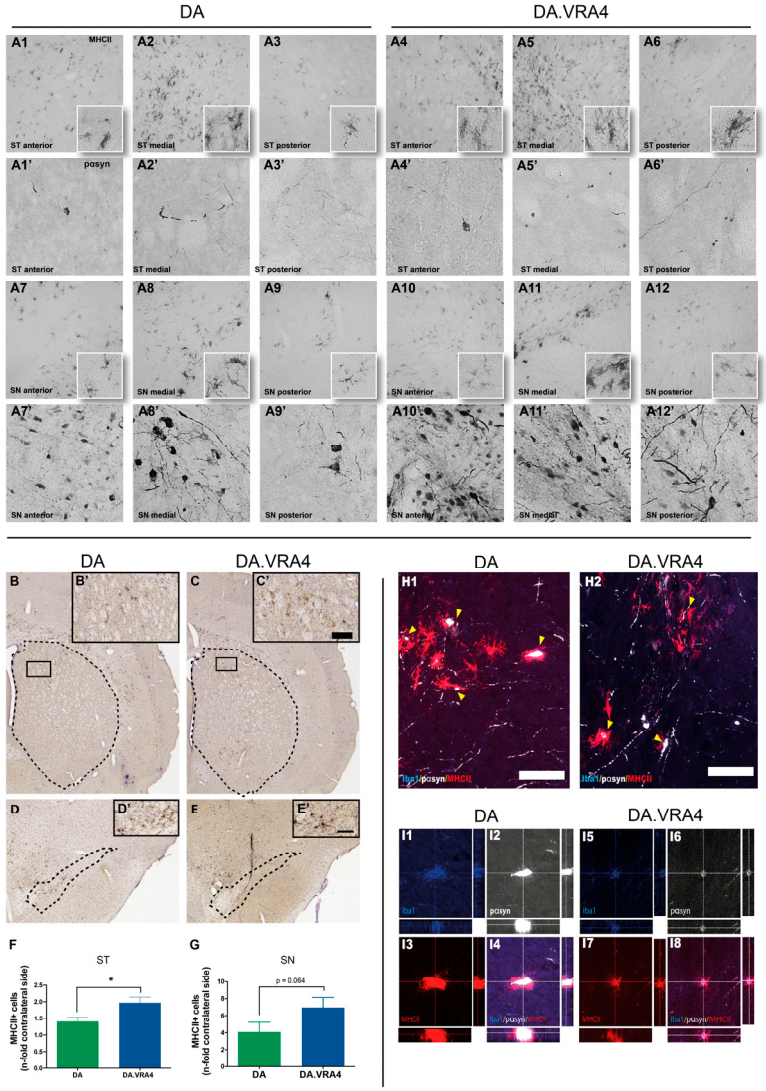


Figure 14 | **Antigen-presenting MHCII+ cells colocalize with pasyn pathology-affected brain regions**. **(A)** Representative photomicrographs of MHCII+ cells of asyn+PFFs groups in regions with pasyn immunoreactivity; 3 striatal **(A1-A6 ; A1´-A6)** and 3 nigral **(A7-A12 ; A7´-A12´)** levels Scale bar= 25µm. Representative **(B-C)** striatal sections and nigral C´scalebar=200µm **(D-E)** sections of MHCII+ immunohistochemical staining.E´scalebar=100µm **(F-G)** Ratio of MHCII+ cells in the ipsilateral vs contralateral side, DA.VRA4 overexpressing asyn showed a significant increased of MHCII+ cells compared to DA. **(H1-H2)** Representative images of Iba1+/MHCII+ cells close to pasyn immunoreactive cells and/or deposits in striatum of DA and DA.VRA4, cells with advanced pasyn pathology were frequently seen to be completelty enclosed or engulfed by one or several Iba1+/MHCII+ but not Iba1+/MHCII- cells. Scale bar =100µm**(I1-I8)** Representative images of Iba1+/MHCII+ cells colocalizating with pasyn inclusions.

Increased peripheral pro-inflammatory state and altered activation phenotype of local microglia in *Mhc2ta* allelic variant rats

It is known that upon activation, microglial cells express MHCII molecules, which are rarely observed in the CNS in a homeostatic state. Along with that microglia undergo morphological changes by increasing their ramifications and cell body size and increase in numbers.

In the αsyn-overexpression model we found that despite not showing an effect on the total number of CD11b+ cells in ST and SN (Figure 15A), *Mhc2ta* allelic variant rats (DA.VRA4) presented a higher percentage of hypertrophic cells (Type C) in the ipsilateral striatum (11.7% vs 7.2% in DA, Figure 15B) and a reduced percentage of surveying phenotype (Type A) in the ipsilateral SN of DA.VRA4 animals compared to DA (40.3% vs 52.7%, Figure 15B) (Paper II).

To investigate the Mhc2ta-mediated effects on the peripheral immune response to intracranial αsyn, we determined the cytokine- and chemokine profiles in serum after rAAV6-αsyn injection. There was up-regulation of pro-inflammatory cytokines in DA.VRA4 animals compared to DA background strain in response to αsyn (Figure 15C). Compared to DA, DA.VRA4 rats displayed increased levels of TNF-α (0.65 +/- 0.055 vs 0.45 +/- 0.044, $p<0.05$), a cytokine involved in systemic inflammation and implicated in multiple signalling pathways promoting exacerbation of the inflammatory response. DA.VRA4 rats also displayed higher levels of IL-2, a cytokine with roles inducing tolerance and immunity, primarily via direct effects on T-cells (0.81 +/- 0.030 vs 0.67 +/- 0.009, $p<0.05$).

These results indicate that the DA.VRA4 strain presents a highly activated microglia profile and a more pro-inflammatory cytokine profile compared to the DA background strain in response to αsyn (Paper III).

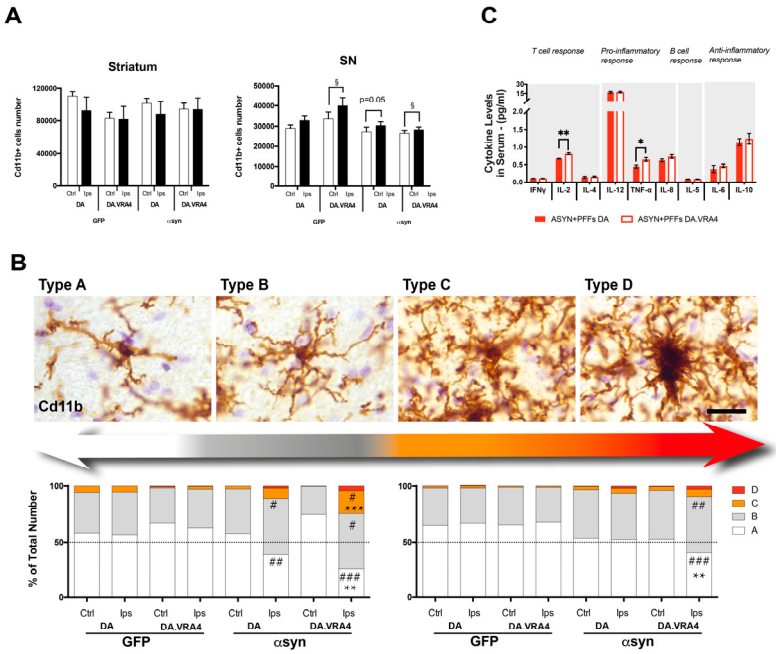


Figure 15 | Effect of Mhc2ta allelic variant on local and peripheral immune reponse. (A) Total number of CD11b+ cells in ST and SN determine by stereological cell counts. **(B)** Photomicrographs under a 100x objective showing the different morphologies used to identify microglial phenotypes. Representative morphology for Type A (Surveying), Type B (Hyper-ramified), Type C (Hypertrophic) and Type D (amoeboid). Proportion of Type A-D microglia from the Cd11b+ stereological estimations in striatum (*left graph*) and SN (*right graph*). Values are shown as mean ± SEM (n=6-8 per group). **(C)** Unilatera injection of rAAV-asyn+PFFs induced increase of IL-2 (p=0.0042) and TNF-a (p=0.030) in serum from DA.VRA4 compared to DA rats. **(A,B)** Two-way ANOVA, results from post-hoc group comparisons (Bonferroni test) are reported as follows: p<0.05 *between strains, # within strains, p<0.01 ** between strains, ## within strain. **(C)** Unpaired t-test, *p<0.05, **p<0.01.

Enhanced αsyn propagation and inclusion formation in rats with reduced antigen-presenting capacity

To further delineate the impact of the differential microglial phenotypes in αsyn seeding and propagation, we administered a low-titer rAAV-αsyn injection in the SN combined with injection of αsyn PFFs in the ST two weeks after (Thakur et al., 2017) in DA.VRA4 and DA rat. We analyzed the αsyn deposition pattern, the type, and the abundance of different αsyn depositions (dot-like, nuclear, perinuclear, filiform neurites, varicose neurites, Lewy neurite-like and Lewy body-like). As can be seen in Figure 16B, the pathology was different in terms of deposition and distribution pattern (forebrain, midbrain, hindbrain). Overall, the lowest levels of αsyn pathology were detected furthest away from the respective injection sites such as the frontal pole and the cerebellum. In the mid and hindbrain DA.VRA4 displayed higher scores compared to DA, indicating that reduced antigen-presenting capacity leads to enhanced seeding and aggregation of αsyn. Although low levels of immunoreactivity could be detected throughout the brain, the expression levels were highest at the site of rAAV-αsyn injection as well as PFF injection. The sites with most pathology were the midbrain as well as the striatum, thalamus and several cortical structures.

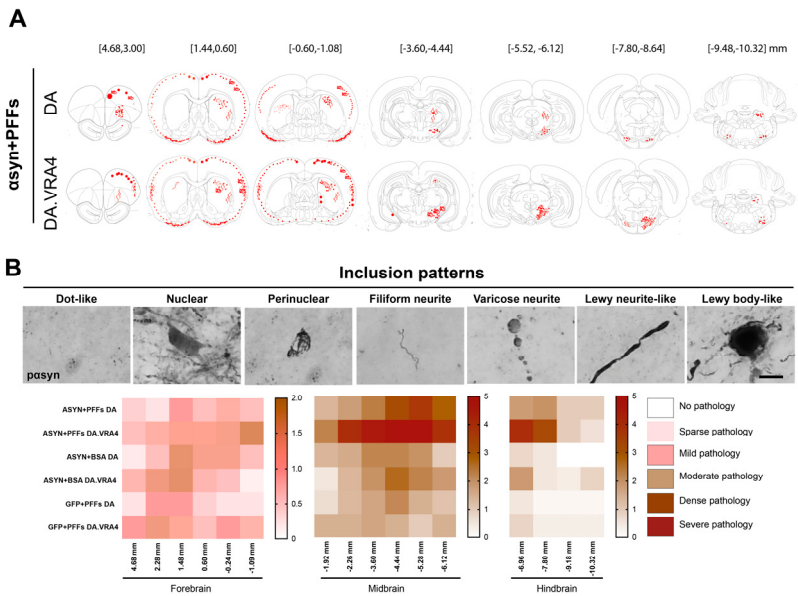


Figure 16 | PFFs seeds induced widespread αsyn pathology (pasyn) in the brain (A) Map showing the distribution of pasyn immunoreactivity (aggregates and neurites (red dots and lines, respectively) in coronal sections from animals sacrificed at 8 weeks post PFFs and immunostained with anti pasyn. **(B)** Scoring colour scheme illustrating the anatomical distribution of pasyn pathology and aggregation pattern after PFFs/BSA injection. Scoring scale 0, no inclusions; 1 sparse (very few neurites, max 1 nuclear deposit, scattered dot-like inclusions); 2 mild (varicose and filiform neurites, with or without presence of nuclear deposits); 3 moderate (many nuclear and perinuclear aggregates, neurites, large area with dot-like inclusions); 4 dense (many nuclear, perinuclear, filiform neurites and Lewy neurite-like; 5 (many perinuclear deposit, Lewy neurite-like and dot-like inclusions; 6 severe (many Lewy bodies-like and Lewy neurites-like). Data is shown as average values from each animal score.

Mhc2ta allelic variants enhances neurodegeneration in DA.VRA4 rats

To address whether *Mh2cta* allelic differences have functional consequences on the dopaminergic neurodegeneration, the amount of striatal axonal projection and nigral dopaminergic neurons were evaluated in the αsyn-overexpression model and the αsyn-seeding double hit model.

Overexpression of αsyn led to a significant loss of TH fibre staining intensity in the dorsal part of ipsilateral striatum of DA.VRA4 (≈30%) rats but not in DA rats (Figure 17B). Further, DA.VRA4 rats showed to be more vulnerable to the αsyn-seeding double hit model and displayed higher levels of TH+ fibre degeneration compared to rats of the DA (Figure 17E). In the DA.VRA4 strain, the TH+ fibre loss reached ≈50%, compared to the effect of the fibrils alone (GFP+PFFs), where there was a reduction of less than 20%. In DA rats, the combination of αsyn+PFFs resulted in a loss of TH+ fibres ≈40% compared to GFP+PFFs (≈20%).

Stereological quantification of TH+ neurons in SNpc did not reveal any significant differences between strains in the αsyn-overexpression model (Figure 17C). In the αsyn-propagation double-hit model, the presence of αsyn-seeds (PFFs) induced a significant loss of DN in DA.VRA4 (GFP+PFFs), which was enhanced by the presence of asyn substrate (αsyn+PFFs) (DA.VRA4 GFP PFFs: 9576 +/- 2200, $p<0.01$, DA.VRA4 αsyn PFFs: 6460 ±1488, $p<0.01$). In DA rats only the combined PFFs-seeded αsyn substrate resulted in a significant cell loss on the side of injection (cells remaining: 6257 +/- 2093, $p<0.01$) whereas there was no significant side bias in the GFP control group. Taken together these results demonstrate that the lower *Mh2cta*-expressing congenic DA.VRA4 strain has a higher susceptibility to the combined insult of αsyn overexpression and PFF injections when compared to DA background strain.

Mhc2ta allelic variants enhances motor impairment in DA.VRA4 rats

To determine if the observed enhanced inflammation and neurodegeneration in the DA.VRA4 strain affects motor impairment, for that we evaluated the contralateral forepaw used on the adjusting steps at every time points. The difference to DA was significantly different at 8 and 12 weeks post rAAV6-asyn delivery.

