From the Department of Medical Epidemiology and Biostatistics
Karolinska Institutet, Stockholm, Sweden

PARKINSON'S DISEASE ETIOLOGY – BEYOND THE BRAIN AND LATE ADULTHOOD

Bojing Liu

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Parkinson's disease etiology – beyond the brain and late adulthood

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Bojing Liu

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Principal Supervisor:
Docent Karin Wirdefeldt
Karolinska Institutet
Department of Medical Epidemiology and Biostatistics

Co-supervisor(s):
Professor Honglei Chen
Michigan State University
Department of Epidemiology and Biostatistics

Docent Fang Fang
Karolinska Institutet
Department of Medical Epidemiology and Biostatistics

Opponent:
Associate Professor Per Borghammer
Aarhus University
Department of Clinical Medicine

Examination Board:
Docent Shahram Bahmanyar
Karolinska Institutet
Department of Medicine

Docent Karin Modig
Karolinska Institutet
Institute of Environmental Medicine

Docent Gesine Paul-Visse
Lund University
Department of Clinical Science, Wallenberg Neuroscience Center

ABSTRACT

Despite much effort investigating the etiology of Parkinson's disease (PD), the causes and the exact mechanisms underlying the disease remain elusive. Braak's hypothesis suggests that PD pathology may start in the enteric nervous system and later spread to the brain via the vagus nerve. This hypothesis is further extended to the dual-hit hypothesis suggesting that environmental neurotropic pathogens may contribute to PD development through nasal and gut gateways that are in direct connection with each other via inhalation and ingestion. Mounting evidence also suggests the importance of neuroinflammation in the pathogenesis of PD. In this thesis, I aimed to explore the etiology of PD focusing on developmental origins related to early infection and inflammation, gastrointestinal aspects, and olfactory function using various register and population based datasets.

In **Study I**, we conducted a cohort study to examine developmental aspects of PD regarding early life infection, parental age at birth, multiple birth. We considered birth order, sibship size, birth seasonality, and flu activity in the year of birth as surrogates for early infection and inflammation. Overall, we found that early life characteristics were not associated with future risk of PD, indicating little support for the importance of early life aspects in PD etiology.

In **Study II**, we evaluated vagotomy and its subtypes (truncal and selective vagotomies) in relation to PD risk in a matched cohort. We found that truncal vagotomy, with the nerve trunk fully resected, appeared to be associated with a decreased risk of PD more than five years after the surgery, while selective vagotomy was not associated with the risk of PD. The results provide preliminary evidence supporting Braak's hypothesis.

In **Study III**, we conducted a nested case-control study in the Swedish total population and a cohort study in Swedish twins to investigate irritable bowel syndrome (IBS) diagnosis as well as IBS based on self-reported symptoms in relation to the risk of PD. The results demonstrated that IBS was linked to an elevated risk of PD. The findings add additional evidence suggesting the importance of gut-brain-axis in PD development.

In **Study IV**, we examined whether poor olfaction is associated with long-term mortality and potential explanations for such association among older adults in a community-based cohort. We found that poor olfaction was associated with higher long-term mortality and that part of the association was explained by neurodegenerative diseases, in particular, PD and dementia, and body weight loss.

In summary, by taking advantage of Swedish nationwide registers, we provide some evidence supporting Braak's hypothesis and the importance of the gut-to-brain axis in PD development. Our data, however, do not support the importance of developmental origins of PD. Additionally, we confirmed the association between poor olfaction and mortality in healthy older adults from the Health, Aging and Body Composition study and identified PD or dementia and body weight loss as part of the potential mechanisms underlying the association.

LIST OF SCIENTIFIC PAPERS

I. **Liu B**, Chen H, Fang F, Tillander A,Wirdefeldt K. Early-Life Factors and Risk of Parkinson's Disease: A Register-Based Cohort Study. PLoS ONE 2016;11(4)

II. **Liu B,** Fang F, Pedersen NL, Tillander A, Ludvigsson JF, Ekbom A, Svenningsson P, Chen H, Wirdefeldt K. Vagotomy and Parkinson disease: a Swedish register-based matched-cohort study. Neurology 2017; 88: 1996-2002.

III. **Liu B**, Sjölander A, Pedersen NL, Ludvigsson JF, Chen H, Fang F, Wirdefeldt K. Irritable Bowel Syndrome and Parkinson's Disease Risk: A Swedish Register-based Study (Manuscript)

IV. **Liu B**, Luo Z, Pinto JM, Shiroma EJ, Tranah GJ, Wirdefeldt K, Fang F, Harris TB, Chen H. Poor olfaction predicts long-term mortality among older adults in a community-based cohort (Submitted)

CONTENTS

LIST OF ABBREVIATIONS

AD	Alzheimer's disease
BSIT	Brief Smell Identification Test
CDR	Cause of Death Register
CI	Confidence interval
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
DAG	Directed acyclic graph
ENS	Enteric nervous system
GI	Gastrointestinal
GWAS	Genome-wide association studies
Health ABC	Health, Aging and Body Composition
HR	Hazard ratio
HP	Helicobacter pylori
IBD	Inflammatoty bowel disease
IBS	Irritable bowel syndrome
ICD	International Classification of Diseases
IL	Interleukin
MGR	Multi-Generation Register
MDS	International Parkinson and Movement Disorder Society
NPR	National Patient Register
NSAID	Nonsteroidal Anti-inflammatory Drug
OR	Odds Ratio
PD	Parkinson's disease
PPV	Positive predictive value
SNpc	Substantia nigra pars compacta
RBD	Rapid eye movement sleep behavioral disorder
SD	Standard deviation
SALT	Screening Across the Lifespan of Twins Study
STR	Swedish Twin Registry
TPR	Swedish Total Population Register
TNF-α	Tumor necrosis factor α

1 BACKGROUND

Two hundred years ago, London doctor James Parkinson first described "paralysis agitans" in his work "An Essay on the shaking Palsy" (1871) [1]. The disease was later renamed as Parkinson's disease (PD). PD is the second most common aging-related neurodegenerative disorder after Alzheimer's disease. Due to selective loss of dopaminergic neurons in the *substantia nigra pars compacta*, PD is characterized by cardinal motor symptoms of tremor, bradykinesia, rigidity, and postural instability [2]. PD also has a long prodromal phase, with non-motor symptoms of constipation, olfactory loss, psychiatric symptoms, and rapid eye movement sleep behavior disorders occurring up to several decades before the onset of motor symptoms [3, 4]. Despite a 200-year effort to understand PD etiology, the exact causes and underlying mechanisms of the disease remain unclear. Genetic factors only account for 5-10% of cases [5], while the majority of PD cases are sporadic – indicating the importance of environmental components in PD etiology. Historically, research on PD etiology has focused on the brain and the period of late adulthood. In the past 15 years, new insights have emerged regarding the concept that PD pathology may originate in peripheral organs during the long prodromal phase and spread to the brain via nasal and gut routes [6, 7]. In this thesis, different epidemiological approaches were used to explore the etiology of PD beyond the brain and late adulthood, with special focus on early life infection or inflammation, gastrointestinal (GI) aspects, and olfaction.

1.1 PARKINSON'S DISEASE (PD) CLINICAL FEATURES

1.1.1 Motor symptoms

Motor manifestations of PD only present after about 60% of dopaminergic neurons have died [2]. Cardinal motor symptoms include 1) tremor (both rest and action tremor), 2) rigidity, 3) bradykinesia (i.e. slowness of voluntary movements), and 4) postural instability (often occurs late in the disease) [8]. Moreover, asymmetry of motor onset is common in most PD patients [8].

1.1.2 Non-motor symptoms

Non-motor symptoms may occur in both clinical and preclinical stages (i.e. prior to the motor onset) of PD [3, 4]. A meta-analysis demonstrates that prevalence of hyposmia is 75.5%, constipation 50%, anxiety 39.9%, rapid eye movement sleep behavior disorders (RBD) 37%, depression 36.6%, and daytime sleepiness 33.9% in PD patients [3, 4]. Strong evidence suggests that constipation, olfactory deficit, RBD, and depression can occur many years before PD diagnosis [3, 4, 9]. Non-motor symptoms that arise in the preclinical stage of PD are commonly referred to as prodromal non-motor or premotor symptoms. Selected PD premotor symptoms

and the corresponding time periods preceding PD diagnosis have been summarized for constipation (up to 20 years), depression (within 5 years), hyposmia (within 4 years), RBD (up to 10-29 years), anxiety (could be > 20 years), and daytime sleepiness (up to 10 years) [9]. Moreover, lower heart rate variability has also been recently linked to an increased PD risk indicating cardiac dysautonomia in the prodromal stage of PD[10].

1.1.3 Clinical diagnosis criteria

At present, reliable biomarkers and specific neuroimaging techniques are not yet available for the clinical diagnosis of PD. Instead, PD diagnosis is generally made upon cardinal motor symptoms and signs, duration of the symptoms, exclusion of alternative diagnoses, and response to antiparkinsonian drugs. Several sets of clinical diagnostic criteria for PD have been proposed: the UK Parkinson's Disease Society Brain Bank criteria include postural instability not caused by other differential diagnosis[11], while Gelb et al. emphasize the asymmetric onset and exclusion of postural impairment [8]. In 2015, the International Parkinson and Movement Disorder Society (MDS) developed clinical diagnostic criteria for PD (MDS-PD criteria) based on elements from the UK Brain Bank Criteria and omitting features that are no longer justified [12]. With increasing recognition of non-motor symptoms in PD, the MDS-PD criteria also began to incorporate non-motor manifestations. It is worth to note that PD diagnoses given by experienced clinicians have been proven to be more accurate than those based on clinical criteria[13]. The goal of the MDS-PD criteria is to facilitate less experienced physicians to assign PD diagnoses as well as utilization in medical research [12].

In general, the MDS-PD criteria classify PD into two certainty levels (i.e. *clinically established PD* and *probable PD*) in two steps: first, to define a parkinsonism, and second, to evaluate whether the parkinsonism is attributed to PD according to supportive, exclusion, and red flag criteria[12]. For illustration, the MDS-PD criteria are summarized below and detailed information on supportive, exclusion, and red flag criteria have been presented previously [12].

Step 1: Define parkinsonism according to bradykinesia, in combination with at least either rest tremor or rigidity

Step 2: Define PD diagnosis into certainty levels based on supportive, exclusion, and red flag criteria.

Clinically Established PD: absence of absolute exclusion criteria; at least two supportive criteria; no red flags

Clinically Probable PD: absence of absolute exclusion criteria; presence of red flags counterbalanced by supportive criteria (i.e. one red flag must be compensated by least one supportive criterion and no more than two red flags are allowed)

1.2 PATHOGENESIS AND PATHOLOGICAL HYPOTHESES

The pathological marks of PD are essentially the selective neurodegeneration of dopaminergic neurons in the *substantia nigra pars compacta* (SNpc) and α-synuclein enriched Lewy bodies and Lewy neurites[14]. A variety of biological processes including increased oxidative stress, mitochondrial dysfunction, impairments in the ubiquitin-proteasome system, and neuroinflammation can also contribute to PD pathogenesis [14-16]. Normal α-synuclein is a soluble small protein mostly located in axons and their presynaptic terminals, and usually binds to membranes of synaptic vesicles [17]. Under certain conditions, α-synuclein loses membrane-attachment and begins to self-aggregate together with some additional proteins, forming spherical and thread-like Lewy bodies or Lewy neurites [17].

1.2.1 Braak's staging system and Braak's hypothesis (dual-hit hypothesis)

Braak and colleagues proposed a staging system that explains the process of pathological development in some PD patients based on autopsy [17, 18]. The staging system suggests that the Lewy pathology may originate in the peripheral enteric nervous system (ENS) and the olfactory bulb, and later spreads to the central nervous system (CNS) following six stages [17, 18]:

Stage 1: lesions in ENS, the dorsal motor nucleus and/or adjoining reticular zone, olfactory bulb and related portions of anterior olfactory nucleus (medulla oblongata and olfactory system)

Stage 2: lesions in caudal raphe nuclei, gigantocellular reticular nucleus, and coeruleus-subcoeruleus complex (medulla oblongata and pontine tegmentum)

Stage 3: lesions in substantia nigra pars compacta (midbrain)

Stage 4: lesions in anteromedial temporal mesocortex (basal prosencephalon and mesocortex)

Stage 5: lesions in high order sensory association areas of the neocortex and prefrontal neocortex (neocortex)

Stage 6: lesions in first order sensory association areas of the neocortex, premotor area, and primary sensory area and motor field (neocortex)

Based on the staging system, Braak and colleagues hypothesized that an unknown pathology may originate in the gut and later enter the ENS and migrate to the CNS via the vagus nerve [19]. Braak's hypothesis was further extended to the dual-hit hypothesis stating that neurotropic pathogens may enter the brain via two gateways: the nose and gut, which are in close contact with the outer environment and in direct connection with each other via inhalation and ingestion[6, 7]; and PD pathology may spread from the olfactory bulb to other parts of the brain and/or from the gut to the brain via the vagus nerve [6, 7]. In line with stage I lesions, epidemiological studies consistently reported that constipation [20, 21] and olfactory dysfunction [22, 23] are common prodromal symptoms occurring prior to PD motor onset [4]. Pathological aggregation of α-synuclein was also found in different GI sites up 20 years before the PD diagnosis [24]. Furthermore, the spread of α-synuclein was also supported by the finding in mice that aggregated α-synuclein was transferred from the olfactory bulb to other interconnected parts of brain [25]. Moreover, coeruleus and subcoeruleus complex that are affected in stage II have been suggested to be involved in mood and sleep regulation, therefore in line with the presence of depression and RBD in the prodromal stage of PD [26]. Criticisms have also been raised, partly because not all PD cases follow the system. However, there is an increasing body of evidence supporting Braak's hypothesis in, at least, some PD patients [27]. Another key point of the hypothesis is that PD-pathology migrates from the gut to the brain via the vagus nerve [7, 19]. We attempted to explore the association between vagotomy, a surgical division of the vagus nerve, and risk of PD in **Study II**. And in **Study IV**, we examined olfactory dysfunction in relation to mortality and evaluated whether this association can be explained by neurodegenerative diseases including PD.

1.2.2 Gut-brain-microbiota axis

The gut-brain axis involves bidirectional neural communication between the ENS and CNS through the vagal nerve [28]. Microbiota regulate the gut-brain axis through immunological, neuroendocrine, and direct neural mechanisms [29]. For instance, lipopolysaccharide synthesized by pathogenic bacteria can induce innate immune system activation; gut bacteria can synthesize human neurotransmitters (e.g. γ-aminobutyric acid, serotonin, and dopamine) and stimulate afferent neurons in the ENS to send signals to the CNS through the vagus nerve [30].

The role of microbiota in modulating gut-to-brain interactions has been investigated in the etiology of PD [29]. There is an increasing recognition of gut microbiota disturbances in PD patients [31-33]. Although, the causal relationship between microbial alterations and PD remains unclear, it has been hypothesized that chronic inflammation induced by gut microbiota and

increased intestinal permeability may lead to systemic and neuro-inflammation, which in turn promote neurodegeneration in PD [30 34]. The proposed involvement of the gut-brain-microbiota axis in PD etiology is in line with early GI dysfunction such as constipation. Interestingly, dysfunction of the axis has also been linked to GI diseases, such as inflammatory bowel disease (IBD)[35] and irritable bowel syndrome (IBS) [36]. It is possible that certain pathological changes in the gut-brain-microbiota axis may underlie both GI diseases and PD. In **Study III,** we explored the association between IBS and subsequent PD risk.

1.2.3 Neuroinflammation and neurodegeneration

Mounting evidence suggests the important role of neuroinflammation in PD neurodegeneration [16]. Dopaminergic neurons undergoing cell death can trigger inflammatory reactions such as microglia activation, astrogliosis, and lymphocyte recruitment, which contribute to a harmful inflammatory loop that drives neuron death further [16]. Such a vicious circle may perpetuate neurodegeneration in PD [16]. McGeer and colleagues first observed microglia activation in the *substantia nigra* of PD patients post-mortem [37]. Clinical studies additionally found increased levels of circulating interleukin (IL)-1β, IL-2, IL-6, high sensitive C-reactive protein, and tumor necrosis factor α (TNF-α) in serum and cerebrospinal fluid of PD patients [38-41]. Findings from animal models indicate that systemic lipopolysaccharide and TNF-α injection in mice can activate microglia and induce delayed and progressive loss of dopaminergic neurons [42]. Moreover, mutations in PD-associated genes such as LRRK2 and HLA-DR have been related to immune functions [43]. Epidemiological evidence has linked nonsteroidal anti-inflammatory drugs (NSAIDs) use, especially ibuprofen, to a 35-45% reduced PD risk [44]. Although convergent evidence supports the involvement of inflammation in PD pathogenesis, the origin of the inflammatory process and its role in PD etiology remains unclear. In **Study I**, we attempted to explore the associations between factors related to early life inflammation or infection and the risk of PD in late adulthood.

1.3 EPIDEMIOLOGY

1.3.1 Incidence, prevalence, and mortality

Incidence of PD varies from 1.5 to 22 per 100 000 person-years for all age groups [45] and estimates may differ by demographic characteristics of the study population, diagnostic criteria, and case-identification source [45]. PD incidence is highly associated with age; disease onset before age 40 is very rare but incidence increases rapidly after the age of 60 [45]. PD incidence in men is about 50% higher than in women [45, 46]. A meta-analysis has reported

pooled incidence (per 100 000 person-years) by age and sex based on 14 studies conducted in 2001-2014 (Table 1.3.1) [46]. *Prevalence* of PD also rises with age due to the increased incidence. Another meta-analysis including 47 studies summarized worldwide PD prevalence (per 100 000 persons) by sex and age group (Table 1.3.1) [47]. Men have twice the prevalence of PD than women, but the difference become less prominent in older age groups [47].

Mortality is approximately doubled in PD patients compared to the general population [45]. Factors regarding disease severity, dementia, and age of onset can influence the mortality, and pneumonia is the most common cause of death in PD patients [45]. Demented PD patients have higher mortality rates than non-demented PD patients, with the age- and sex-specific standardized mortality rate ratios being 3.10 and 1.15, respectively [48]. Life expectancy decreases in nearly all age groups with PD, and particularly in patients with early age at onset. Life expectancy of PD patients vs. the general population was 38 vs. 49 years at age of onset 29-39, 21 vs. 31 years at age of onset 40-64, and 5 vs. 9 years at age of onset ≥65 [49]. Furthermore, adverse outcomes due to weight loss and malnutrition have been increasingly recognized in PD patients and linked to deleterious quality of life, disability, and mortality [50, 51].

Table 1.3.1 Incidence and prevalence of PD reported from two recent meta-analyses[46 47]

Age group	**Pooled incidence (per 100, 000 person years)**		**Pooled prevalence (per 100, 000 persons)**	
	Men	Women	Men	Women
40-49	3.57	3.26	36	45
50-59	14.67	8.43	134	41
60-69	58.22	30.32	389	392
70-79	168.58	93.32	932	813
80+	258.47	103.45	2101	1517

1.3.2 Genetic and selected non-genetic factors in PD etiology

Genetic components of PD have been estimated using linkage analysis, genome-wide association studies (GWAS), and family-based studies. Although cross-sectional twin studies suggested no heritability for PD [52, 53], a Swedish twin study using longitudinal information has estimated PD heritability at 34% [54]. Having a first degree relative or any relative with PD has been associated with higher PD risk [55], indicating influence of both genetics and shared environment. Six monogenic forms of PD have been identified with alpha synuclein, Parkin, PINK1, and DJ-1 being related to early-onset PD, and LRRK2 and VPS35 being related to late-onset PD [5]. GWAS have identified 26 genomic loci related to late-onset sporadic PD [56]. However, evidence jointly suggests that genetics only explain 5-10% of PD cases [5], prompting the importance of environmental influence in PD etiology.

Strong evidence suggests a lower PD risk for smoking and caffeine intake and higher PD risk for exposure to pesticide. A large meta-analysis has estimated relative risk of PD comparing ever- versus never-smokers at 0.64 (95% CI=0.60-0.69) [55]. Similarly, a lower relative risk of PD for caffeine intake has been estimated at 0.67 (95% CI=0.58-0.76) [55]. History of pesticide use has been linked to a higher relative risk of PD equal to 1.78 (95% CI=1.50-2.10) [55]. Several other non-genetic factors have also been studied but evidence is limited and inconclusive (Table 1.3.2).