In the double hit model DA.VRA4 animals shown also a reduction of the forepaw use at all time points, but this difference became significant at 8 weeks compared to sham control. No significant motor impairment was observed in the DA groups.

In Paper II and III, we observed how *Mhc2ta* allelic variants regulating inflammation increased the aggregation, spread, and toxicity of αsyn on dopaminergic neurons and motor impairment in DA.VRA4 animals.

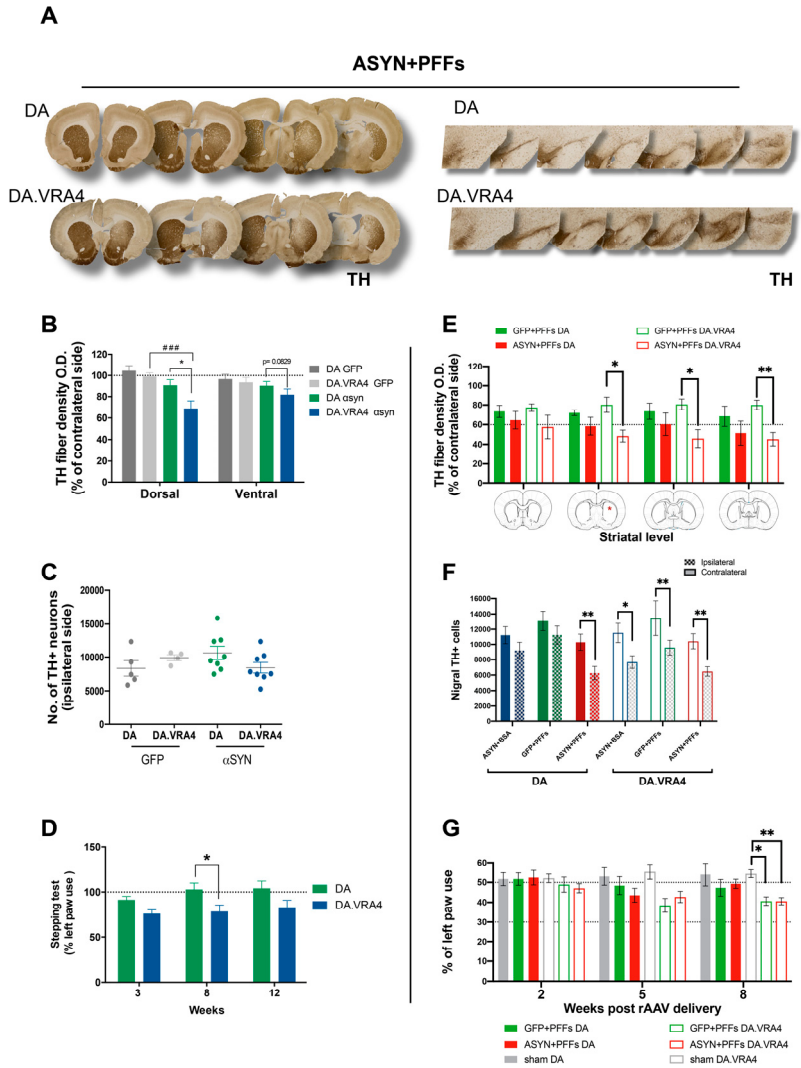


Figure 17 | αsyn PFFs-seeded pathology exacerbate dopaminergic neurodegeration. (A) Representative photomicrographs showing TH immunohistochemical staining throughout the brain for rats of the DA and DA.VRA4 strain after combined PFFs and asyn injection. **(B)** αsyn, but not GFP, overexpression induced increased loss of TH+ fibers in the dorsal striatum of DA.VRA4 compared to DA rats. **(C)** Stereological quantification of TH+ neurons in SNPc of DA.VRA4 and DA rats after overexpression of GFP and asyn. Data are presented as mean SEM percentage of neurons in the ipsilateral vs contralateral side (n=6-8 per group) **(D)** Decreased use of contralateral front paw in the stepping test for DA.VRA4 compared to DA rats. Data are expressed as mean SEM (n=7-8 per group) **(E)** Unilateral injection of rAAV-asyn+PFFs induced significant TH+ fiber loss at 3 different striatal levels of DA.VRA4 animals compared to GFP+PFFs controls **(F)** Stereological assessment of TH+ loss at 8 weeks post PFFs injection. **(G)** Behavioural assessment of forelimb akinesia. Two-way ANOVA post-doc group comparisons (Bonferroni or Tukey´s test) * $p<0.05$, ** $p<0.01$

Paper IV

Low prevalence of known pathogenic mutations in dominant PD genes in Swedish population

Immunogenetics of monogenic PD

Here, we analyzed the prevalence of pathogenic mutations in *SNCA* (duplications/triplications, p.A30P, p.A53T) and *LRRK2* (p.N1437H, p.R1441H, p.Y1699C, p.G2019S, p.I2020T) in the Swedish PD Genetics Network. These genes have been reported to play a role in modulating inflammatory responses.

Swedish PD patient sample collection

The *Swedish PD Genetics Network* is a national multicenter consortium, including 2.259 patients with PD, which represents >10% of the entire patient population estimated PD cases. The sample collection includes seven cohorts from 4 different regions in Sweden (Lund: MPBC and PARLU, Umeå, Stockholm: Parkinson_Karolinska, BioPark and BPS and Gothenburg), reflecting wide geographical distribution across the country (Figure 9, Table 8). Analysis of point mutations in *LRRK2* and *SNCA* was performed in DNA from 2,206 unique individuals, 98% of which yielded results

Table 8 | Case series included in this study

§ Based on information on 544patients for whom these data were available. Average age at onset was 60.7 years for all those 1,383 patients for whom this data was avialable. Average age at diagnosis was 64.4 years for 717 additional patients. Total of patients 2,206

Location (study, PI)	Number of samples from unique patients	Inclusion	Years of inclusion	Means of collecting clinical data	Mean age at onset / diagnosis (years)	Self-reported positive family history: relatives with PD
Lund (MPBC)	658	Population-based / geographical diagnosis registry	2014-2017	Study visit to research nurse, record review	64.9 (AD)§	1st degree: 59 patients (9.0%) 2nd degree (only): 65 patients (9.9%)
Umeå (NYPUM)	643	Population-based / geographical diagnosis registry	2000-2016	Study visit to neurologist, record review	62.9 (AO)	1st degree: 69 patients (10.7%) 2nd degree (only): 59 patients (9.2%)
Stockholm (Parkinson_ Karolinska)	361*	Population-based / geographical diagnosis registry	1997-2014	In conjunction to ordinary visit to neurology clinic	59.0 (AO)	1st degree: 53 patients (14.7%) 2nd degree (only): 23 patients (6.4%)
Gothenburg	228*	Service-based	2000-2012	Study visit to research nurse	57.0 (self- reported AO)	1st degree: 22 patients (9.6%) 2nd degree (only): 20 patients (8.8%)
Stockholm (BioPark)	165	Service-based	2013-ongoing	As part of regular outpatient visit	63.0 (AD)	1st degree: 31 patients (17.9%) 2nd degree: NA
Lund (PARLU)	127**	Population-based portion; portion patients with heredity	2008-ongoing	Study visit to neurologist/ neurology registrar, record review	60.6 (AO)	1st degree: 41 patients (32.3%) 2nd degree (only): 13 patients (10.2%)
Stockholm (BPS)	24	Service-based	2012-2014	As part of regular outpatient visit	61.1 (AO)	1st degree: 2 patients (8.3%) 2nd degree (only): 2 patients (8.3%)

LRRK2-G2019S is the most common variation in Swedish Population

Genetic screening was performed on 2,206 Swedish PD patients for six different known mutations in LRRK2; the internationally common mutation LRRK2 p.G2019S was present in 0.54% of all patients (13 patients). Using the data available, we report that 21.6% of the LRRK2 carriers had at least one first-or second degree relative with PD.

LRRK2 p.G2019S has an incomplete penetrance, which has been reported to differ based on ethnic group, sex, and the presence of other genetic or environmental modifiers. Interestingly, LRRK2 p.G2019S was detected in 1/942 (0.11%) population-based controls from southern Sweden matched to the MPBC. All mutations carriers were of Swedish origin, as were 85.5% of the entire control cohort. Likewise, LRRK2 p.G2019S represented the vast majority (90.4%) of known pathogenic mutations in the gnomAD databases. Although, LRRK2 p.G2019S is the most common mutation associated with PD, but it only accounts for a minute fraction of PD in the population. Importantly in the Swedish population it is also present in controls.

Presence of *SNCA* Copy number variation in Swedish Population

Analysis of *SNCA* copy number variation (CNV) in 2,206 samples yielded results with a call rate of 98.8% and showed eight tentatively positive or ambiguous results using both digital PCR and TaqMan; MLPA analyses confirmed *SNCA* duplication in one individual sample (0.045%).

This carrier belonged to the 21.6% with positive family history (Table 9.)

Presence of *LRRK2* and *SNCA* mutations in the Swedish population compared to other population

We have compiled published studies that examine variants in more than one dominant PD gene in PD patients, and found only three previous studies that analysed both SNCA CNVs and LRRK2 p.G2019S in the same patient series, allowing for direct comparison of the frequency of these two dominant PD variants (Bentley et al., 2018; Nuytemans et al., 2009; Yonova-Doing et al., 2012). Similar to what we report in Paper IV, LRRK2 p.G2019S variants are more often than SNCA duplications. The frequency of LRRK2 p.G2019S was between 0 and 4.3%.

In paper IV, we have shown that *LRRK2* p.G2019S prevalence is very low, despite being the most frequently encountered variant in dominant PD genes, followed by *SNCA* duplications. However, our results may not support routine genetic screening of the established pathogenic variants in dominant PD genes, since the likelihood of a positive

result in most circumstance will be very low in the Swedish PD patient population. The relevance of *LRRK2* and *SNCA* mutation screening is that these genes are targeted by many ongoing clinical trials. Thus, patients with mutations in these genes may be the ones that benefit the most from these novel therapies

Table 9 | PD patients with mutations in dominant PD genes

This table summarizes the clinical on the 13 patients carrying one of the known pathogenic mutations tested N.A:, not avalable

Individual	Site (Study)	Mutation	Sex	AO	Age at inclusion	Pos. family history	Brady-kinesia	Rigi-dity	Tremor	RBD symptoms	Cognitive dysfunction	Ortho-statism	Comment
1906-1119	Lund (MPBC)	LRRK2 p.(Gly2019Ser)	M	53 (AD)	59	Yes	Yes	Yes	No	No	No	No	Parent had dementia, other relative PD
1906-1767	Lund (MPBC)	LRRK2 p.(Gly2019Ser)	F	50 (AD)	75	No	Yes	Yes	No	Yes	Yes	Yes	No self-reported family history of PD or AD
1906-1150	Lund (MPBC)	LRRK2 p.(Gly2019Ser)	F	45 (AD)	49	Yes	Yes	Yes	No	No	No	No	Parent and grandparent had PD
1906-1211	Lund (MPBC)	LRRK2 p.(Gly2019Ser)	M	59 (AD)	63	Yes	Yes	Yes	No	N.A.	No	No	Grandparent had PD
1906-1210	Lund (MPBC)	LRRK2 p.(Gly2019Ser)	F	56 (AD)	63	No	Yes	Yes	Yes	N.A.	No	No	No self-reported family history of PD or AD
1906-1645	Lund (MPBC)	LRRK2 p.(Gly2019Ser)	M	64 (AD)	66	No	N.A.	Yes	Yes	No	No	No	No self-reported family history of PD or AD
PD1-A12	Stockholm (Parkinson_Karolinska)	LRRK2 p.(Gly2019Ser)	M	75	79	No	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	Hemiparkinsonsism
PD2-E07	Stockholm (Parkinson_Karolinska)	LRRK2 p.(Gly2019Ser)	F	58	74	No	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	-
PD2-H07	Stockholm (Parkinson_Karolinska)	LRRK2 p.(Gly2019Ser)	M	47	51	No	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	Heart condition, has had a stroke
PD3-E09	Stockholm (Parkinson_Karolinska)	LRRK2 p.(Gly2019Ser)	M	53	58	Yes	N.A.	Yes	Yes	N.A.	N.A.	N.A.	-
PD4-A11	Stockholm (Parkinson_Karolinska)	LRRK2 p.(Gly2019Ser)	M	54	56	No	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	-
10793	Umeå (NYPUM)	LRRK2 p.(Gly2019Ser)	F	48	60	No	Y	Y	Y	N	N	Y	-
19061750	Lund (MPBC)	*SNCA* duplication	F	52	54	Yes	Y	Y	N	Y	Y	Y	*

This table summarizes the clinical data on the 13 patients carrying one of the known pathogenic mutations tested. N.A., not available. *This patient belongs to the Swedish Lister Family, a large kindred with *SNCA* multiplications . Her carrier status was known from the PARLU study, why her DNA was excluded from the present analyses. She turned out to be included in MPBC as well.

Discussion and future perspectives

General discussion

Our understanding of PD pathophysiology and associated risk factors (genetic and environmental) has greatly improved in the past 10 years, since a handful of casual Parkinson´s genes have identified.

However, despite this remarkable effort, the vast amount of variants identified through GWAS (genome-wide association studies) does not account for the entire estimated genetic component of PD: furthermore there are still no current treatments that have been proven to slow down disease progression. Early investigations into the role of genetic factors in PD focused on the identification of rare mutations underlying familial forms of the disease (Kitada et al., 1998; Polymeropoulos et al., 1997; Singleton et al., 2003; Valente et al., 2004), but in recent times there has been a growing appreciation for the important contribution of genetics in sporadic disease (Chang et al., 2017; Nalls et al., 2014). The largest-to-date meta-analysis GWAS for PD, has identified 90 independent genome-wide significant signals across 78 loci, and identifying 70 putatively causal genes for risk signals, using 37700 PD cases and GWAS data generated by 23andMe (https://www.biorxiv.org/content/biorxiv/early/2019/02/11/388165.full.pdf).

The presence of inflammatory changes occurring in the brain of patients with PD has been registered for more than thirty years (McGeer, Itagaki, Boyes, et al., 1988); however, the mechanisms underlying inflammation, as a cause of the progressive nature of the disease, have not been fully understood.