Table 1.3.2 Summary of selected non-genetic factors related to PD risk

Positive association	Inverse association
Pesticides[55]	Smoking[55]
Farming/agriculture[55]	Caffeine[55]
Rural living [55]	Alcohol [55, 61]
Head injury[57, 58]	Physical activity [62]
Anti-hypertensive drugs[55, 59]	NSAIDs [55, 63]
Well water [55]	Hypertension [55]
Rosacea [60]	

1.4 NON-GENETIC FACTORS IN PD ETIOLOGY STUDIED IN THE THESIS

1.4.1 Sibling structure, birth season and month, influenza activity in the year of birth, and parental age

According to the *developmental origin of disease* hypothesis, environmental exposures such as infection occurring during pre- and neo-natal periods may alter brain development and in turn affect the susceptibility to neurodegenerative diseases[64, 65]. Evidence supporting this hypothesis has been reported for the association of birth order, sibship size with Alzheimer's disease (AD) [66], amyotrophic lateral sclerosis [67] and schizophrenia [68]. It is likely that birth order and sibship size are merely surrogates for childhood common infections within the household. The developmental origin could be equally important for PD etiology and yet has been rarely tested in epidemiological studies. Early-life inflammatory triggers, such as infections, may result in continuing changes of cytokine production, number and function of microglial cells [69]. Animal models have shown that mice exposed to lipopolysaccharide *in utero* were born with fewer dopamine producing neurons [70]. Two epidemiological studies reported no association between birth order, sibship size, and PD risk [71, 72], but results had large statistical uncertainty. Although controversial[73-75], season and year of birth, as indicators of intrauterine infection, revealed a peak of PD in births between March to June and births clustering in influenza pandemic years [76], while another study reported a moderate but non-significant increased PD risk among those born in summer versus spring seasons [72]. Moreover, older parental age at birth might relate to future disease susceptibilities due to the higher mutation rate and oxidative stress in germ cells [77, 78]. Taken together, the potential role

of early life factors and a related developmental origin of PD has not been adequately investigated.

1.4.2 Vagotomy

One important aspect of Braak's hypothesis is that PD pathology may spread from the gut to the brain via the vagus nerve. Supporting evidence has been reported from animal models: in mice, intragastrical rotenone administration induced α-synuclein accumulation in the ENS and subsequent PD-like pathology in the vagal dorsal motor nuclei, spinal cord, and SNpc[79], while resection of the vagal nerves before rotenone treatment delayed the spread of PD-like pathology [80]. Vagotomy was the gold standard surgical treatment for peptic ulcer to decrease gastric acid secretion before the recognition of *Helicobacter pylori* [81]. Nowadays, the indication for vagotomy has been shifted to complications due to peptic ulcer [81]. There are three types of vagotomy: truncal, selective, and highly selective (Fig 1.4.2) [81]. In truncal vagotomy, resection of nerve trunks is performed above the celiac and hepatic branches, while selective vagotomy spares the celiac and hepatic braches and highly selective vagotomy only eradicates innervation to the acid and pepsin-producing cells [81]. According to Braak's hypothesis, if aggregated α-synuclein migrates from the gut to the brain via the vagus nerve, it is conceivable that truncal vagotomy may reduce PD risk, while selective and highly selective vagotomy may have a smaller or null effect. A Danish register-based study reported a lower risk for PD among individuals with truncal vagotomy more than 20 years after the surgery [82]. The results however could not be replicated in a re-analysis of the same study population [83]. Hence, further investigation on vagotomy and PD risk is warranted.

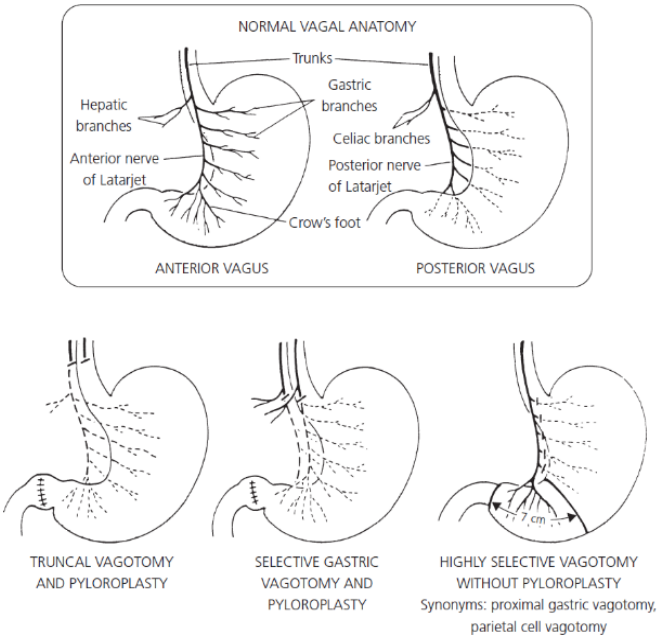


Fig 1.4.2. Illustration of normal vagal anatomy and types of vagotomy (Reprinted from Glasgow and Mulvihill, with permission from Wiley) [81]

1.4.3 Irritable bowel syndrome (IBS)

IBS is a common disorder characterized by a group of symptoms consisting of recurrent abdominal pain, discomfort, and alterations of bowel habits [36, 84]. According to prevailing stool patterns, IBS is classified into three subtypes: IBS-diarrhea, IBS-constipation, and IBS-mixed with both symptoms or neither[85]. Mechanisms related to IBS development involve genetic factors, post-infectious changes in the gut, brain-gut interaction, activation of the immune system, alterations in the gut microbiome, increased intestinal permeability, altered gut motility, visceral sensation, or bile salt metabolism [36, 84]. IBS and PD may have certain shared symptoms and etiology including constipation, dysregulation of gut-brain axis, disturbance of gut microbiota and related intestinal inflammation, and increased intestinal permeability [34, 36]. Evidence on IBS and subsequent PD risk is sparse. A cohort study based on data from the Taiwan National Health Insurance program reported a 48% higher risk of PD for IBS patients compared to individuals without IBS [86]. Several limitations may exist in this study, including a limited follow-up period, a possible under-diagnosis of IBS, potential confounding by life style factors (e.g. smoking and alcohol consumption), and surveillance bias.

1.4.4 Olfaction

Olfaction impairment is common in older adults; the prevalence was estimated at 20-30% among people over 50 years and 60% among those over 80 years[87-89]. Poor sense of smell has a deleterious impact on safety [90], nutrition [91], and quality of life [92]. Converging lines of evidence also suggest that impaired olfaction in particular predicts PD, AD, dementia, and cognitive decline[9, 93, 94]. Several studies have linked olfactory dysfunction to higher mortality [95-100], however these studies have mainly focused on all-cause mortality and had a limited follow-up period. Further, little is known about mechanisms underlying the association between olfaction and higher mortality among older adults. Although neurodegenerative diseases [95-98, 100], poor nutrition [96-98], food or gas poisoning [97, 98], fire or hazardous environment [101] have been hypothesized to affect mortality in individuals with olfactory dysfunction, little empirical data on this research area exists.

2 AIMS

In this thesis, we aim to first, explore PD etiology based on Braak's hypothesis, gut-to-brain-microbiota axis, and neuroinflammatory mechanisms; and second, examine whether poor olfaction is related to mortality and explore the potential explanations including neurodegeneration due to PD.

Specific objectives include:

I. To study sibling structure, birth season and month, influenza activities in the year of birth, as proxies for early infection and inflammation, and parental age in relation to PD risk

II. To examine the association between vagotomy and risk of PD

III. To examine the association between IBS and risk of PD

IV. To examine olfactory impairment is associated with long term all-cause and cause-specific mortalities; second, what are the potential explanations for the association between olfactory function and death.

3 DATA SOURCES AND MEASUREMENTS

3.1 SWEDISH NATIONAL HEALTH AND POPULATION REGISTERS (STUDIES I-III)

Studies I-III were conducted based on linkage of multiple Swedish national health and population registers via the unique personal identity number available for all residents in Sweden.

The *Swedish Total Population Register (TPR)* was established in 1968 and is maintained by Statistics Sweden. It contains information on, for instance, birth, death, marital status, family relationships, and dates of immigration (from 1969) and emigration (from 1961) [102].

The *Swedish Multi-Generation Register (MGR)* is a part of the TPR and holds information on biological and adoptive parents for individuals (index persons) who were born in 1932 or later and lived in Sweden in 1961[102, 103]. The MGR has 97% coverage of all mothers and 95% coverage of all fathers for Swedish born index persons [103]. The corresponding coverage for foreign-born residents is 27% and 22%, respectively [103]. The register provides a unique resource to study the influence of family structure on diseases and conditions [103]. In **Study I**, we retrieved information on sibling structure and parental age from the MGR.

The S*wedish Population and Housing Censuses* were conducted by Statistics Sweden every five years from 1960 to 1990 to collect information on housing, civil status, educational attainment, income, occupation, and social class [104]. **Studies II** and **III** were based on the censuses targeting the entire Swedish population in 1970 and 1980, respectively.

The *Swedish Twin Registry (STR)* was established in the late 1950s. It is a nationwide population-based register including more than 194,000 twins who were born between 1886 and 2008 [105, 106]. Data collection has been done through several waves of questionnaires [105, 106] and linkage to different national health and population registers [105, 106]. In the *Screening Across the Lifespan of Twins Study* (SALT), telephone interviews were carried out in 1998-2002 with twins born before 1958 [105, 106] to collect information on a broad spectrum of diseases and conditions, medication use, life style factors (e.g. smoking, alcohol consumption, and diet), occupation, and education [105, 106]. In **Study III**, we obtained information on abdominal symptoms related to IBS and other covariates from SALT**.**

The *Swedish National Patient Register (NPR)* was established in 1964 to collect information on medical diagnoses and dates of admission and discharge of hospitalization. Data collection started in six counties of Sweden and the NPR became nationwide in 1987 [107]. Psychiatric

care was included in 1973[107]. Surgical day-care procedures were included in 1997 and >80% outpatient specialist care is covered since 2001. Although private caregivers are not included, the NPR covers nearly 100% of outpatient records from public caregivers [107]. Primary care is currently not included in the NPR[107]. We identified PD diagnoses (**Studies I-III**), vagotomy (**Study II**), IBS diagnoses (**Study III**), and comorbidities (**Study II and III**) from the NPR.

The *Cause of Death Register (CDR)* was established to collect nationwide information on deaths since 1952 and became digitalized in 1961[108]. Coverage is virtually complete for all deaths since 1952 and records information on date of death, underlying and contributing causes of death, place of death, and death abroad [108].

3.2 THE HEALTH, AGING, AND BODY COMPOSITION STUDY (STUDY IV)

The Health, Aging and Body Composition (Health ABC) study is an interdisciplinary study designed to investigate risk factors of health outcomes among healthier older adults[109]. The study recruited 3,075 well-functioning community-dwelling older adults (48.4% men and 41.6% blacks) aged 70-79 years during 1997-1998 in Pittsburgh, Pennsylvania, and Memphis, Tennessee[109]. Eligibility criteria were 1) no difficulty to walk a quarter mile or climb ten steps, or to perform activities of daily living, 2) no history of life-threatening cancer in the past three years, and 3) no plan to move out of the area in the next three years [109]. Participants in the Health ABC study were followed with annual clinical or home visits through year 6 and then in year 8, 10, 11, and 16 [109]. Semi-annually telephone interviews were performed to update health status between enrollment and year 15 and then quarterly through year 17[109]. Data collection and follow-up were completed in 2014 [109]. **Study IV** was based on data retrieved from the Health ABC Study.