In this thesis I set out to explore this question, since understanding inflammation in PD opens up a whole array of potential treatment options.

Furthermore, since the loss of nigral dopaminergic neurons is the major histopathological feature of PD, I decided to map the genetic factors that influence this trait. Here I present a systematic and thorough characterization of how genetic factors regulate susceptibility to PD through immune mechanisms, to map these to distinct genetic loci and investigate how these are associated to human disease.

Identification of new genetic regions linked to PD susceptibility

The starting point of this thesis was to identify new genetic regions regulating susceptibly to dopaminergic neuron loss and axonal pathology (Paper I). I used the linkage analyses strategy; this strategy relies on the principles of linkage disequilibrium, which is based on the co-segregation of markers (e.g. SNPs, tandem repeats etc) with nearby alleles. Linkage analyses are use for finding out the genetic basis of quantitative traits.

en1, engrailed 1 is part of a complex network of transcription factors that has a pivotal role in the development and maintenance of dopaminergic neurons (Alvarez-Fischer et al., 2011; Veenvliet et al., 2013). *en1* hemizygosity has been shown to induce features reminiscent of PD in mice, such as progressive nigrostriatal dysfunction and loss of dopaminergic neurons in SNpc in SwissOF1 mice (Le Pen et al., 2008; Nordstroma et al., 2015; Sonnier et al., 2007). In contrast, C57Bl/6 mice with the same hemizygous loss of *en1* do not display the neurodegenerative phenotype (Sgado et al., 2006*).* C57Bl/6 mice thus harbour genetic factors outside the *en1* locus regulating both the number of dopaminergic cells and their survival. This observation motivated a linkage analysis in an F2 C57Bl/6xSwissOF1-en1+/- intercross to map QTLs regulating PD-like neurodegenerative changes in response to *en1* hemizygous loss. Interestingly, the number of DN in the SNpc of C57Bl/6-WT mice was significantly lower compared to SwissOF1-WT mice. These differences in DN numbers between adult mice of different inbred strains could be attributed to a different rate of early developmental neurogenesis or age-related cell death in the mesencephalon in the two strains.

Our model develops spontaneous PD in the absence of one *en1* allele in a SwissOF1 genetic background, where we observed a reduction around 20% of dopaminergic neurons, increased load of axonal swellings and bigger size, compared to C57Bl/6-*en1*$^{+/-}$. We identified 8 interacting QTLs that explain the vast majority of the variation in dopamine neuron loss observed in the F2 population. This indicates that no single locus is sufficient to explain the phenotypic differences, but rather a combination of alleles from different loci.

Over the past few years, there has been increasing evidence that striatal terminal dysfunction precedes neuronal cell loss in the SNpc and post-mortem studies support the notion that it is indeed an early neuropathological change in PD (Galvin et al., 1999; Kordower et al., 2013). This is further supported by some animal PD models (Chu et al., 2012; Kordower et al., 2013). The proposed underlying mechanism is that neurodegeration starts at the nerve terminals and protracts retrogradely to the dopaminergic cell bodies in SNpc. In the context of partial loss of *en1*, the presence of dystrophic dopaminergic axon terminals has been reported in the dorsal portion of the striatum as early as 8 days of age in SwissOF1 (Nordstroma et al., 2015) but not in C57Bl/6-*en1*$^{+/-}$ (Sgado et al., 2006; Veenvliet et al., 2013). We detected signs of nigrostriatal terminal dysfunction at 17 weeks in SwissOF1-*en1*$^{+/-}$ and C57Bl/6j-*en1*$^{+/-}$,

without dopaminergic neuronal loss in the latter. Therefore, we conclude that alleles from C57Bl/6J genetic background confer resilience to dopaminergic cell loss despite initial axonal dysfunction.

We were able to link the load of axonal swellings and their size to 7 and 8 QTLs respectively. From the 23 QTLs we have identified, 10 are overlapping. We found indication of this overlap on chr2 (En1e, En1s), chr4 (En1j, En1v), chr6 (En1k and En1p), chr15 (Enli and En1q) and chr18 (En1h and En1n). Therefore, our results indicate that the number of QTLs reported in this thesis is likely an over-estimation. However, the 10 loci linked to both axonal pathology and DN survival may regulate key features of PD-like neurodegeneration.

It is noteworthy to mention that some of the identified QTLs may be specific to F2 C57Bl/6xSwissOF1-*en1*$^{+/-}$ male animals, since F2 females were not analysed. In addition, possibly occuring imprinintg effects, i.e. that either the maternal or paternal allele at a specific locus is expressed, may have been overlooked; however those effects can not be mapped without reciprocal cross. i.e.C57Bl/6- *en1*$^{+/-}$ male with SwissOF1 female.

In summary, in paper I, I have use linkage analysis for dissecting the genetic architecture of key traits of dysfunction and degeneration of DNs. Although a porwerful technique, the specific genetic loci still need to be further delineated, for which QTL fine-mapping is necessary.

Looking ahead

RNAseq-based transcriptome profiling of en1$^{+/-}$ *mice*

In this on going follow-up study, we have made a significant effort to complement our identified QTLs and further delineate the genetic factors conferring a decreased susceptibility to PD-like pathology in C57Bl/6 mice upon *en1* hemizygosity. To do so, we analysed how the changes in gene expression induced by the hemizygous loss of *en1* differ between SwissOF1 and C57Bl/6 mice.

We coupled histological analysis and unbiased genome-wide transcriptome profiling of DNs in laser-microdissected SNpc from 1-week-old wild-type and *en1* hemizygous mice on the C57Bl/6 and SwissOF1 background genomes. We relied on the Laser Capture Microdissection technique for accurate SNpc isolation followed by construction of high-quality RNA-seq libraries using the SMART-Seq method optimized for ultra-low amounts of RNA. We decided to study the expression profiles within this anatomical region in light of the recent description of complex traits as omnigenic, which states that genetic variants underlying a complex disease are most likely expressed in the disease-related tissue (Boyle et al., 2017). Our preliminary data show both transgene-induced and strain-dependent early transcriptomic patterns; importantly, our preliminary analysis reveals enrichment of gene products involved in cellular oxidant detoxification and oxidative phosphorylation in C57Bl/6-*en1*$^{+/-}$

compared to WT C57Bl/6 mice, thereby indicating potential candidate genes and pathways which could underlie neuroprotection in C57Bl/6J mice.

Interestingly, when looking at the strain effect upon *en1* hemizygozity (i.e. C57Bl/6-*en1*$^{+/-}$ vs SwissOF1-*en1*$^{+/-}$). Regarding the observed up-regulation of HLA genes, we cannot discard the possibility of an artefact due to a better mapping of the reads to the reference transcriptome, annotated based on the C57Bl/6 strain. However, as no long-read sequencing was carried out, discerning haplotype-based effects is a difficult task.

Functional evaluation of *Mh2cta* as a regulator of αsyn pathology in PD

In the second stage of the thesis, I focused on the functional evaluation of *Mhc2ta*, the major regulator of MHCII expression, and a target gene that was identified in a previous QTL mapping in rats. Common genetic variants affecting expression levels of MHCII have been reported to be associated with PD risk (Kannarkat et al., 2015; Pierce et al., 2017). The presence of MHCII+ microglia in the brain of PD patients (McGeer, Itagaki, Boyes, et al., 1988) closely correlates with αsyn depositions in neurons (Imamura et al., 2003) at early stages of PD (Croisier et al., 2005) thereby supporting further the role of MHCII in disease susceptibility and possibly disease progression.

Our congenic rat strains (DA and DA.VRA4) are a relevant model to study the impact of MHCII on PD. The naturally occurring allelic differences found in the *Mhc2ta* promoter leading to differential expression levels of *Mhc2ta* protein have an orthologous counterpart in the human genome, where a corresponding promoter polymorphism was found to regulate *MHC2TA* and *MHCII* expression in humans (Harnesk et al., 2008; Swanberg et al., 2005).

Immune effects

It has been previously shown that the activation of microglia in response to αsyn overexpression requires MHCII (Harms et al., 2013). In paper II, using the rAAV-αsyn model, we have shown that naturally occurring allelic variants of *Mhc2ta* differentially regulate the expression of MHCII in response to αsyn. Furthermore, we found that the DA.VRA4 rat strain displayed lower MHCII and CD74 expression levels, thus confirming that *Mhc2ta* is crucial for the induction of components of the antigen presenting process. Follow-up *in vitro* studies support this notion by showing that the completely ablation of *Mhc2ta* disrupts the antigen presenting capacity of microglia in response to αsyn fibrils (Williams et al., 2018). It is important to mention that while the knockout models completely abolished MHCII expression, the model use in this thesis

is immunologically functional. These observations suggest *Mhc2ta* as an immune-modulator of the inflammation on PD.

Microglia activation is an early event that progresses as PD pathology develops in areas where asyn and neurodegeneration occurs, irrespectively of the presence or absence of cell death (Croisier et al., 2005; Doorn et al., 2014; Halliday et al., 2011; Hunot et al., 1996; Imamura et al., 2003; Knott et al., 2000). Whether microglial activation is detrimental or whether some inflammatory processes may be acting to limit injury and promote αsyn clearing is still an open question. A valid argument is that it is not detrimental at all stages of the disease, but rather it is a highly timely regulating process. In Paper II, we studied the effect of *Mhc2ta* allelic variants in early stages of the neurodegenerative process, since we observed a very moderate loss of TH+ cells in SNpc.

Our data showed that *Mhc2ta* allelic variants are affecting microglial activation (morphological changes and cytokine release) without microgliosis (expansion of microglia) since we observed a moderate increase in the numbers of CD11b+ cells in response to αsyn. The activation and phenotype of microglia is complex, microglia activation has been classified using a nomenclature from macrophages, in M1 and M2 activation (Mills et al., 2000). However, the M1-M2 dichotomous paradigm represents just the extremes of a rather large and complex spectrum of activation states. In this context, we determined the activation profile of microglia in response to αsyn. Although not covering the full spectrum we used a classification, which provides significant insight into the functional activation state of microglia.

We categorized four different types of microglial cells (surveying, hyper ramified, hypertrophic or bushy, amoeboid) based on morphological characteristics, and found that αsyn overexpression induced a shift towards more activated (hyperamified and amoeboid) microglia. DA.VRA4 animals presented higher percentage of amoeboid and bushy microglia, and a drastic reduction of surveying microglia in response to αsyn. Interestingly, morphological similarities between human and rodent microglia suggest highly conserved functions between species (Torres-Platas et al., 2014). In this regard, recent studies report progressive changes in morphology of MHCII-expressing microglia in neurodegenerative disease (Walker et al., 2015).

It is well known that the activation of microglia is a finely regulated process, which depends on the nature of the stimulus and the prior state of the cell (Gomez-Nicola et al., 2015). Previous studies have proposed to identify microglial cell phenotypes based on their inducing stimuli, as read out from their transcriptome signature, such as M_{LPS} or M_{IL4} after been stimulated respectively, with LPS+IFNγ or IL4 (Beins et al., 2016).

The fact that we do not see any difference in the total population of Cd11b+ cells, but in the percentage of different types of microglia and in the counts of MHCII+ cells in the striatum of DA.VRA4 rats suggest that the microglial activation present in our congenic rat strain can be identified as *Mhc2ta*-induced $M_{\alpha syn}$ phenotype, while DA.VRA4 are more prone to become activated. At a molecular level we observed that DA.VRA4 animals, compared to DA, present lower expression levels of Arginase 1 (Arg1), which is a well-studied anti-inflammatory marker that can contribute to wound healing and matrix deposition (Munder, 2009). These findings suggest that DA.VRA4

Mhc2ta-variant carriers differentially gear towards less effective antigen presentation and less anti-inflammatory response in response to αsyn.

αsyn propagation

One of the main questions that remain open regarding PD progression is how the pathology spreads. Progress has been made regarding the pattern of spreading: the αsyn propagation is proposed to start in the dorsal motor nucleus of the vagal nerve and olfactory bulb prior to the appearance of classic motor symptoms in PD, followed by interconnected brain regions (Braak et al., 2003). This pathological propagation matches the symptomatic progression and provides the basis for a plausible spreading mechanism. In this regard a prion-like propagation pattern has been hypothesized (Brundin et al., 2017); the hypothesis has been questioned (Surmeier et al., 2017) but has also led to in vitro and in vivo models mimicking the progressive αsyn pathology and PD-like symptomology. In our endeavour to understand how *Mhc2ta* allelic variants could affect the propagation of αsyn in Paper III, we used a human αsyn-seeding model that recapitulates progressive and widespread propagation of pαsyn, the most pathogenic form of αsyn.

The αsyn propagation model combines a low concentration of human αsyn overexpression in the nigrostriatal system, prior to the striatal injection of human αsyn-seeds (PFFs). The combination of these two factors (αsyn increased levels and the seed of αsyn) led to pαsyn immunoreactivity in regions connected to the site of injection, and spread to the contralateral side. These findings are consistent with previous observations from PFF-based models, in which striatal PFFs seeds are injected (Abdelmotilib et al., 2017; Luk et al., 2009; Paumier et al., 2015). In addition, the inclusion pattern showed different forms of aggregation (dot-like, Lewy neurite, Lewy body-like), which have been reported previously (Rey et al., 2016). However, the main difference with these studies is that the PFFs are from a different species to the animals used. It has been shown that partial species barriers could interfere with the efficiency of the PFFs to propagate. Therefore, in our studies we have used a combination of human substrate overexpression and injection of human PFFs seeds describe previously (Thakur et al., 2017). In Thakur et al., both injections were performed in the same region SN. In contrast, in our study we injected the rAAV in SN and after two weeks PFFs were injected in the striatum. In this manner, we ensured a stable expression of the transgene and local transient inflammatory effects of the injection, so as to avoid confound inflammatory effects due to the injection in the same region.