3.3 OUTCOME ASCERTAINMENT

3.3.1 PD ascertainment (Studies I-III)

PD risk is the primary outcome in **Studies I, II, and III**. We identified PD cases from the NPR based on primary and secondary diagnoses at hospital discharge or outpatient visit using the Swedish revision of the International Classification of Diseases (ICD) codes (ICD-7: 350 in 1964-1968; ICD-8: 342 in 1969-1986; ICD-9: 332.0 in 1987-1996; and ICD-10: G20 from 1997 onward). Date of PD identification was defined as date of first hospital admission or outpatient contact. In **Study IV**, we examined PD as a potential mediator of the association between olfactory impairment and all-cause mortality. PD diagnoses were adjudicated by two

movement disorder specialists in agreement after comprehensive reviews of self-reported PD diagnoses, medication use, hospitalization surveillance, and cause of death [23].

3.3.2 All-cause and cause-specific mortality (Study IV)

In **Study IV,** we examined the association between olfaction and all-cause as well as cause-specific mortality. Investigators from the Health ABC study closely recorded participants' survival through comprehensive hospitalization records and death certificates. For each death, a knowledgeable proxy was interviewed to obtain detailed information on the death event and physical functioning of the study participant while alive. A team of medical experts reviewed information from interviews, hospitalization records, death certificates, and autopsy data and adjudicated the underlying cause of death by consensus. We examined all-cause mortality and underlying causes of death due to 1) dementia or PD; 2) cardiovascular diseases; 3) cancer; and 4) respiratory diseases.

3.4 EXPOSURE AND OTHER VARIABLE ASCERTAINMENT

3.4.1 Sibling structure, birth season and month, parental age, and influenza activity in the year of birth (Study I)

In **Study I**, we retrieved information on sibling structure, parental age, season and month of birth from the MGR. For each participant, we identified full and maternal half siblings. Exposure variables were subsequently calculated as follows: sibship size (1, 2, 3, or ≥4), number of older/younger siblings (0, 1, 2, or ≥3), birth interval between nearest older/younger siblings (<2, 2–6, or >6 years), parental age at birth (≤20, 21–25, 26–30, 31–35, 36–40, or >40 years), multiple births (yes/no), birth month, and birth season. We obtained historical information about influenza-like illness activities in 1932-1970s from the Swedish Public Health Agency. Since 1911, the Swedish Public Health Agency requested general practitioners in most parts of Sweden to report influenza-like illness every week or month. Annual burden of influenza-like illness was calculated by dividing the number of cases by the corresponding mid-year population size[110] and subsequently categorized into low, intermediate, and high burden using the cut-offs of 500/100,000 and 1,500/100,000 person-years. We considered variables regarding sibling structure, birth season and month, and flu activity in the year of birth as surrogates for early life infection and inflammation.

3.4.2 Vagotomy (Study II)

In **Study II**, We identified vagotomized patients from the NPR using codes from the Swedish Classification of Operations and Major Procedures: 4471-4478, 4411-4416, 4418-4419, 4451, and 4453 during the period 1964-1996, and JDG00, JDG01, JDG10, JDG11, JDG96 and

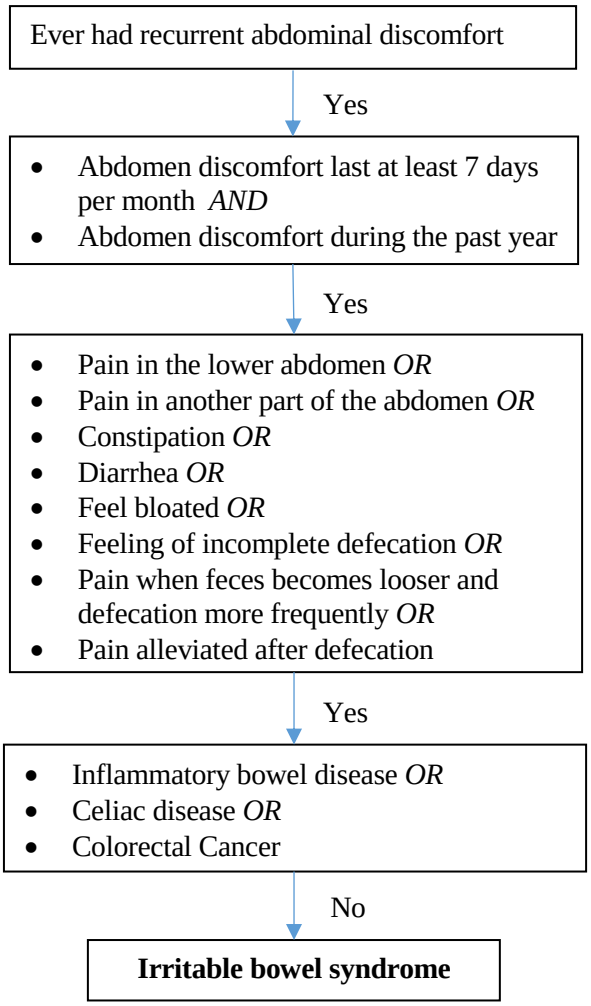
JDG97 from 1997 onward. Following review of the surgical codes by an expert in gastroenterological surgery, we further categorized vagotomy into truncal and selective (i.e. including both selective and highly selective). The date of hospital discharge was defined as the date of the surgery.

3.4.3 IBS (Study III)

In **Study III**, we examined both register-based IBS diagnosis as well as self-reported symptoms based IBS in relation to PD risk. IBS diagnoses were identified from the NPR according to the Swedish revisions of ICD codes (ICD-7: 573.10, 573.21, 573.22 in 1964–1968; ICD-8: 564.10, 564.11, 564.19 in 1969–1986; ICD-9: 564B in 1987–1996; and ICD-10: K58 from 1997 onward). Patients with alternative diagnosis of inflammatory bowel disease, celiac disease, or colorectal cancer in the NPR were excluded.

In SALT, we ascertained IBS patients using a previously reported algorithm based on self-reported abdominal symptoms (Fig 3.4.3) [111]. Similarly, patients with diagnoses of inflammatory bowel disease, celiac disease, or colorectal cancer based on the NPR were excluded.

Fig 3.4.3 Definition of IBS in SALT Swedish twins



3.4.4 Olfaction (Study IV)

In the Health ABC study, sense of smell was evaluated at the year 3 clinical examination in 1999-2000 using the 12-item Brief Smell Identification Test (BSIT) [112]. The BSIT is a simple and cost-effective test for smell identification and has been validated and widely used in clinical and epidemiological settings [112, 113]. Participants scratched and smelled one of twelve odorants common in daily life, and then identified the odor from four alternatives written in a paper booklet [112]. Each correct answer was scored as one point with a total score ranging from 0 to 12 [112]. We further categorized the total BSIT score into poor, moderate, and good

olfactory function using cut-offs of 8, 9-10, 11-12. The cut-offs correspond to the tertiles of BSIT distribution in the sample.

3.4.5 Covariates

In the statistical analyses, we adjusted for several covariates to control for confounding based on evidence from the literature and subject knowledge. We considered age and sex in **Studies I-IV**, family history of PD and parental socioeconomic status in **Study I**; educational attainment in **Study III**; country of birth, comorbidity, and chronic obstructive pulmonary disease (COPD) as a proxy for smoking in **Studies II** and **III;** Osteoarthritis and rheumatologic disease as proxies for use of NSAIDs in **Study II**; smoking and alcohol consumption in **Study III;** demographics (e.g., age, sex, race, and education), anthropometrics (e.g. weight and height), lifestyle factors (e.g., smoking, alcohol drinking, and physical activity), and self-reported health status (i.e., excellent, very good, good, fair, and poor), a list of baseline diseases, and selective biomarkers in **Study IV**.

4 METHODS

4.1 TYPES OF STUDY DESIGN

4.1.1 Standard cohort study (Studies I, III, IV)

In a cohort study, the general goal is to compare incidence, rate, or time to an outcome among sub-cohorts that are made up of people who share a common condition, e.g. exposed and unexposed subgroups[114]. It is a longitudinal design, in which exposed and unexposed cohorts are prospectively followed for the occurrence of an outcome.

In **Study I**, we identified more than 3.5 million individuals in the MGR, who were born between 1932 and 1970 in Sweden, alive, and had no previous diagnosis of PD on January 1, 2002. The cohort was followed until PD diagnosis, death, emigration out of Sweden, or December 31, 2010, whichever occurred first. We compared PD risk among individuals with different sibling structure, birth month and season, and flu activity in the year of birth, and parental age.

In **Study III**, we examined the association between IBS and subsequent PD risk using both register-based and self-reported symptoms based IBS cases. Self-reported IBS in relation to PD risk was explored in a cohort study based on all twins in the STR who responded to the SALT interview during 1998-2002 (N= 44,919). We excluded twins who discontinued participation, or emigrated, or had missing on linkage information or date of emigration (N=61), or had pre-existing PD according to self-report or a diagnosis from NPR (N=96), or had missing on self-reported IBS (N=529). In total, we followed 44,233 twins until date of PD diagnosis, death, emigration out of Sweden, or December 31, 2014, whichever occurred first.

In **study IV**, we elucidated the association between olfactory impairment and all-cause as well as cause-specific mortality. We also investigated possible explanations to the associations. A total of 2,544 participants had their sense of smell measured at the year 3 clinical examination in 1999-2000 (baseline) using the 12-item BSIT[112]. Seven participants with missing BSIT score were excluded leaving 2,537 participants who were followed from baseline until date of death, last alive contact, or September 30, 2014.

4.1.2 Matched cohort study (Study II)

In contrast to randomized trials, a causal effect that one aims to evaluate in observational studies is usually confounded by causes shared by the exposure and outcome[115]. Different methods are available to adjust for confounding, for example standardization, stratification,

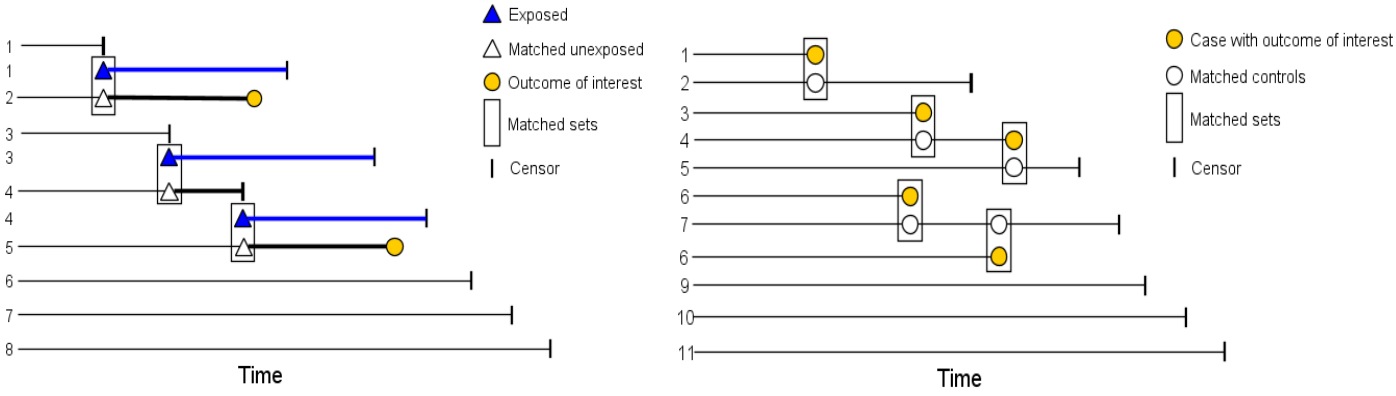
regression modelling, etc. [115, 116]. In the presence of strong confounding, strategies applied to study designs usually achieve higher efficiency compared to the statistical methods mentioned above [116]. One common way is to match by confounders, for instance, age and sex [116]. An example of 1:1 matched cohort design is depicted in Fig 4.1A.

In **Study II**, we conducted a matched cohort study to explore the relationship between vagotomy and PD risk. Among 10,006 vagotomized patients ascertained from the NPR in 1970-2010, 9,430 were included for fulfilling inclusion criteria of being born before 1970, living in Sweden, and free-of-PD before vagotomy. For each vagotomized patient, we randomly selected 40 PD-free reference individuals (from the 1970 census) individually matched on sex and year of birth on the date of vagotomy (index date). To minimize potential bias due to incomplete coverage of the NPR, we further restricted reference individuals to live in the counties covered by the NPR in 1977 according to their residential information in the census. In total, 9,430 vagotomized patients and 377,200 reference individuals were included in the study and followed from the index date to the date of PD diagnosis, death, emigration out of Sweden, or December 31, 2010, whichever occurred first.

Fig 4.1 Example of matched cohort and nested case-control design.

A. 1:1 matched cohort design (follow-up indicated by the bolded lines)

B. 1:1 nested case-cohort design



4.1.3 Nested case-control study (Study III)

In a nested case-control study, the matching approach can also be used as a means of accounting for confounding. In a defined cohort, at each time point of case occurrence, a specified number of controls are selected among individuals who have not developed a certain disease [117]. Key features of the nested case-control design include matching on time; cases and controls matched on confounding factors (e.g. age and sex), a selected control may later become a case, and a control can serve several cases [117]. The nested case-control design

is popular since the sampling allows efficient analysis using a subset of a large cohort with substantial savings in resources and time [118]. An example of 1:1 nested case-control design is depicted in Fig 4.1B.

In **Study III**, we examined the relationship between IBS diagnosis and PD risk in a nested case-control study based on the 1980 census. PD cases were ascertained from the NPR between 1987 and 2010. For each PD case, we randomly selected 30 controls who were alive and living in Sweden, and currently without PD diagnosis, individually matched to the PD case on sex and year of birth on the date of PD diagnosis (index date). In total, 56,564 PD cases and 1,696,920 controls were included and compared regarding history of IBS diagnosis.

4.2 STATISTICAL METHODS

4.2.1 Survival analysis

4.2.1.1 Cox proportional hazard model

An important feature of time-to-event data is censoring. In standard survival analysis, it is important to assume that the censoring is non-informative, meaning that at any time, individuals who are censored have the same survival probability as those who are still at risk and being followed [119]. This assumption is however difficult to test [119]. The Cox proportional hazard model is commonly applied to analyze time-to-event data [120]. Researchers often compare the hazard of a certain disease between two groups using the Cox proportional hazard model to estimate the hazard ratios (HRs). The model does not require any assumptions regarding the shape of underlying hazards, but assume that the ratio of the hazard function to a baseline hazard is constant over time (e.g. hazards of exposed and non-exposed groups are proportional over time) [121]. It has been discussed that in the presence of time-varying effects (i.e. HRs changing over time), presenting period specific HRs may be problematic due to certain selection bias [122]. Alternatively, standardized survival or cumulative failure curves controlling for confounders are suggested to overcome this problem [122]. For example, one can calculate and generate standardized survival curves based on the Cox models using the stdReg R package[123]. Cox proportional hazard models were used in **Studies I-IV**, and, we presented age and sex adjusted cumulative incidence in **Study II** and standardized survival curves in **Studies III** and **IV**.

4.2.1.2 Handling cluster data

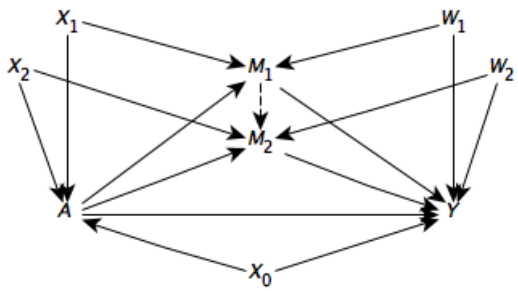
Most statistical regressions assume that observations are independent. This assumption however does not hold in studies including siblings, twins, or other types of correlated data.

Several analytical methods are available to address this issue for different purposes. When performing between-cluster analyses, one can estimate the robust standard error. We estimated the robust standard error in **Studies I** and **III** to account for relatedness in a family or twin pair. On the other hand, conditional effect within clusters can be estimated in matched cohort and nested case-control studies. We used stratified Cox models in **Study II** to estimate HRs and conditional logistic regressions in **Study III** to estimate odds ratios (ORs) conditional on sex and birth year matching pairs.

4.2.2 Mediation analysis

Mediation analyses performed in **Study IV** assumes that the causal directed acyclic graph (DAG) in Figure 4.2.2 depicts the true causal relationships between the exposure, mediators, and outcomes. The exposure was olfactory function categorized into three groups: poor, moderate, and good. The two mediators are baseline prevalent and incident dementia and/or PD (yes/no) (M_1) and on-average yearly body weight loss ≥ 2% (M_2).

Figure 4.2.2. Causal DAG for the Mediation Model where *A* = Olfaction, M_1 = Dementia and/or Parkinson's disease, M_2 = on-average yearly weight loss ≥ 2%, *Y* = all-cause mortality, (X_0, X_1, X_2, W_1, W_2) are confounding factors collectively called *C*.



A sequential approach described by Steen et al.[124] was used to decompose the total effect of olfaction on all-cause mortality into a natural direct effect (i.e. A→Y), an indirect effect through M_1 (i.e. combined paths of A→M_1→Y and A→M_1→M_2→Y), and an indirect effect only mediated by M_2 (i.e. A→M_2→Y). To estimate the direct and indirect effects, we used a weighting approach described by Lange et al.[125] that is suitable for common survival outcomes. Of note, regarding the mediation analysis, we have assumed [126]: 1) no unmeasured exposure-outcome confounders given X_0; 2) no unmeasured mediator-outcome confounders given W_1 and W_2; 3) no unmeasured exposure-mediator confounders given X_1 and X_2; 4) no mediator-outcome confounder W_1 and W_2 affected by A.

5 MAIN RESULTS

5.1 EARLY LIFE CHARACTERISTICS AND RISK OF PD (STUDY I)

In **Study I**, we identified 8,779 PD cases, of which 80% were primary diagnoses, during a follow-up time of 27.1 million person-years. The overall crude incidence rate (per 100,000 years) was 32.4 (39.5 for men and 25.2 for women). Mean age (±SD) at PD diagnosis was 65.1 (±7.6) years. Overall, we did not observed any association between sibling structure and PD risk, except for a slightly decreased PD risk linked to having older siblings (Table 5.1.1). Compared to first-born children, those with at least one older sibling had 7% lower PD risk (HR=0.93, 95% CI: 0.89, 0.98). No association was observed between parental age at birth and the risk of PD (Table 5.1.2). We did not find any clear pattern of PD risk related to month and season of birth or flu activity in the year of birth (Table 5.1.3).

Table 5.1.1 Sibling structure and PD risk

	HR (95% CI)[1]	**Wald test (p-value)**
Sibship size		0.57
1	1	
2	0.97 (0.91-1.04)	
3	0.96 (0.90-1.03)	
≥4	0.95 (0.89-1.02)	
Older siblings		**0.01**
0	1	
1	0.92 (0.87-0.97)	
2	0.98 (0.90-1.06)	
≥3	0.91 (0.82-1.01)	
Birth interval from the nearest elder sibling (years)		**0.02**
No elder siblings	1	
<2	0.91 (0.84-0.98)	
2-6	0.94 (0.89-1.00)	
>6	0.93 (0.84-1.04)	
Younger siblings		0.52
0	1	
1	1.01 (0.96-1.07)	
2	1.02 (0.95-1.09)	
≥3	0.96 (0.90-1.03)	
Birth interval from the nearest younger sibling (years)		0.94
No younger siblings	1	
<2	0.99 (0.92-1.06)	
2-6	1.00 (0.95-1.06)	
>6	1.02 (0.95-1.09)	
Multiple birth		-
No	1	
Yes	0.98 (0.83-1.16)	

1 Adjusting for attained age, sex, birth year category, parental socioeconomic status, and stratified by family PD history

Table 5.1.2 Parental age and PD risk

	HR (95% CI)[1]	Wald test (p-value)
Maternal age category (years)		0.55
≤20	1.01 (0.93-1.11)	
21-25	1	
26-30	1.05 (0.99-1.11)	
31-35	1.04 (0.97-1.11)	
36-40	1.05 (0.97-1.14)	
>40	0.98 (0.85-1.13)	
Paternal age category (years)		0.82
≤20	0.91 (0.76-1.09)	
21-25	1.02 (0.95-1.09)	
26-30	1	
31-35	1.03 (0.97-1.09)	
36-40	1.01 (0.95-1.09)	
>40	1.03 (0.95-1.11)	

1 Adjusted for attained age, sex, birth year category, parental socioeconomic status, and stratified by family PD history.