MHCII response and immune peripheral effects

The presence of MHCII+ cells has been seen in the vicinity to αsyn in PD brains (Croisier et al., 2005), αsyn-overexpression PD models (Jimenez-Ferrer et al., 2017; Sanchez-Guajardo et al., 2010) and after striatal injection of PFFs (Duffy et al., 2018). In our congenic rat strains MHCII+ cells followed the same spatial distribution as the pαsyn pathology, and microglial co-localized with pαsyn already at 8 weeks after PFFs injections, something that in models using solely the PFFs takes around 6 months (Harms et al., 2017). In the context of pαsyn-induced immune response, we observed that in addition to the local effects on microglial activation, the administration of αsyn fibrillar forms has effects on the peripheral immune response with and increase pro-inflammatory response in the presence of lower *Mhc2ta* expression levels. In general, DA.VRA4 congenic animals presented a maintained pro-inflammatory state in serum. DA.VRA4 animals treated with αsyn+PFFs presented higher levels of IL-2, a cytokine critical for the development of T-cells. Activated T-cells produce and secrete IL-2 cytokine that binds their own IL-2R to enhance proliferation in an autocrine fashion (Ozaki et al., 2000). It plays a key role in T-cell differentiation and is a critical determinant of the fate decisions of antigen receptor-activated into to proinflammatory subsets with differential cytokine secretion profile; Th1 (e.g. IFN-γ, IL-2 and TNF-α) and Th2 (e.g. IL-4, IL-5 and IL-13)(Cote-Sierra et al., 2004; Liao et al., 2011). Interestingly, CSF of PD patients have shown higher levels of IL-2 (Mogi, Harada, Kondo, et al., 1996; Mogi, Harada, Narabayashi, et al., 1996). A meta-analysis of note showed that peripheral levels of IL-2 are also increased in PD patients compared to control (Qin et al., 2016).

Our results thus suggest that the presence of lasting effects on the peripheral immune response to αsyn pathology can have an effect in the magnitude and the type of T cell response. However, further experiments are needed to fully characterize the type of T-cell response.

Neurodegeneration

Over the past few years, a growing number of studies have highlighted the relevance of striatal terminal dysfunction as an early neuropathological feature in PD (Kordower et al., 2013). The functional effects of *Mhc2ta* allelic variants were determined in the αsyn-overexpression model and the αsyn-propagation model. The DA.VRA4 strain showed to be more susceptible to striatal dopaminergic fiber degeneration and displayed enhanced behavioral impairment in both αsyn models. However, in the model where we only overexpressed αsyn using an rAAV6 vector, there was motor impairment without any dopaminergic loss in SNpc. The impact of striatal TH+ fiber loss on performance in behavioral test without cell loss is supported by the fact that αsyn-induced changes of dopamine release and reuptake patterns can be detected before any sign of cell loss

(Lundblad et al., 2012). Thus, striatal denervation is an early sign of dopaminergic neurodegeneration, which is reflected in behavioural impairment. *Mhc2ta* allelic variant carriers, DA.VRA4 are more susceptible to these early changes as well as to cell loss. In the αsyn-seeding model, the fact that DA.VRA4 animals showed behavioural impairment in all groups regardless of the treatment strengthens this notion: DA animals, are less susceptible, only displaying motor impairment in the combined group, hence suggesting that *Mhc2ta* variants regulates the threshold to develop pαsyn pathology and motor deficits.

These results seem to contradict the notion that complete absence of *Mhc2ta* is neuroprotective in the αsyn overexpression mouse model (Williams et al., 2018) and MHCII is neuroprotective in the αsyn overexpression mouse model (Harms et al., 2013). The discrepant results could be explained as follows; firstly, the difference in the genetic organization of the MHC locus (Rolstad, 2014), which could have an impact in immune pathways being different in these rodent species. Secondly, that MHCII knock out has a deficiency of CD4 T-cells, making it difficult to conclude whether it is the deficiency of MHCII or the lack of CD4 T-cells that is responsible for the neuroprotective effects. Furthermore, in humans, a loss of function in MHC2TA induces bare syndrome and severe immunodeficiency (Steimle et al., 1993), thereby, making knockout models difficult to interpret in a physiologically relevant way. In our case, we do know that the DA.VRA4 strain has a functional immune system and CD4+ cells. However, the response CD4+ cells has not been characterized yet in our model, and hence the question whether there is a difference in the T cell response in DA.VRA4 animals is still to be answered, for which further evaluation of molecular and cellular parameters are required. The full characterization of T cell response by means of histochemical techniques is limited due to the sensitivity of the technique. Therefore, flow-cytometry experiments *in vivo* and *in vitro* will be used to further dissect the effects of *Mhc2ta* allelic variants in microglial activation and MHCII+ cells and T cell interaction in the αsyn PFFs combined model.

We hypothesize that differential allelic variants in *Mhc2ta* can exert effects in quantitative (i.e. number of molecules per cell) *MHCII*, which could synergize with *MHCII* alleles qualitative effects (i.e. peptide binding affinity, biochemical properties, size of the cleft) thereby having an impact on the nature of the αsyn-induced antigen being presented resulting in a differential T cell profile.

It is important to bear in mind the limitations of these studies; *Mhc2ta* allelic variants may also affect MHCII expression of non-microglial cells such as astrocytes and B cells (Constantinescu et al., 2005; Reith et al., 2005; Waldburger et al., 2001) that we might have overlooked. It is noteworthy that in an EAE model (experimental autoimmune encephalomyelitis) allelic differences of *Mhc2ta* regulate levels of MHCII in APCs, and to a great extend in B cell subset (Harnesk et al., 2008). It is also relevant to bring to discussion the potential effect of differential microglial activation in the type of activation of astrocytes. Recently it has been shown that activated microglia induce a differential activation profile of astrocytes, thereby losing the ability to promote neuronal survival, outgrowth, synaptogenesis and phagocytosis (Liddelow et al., 2017).

We cannot rule out that DA.VRA4 microglia and lymphocytes are "primed" due to the lower expression levels of Mhct2a and display altered inflammatory profiles prior to nigral transgene expression and injection of PFFs. Furthermore, we are interested in investigating the effect of *CLEC16* in linkage disequilibrium with *MHC2TA* and *IFNγ*, the strongest inducer of *MHC2TA*-regulated genes which have influence on inflammatory factors (Swanberg et al., 2012).

In Paper III, we have shown that DA.VRA4 rats present a more pro-inflammatory state, which might be detrimental for neurons. Antigen–presentation by MHCII+ cells typically occurs after processing of foreign agents and leads to the presentation of antigen to lymphocytes; usually this response protects the host from invading pathogens or foreign proteins. In our paradigm of *Mhc2ta*-regulated response to αsyn, this engulfing and degradation of pαsyn fibrils and inclusions may be beneficial, increasing clearance of pαsyn pathogenic aggregates, but at a higher inflammatory cost, which could affect the microenvironment amplifying several steps in the pathogenic cascade observed in PD. Thus, this inflammatory response can presumably affect neuronal integrity and might also enhance further aggregation of pαsyn in neighbouring cells. Furthermore, the observed widespread αsyn pathology and dopaminergic neurodegeneration, in combination with increased susceptibility in the DA.VRA4 strain strongly point out *Mhc2ta* as a facilitator and aggravator of PD-like αsyn pathology.

Looking ahead

Genetic and environmental modifiers of PD risk disease

Using a translational approach and based on the finding that functional polymorphisms in Mhc2ta reduce MHCII expression in DA.VRA4 and enhance the αsyn-induced neuroinflammation, neurodegeneration and motor impairment response, we have started to study the prevalence of SNPs associated with lower expression of MHC2TA in humans (Swanberg et al., 2005) as well as HLA SNPs that have been associated to risk of PD (Chang et al., 2017; Hamza et al., 2010; Hill-Burns et al., 2011; Nalls et al., 2014; Simon-Sanchez et al., 2011) as well as certain HLA alleles (Lampe et al., 2003; Wissemann et al., 2013).

To study the association of MHC2TA and HLA alleles to PD, including an interaction between alleles, we have used the MPBC sample collection, a cross-sectional Swedish case-control cohort (the local counterpart of the Swedish PD Genetics Network), described in Paper IV, including 954 PD patients and 954 population-based controls matched on gender, age and area of residence. Participants are 64% male with a mean year of birth of 1944, and 86% have Swedish ancestry. To do this, we will use genotype information obtained from whole-genome genotyping (From Illumina Global Screening Array-24v1+MD).

At this first stage of the project, 13 *MHC2TA* SNPs and 7 HLA SNPs have been tested for association to PD by comparing allele frequencies between cases and controls. Linear regression was used to identify cofounding factors and interactions were assessed

by logistic regression (SPSS). Of the 13 *MHC2TA* SNPs tested, there was no evidence for association with PD, using single regression (Table 10). We have found 2 HLA SNPs associated to PD previously (Ahmed et al., 2012; Hamza et al., 2010; Simon-Sanchez et al., 2011). Association was observed for rs660895 associated to PD (p=0.030) and rs4248166 (p=0.024) when using a dominant model. For the previously reported rs3129882 no association was found it (p=0.089); interestingly, it is important to note that in a previous study, no association was found it in a Swedish population (Ran et al., 2013).

In that respect, genetic and experimental studies point out antigen presentation capacity as a factor in PD pathogenesis. Several variants in the Human Leukocyte Antigen (HLA) locus encoding MHCII molecules are associated to PD and can double the risk (Hamza et al., 2010). Risk HLA-variants have been suggested to cause a shift towards a proinflammatory response to certain environmental challenge (Kannarkat et al., 2015). It is important to note, however, that interaction analysis is still going on and the interaction between MHC2TA SNPs and HLA SNPs or haplotypes cannot be excluded at this moment. Strengths of this whole-genome genotyping data from PD patient and matched aged control, include that we have epidemiological information. This epidemiological information includes caffeine and tobacco consumption and exposition to pesticides. Further analysis, using this epidemiological data in the MPBC cohort, can give insight into whether certain *MHC2TA* variants or *HLA* SNPs can predict any specific symptoms. Since has been reported that increased proinflammatory cytokines correlates with non-motor symptoms and cognitive decline in PD patients, thus neuroinflammation can act as predictive indicator for symptoms (Menza et al., 2010).

The genetic architecture of PD is complex, and interplay of more than one genetic factor such as in oligogenic and digenic inheritance is likely. Recently polygenic risk scores have been (Escott-Price et al., 2015) shown to capture the overall genetic architecture of complex genetics and aggregate the effects of multiple genetic markers (both protective and risk variants) (Escott-Price et al., 2015). Thus, the fact that we do not observed any association in the Swedish patients cannot rule out the role of *MHC2TA* as a genetic modifier, which could be affecting the penetrance, dominance and expressivity of another genetic variant with incomplete penetrance such as *LRRK2*, studied in paper IV. Future studies with the information retrieved from the MPBC whole genome genotyping can be used to calculate polygenic risk score of incomplete penetrant variants.

Despite not finding correlation of *MHC2TA* in MPBC cohort, our results from rodent model support the idea that *MHC2TA* could be a viable target to modulate *MHCII*-mediated inflammatory processes and slow disease progression in PD. Therefore, this *MHC2TA*-induced mechanism is still functionally interesting.

Table 10 | SNPs for HLA
*Pearson Chi-square rs75855844 (7) is not in Illumina array

	SNP	Allelles (Major/Minor)	N (PD/Control)	MAF PD	MAF Controls	MAF SweGene	P-value PD vs ControlAdditive model*	P-value PD vs Control Dominant model
HLA								
1	rs3129882	A/G	942/941	0.3769	0.4118	0.4025	0.089	0.058
2	rs660895	A/G	942/942	0.2102	0.2399	0.2635	0.089	0.030
3	rs2395163	T/C	942/942	0.2240	0.2282	0.2285	0.780	0.605
4	rs9275326	C/T	942/942	0.1205	0.1465	0.1315	0.112	0.079
5	rs9268515	G/C	942/941	0.1959	0.2024	0.2085	0.841	0.723
6	rs4248166	T/C	940/941	0.1957	0.1695	0.2265	0.077	0.024
7	rs75855844	G/A	---	---	---	0.2985		

Genetic population-based studies

In the last stage of this thesis, we have performed a mutation screening in the *LRRK2* and *SNCA* genes. These mutations are found in monogenic PD, and today constitute the most important candidate genes.

In Paper IV, we investigated 8 mutations in 2,206 Swedish PD Patients. This number represents around 10% of the total PD patients in Sweden, based on prevalence estimates (von Campenhausen et al., 2005). We have combined participants with unknown disease etiology from cohorts in 4 different geographic regions. Among the 2,206 participants, 81% were from population-based cohorts at geographically dispersed sites across the country which-allowed us to study possible regional difference in prevalence of PD.

The background population from where the patients included can be defined based on Sweden´s national population database. The majority of patients in the *Swedish Parkinson´s Genetics Network* presented a late-onset PD with an average age at onset of 60.7 years, and 64.4 years at diagnosis. Thus, we used population data from residents 50 year or older. In Swedish population aged 50 years or older, 14.6% were born abroad or had both parent born abroad, 3.5% had one parent born abroad. Taking these observations together and with the self-reported Swedish origin in 86% of the PD patients in the MPBC cohort. We therefore conclude the combined cohort represents well the Swedish PD population, further emphasizing the representative nature of our data.

The 8 mutations were selected to include the ones that have been previously reported with prevalence in Sweden (*SNCA* duplications and p.A53T variants as well as p.N1437H and p.G2019S) (Carmine Belin et al., 2006; Fuchs et al., 2007; Puschmann et al., 2012; Puschmann, Ross, et al., 2009) or historically related population (Norway, Denmark, United Kingdom or Germany) (*SNCA* pA30P, LRRK2 p.R1441H, p.Y1699C

and p.I2020T) (Lee et al., 2017; Seidel et al., 2010; Zimprich et al., 2011). In addition, the biological and neuropathological functions of *SNCA* and *LRRK2* in the pathogenic cascade in PD, and their relevance in immune responses as well as their potential as drug targets in PD motivated the investigation of mutation in these genes.

We identified mutations in 13 (0.59%) individuals. Of the 13 mutations, 12 were LRRK2 p.G2019S and 1 *SNCA* duplication. Four of the 12 *LRRK2* p.G2019S carriers, have reported positive family history. LRRK2 p.G2019S is known to have a markedly incomplete and varying penetrance (Lee et al., 2017; Marder et al., 2015) and was found in 0,11% of population-based controls from a subset of the *Swedish Parkinson Genetic Network*, the MPBC cohort. It has been reported a regional gradient in the prevalence of this mutation (Correia Guedes et al., 2010), where higher frequencies are observed in Italian population (1.6%) of 2976 patients from Italy (Cilia et al., 2014) , North Africa Arabic patients (41%) of 49 patients with sporadic PD (Gorostidi et al., 2009). It is important to note, that the founder effects can be very marked in limited-size samples. In contrast, low frequency of this variants is observed in large-size international cohorts (Correia Guedes et al., 2010). Thus, we have confirmed what has been found in terms of mutation rates in other North-European PD patient cohorts.

The low prevalence of LRRK2 p.G2019S pathogenic mutation in our multi-center cohort, when compared to other previous studies, might be an indication of a marked selection bias, a publication bias, or reflect true differences in the presence of these mutations between populations.

We have compare the frequency of these variants with GnomAD and the published literature, to address this question. Data from GnomAD, includes large number of studies on Alzheimer disease, migraine, and psychiatric disorders but not on other neurodegenerative disorders. We found that 0.11% of the individuals included in gnomAD carried one of the 5 most common *LRRK2* variants, almost exclusively *LRRK2* p.G2019S, whereas none at all of the pathogenic SNCA point mutations were found.

There was a marked 42-fold difference in the frequency of LRRK2 p.G2019S in gnomAD between 1.64% in Ashkenazi Jews and 0.039% in all other ethnicities , confirming the presence of a relatively ancient founder in Mediterranean populations. Furthermore, *LRRK2* p.G2019S also represented the vast majority (90.4%) of known pathogenic mutations in gnomAD datasets. Thus, our results are in line with the notion that *LRRK2* p.G2019S is the relatively most common pathogenic mutation, however as it displays a weak penetrance, it can only explain a small fraction of PD in the population.

Thus, environment factors and/or other genes modify the threshold for the penetrance and can alter disease-causing effect of *LRRK2* mutations.

A limitation to the study is that not all dominant pathogenic variants were analyzed, including *LRRK2* p(Arg1441His), p(Arg1141Cys) and *VPS35* p.(Asp620Asn), which are relatively more common variants in other populations. However, these has not been documented in Swedish population or neighbouring countries. Despite being the most common risk factor for PD , *GBA* mutations were not suitable to test in our multi-center cohort, due to low predictive value of this variants, since it has been reported that *GBA*

homozygotes and heterozygotes (i.e. Patients with Gaucher disease) never develop PD (Alcalay et al., 2014; Rosenbloom et al., 2011).

Familial autosomal dominant of PD have been documented in families with SNCA mutations or gene duplication/triplication. In general, triplication generates very high expression of mRNA and protein molecules and is associated with causing severe forms of PD. In contrast, patients with duplicated SNCA resemble idiopathic PD, with similar age at onset, and slower disease progression (La Cognata et al., 2017).

For our SNCA CNV screening, we used a commercial TaqMan Assays design to identified copy number variation. We tested 2,206 patients and identify one carrier (0.045%). This results, are lower compared to what has been report in two previous studies, where the frequency for CNVs is between 0.23-0.32% (Bentley et al., 2018; Nuytemans et al., 2009). In this regard duplication and triplication of SNCA gene, have been reported in families with different ethnic background. The cases were reported in Australia (n=2), Belgium (n=1), Canada (n=1), France (n=13), Germany (n=4), Italy (n=5), Japan (n=13), Korea (n=5), South Africa (n=1), Sweden (n=1), Tunisia (n=1), Turkey (n=4), the United Kingdom (n=3), and the United States (n=5) (La Cognata et al., 2017).

Along with the familiar forms, sporadic PD patients have been reported to carry de novo duplication of SNCA (Ahn et al., 2008; Nuytemans et al., 2009). However, in our study, the patient identified with duplication is part of the Swedish Lister Family, a large kindred with SNCA multiplications (Puschmann, Wszolek, et al., 2009).

No other genetic variants tested were found, indicating even lower frequencies. To our knowledge, this is the largest patient series tested for SNCA CNVs reported to date.

Looking ahead

Based on our results and the rapid development of the field of immunogenetics, we envision that more personalized therapeutic strategies that account for the immune satus of PD patients will be designed.

Concluding remarks

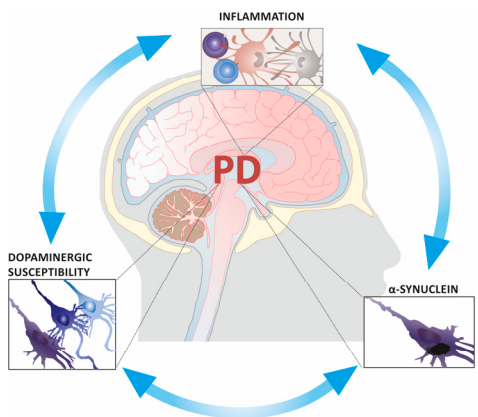
Our results highlight the complexity of defining the genetic risk variants and cellular mechanisms involved in PD. In this thesis we have aimed to dissect the link between genetic variants and cellular responses in neurodegeneration, with a focus in the concomitant inflammatory process in PD.

* Strain differences can be employed to map disease susceptibility. Several mouse loci are linked to dopaminergic neurodegeneration in the hemizygous *en1*$^{+/-}$ model.

* Common genetic variants affecting *Mhc2ta* expression levels regulates MHCII expression, reduced antigen presentation capacity and highly reactive microglial phenotype in DA.VRA4 animals in response to αsyn.

* *Mhc2ta*-induced differential MHCII response, enhance αsyn pathology and neurodegeneration in DA.VRA4 animals.

In light of our findings, we conclude that the mechanisms described here and the corresponding genes studied in the present study lay the foundation for further analyses whose results may be translated into novel therapeutic disease-modifying strategies for PD.



References

Aarsland, D., Pahlhagen, S., Ballard, C. G., Ehrt, U., & Svenningsson, P. (2011). Depression in Parkinson disease--epidemiology, mechanisms and management. *Nat Rev Neurol, 8*(1), 35-47. doi:10.1038/nrneurol.2011.189

Abdelmotilib, H., Maltbie, T., Delic, V., Liu, Z., Hu, X., Fraser, K. B., . . . West, A. (2017). alpha-Synuclein fibril-induced inclusion spread in rats and mice correlates with dopaminergic Neurodegeneration. *Neurobiol Dis, 105*, 84-98. doi:10.1016/j.nbd.2017.05.014

Ahmed, I., Tamouza, R., Delord, M., Krishnamoorthy, R., Tzourio, C., Mulot, C., . . . Elbaz, A. (2012). Association between Parkinson's disease and the HLA-DRB1 locus. *Mov Disord, 27*(9), 1104-1110. doi:10.1002/mds.25035

Ahn, T. B., Kim, S. Y., Kim, J. Y., Park, S. S., Lee, D. S., Min, H. J., . . . Jeon, B. S. (2008). alpha-Synuclein gene duplication is present in sporadic Parkinson disease. *Neurology, 70*(1), 43-49. doi:10.1212/01.wnl.0000271080.53272.c7

Albert, K., Voutilainen, M. H., Domanskyi, A., Piepponen, T. P., Ahola, S., Tuominen, R. K., . . . Airavaara, M. (2019). Downregulation of tyrosine hydroxylase phenotype after AAV injection above substantia nigra: Caution in experimental models of Parkinson's disease. *J Neurosci Res, 97*(3), 346-361. doi:10.1002/jnr.24363

Alcalay, R. N., Dinur, T., Quinn, T., Sakanaka, K., Levy, O., Waters, C., . . . Zimran, A. (2014). Comparison of Parkinson risk in Ashkenazi Jewish patients with Gaucher disease and GBA heterozygotes. *JAMA Neurol, 71*(6), 752-757. doi:10.1001/jamaneurol.2014.313

Aldrin-Kirk, P., Davidsson, M., Holmqvist, S., Li, J. Y., & Bjorklund, T. (2014). Novel AAV-based rat model of forebrain synucleinopathy shows extensive pathologies and progressive loss of cholinergic interneurons. *PLoS One, 9*(7), e100869. doi:10.1371/journal.pone.0100869

Alvarez-Fischer, D., Fuchs, J., Castagner, F., Stettler, O., Massiani-Beaudoin, O., Moya, K. L., . . . Prochiantz, A. (2011). Engrailed protects mouse midbrain dopaminergic neurons against mitochondrial complex I insults. *Nat Neurosci, 14*(10), 1260-1266. doi:10.1038/nn.2916

Baba, Y., Kuroiwa, A., Uitti, R. J., Wszolek, Z. K., & Yamada, T. (2005). Alterations of T-lymphocyte populations in Parkinson disease. *Parkinsonism Relat Disord, 11*(8), 493-498. doi:10.1016/j.parkreldis.2005.07.005

Beins, E., Ulas, T., Ternes, S., Neumann, H., Schultze, J. L., & Zimmer, A. (2016). Characterization of inflammatory markers and transcriptome profiles of differentially activated embryonic stem cell-derived microglia. *Glia, 64*(6), 1007-1020. doi:10.1002/glia.22979

Benjamin, J. L., Hedin, C. R., Koutsoumpas, A., Ng, S. C., McCarthy, N. E., Prescott, N. J., . . . Whelan, K. (2012). Smokers with active Crohn's disease have a clinically relevant dysbiosis of the gastrointestinal microbiota. *Inflamm Bowel Dis, 18*(6), 1092-1100. doi:10.1002/ibd.21864

Bentley, S. R., Bortnick, S., Guella, I., Fowdar, J. Y., Silburn, P. A., Wood, S. A., . . . Mellick, G. D. (2018). Pipeline to gene discovery - Analysing familial Parkinsonism in the Queensland Parkinson's Project. *Parkinsonism Relat Disord, 49*, 34-41. doi:10.1016/j.parkreldis.2017.12.033

Biedermann, L., Brulisauer, K., Zeitz, J., Frei, P., Scharl, M., Vavricka, S. R., . . . Schuppler, M. (2014). Smoking cessation alters intestinal microbiota: insights from quantitative investigations on human fecal samples using FISH. *Inflamm Bowel Dis, 20*(9), 1496-1501. doi:10.1097/MIB.0000000000000129

Bilovocky, N. A., Romito-DiGiacomo, R. R., Murcia, C. L., Maricich, S. M., & Herrup, K. (2003). Factors in the genetic background suppress the engrailed-1 cerebellar phenotype. *J Neurosci, 23*(12), 5105-5112.

Bjorklund, A., & Dunnett, S. B. (2007). Dopamine neuron systems in the brain: an update. *Trends Neurosci, 30*(5), 194-202. doi:10.1016/j.tins.2007.03.006

Blum-Degen, D., Muller, T., Kuhn, W., Gerlach, M., Przuntek, H., & Riederer, P. (1995). Interleukin-1 beta and interleukin-6 are elevated in the cerebrospinal fluid of Alzheimer's and de novo Parkinson's disease patients. *Neurosci Lett, 202*(1-2), 17-20. doi:10.1016/0304-3940(95)12192-7

Bonifati, V., Rizzu, P., Squitieri, F., Krieger, E., Vanacore, N., van Swieten, J. C., . . . Heutink, P. (2003). DJ-1(PARK7), a novel gene for autosomal recessive, early onset parkinsonism. *Neurol Sci, 24*(3), 159-160. doi:10.1007/s10072-003-0108-0

Boyle, E. A., Li, Y. I., & Pritchard, J. K. (2017). An Expanded View of Complex Traits: From Polygenic to Omnigenic. *Cell, 169*(7), 1177-1186. doi:10.1016/j.cell.2017.05.038

Braak, H., & Braak, E. (2000). Pathoanatomy of Parkinson's disease. *J Neurol, 247 Suppl 2*, II3-10. doi:10.1007/PL00007758

Braak, H., Rub, U., Gai, W. P., & Del Tredici, K. (2003). Idiopathic Parkinson's disease: possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. *J Neural Transm (Vienna), 110*(5), 517-536. doi:10.1007/s00702-002-0808-2

Brundin, P., & Melki, R. (2017). Prying into the Prion Hypothesis for Parkinson's Disease. *J Neurosci, 37*(41), 9808-9818. doi:10.1523/JNEUROSCI.1788-16.2017

Burke, R. E., & O'Malley, K. (2013). Axon degeneration in Parkinson's disease. *Exp Neurol, 246*, 72-83. doi:10.1016/j.expneurol.2012.01.011

Cacabelos, R. (2017). Parkinson's Disease: From Pathogenesis to Pharmacogenomics. *Int J Mol Sci, 18*(3). doi:10.3390/ijms18030551

Campelo, C., & Silva, R. H. (2017). Genetic Variants in SNCA and the Risk of Sporadic Parkinson's Disease and Clinical Outcomes: A Review. *Parkinsons Dis, 2017*, 4318416. doi:10.1155/2017/4318416

Cantor, R. M. (1995). A genetic analysis of common disease data. *Genet Epidemiol, 12*(6), 735-739. doi:10.1002/gepi.1370120634

Caraceni, T., Scigliano, G., & Musicco, M. (1991). The occurrence of motor fluctuations in parkinsonian patients treated long term with levodopa: role of early treatment and disease progression. *Neurology, 41*(3), 380-384. doi:10.1212/wnl.41.3.380

Carmine Belin, A., Westerlund, M., Sydow, O., Lundstromer, K., Hakansson, A., Nissbrandt, H., . . . Galter, D. (2006). Leucine-rich repeat kinase 2 (LRRK2) mutations in a Swedish Parkinson cohort and a healthy nonagenarian. *Mov Disord, 21*(10), 1731-1734. doi:10.1002/mds.21016

Chang, D., Nalls, M. A., Hallgrimsdottir, I. B., Hunkapiller, J., van der Brug, M., Cai, F., . . . Graham, R. R. (2017). A meta-analysis of genome-wide association studies identifies 17 new Parkinson's disease risk loci. *Nat Genet, 49*(10), 1511-1516. doi:10.1038/ng.3955

Chu, J. M., Chan, Y. S., Chen, L. W., & Yung, K. K. (2012). Neurokinin receptor 3 peptide exacerbates 6-hydroxydopamine-induced dopaminergic degeneration in rats through JNK pathway. *J Neurochem, 123*(3), 417-427. doi:10.1111/j.1471-4159.2012.07858.x