Table 5.1.3 Birth month, season, and influenza-like illness incidence in the year of birth in relation to PD risk

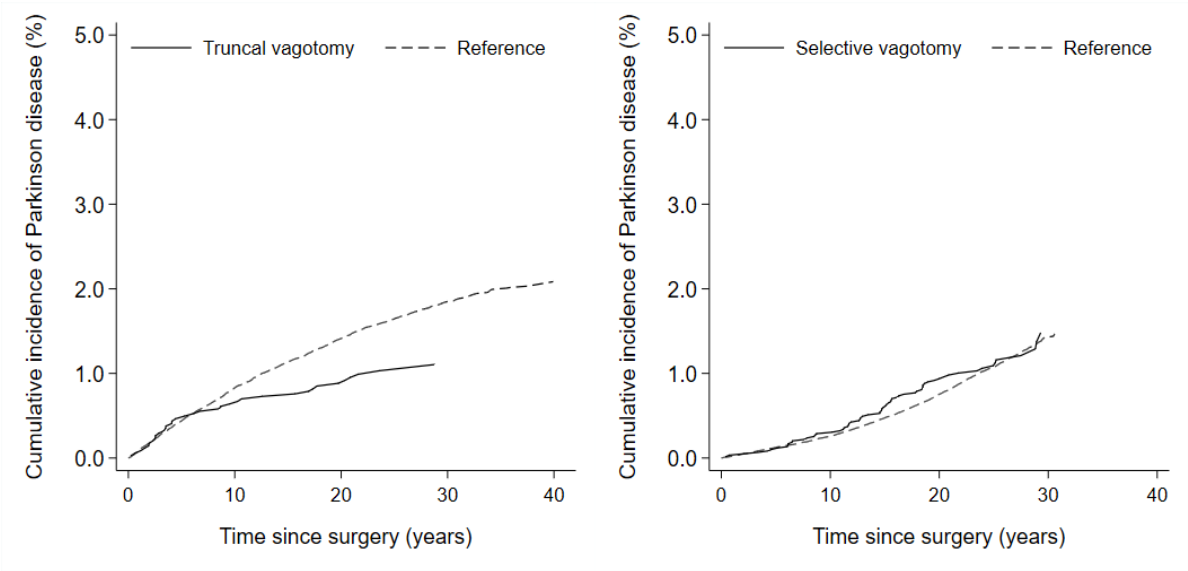
	HR (95% CI)[1]	Wald test (p-value)
Birth month		0.17
JAN	1.05 (0.94-1.18)	
FEB	1.10 (0.99-1.23)	
MAR	1.16 (1.04-1.29)	
APR	1.10 (0.99-1.22)	
MAY	1.02 (0.92-1.14)	
JUN	1.03 (0.92-1.15)	
JUL	1.13 (1.01-1.26)	
AUG	1.14 (1.02-1.27)	
SEP	1	
OCT	1.09 (0.97-1.22)	
NOV	1.05 (0.93-1.18)	
DEC	1.07 (0.96-1.20)	
Season		0.44
Spring (MAR-MAY)	1.05 (0.98-1.12)	
Summer (JUN-AUG)	1.05 (0.98-1.12)	
Autumn (SEP-NOV)	1	
Winter (DEC-FEB)	1.03 (0.97-1.10)	
Influenza-like illness category (cases/100 000 person years)		0.56
Low (<=500)	1	
Intermediate (500-1500)	0.98 (0.93-1.03)	
High (>1500)	1.07 (0.87-1.32)	

1 Adjusted for attained age, sex, birth year category, parental socioeconomic status, and stratified by family PD history.

5.2 VAGOTOMY AND RISK OF PD (STUDY II)

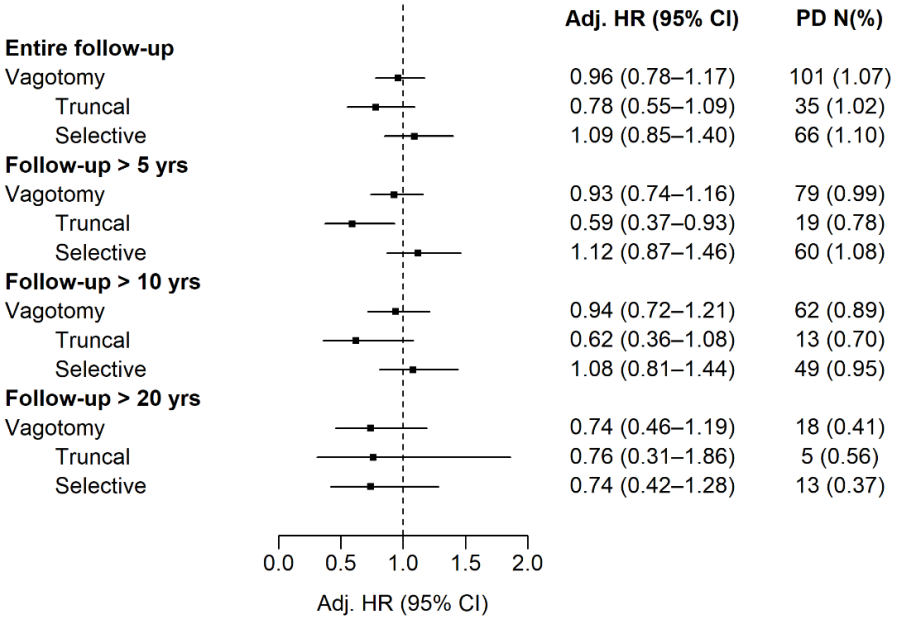
In **Study II**, we compared the risk of PD among 9,430 vagotomized patients (3,445 truncal, 5,978 selective) and 377,200 age and sex matched reference individuals. Mean age (±SD) on the index date (date of vagotomy) was 54.3 (±15.2) years and mean age at PD diagnosis was 75.6 (±8.4) years. We identified 4,930 incident PD cases during 7.3 million person-years of follow-up time. Crude incidence (per 100,000 person-years) of PD was 61.8 for vagotomized patients (80.4 for truncal, and 55.1 for selective) and 67.5 for reference individuals. Fig 5.2.1 depicts cumulative incidence of PD comparing truncal and selective vagotomized patients to their matched non-vagotomized individuals.

Figure 5.2.1 Cumulative incidence of PD comparing vagotomized to the matched non-vagotomized individuals



We observed that vagotomy was not associated with PD risk during the entire follow-up (Figure 5.2.2, HR=0.96, 95% CI=0.78-1.17), although truncal vagotomized patients appeared to have a lower risk than references. When restricted to > 5 year of follow-up, we found a lower risk of PD in truncal vagotomized patients (HR=0.59, 95% CI=0.37-0.93). Similar results were observed >10 and > 20 years after truncal vagotomy, but the risk reduction did not reach the statistical significance. On the other hand, we did not observe any association between selective vagotomy and risk of PD.

Figure 5.2.2 Overall and temporal relationship between vagotomy and risk of PD*



* Models conditional on sex and birth year matching pairs, adjusted for country of birth, chronic obstructive pulmonary, diabetes, vascular diseases, rheumatologic disease, osteoarthritis, and comorbidity index.

5.3 IBS AND RISK OF PD (STUDY III)

In **Study III**, we aimed to explore register-based IBS diagnosis and IBS based on self-reported symptoms in relation to PD risk in two complementary data sources. In the nested case-control study based on the entire Swedish population, we included 56,564 PD cases diagnosed by hospital specialists and 1,691,978 sex and birth year matched controls. IBS was associated with a 43% higher risk of PD during the entire observational time (Table 5.3.1). We also observed a 50% increased PD risk ≥5 years and a 39% increased PD risk more than 10 years after IBS diagnosis. The association appeared to be stronger among IBS patients diagnosed at older (≥ 50 years) than younger age (<50 years), but the difference was not statistically significant (p for interaction=0.11). Stratified analyses showed similar results by sex and age on the index date. Sensitivity analyses yielded attenuated associations when restricting to primary PD diagnosis, or when adjusting for number of hospital visits, or when adjusting for comorbidity with depressive disorders and anxiety (eTable 4 in **Study III** supplemental data).

In the cohort study based on the STR, we followed 2,962 self-reported IBS patients and 41,271 non-IBS persons. During an average of 13.7 years of follow-up, 440 persons developed PD (31 with IBS, 209 without IBS). A similar magnitude of risk increase in PD was noted for self-reported IBS (HR=1.39, 95% CI=0.95-2.03) after adjusting for attained age, sex, educational attainment, history of smoking, alcohol consumption in the last month,

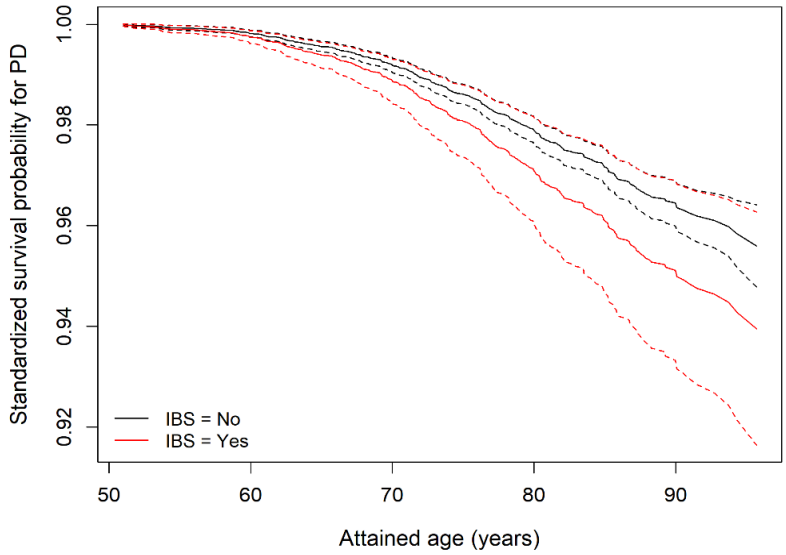
and comorbidity index. Standardized survival curves (over the distribution of covariates) also showed higher PD risk in IBS patients compared to non-IBS individuals (Fig. 5.3.1).

Table 5.3.1 Irritable bowel syndrome diagnosis and risk of PD, nationwide nested case-control analysis

	PD cases N (%)	Controls N (%)	OR (95% CI)[1]
Non IBS	56325 (99.6)	1691978 (99.7)	1
IBS	239 (0.4)	4942 (0.3)	1.43 (1.26-1.63)
Years before index date			
<5	91 (0.2)	2019 (0.1)	1.33 (1.08-1.65)
≥5	148 (0.3)	2923 (0.2)	1.50 (1.27-1.77)
≥10	82 (0.1)	1751 (0.1)	1.39 (1.11-1.74)
Age at IBS diagnosis, years			
<50	26 (0.0)	725 (0.0)	1.07 (0.72-1.58)
≥50	213 (0.4)	4217 (0.2)	1.49 (1.30-1.72)
Stratified by Sex			
Male	85 (0.2)	1682 (0.1)	1.49 (1.20-1.86)
Female	154 (0.3)	3260 (0.2)	1.40 (1.19-1.65)
Stratified by age on the index date, years			
≤69	75 (0.1)	1475 (0.1)	1.52 (1.20-1.91)
70-79	97 (0.2)	2065 (0.1)	1.39 (1.13-1.70)
≥80	67 (0.1)	1402 (0.1)	1.42 (1.11-1.81)

1. Conditional on birth year and sex matching pairs, country of birth, educational attainments, chronic obstructive pulmonary, comorbidity index

Fig. 5.3.1 Standardized survival functions and confidence intervals for PD among IBS and non-IBS subjects in SALT Swedish twins



5.4 POOR OLFACTION AND LONG-TERM MORTALITY (STUDY IV)

Study IV examined the association between olfaction, all-cause, and cause-specific mortalities among 2,537 older adults in the Heath ABC study. A total of 1,565 participants had died during a median of 12.0 years of follow-up. Participants with poor and moderate olfaction had 51%

and 17% higher all-cause mortality, respectively (Table 4.5.1). Fig 5.4.1 depicts standardized survival curves indicating higher all-cause mortality in older adults with poor and moderate sense of smell compared to those with good sense of smell. Regarding cause-specific mortalities, poor sense of smell was strongly linked deaths due to dementia or PD and moderately linked to death due to cardiovascular diseases (Table 5.4.1). Olfactory function, however, was not associated with cancer or respiratory disease mortality. Fig 5.4.2 shows the standardized survival of cause-specific mortalities for different olfactory function groups.