Cilia, R., Siri, C., Rusconi, D., Allegra, R., Ghiglietti, A., Sacilotto, G., . . . Goldwurm, S. (2014). LRRK2 mutations in Parkinson's disease: confirmation of a gender effect in the Italian population. *Parkinsonism Relat Disord, 20*(8), 911-914. doi:10.1016/j.parkreldis.2014.04.016

Constantinescu, C. S., Tani, M., Ransohoff, R. M., Wysocka, M., Hilliard, B., Fujioka, T., . . . Rostami, A. (2005). Astrocytes as antigen-presenting cells: expression of IL-12/IL-23. *J Neurochem, 95*(2), 331-340. doi:10.1111/j.1471-4159.2005.03368.x

Cook, D. A., Kannarkat, G. T., Cintron, A. F., Butkovich, L. M., Fraser, K. B., Chang, J., . . . Tansey, M. G. (2017). LRRK2 levels in immune cells are increased in Parkinson's disease. *NPJ Parkinsons Dis, 3*, 11. doi:10.1038/s41531-017-0010-8

Correia Guedes, L., Ferreira, J. J., Rosa, M. M., Coelho, M., Bonifati, V., & Sampaio, C. (2010). Worldwide frequency of G2019S LRRK2 mutation in Parkinson's disease: a systematic review. *Parkinsonism Relat Disord, 16*(4), 237-242. doi:10.1016/j.parkreldis.2009.11.004

Cote-Sierra, J., Foucras, G., Guo, L., Chiodetti, L., Young, H. A., Hu-Li, J., . . . Paul, W. E. (2004). Interleukin 2 plays a central role in Th2 differentiation. *Proc Natl Acad Sci U S A, 101*(11), 3880-3885. doi:10.1073/pnas.0400339101

Croisier, E., Moran, L. B., Dexter, D. T., Pearce, R. K., & Graeber, M. B. (2005). Microglial inflammation in the parkinsonian substantia nigra: relationship to alpha-synuclein deposition. *J Neuroinflammation, 2*, 14. doi:10.1186/1742-2094-2-14

Davis, E. J., Foster, T. D., & Thomas, W. E. (1994). Cellular forms and functions of brain microglia. *Brain Res Bull, 34*(1), 73-78. doi:10.1016/0361-9230(94)90189-9

de Lau, L. M., & Breteler, M. M. (2006). Epidemiology of Parkinson's disease. *Lancet Neurol, 5*(6), 525-535. doi:10.1016/S1474-4422(06)70471-9

Decressac, M., Mattsson, B., Lundblad, M., Weikop, P., & Bjorklund, A. (2012). Progressive neurodegenerative and behavioural changes induced by AAV-

mediated overexpression of alpha-synuclein in midbrain dopamine neurons. *Neurobiol Dis, 45*(3), 939-953. doi:10.1016/j.nbd.2011.12.013

Devi, L., Raghavendran, V., Prabhu, B. M., Avadhani, N. G., & Anandatheerthavarada, H. K. (2008). Mitochondrial import and accumulation of alpha-synuclein impair complex I in human dopaminergic neuronal cultures and Parkinson disease brain. *J Biol Chem, 283*(14), 9089-9100. doi:10.1074/jbc.M710012200

Doorn, K. J., Moors, T., Drukarch, B., van de Berg, W., Lucassen, P. J., & van Dam, A. M. (2014). Microglial phenotypes and toll-like receptor 2 in the substantia nigra and hippocampus of incidental Lewy body disease cases and Parkinson's disease patients. *Acta Neuropathol Commun, 2*, 90. doi:10.1186/s40478-014-0090-1

Duffy, M. F., Collier, T. J., Patterson, J. R., Kemp, C. J., Luk, K. C., Tansey, M. G., . . . Sortwell, C. E. (2018). Correction to: Lewy body-like alpha-synuclein inclusions trigger reactive microgliosis prior to nigral degeneration. *J Neuroinflammation, 15*(1), 169. doi:10.1186/s12974-018-1202-9

Erro, R., Vitale, C., Amboni, M., Picillo, M., Moccia, M., Longo, K., . . . Barone, P. (2013). The heterogeneity of early Parkinson's disease: a cluster analysis on newly diagnosed untreated patients. *PLoS One, 8*(8), e70244. doi:10.1371/journal.pone.0070244

Escott-Price, V., International Parkinson's Disease Genomics, C., Nalls, M. A., Morris, H. R., Lubbe, S., Brice, A., . . . members, I. c. (2015). Polygenic risk of Parkinson disease is correlated with disease age at onset. *Ann Neurol, 77*(4), 582-591. doi:10.1002/ana.24335

Foltynie, T., Brayne, C., & Barker, R. A. (2002). The heterogeneity of idiopathic Parkinson's disease. *J Neurol, 249*(2), 138-145.

Fuchs, J., Mueller, J. C., Lichtner, P., Schulte, C., Munz, M., Berg, D., . . . Gasser, T. (2009). The transcription factor PITX3 is associated with sporadic Parkinson's disease. *Neurobiol Aging, 30*(5), 731-738. doi:10.1016/j.neurobiolaging.2007.08.014

Fuchs, J., Nilsson, C., Kachergus, J., Munz, M., Larsson, E. M., Schule, B., . . . Farrer, M. J. (2007). Phenotypic variation in a large Swedish pedigree due to SNCA duplication and triplication. *Neurology, 68*(12), 916-922. doi:10.1212/01.wnl.0000254458.17630.c5

Fuchs, J., Stettler, O., Alvarez-Fischer, D., Prochiantz, A., Moya, K. L., & Joshi, R. L. (2012). Engrailed signaling in axon guidance and neuron survival. *Eur J Neurosci, 35*(12), 1837-1845. doi:10.1111/j.1460-9568.2012.08139.x

Galvin, J. E., Uryu, K., Lee, V. M., & Trojanowski, J. Q. (1999). Axon pathology in Parkinson's disease and Lewy body dementia hippocampus contains alpha-, beta-, and gamma-synuclein. *Proc Natl Acad Sci U S A, 96*(23), 13450-13455. doi:10.1073/pnas.96.23.13450

Geurtsen, G. J., Hoogland, J., Goldman, J. G., Schmand, B. A., Troster, A. I., Burn, D. J., . . . Criteria, M. D. S. S. G. o. t. V. o. P.-M. (2014). Parkinson's disease mild cognitive impairment: application and validation of the criteria. *J Parkinsons Dis, 4*(2), 131-137. doi:10.3233/JPD-130304

Gomez-Nicola, D., & Perry, V. H. (2015). Microglial dynamics and role in the healthy and diseased brain: a paradigm of functional plasticity. *Neuroscientist, 21*(2), 169-184. doi:10.1177/1073858414530512

Gorostidi, A., Ruiz-Martinez, J., Lopez de Munain, A., Alzualde, A., & Marti Masso, J. F. (2009). LRRK2 G2019S and R1441G mutations associated with Parkinson's disease are common in the Basque Country, but relative prevalence is determined by ethnicity. *Neurogenetics, 10*(2), 157-159. doi:10.1007/s10048-008-0162-0

Grisel, J. E. (2000). Quantitative trait locus analysis. *Alcohol Res Health, 24*(3), 169-174.

Halliday, G. M., & Stevens, C. H. (2011). Glia: initiators and progressors of pathology in Parkinson's disease. *Mov Disord, 26*(1), 6-17. doi:10.1002/mds.23455

Hamza, T. H., Zabetian, C. P., Tenesa, A., Laederach, A., Montimurro, J., Yearout, D., . . . Payami, H. (2010). Common genetic variation in the HLA region is associated with late-onset sporadic Parkinson's disease. *Nat Genet, 42*(9), 781-785. doi:10.1038/ng.642

Hanks, M., Wurst, W., Anson-Cartwright, L., Auerbach, A. B., & Joyner, A. L. (1995). Rescue of the En-1 mutant phenotype by replacement of En-1 with En-2. *Science, 269*(5224), 679-682. doi:10.1126/science.7624797

Harms, A. S., Cao, S., Rowse, A. L., Thome, A. D., Li, X., Mangieri, L. R., . . . Standaert, D. G. (2013). MHCII is required for alpha-synuclein-induced activation of microglia, CD4 T cell proliferation, and dopaminergic neurodegeneration. *J Neurosci, 33*(23), 9592-9600. doi:10.1523/JNEUROSCI.5610-12.2013

Harms, A. S., Delic, V., Thome, A. D., Bryant, N., Liu, Z., Chandra, S., . . . West, A. B. (2017). alpha-Synuclein fibrils recruit peripheral immune cells in the rat brain prior to neurodegeneration. *Acta Neuropathol Commun, 5*(1), 85. doi:10.1186/s40478-017-0494-9

Harnesk, K., Swanberg, M., Ockinger, J., Diez, M., Lidman, O., Wallstrom, E., . . . Piehl, F. (2008). Vra4 congenic rats with allelic differences in the class II transactivator gene display altered susceptibility to experimental autoimmune encephalomyelitis. *J Immunol, 180*(5), 3289-3296. doi:10.4049/jimmunol.180.5.3289

Haubenberger, D., Reinthaler, E., Mueller, J. C., Pirker, W., Katzenschlager, R., Froehlich, R., . . . Zimprich, A. (2011). Association of transcription factor polymorphisms PITX3 and EN1 with Parkinson's disease. *Neurobiol Aging, 32*(2), 302-307. doi:10.1016/j.neurobiolaging.2009.02.015

Hernan, M. A., Takkouche, B., Caamano-Isorna, F., & Gestal-Otero, J. J. (2002). A meta-analysis of coffee drinking, cigarette smoking, and the risk of Parkinson's disease. *Ann Neurol, 52*(3), 276-284. doi:10.1002/ana.10277

Hernandez, D. G., Reed, X., & Singleton, A. B. (2016). Genetics in Parkinson disease: Mendelian versus non-Mendelian inheritance. *J Neurochem, 139 Suppl 1*, 59-74. doi:10.1111/jnc.13593

Hill-Burns, E. M., Factor, S. A., Zabetian, C. P., Thomson, G., & Payami, H. (2011). Evidence for more than one Parkinson's disease-associated variant within the HLA region. *PLoS One, 6*(11), e27109. doi:10.1371/journal.pone.0027109

Hunot, S., Bernard, V., Faucheux, B., Boissiere, F., Leguern, E., Brana, C., . . . Hirsch, E. C. (1996). Glial cell line-derived neurotrophic factor (GDNF) gene expression in the human brain: a post mortem in situ hybridization study with

special reference to Parkinson's disease. *J Neural Transm (Vienna), 103*(8-9), 1043-1052. doi:10.1007/BF01291789

Imamura, K., Hishikawa, N., Sawada, M., Nagatsu, T., Yoshida, M., & Hashizume, Y. (2003). Distribution of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson's disease brains. *Acta Neuropathol, 106*(6), 518-526. doi:10.1007/s00401-003-0766-2

Jewett, M., Dickson, E., Brolin, K., Negrini, M., Jimenez-Ferrer, I., & Swanberg, M. (2018). Glutathione S-Transferase Alpha 4 Prevents Dopamine Neurodegeneration in a Rat Alpha-Synuclein Model of Parkinson's Disease. *Front Neurol, 9*, 222. doi:10.3389/fneur.2018.00222

Jimenez-Ferrer, I., Jewett, M., Tontanahal, A., Romero-Ramos, M., & Swanberg, M. (2017). Allelic difference in Mhc2ta confers altered microglial activation and susceptibility to alpha-synuclein-induced dopaminergic neurodegeneration. *Neurobiol Dis, 106*, 279-290. doi:10.1016/j.nbd.2017.07.016

Jimenez-Ferrer, I., & Swanberg, M. (2018). Immunogenetics of Parkinson's Disease. In T. B. Stoker & J. C. Greenland (Eds.), *Parkinson's Disease: Pathogenesis and Clinical Aspects*. Brisbane (AU).

Kannarkat, G. T., Cook, D. A., Lee, J. K., Chang, J., Chung, J., Sandy, E., . . . Tansey, M. G. (2015). Common Genetic Variant Association with Altered HLA Expression, Synergy with Pyrethroid Exposure, and Risk for Parkinson's Disease: An Observational and Case-Control Study. *NPJ Parkinsons Dis, 1*. doi:10.1038/npjparkd.2015.2

Kim, C., Ho, D. H., Suk, J. E., You, S., Michael, S., Kang, J., . . . Lee, S. J. (2013). Neuron-released oligomeric alpha-synuclein is an endogenous agonist of TLR2 for paracrine activation of microglia. *Nat Commun, 4*, 1562. doi:10.1038/ncomms2534

Kirik, D., Rosenblad, C., Burger, C., Lundberg, C., Johansen, T. E., Muzyczka, N., . . . Bjorklund, A. (2002). Parkinson-like neurodegeneration induced by targeted overexpression of alpha-synuclein in the nigrostriatal system. *J Neurosci, 22*(7), 2780-2791. doi:20026246

Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., . . . Shimizu, N. (1998). Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature, 392*(6676), 605-608. doi:10.1038/33416

Klein, C., & Westenberger, A. (2012). Genetics of Parkinson's disease. *Cold Spring Harb Perspect Med, 2*(1), a008888. doi:10.1101/cshperspect.a008888

Knott, C., Stern, G., & Wilkin, G. P. (2000). Inflammatory regulators in Parkinson's disease: iNOS, lipocortin-1, and cyclooxygenases-1 and -2. *Mol Cell Neurosci, 16*(6), 724-739. doi:10.1006/mcne.2000.0914

Kordower, J. H., Olanow, C. W., Dodiya, H. B., Chu, Y., Beach, T. G., Adler, C. H., . . . Bartus, R. T. (2013). Disease duration and the integrity of the nigrostriatal system in Parkinson's disease. *Brain, 136*(Pt 8), 2419-2431. doi:10.1093/brain/awt192

La Cognata, V., Morello, G., D'Agata, V., & Cavallaro, S. (2017). Copy number variability in Parkinson's disease: assembling the puzzle through a systems biology approach. *Hum Genet, 136*(1), 13-37. doi:10.1007/s00439-016-1749-4

Lampe, J. B., Gossrau, G., Herting, B., Kempe, A., Sommer, U., Fussel, M., . . . Reichmann, H. (2003). HLA typing and Parkinson's disease. *Eur Neurol, 50*(2), 64-68. doi:10.1159/000072500

Le Pen, G., Sonnier, L., Hartmann, A., Bizot, J. C., Trovero, F., Krebs, M. O., & Prochiantz, A. (2008). Progressive loss of dopaminergic neurons in the ventral midbrain of adult mice heterozygote for Engrailed1: a new genetic model for Parkinson's disease? *Parkinsonism Relat Disord, 14 Suppl 2*, S107-111. doi:10.1016/j.parkreldis.2008.04.007

Lee, A. J., Wang, Y., Alcalay, R. N., Mejia-Santana, H., Saunders-Pullman, R., Bressman, S., . . . Michael, J. F. L. C. C. (2017). Penetrance estimate of LRRK2 p.G2019S mutation in individuals of non-Ashkenazi Jewish ancestry. *Mov Disord, 32*(10), 1432-1438. doi:10.1002/mds.27059

Liao, W., Lin, J. X., Wang, L., Li, P., & Leonard, W. J. (2011). Modulation of cytokine receptors by IL-2 broadly regulates differentiation into helper T cell lineages. *Nat Immunol, 12*(6), 551-559. doi:10.1038/ni.2030

Liddelow, S. A., Guttenplan, K. A., Clarke, L. E., Bennett, F. C., Bohlen, C. J., Schirmer, L., . . . Barres, B. A. (2017). Neurotoxic reactive astrocytes are induced by activated microglia. *Nature, 541*(7638), 481-487. doi:10.1038/nature21029

Lidman, O., Swanberg, M., Horvath, L., Broman, K. W., Olsson, T., & Piehl, F. (2003). Discrete gene loci regulate neurodegeneration, lymphocyte infiltration, and major histocompatibility complex class II expression in the CNS. *J Neurosci, 23*(30), 9817-9823.

Lo Bianco, C., Ridet, J. L., Schneider, B. L., Deglon, N., & Aebischer, P. (2002). alpha -Synucleinopathy and selective dopaminergic neuron loss in a rat lentiviral-based model of Parkinson's disease. *Proc Natl Acad Sci U S A, 99*(16), 10813-10818. doi:10.1073/pnas.152339799

Luk, K. C., Kehm, V., Carroll, J., Zhang, B., O'Brien, P., Trojanowski, J. Q., & Lee, V. M. (2012). Pathological alpha-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. *Science, 338*(6109), 949-953. doi:10.1126/science.1227157

Luk, K. C., Kehm, V. M., Zhang, B., O'Brien, P., Trojanowski, J. Q., & Lee, V. M. (2012). Intracerebral inoculation of pathological alpha-synuclein initiates a rapidly progressive neurodegenerative alpha-synucleinopathy in mice. *J Exp Med, 209*(5), 975-986. doi:10.1084/jem.20112457

Luk, K. C., Song, C., O'Brien, P., Stieber, A., Branch, J. R., Brunden, K. R., . . . Lee, V. M. (2009). Exogenous alpha-synuclein fibrils seed the formation of Lewy body-like intracellular inclusions in cultured cells. *Proc Natl Acad Sci U S A, 106*(47), 20051-20056. doi:10.1073/pnas.0908005106

Lundberg, C., Lidman, O., Holmdahl, R., Olsson, T., & Piehl, F. (2001). Neurodegeneration and glial activation patterns after mechanical nerve injury are differentially regulated by non-MHC genes in congenic inbred rat strains. *J Comp Neurol, 431*(1), 75-87.

Lundblad, M., Decressac, M., Mattsson, B., & Bjorklund, A. (2012). Impaired neurotransmission caused by overexpression of alpha-synuclein in nigral dopamine neurons. *Proc Natl Acad Sci U S A, 109*(9), 3213-3219. doi:10.1073/pnas.1200575109

Macdonald, P. A., & Monchi, O. (2011). Differential effects of dopaminergic therapies on dorsal and ventral striatum in Parkinson's disease: implications for cognitive function. *Parkinsons Dis, 2011*, 572743. doi:10.4061/2011/572743

Manichaikul, A., Dupuis, J., Sen, S., & Broman, K. W. (2006). Poor performance of bootstrap confidence intervals for the location of a quantitative trait locus. *Genetics, 174*(1), 481-489. doi:10.1534/genetics.106.061549

Marder, K., Wang, Y., Alcalay, R. N., Mejia-Santana, H., Tang, M. X., Lee, A., . . . Consortium, L. A. J. (2015). Age-specific penetrance of LRRK2 G2019S in the Michael J. Fox Ashkenazi Jewish LRRK2 Consortium. *Neurology, 85*(1), 89-95. doi:10.1212/WNL.0000000000001708

Martino, R., Candundo, H., Lieshout, P. V., Shin, S., Crispo, J. A. G., & Barakat-Haddad, C. (2017). Onset and progression factors in Parkinson's disease: A systematic review. *Neurotoxicology, 61*, 132-141. doi:10.1016/j.neuro.2016.04.003

McGeer, P. L., Itagaki, S., Akiyama, H., & McGeer, E. G. (1988). Rate of cell death in parkinsonism indicates active neuropathological process. *Ann Neurol, 24*(4), 574-576. doi:10.1002/ana.410240415

McGeer, P. L., Itagaki, S., Boyes, B. E., & McGeer, E. G. (1988). Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. *Neurology, 38*(8), 1285-1291. doi:10.1212/wnl.38.8.1285

Menza, M., Dobkin, R. D., Marin, H., Mark, M. H., Gara, M., Bienfait, K., . . . Kusnekov, A. (2010). The role of inflammatory cytokines in cognition and other non-motor symptoms of Parkinson's disease. *Psychosomatics, 51*(6), 474-479. doi:10.1176/appi.psy.51.6.474

Mills, C. D., Kincaid, K., Alt, J. M., Heilman, M. J., & Hill, A. M. (2000). M-1/M-2 macrophages and the Th1/Th2 paradigm. *J Immunol, 164*(12), 6166-6173. doi:10.4049/jimmunol.164.12.6166

Miyadera, H., & Tokunaga, K. (2015). Associations of human leukocyte antigens with autoimmune diseases: challenges in identifying the mechanism. *J Hum Genet, 60*(11), 697-702. doi:10.1038/jhg.2015.100

Mogi, M., Harada, M., Kondo, T., Riederer, P., & Nagatsu, T. (1996). Interleukin-2 but not basic fibroblast growth factor is elevated in parkinsonian brain. Short communication. *J Neural Transm (Vienna), 103*(8-9), 1077-1081. doi:10.1007/BF01291792

Mogi, M., Harada, M., Narabayashi, H., Inagaki, H., Minami, M., & Nagatsu, T. (1996). Interleukin (IL)-1 beta, IL-2, IL-4, IL-6 and transforming growth factor-alpha levels are elevated in ventricular cerebrospinal fluid in juvenile parkinsonism and Parkinson's disease. *Neurosci Lett, 211*(1), 13-16. doi:10.1016/0304-3940(96)12706-3

Montgomery, S. B., Sammeth, M., Gutierrez-Arcelus, M., Lach, R. P., Ingle, C., Nisbett, J., . . . Dermitzakis, E. T. (2010). Transcriptome genetics using second generation sequencing in a Caucasian population. *Nature, 464*(7289), 773-777. doi:10.1038/nature08903

Mu, J., Chaudhuri, K. R., Bielza, C., de Pedro-Cuesta, J., Larranaga, P., & Martinez-Martin, P. (2017). Parkinson's Disease Subtypes Identified from Cluster Analysis of Motor and Non-motor Symptoms. *Front Aging Neurosci, 9*, 301. doi:10.3389/fnagi.2017.00301

Munder, M. (2009). Arginase: an emerging key player in the mammalian immune system. *Br J Pharmacol, 158*(3), 638-651. doi:10.1111/j.1476-5381.2009.00291.x

Nalls, M. A., Pankratz, N., Lill, C. M., Do, C. B., Hernandez, D. G., Saad, M., . . . Singleton, A. B. (2014). Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. *Nat Genet, 46*(9), 989-993. doi:10.1038/ng.3043

Nordstroma, U., Beauvais, G., Ghosh, A., Pulikkaparambil Sasidharan, B. C., Lundblad, M., Fuchs, J., . . . Brundin, P. (2015). Progressive nigrostriatal terminal dysfunction and degeneration in the engrailed1 heterozygous mouse model of Parkinson's disease. *Neurobiol Dis, 73*, 70-82. doi:10.1016/j.nbd.2014.09.012

Noyce, A. J., Bestwick, J. P., Silveira-Moriyama, L., Hawkes, C. H., Giovannoni, G., Lees, A. J., & Schrag, A. (2012). Meta-analysis of early nonmotor features and risk factors for Parkinson disease. *Ann Neurol, 72*(6), 893-901. doi:10.1002/ana.23687

Nuytemans, K., Meeus, B., Crosiers, D., Brouwers, N., Goossens, D., Engelborghs, S., . . . Theuns, J. (2009). Relative contribution of simple mutations vs. copy number variations in five Parkinson disease genes in the Belgian population. *Hum Mutat, 30*(7), 1054-1061. doi:10.1002/humu.21007

Olsson, M., Nikkhah, G., Bentlage, C., & Bjorklund, A. (1995). Forelimb akinesia in the rat Parkinson model: differential effects of dopamine agonists and nigral transplants as assessed by a new stepping test. *J Neurosci, 15*(5 Pt 2), 3863-3875.

Ozaki, K., Kikly, K., Michalovich, D., Young, P. R., & Leonard, W. J. (2000). Cloning of a type I cytokine receptor most related to the IL-2 receptor beta chain. *Proc Natl Acad Sci U S A, 97*(21), 11439-11444. doi:10.1073/pnas.200360997

Paisan-Ruiz, C., Jain, S., Evans, E. W., Gilks, W. P., Simon, J., van der Brug, M., . . . Singleton, A. B. (2004). Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron, 44*(4), 595-600. doi:10.1016/j.neuron.2004.10.023

Papagno, C., & Trojano, L. (2018). Cognitive and behavioral disorders in Parkinson's disease: an update. I: cognitive impairments. *Neurol Sci, 39*(2), 215-223. doi:10.1007/s10072-017-3154-8

Parkinson, J. (2002). An essay on the shaking palsy. 1817. *J Neuropsychiatry Clin Neurosci, 14*(2), 223-236; discussion 222. doi:10.1176/jnp.14.2.223

Paumier, K. L., Luk, K. C., Manfredsson, F. P., Kanaan, N. M., Lipton, J. W., Collier, T. J., . . . Sortwell, C. E. (2015). Intrastriatal injection of pre-formed mouse alpha-synuclein fibrils into rats triggers alpha-synuclein pathology and bilateral nigrostriatal degeneration. *Neurobiol Dis, 82*, 185-199. doi:10.1016/j.nbd.2015.06.003

Paxinos, G., Watson, C. R., & Emson, P. C. (1980). AChE-stained horizontal sections of the rat brain in stereotaxic coordinates. *J Neurosci Methods, 3*(2), 129-149.

Piehl, F., Lundberg, C., Khademi, M., Bucht, A., Dahlman, I., Lorentzen, J. C., & Olsson, T. (1999). Non-MHC gene regulation of nerve root injury induced spinal cord inflammation and neuron death. *J Neuroimmunol, 101*(1), 87-97.

Pierce, S., & Coetzee, G. A. (2017). Parkinson's disease-associated genetic variation is linked to quantitative expression of inflammatory genes. *PLoS One, 12*(4), e0175882. doi:10.1371/journal.pone.0175882

Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A., . . . Nussbaum, R. L. (1997). Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science, 276*(5321), 2045-2047. doi:10.1126/science.276.5321.2045

Postuma, R. B., Berg, D., Stern, M., Poewe, W., Olanow, C. W., Oertel, W., . . . Deuschl, G. (2015). MDS clinical diagnostic criteria for Parkinson's disease. *Mov Disord, 30*(12), 1591-1601. doi:10.1002/mds.26424

Pringsheim, T., Jette, N., Frolkis, A., & Steeves, T. D. (2014). The prevalence of Parkinson's disease: a systematic review and meta-analysis. *Mov Disord, 29*(13), 1583-1590. doi:10.1002/mds.25945

Prytz, H., Benoni, C., & Tagesson, C. (1989). Does smoking tighten the gut? *Scand J Gastroenterol, 24*(9), 1084-1088.