Table 5.4.1 Olfaction, total mortality, and selected cause-specific mortality in the Health ABC study

Mortality		Death(N)	Rate[1]	HR (95% CI)[2]
All-cause mortality				
	Good	433	44.6	1
	Moderate	524	55.6	1.17 (1.02-1.33)
	Poor	608	77.4	1.51 (1.33-1.72)
Cause-specific mortality				
Dementia or Parkinson's disease				
	Good	56	5.8	1
	Moderate	70	7.4	1.23 (0.86-1.77)
	Poor	138	17.6	3.24 (2.34-4.49)
Cardiovascular diseases				
	Good	168	17.3	1
	Moderate	202	21.4	1.17 (0.95-1.44)
	Poor	219	27.9	1.39 (1.12-1.72)
Cancers				
	Good	119	12.3	1
	Moderate	130	13.8	1.04 (0.80-1.34)
	Poor	129	16.4	1.09 (0.84-1.42)
Respiratory diseases				
	Good	24	2.5	1
	Moderate	37	3.9	1.47 (0.86-2.48)
	Poor	29	3.7	1.37 (0.77-2.42)

1: Mortality rate per 1000 person-years; 2: for all-cause mortality, adjusted for age, sex, education, BMI, drinking, briskly walking, self-reported general health status, cardiovascular diseases, cancer, diabetes, hypertension, depressive symptoms, stratified by race, smoking, chronic kidney disease; for dementia or Parkinson's disease mortality stratified by race, smoking, and depressive symptoms; for the rest of the cause-specific mortalities: stratified by race and smoking.

Fig 5.4.1 Standardized survivals curves and confidence intervals of all-cause mortality for different olfactory function groups

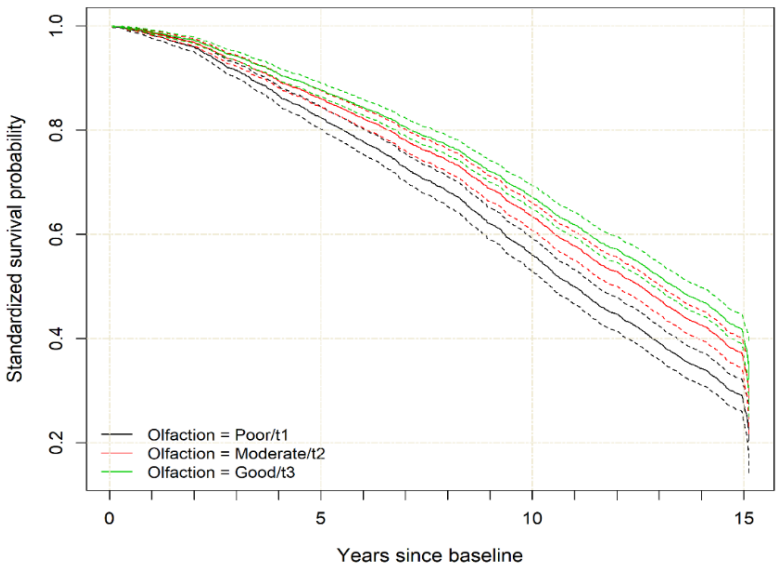
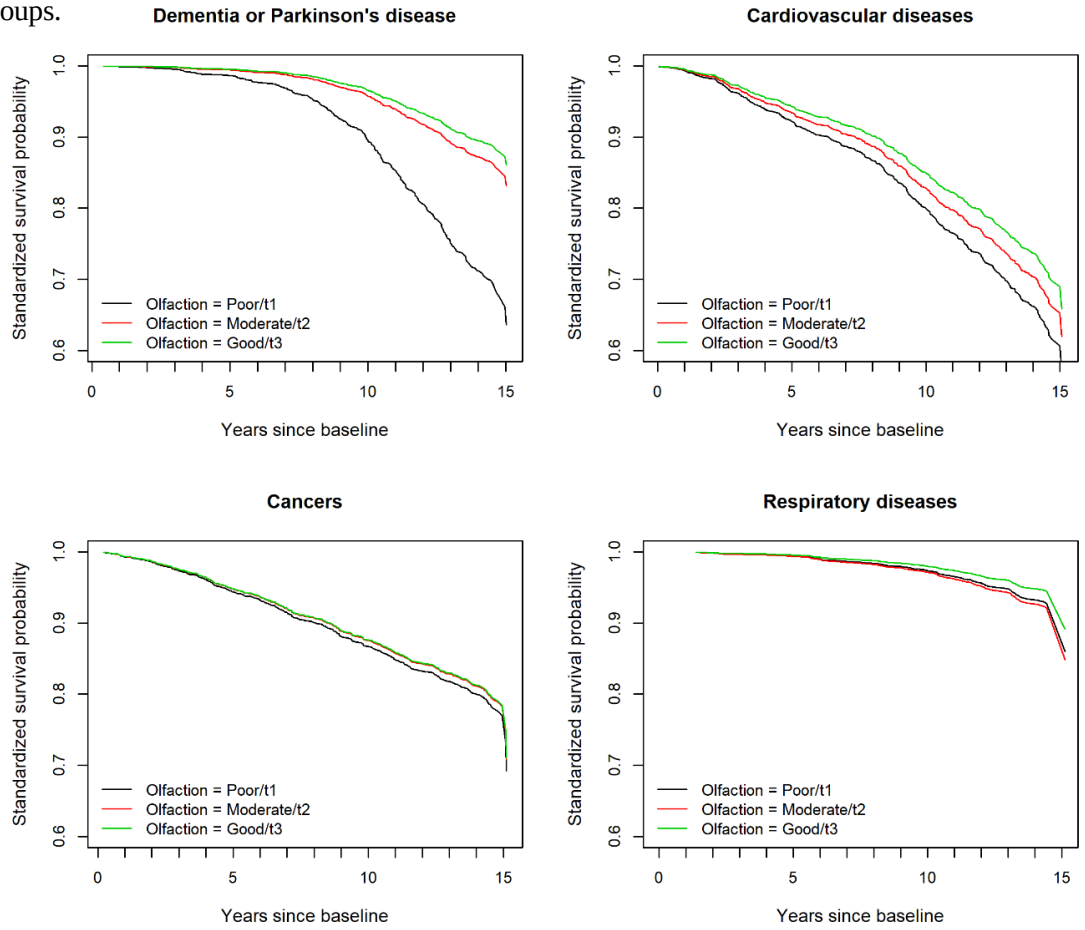


Fig 5.4.2 Standardized survivals curves of cause-specific mortalities for different olfactory function groups.



In mediation analyses (Table 5.4.2), 26.4% of the increased all-cause mortality associated with poor olfaction was mediated by paths through dementia or PD (i.e. path through dementia/PD only and path through dementia/PD and subsequent weight loss), whereas the proportion decreased to 15.3% among those with moderate olfaction. The corresponding proportion mediated only through weight loss was 18.3% and 33.0% for poor and moderate olfactory function groups, respectively.

Table 5.4.2 Mediation analyses between olfaction and all-cause mortality

Effect[1]	**Olfaction Poor vs. Good HR (95% CI)**	**% Mediated**	**Olfaction Moderate vs. Good HR (95% CI)**	**% Mediated**
Effect not through any mediator	1.32 (1.15-1.54)	-	1.12 (0.99-1.28)	-
Indirect effect through dementia/PD	1.09 (1.05-1.12)	26.4%	1.02 (1.00-1.04)	15.3%
Indirect effect only through mean yearly body weight loss ≥2%	1.05 (1.03-1.09)	18.3%	1.05 (1.02-1.08)	33.0%

1: Adjusted for age, sex, education, BMI, drinking, briskly walking, self-reported general health status, cardiovascular diseases, cancer, diabetes, hypertension, depressive symptoms, stratified by race, smoking, chronic kidney disease

6 GENERAL DISCUSSION AND CONCLUSION

6.1 RESULTS AND IMPLICATIONS

In **Studies I-IV**, we investigated factors related to PD etiology beyond the brain and late adulthood, including early life characteristics, vagotomy, IBS, and olfaction. In **Study I**, we demonstrated that early life characteristics of sibling structure, parental age at birth, month and season of birth, or influenza burden in the year of birth had little influence on the risk for PD late in life. These results are generally in line with previous findings [71-75].

In **Study II**, beyond the 5 year period post-surgery, truncal, but not selective, vagotomy appeared to be associated with a lower risk of PD compared to non-vagotomy individuals. A potential explanation may be that the spread of α-synuclein via the vagus nerve was reduced further after truncal versus selective vagotomy, because the truncal vagotomy targets anatomically much broader than the selective vagotomy. Our results are consistent with Svensson et al.'s paper that reported lower PD risk 20 years after the truncal vagotomy using the Danish registers [82]. In line with these findings, phosphorylated a-synuclein was detected in the gut of prodromal PD patients on average seven years (ranging from four months to twenty years) prior to PD diagnosis [24]. Additional investigations are needed to replicate the results and to further explore the potential temporal-spatial pattern in the spreading of α-synuclein. In contrast, Tysnes et al. did not replicate the reported lower PD risk after truncal vagotomy in the same Danish population [127]. Discrepancy in the Danish data may partly be attributed to difference in methodology as well as large statistical uncertainty. Although preliminary, evidence from two independent studies supports Braak's hypothesis that PD pathology may migrate from the gut to the brain through the vagus nerve.

Despite growing supporting evidence for Braak's hypothesis, factors promoting α-synuclein aggregation and the mechanism underlying PD neurodegeneration remain elusive. One proposed mechanism suggests that, through one path, GI inflammatory triggers exert deleterious effects on gut microbiota, intestinal permeability, and induce α-synuclein aggregation in the ENS, which later migrates to the CNS; and through another path, intestinal inflammation and altered permeability may promote systemic inflammation and subsequently increase the blood-brain barrier permeability [34]. Together, the two paths may jointly lead to neuroinflammation and neurodegeneration[34].