Puschmann, A., Englund, E., Ross, O. A., Vilarino-Guell, C., Lincoln, S. J., Kachergus, J. M., . . . Nilsson, C. (2012). First neuropathological description of a patient with Parkinson's disease and LRRK2 p.N1437H mutation. *Parkinsonism Relat Disord, 18*(4), 332-338. doi:10.1016/j.parkreldis.2011.11.019

Puschmann, A., Ross, O. A., Vilarino-Guell, C., Lincoln, S. J., Kachergus, J. M., Cobb, S. A., . . . Nilsson, C. (2009). A Swedish family with de novo alpha-synuclein A53T mutation: evidence for early cortical dysfunction. *Parkinsonism Relat Disord, 15*(9), 627-632. doi:10.1016/j.parkreldis.2009.06.007

Puschmann, A., Wszolek, Z. K., Farrer, M., Gustafson, L., Widner, H., & Nilsson, C. (2009). Alpha-synuclein multiplications with parkinsonism, dementia or progressive myoclonus? *Parkinsonism Relat Disord, 15*(5), 390-392. doi:10.1016/j.parkreldis.2008.08.002

Qin, X. Y., Zhang, S. P., Cao, C., Loh, Y. P., & Cheng, Y. (2016). Aberrations in Peripheral Inflammatory Cytokine Levels in Parkinson Disease: A Systematic Review and Meta-analysis. *JAMA Neurol, 73*(11), 1316-1324. doi:10.1001/jamaneurol.2016.2742

Ran, C., Willows, T., Sydow, O., Johansson, A., Soderkvist, P., Dizdar, N., . . . Belin, A. C. (2013). The HLA-DRA variation rs3129882 is not associated with Parkinson's disease in Sweden. *Parkinsonism Relat Disord, 19*(7), 701-702. doi:10.1016/j.parkreldis.2013.03.001

Rees, K., Stowe, R., Patel, S., Ives, N., Breen, K., Clarke, C. E., & Ben-Shlomo, Y. (2011). Non-steroidal anti-inflammatory drugs as disease-modifying agents for Parkinson's disease: evidence from observational studies. *Cochrane Database Syst Rev*(11), CD008454. doi:10.1002/14651858.CD008454.pub2

Reith, W., LeibundGut-Landmann, S., & Waldburger, J. M. (2005). Regulation of MHC class II gene expression by the class II transactivator. *Nat Rev Immunol, 5*(10), 793-806. doi:10.1038/nri1708

Rey, N. L., Steiner, J. A., Maroof, N., Luk, K. C., Madaj, Z., Trojanowski, J. Q., . . . Brundin, P. (2016). Widespread transneuronal propagation of alpha-synucleinopathy triggered in olfactory bulb mimics prodromal Parkinson's disease. *J Exp Med, 213*(9), 1759-1778. doi:10.1084/jem.20160368

Rolstad, B. (2014). The early days of NK cells: an example of how a phenomenon led to detection of a novel immune receptor system - lessons from a rat model. *Front Immunol, 5*, 283. doi:10.3389/fimmu.2014.00283

Rosenbloom, B., Balwani, M., Bronstein, J. M., Kolodny, E., Sathe, S., Gwosdow, A. R., . . . Weinreb, N. J. (2011). The incidence of Parkinsonism in patients with type 1 Gaucher disease: data from the ICGG Gaucher Registry. *Blood Cells Mol Dis, 46*(1), 95-102. doi:10.1016/j.bcmd.2010.10.006

Ross, G. W., Petrovitch, H., Abbott, R. D., Nelson, J., Markesbery, W., Davis, D., . . . White, L. R. (2004). Parkinsonian signs and substantia nigra neuron density in decendents elders without PD. *Ann Neurol, 56*(4), 532-539. doi:10.1002/ana.20226

Sachidanandam, R., Weissman, D., Schmidt, S. C., Kakol, J. M., Stein, L. D., Marth, G., . . . International, S. N. P. M. W. G. (2001). A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature, 409*(6822), 928-933. doi:10.1038/35057149

Sanchez-Guajardo, V., Febbraro, F., Kirik, D., & Romero-Ramos, M. (2010). Microglia acquire distinct activation profiles depending on the degree of alpha-synuclein neuropathology in a rAAV based model of Parkinson's disease. *PLoS One, 5*(1), e8784. doi:10.1371/journal.pone.0008784

Schallert, T., Fleming, S. M., Leasure, J. L., Tillerson, J. L., & Bland, S. T. (2000). CNS plasticity and assessment of forelimb sensorimotor outcome in unilateral rat models of stroke, cortical ablation, parkinsonism and spinal cord injury. *Neuropharmacology, 39*(5), 777-787.

Schrag, A., Horsfall, L., Walters, K., Noyce, A., & Petersen, I. (2015). Prediagnostic presentations of Parkinson's disease in primary care: a case-control study. *Lancet Neurol, 14*(1), 57-64. doi:10.1016/S1474-4422(14)70287-X

Seidel, K., Schols, L., Nuber, S., Petrasch-Parwez, E., Gierga, K., Wszolek, Z., . . . Kruger, R. (2010). First appraisal of brain pathology owing to A30P mutant alpha-synuclein. *Ann Neurol, 67*(5), 684-689. doi:10.1002/ana.21966

Sgado, P., Alberi, L., Gherbassi, D., Galasso, S. L., Ramakers, G. M., Alavian, K. N., . . . Simon, H. H. (2006). Slow progressive degeneration of nigral dopaminergic neurons in postnatal Engrailed mutant mice. *Proc Natl Acad Sci U S A, 103*(41), 15242-15247. doi:10.1073/pnas.0602116103

Shimozawa, A., Ono, M., Takahara, D., Tarutani, A., Imura, S., Masuda-Suzukake, M., . . . Hasegawa, M. (2017). Propagation of pathological alpha-synuclein in marmoset brain. *Acta Neuropathol Commun, 5*(1), 12. doi:10.1186/s40478-017-0413-0

Simon-Sanchez, J., van Hilten, J. J., van de Warrenburg, B., Post, B., Berendse, H. W., Arepalli, S., . . . Heutink, P. (2011). Genome-wide association study confirms extant PD risk loci among the Dutch. *Eur J Hum Genet, 19*(6), 655-661. doi:10.1038/ejhg.2010.254

Singleton, A. B., Farrer, M., Johnson, J., Singleton, A., Hague, S., Kachergus, J., . . . Gwinn-Hardy, K. (2003). alpha-Synuclein locus triplication causes Parkinson's disease. *Science, 302*(5646), 841. doi:10.1126/science.1090278

Sonnier, L., Le Pen, G., Hartmann, A., Bizot, J. C., Trovero, F., Krebs, M. O., & Prochiantz, A. (2007). Progressive loss of dopaminergic neurons in the ventral

midbrain of adult mice heterozygote for Engrailed1. *J Neurosci, 27*(5), 1063-1071. doi:10.1523/JNEUROSCI.4583-06.2007

Spillantini, M. G., Schmidt, M. L., Lee, V. M., Trojanowski, J. Q., Jakes, R., & Goedert, M. (1997). Alpha-synuclein in Lewy bodies. *Nature, 388*(6645), 839-840. doi:10.1038/42166

Steimle, V., Otten, L. A., Zufferey, M., & Mach, B. (1993). Complementation cloning of an MHC class II transactivator mutated in hereditary MHC class II deficiency (or bare lymphocyte syndrome). *Cell, 75*(1), 135-146.

Stevens, C. H., Rowe, D., Morel-Kopp, M. C., Orr, C., Russell, T., Ranola, M., . . . Halliday, G. M. (2012). Reduced T helper and B lymphocytes in Parkinson's disease. *J Neuroimmunol, 252*(1-2), 95-99. doi:10.1016/j.jneuroim.2012.07.015

Stranger, B. E., Nica, A. C., Forrest, M. S., Dimas, A., Bird, C. P., Beazley, C., . . . Dermitzakis, E. T. (2007). Population genomics of human gene expression. *Nat Genet, 39*(10), 1217-1224. doi:10.1038/ng2142

Sugita, M., Izuno, T., Tatemichi, M., & Otahara, Y. (2001). Meta-analysis for epidemiologic studies on the relationship between smoking and Parkinson's disease. *J Epidemiol, 11*(2), 87-94. doi:10.2188/jea.11.87

Sulzer, D., Alcalay, R. N., Garretti, F., Cote, L., Kanter, E., Agin-Liebes, J., . . . Sette, A. (2017). Erratum: T cells from patients with Parkinson's disease recognize alpha-synuclein peptides. *Nature, 549*(7671), 292. doi:10.1038/nature23896

Surmeier, D. J., Obeso, J. A., & Halliday, G. M. (2017). Parkinson's Disease Is Not Simply a Prion Disorder. *J Neurosci, 37*(41), 9799-9807. doi:10.1523/JNEUROSCI.1787-16.2017

Swanberg, M., Harnesk, K., Strom, M., Diez, M., Lidman, O., & Piehl, F. (2009). Fine mapping of gene regions regulating neurodegeneration. *PLoS One, 4*(6), e5906. doi:10.1371/journal.pone.0005906

Swanberg, M., Lidman, O., Padyukov, L., Eriksson, P., Akesson, E., Jagodic, M., . . . Olsson, T. (2005). MHC2TA is associated with differential MHC molecule expression and susceptibility to rheumatoid arthritis, multiple sclerosis and myocardial infarction. *Nat Genet, 37*(5), 486-494. doi:10.1038/ng1544

Swanberg, M., McGuigan, F. E., Ivaska, K. K., Gerdhem, P., & Akesson, K. (2012). Polymorphisms in the inflammatory genes CIITA, CLEC16A and IFNG influence BMD, bone loss and fracture in elderly women. *PLoS One, 7*(10), e47964. doi:10.1371/journal.pone.0047964

Thakur, P., Breger, L. S., Lundblad, M., Wan, O. W., Mattsson, B., Luk, K. C., . . . Bjorklund, A. (2017). Modeling Parkinson's disease pathology by combination of fibril seeds and alpha-synuclein overexpression in the rat brain. *Proc Natl Acad Sci U S A, 114*(39), E8284-E8293. doi:10.1073/pnas.1710442114

Torres-Platas, S. G., Comeau, S., Rachalski, A., Bo, G. D., Cruceanu, C., Turecki, G., . . . Mechawar, N. (2014). Morphometric characterization of microglial phenotypes in human cerebral cortex. *J Neuroinflammation, 11*, 12. doi:10.1186/1742-2094-11-12

Trojano, L., & Papagno, C. (2018). Cognitive and behavioral disorders in Parkinson's disease: an update. II: behavioral disorders. *Neurol Sci, 39*(1), 53-61. doi:10.1007/s10072-017-3155-7

Valente, E. M., Abou-Sleiman, P. M., Caputo, V., Muqit, M. M., Harvey, K., Gispert, S., . . . Wood, N. W. (2004). Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science, 304*(5674), 1158-1160. doi:10.1126/science.1096284

Van Maele-Fabry, G., Hoet, P., Vilain, F., & Lison, D. (2012). Occupational exposure to pesticides and Parkinson's disease: a systematic review and meta-analysis of cohort studies. *Environ Int, 46*, 30-43. doi:10.1016/j.envint.2012.05.004

Vawter, M. P., Dillon-Carter, O., Tourtellotte, W. W., Carvey, P., & Freed, W. J. (1996). TGFbeta1 and TGFbeta2 concentrations are elevated in Parkinson's disease in ventricular cerebrospinal fluid. *Exp Neurol, 142*(2), 313-322. doi:10.1006/exnr.1996.0200

Veenvliet, J. V., Dos Santos, M. T., Kouwenhoven, W. M., von Oerthel, L., Lim, J. L., van der Linden, A. J., . . . Smidt, M. P. (2013). Specification of dopaminergic subsets involves interplay of En1 and Pitx3. *Development, 140*(16), 3373-3384. doi:10.1242/dev.094565

Volpicelli-Daley, L. A., Luk, K. C., & Lee, V. M. (2014). Addition of exogenous alpha-synuclein preformed fibrils to primary neuronal cultures to seed recruitment of endogenous alpha-synuclein to Lewy body and Lewy neurite-like aggregates. *Nat Protoc, 9*(9), 2135-2146. doi:10.1038/nprot.2014.143

Volpicelli-Daley, L. A., Luk, K. C., Patel, T. P., Tanik, S. A., Riddle, D. M., Stieber, A., . . . Lee, V. M. (2011). Exogenous alpha-synuclein fibrils induce Lewy body pathology leading to synaptic dysfunction and neuron death. *Neuron, 72*(1), 57-71. doi:10.1016/j.neuron.2011.08.033

von Campenhausen, S., Bornschein, B., Wick, R., Botzel, K., Sampaio, C., Poewe, W., . . . Dodel, R. (2005). Prevalence and incidence of Parkinson's disease in Europe. *Eur Neuropsychopharmacol, 15*(4), 473-490. doi:10.1016/j.euroneuro.2005.04.007

Wakeland, E., Morel, L., Achey, K., Yui, M., & Longmate, J. (1997). Speed congenics: a classic technique in the fast lane (relatively speaking). *Immunol Today, 18*(10), 472-477.

Waldburger, J. M., Suter, T., Fontana, A., Acha-Orbea, H., & Reith, W. (2001). Selective abrogation of major histocompatibility complex class II expression on extrahematopoietic cells in mice lacking promoter IV of the class II transactivator gene. *J Exp Med, 194*(4), 393-406. doi:10.1084/jem.194.4.393

Walker, D. G., & Lue, L. F. (2015). Immune phenotypes of microglia in human neurodegenerative disease: challenges to detecting microglial polarization in human brains. *Alzheimers Res Ther, 7*(1), 56. doi:10.1186/s13195-015-0139-9

West, M. J., & Gundersen, H. J. (1990). Unbiased stereological estimation of the number of neurons in the human hippocampus. *J Comp Neurol, 296*(1), 1-22. doi:10.1002/cne.902960102

Williams, G. P., Schonhoff, A. M., Jurkuvenaite, A., Thome, A. D., Standaert, D. G., & Harms, A. S. (2018). Targeting of the class II transactivator attenuates inflammation and neurodegeneration in an alpha-synuclein model of Parkinson's disease. *J Neuroinflammation, 15*(1), 244. doi:10.1186/s12974-018-1286-2

Wissemann, W. T., Hill-Burns, E. M., Zabetian, C. P., Factor, S. A., Patsopoulos, N., Hoglund, B., . . . Payami, H. (2013). Association of Parkinson disease with

structural and regulatory variants in the HLA region. *Am J Hum Genet, 93*(5), 984-993. doi:10.1016/j.ajhg.2013.10.009

Wurst, W., Auerbach, A. B., & Joyner, A. L. (1994). Multiple developmental defects in Engrailed-1 mutant mice: an early mid-hindbrain deletion and patterning defects in forelimbs and sternum. *Development, 120*(7), 2065-2075.

Yang, F., Pedersen, N. L., Ye, W., Liu, Z., Norberg, M., Forsgren, L., . . . Wirdefeldt, K. (2017). Moist smokeless tobacco (Snus) use and risk of Parkinson's disease. *Int J Epidemiol, 46*(3), 872-880. doi:10.1093/ije/dyw294

Yonova-Doing, E., Atadzhanov, M., Quadri, M., Kelly, P., Shawa, N., Musonda, S. T., . . . Bonifati, V. (2012). Analysis of LRRK2, SNCA, Parkin, PINK1, and DJ-1 in Zambian patients with Parkinson's disease. *Parkinsonism Relat Disord, 18*(5), 567-571. doi:10.1016/j.parkreldis.2012.02.018

Zimprich, A., Benet-Pages, A., Struhal, W., Graf, E., Eck, S. H., Offman, M. N., . . . Strom, T. M. (2011). A mutation in VPS35, encoding a subunit of the retromer complex, causes late-onset Parkinson disease. *Am J Hum Genet, 89*(1), 168-175. doi:10.1016/j.ajhg.2011.06.008

Zimprich, A., Biskup, S., Leitner, P., Lichtner, P., Farrer, M., Lincoln, S., . . . Gasser, T. (2004). Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron, 44*(4), 601-607. doi:10.1016/j.neuron.2004.11.005

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