Limited epidemiological evidence exists on the possible prospective association of gut microbiota and intestinal inflammation with PD risk. Few studies have focused on *Helicobacter pylori* (HP) infection in relation to PD risk and results are somewhat controversial [128 129]. A Danish register-based study reported an increased PD risk for treatment to HP infection but not for gastritis or peptic ulcers [128]. A later study reported that HP infection, but not the treatment, was associated with a higher subsequent PD risk [129], in which the authors appeared to neglect the potential reverse causation between PD and HP infection [129]. Indeed, HP infection[130], small intestinal bacterial overgrowth[131] and dysbiosis[32] were more prevalent in PD. Intestinal inflammation and elevated levels of pro-inflammatory cytokines were also observed in PD patients and linked to the disease duration[132]. Overall, evidence from epidemiological studies suggests an involvement of bacterial infection and intestinal inflammation in PD, but the potential causal direction remains to be explored. A recent study reported that viral infection could induce the expression of α-synuclein in the gut among children who received intestinal transplants and showed a positive correlation between α-synuclein expression and degree of upper GI inflammation [133]. Another intriguing study based on animal models of PD reported the role of the microbiome in promoting neuroinflammation and α-synuclein mediated motor deficits [134] supporting microbiota as a risk factor for PD. From an epidemiological point of view, one may focus on GI diseases characterized by microbiota alteration and chronic inflammation, such as IBS and IBD [34, 36, 135], to address the involvement of gut-brain-microbiota axis in PD etiology. Previous results on IBD are inconsistent[136-138], while evidence on IBS in relation to PD risk is rare [86].

In **Study III**, we found an elevated risk of PD associated with IBS. Similar results were noted for IBS identified from the NPR and by the symptom-based algorithm. These associations cannot be entirely attributed to confounding by age, sex, life style factors, and surveillance bias. Although the potential underlying mechanisms are unknown, the results in line with the proposed mechanism that gut microbiota and inflammation might contribute to PD etiology. On the other hand, we cannot rule out the possibility that PD prodromal symptoms such as constipation might be misdiagnosed as IBS, and that residual confounding as an alternative explanation to the results.

According to the dual-hit hypothesis, neurotropic pathogens may invade the brain through the nasal routes and α-synuclein pathology may spread from the olfactory bulb to the connected regions of the brain [17, 18]. In line with this, in **Study II** we found that truncal vagotomy was related to a 40% but not an entire reduction of PD risk. Poor sense of smell is the most

prevalent non-motor prodromal symptom in PD [4] and is strongly linked to higher risk of PD in the general population [139]. Olfactory dysfunction is also considered an early sign of neurodegeneration in general, which can precede clinical diagnosis of PD, AD, Lewy body dementia, and cognitive decline by many years [140]. In **Study IV**, we found that the association between poor sense of smell and higher all-cause mortality was partly explained by PD/dementia and body weight loss. These results supports the pathway from poor olfaction, to neurodegeneration and related body weight loss, and ultimately to death. The study indicates the relevance of using olfaction test as a tool to detect early PD and revealed that neurodegeneration and body weight loss constitute part of the mechanisms underlying the association between poor olfaction and mortality.

6.2 METHODOLOGICAL CONSIDERATIONS

6.2.1 Bias

Bias is defined as "systematic deviation of results or inference from the truth" [141] and exist in nearly all observational studies. Biases can arise due to, for example, systematic errors in study design (e.g. sampling bias, selection bias), data collection (e.g. information bias, recall bias), analysis (e.g. confounding, selection bias). As systematic errors may distort results towards both directions, the magnitude of the bias is usually not straightforward to quantify. Therefore, it is crucial to understand potential biases before interpreting results and drawing conclusions. Given the longitudinal design and use of register-based data, the four studies compiled in the thesis are insusceptible to recall bias. However, other types of bias may be present and deserve awareness and reflection.

6.2.1.1 Measurement error and misclassification

Measurement error may result in misclassifications. A misclassification that is independent of other variables is usually described as non-differential misclassification while its counterpart is called differential misclassification. Non-differential misclassification generally drives the association between exposure and outcome towards null, while differential misclassification can bias the results in both directions.

In **Study I**, we obtained information on early life factors from the MGR and nationwide data on influenza-like-illness from the Swedish Public Health Agency. Coverage of these data are high making the concern of measurement error minor. However, the use of sibling structure, birth season and month, as surrogates of overall childhood infection certainly leads to some degree

of misclassifications. Ecologic analysis on the integrated influenza activity data also hinders the ability to assess the relationship between prenatal infection and PD risk. We however speculate that such misclassification of exposure is likely to be non-differential. Therefore, null associations of early life characteristics with PD cannot rule out a potential influence of prenatal and neonatal infections on later PD risk.

In **Study II**, we ascertained vagotomy from the NPR. Although the validity of the vagotomy procedure has not been evaluated, high accuracy of other surgeries in the GI, such as appendicitis has been reported [142]. Moreover, classification of truncal and selective subtypes was made by an abdominal surgeon in our team after reviewing the surgical codes. Nevertheless, since coverage of the NPR increased over time and was not nationwide until 1987, it is possible that some vagotomized patients may have been misclassified as vagotomy-free individuals. Such under-diagnosis could also happen in PD identification during the earlier years of the NPR. Incomplete coverage may lead to a spurious positive relationship between vagotomy and PD, namely, vagotomy and PD diagnoses are likely to be captured coincidentally among people covered by the NPR. To minimize this bias, we generally restricted the study population to those living in counties covered by NPR in the 1970s. However, as we observed a lower risk of PD for vagotomy, bias due to incomplete coverage most likely would have driven the association towards null.

It has been reported that only a proportion of IBS patients seek care and patients treated by specialists are more severe cases compared to those treated in primary care [143 144, 145]. In **Study III**, IBS patients treated by specialists were captured by the NPR. Those who did not seek medical care or were treated in primary care might be identified by self-reported symptoms from the STR. However, under-diagnosis and misclassification of IBS are inevitable for both sources and may result in either an over- or under-estimation of the association.

PD patients in **Studies I-III** were ascertained from the NPR. In a validation study of the NPR, the positive predictive value (PPV) of any inpatient PD diagnosis (primary or secondary) was 70.8% compared to the gold-standard clinical work-up, and the PPV increased to 80.3% when restricted to primary inpatient diagnoses [146]. In **Studies I** and **III**, when restricting the PD outcome to primary diagnoses only, the results remained similar. Although outpatient PD has not been validated, 92.5% of outpatient PD are primary diagnoses compared to 27% of inpatient diagnoses. Therefore, it is reasonable to assume that PD outpatient diagnoses are more accurate than inpatient ones.

In **Study IV**, olfaction was measured using the BSIT score. This test has been validated and is widely used in epidemiological studies [112, 113]. Cut-offs for the BSIT score (≤8, 9-10, 11-12) were based on both the test norm [112] and their previous applications in epidemiological studies [147 148]. However, we were not able to differentiate causes of olfactory impairments either due to aging or other diseases. Moreover, olfactory function was only measured once, thus the trajectory in association with mortality was unknown. Nevertheless, measurement error on covariates and mortality is likely minor, since information on diseases and conditions collected in the Heath ABC study has been adjudicated by a consensus panel of physicians.

6.2.1.2 Confounding

Due to lack of information, analyses based on Swedish nationwide registers usually only consider a limited number of covariates. As we did not have information on potentially important confounders such as smoking, alcohol consumption, coffee drinking, medication use, and genetics, we attempted to address unmeasured confounding by using proxies, for example, COPD as a proxy for smoking. However, such analyses have been criticized because potential misclassification may be substantial. Indeed, life-long smokers may have a 50% probability of developing COPD [149] and 25–45% of COPD patients have never smoked [150]. Although adjusting for proxies of unmeasured confounders is not optimal, it may still provide some useful indication on the direction of the confounding effect. New methods have been developed to quantify the robustness of the results to unmeasured confounding. For instance, one can calculate the E-value, a measure of the minimum strength of association that an unmeasured confounder would have with both exposure and outcome, in order to explain away the observed association [151]. A large E-value implies that results are robust to a large unmeasured confounding effect [151].

6.2.1.3 Selection bias

Selection bias and sampling error is unlikely in **Studies I-III** which are based on the entire population of Sweden, but likely in **Study IV**, which is based on the Health ABC cohort comprising relatively healthy and well-functioning older adults. Selection bias may also arise from statistical analysis. In **Study II**, we evaluated the temporal relationship between vagotomy and PD risk and presented period-specific HR after 5, 10, and 20 years of follow-up. Since a period-specific HR is conditional on survival at the previous period of time, selection bias may be introduced if an unmeasured confounding has an impact on both previous survival and later outcome [152]. The direction of such bias is however unknown. In contrast to HR, standardized

survival or failure curves are not susceptible to such shortcoming. The cumulative incidence plot in **Study II** demonstrated a lower PD risk after truncal vagotomy indicating potential selection bias was likely not an issue. In **Studies III** and **IV**, we presented the standardized survival curves in addition to the HRs.

6.2.2 Assumptions for mediation analysis

In **Study IV**, we performed mediation analysis to explore whether the association between olfaction and mortality can be explained by PD/dementia or weight loss. As mentioned in Fig 4.2.2 we need four assumptions in order to estimate the direct and indirect effects: 1) no unmeasured exposure-outcome confounders given X_0; 2) no unmeasured mediator-outcome confounders given W_1 and W_2; 3) no unmeasured exposure-mediator confounders given X_1 and X_2; 4) no mediator-outcome confounder W_1 and W_2 affected by A.

Assumptions 1 and 3 are likely to hold because exposure-outcome and exposure-mediator confounders (X_0, X_1 and X_2) are likely to be included in the wide range of baseline variables regarding demographic, behavior and life-style, and comorbidities. Further, assumption 2 may also be fulfilled. We attempted to incorporate most of the suggested confounders for the association between PD/dementia and mortality (such as age, sex, race, life-style factors and baseline comorbidities) in W_1 [153, 154]. Important confounders for the association between body weight loss and mortality[155], such as age, sex, race, baseline BMI, smoking and alcohol consumption, and metabolic diseases have been considered in W_2. We also assume that no mediator-outcome confounder is affected by exposure (assumption 4). However, violation of this assumption is difficult to assess.

6.3 ETHICAL CONSIDERATIONS

Studies I-III are register-based studies and have been approved by the Regional Ethics Committee in Stockholm. Informed consent is not required for the use of register data. Researchers who work with these data are obligated to take care to de-identify individuals as to protect the anonymity of the person. The National Board of Health and Welfare has replaced the personal identity number with a serial number, meaning that all individuals in the register are anonymized. Furthermore, register data are secured by Karolinska Institutet through strict safety control and regulations of data access. In **Study IV,** the study protocol was approved by all relevant Institutional Review Boards (IRB). All study participants provided informed consent at enrollment and all the analyses were based on de-identified data.

6.4 CONCLUSION

Taken together, this thesis provides several important findings: 1) we found little evidence supporting the development origin of PD, while the influence of specific intrauterine, pre- and neonatal infections remains to be elucidated and clarified; 2) lower PD risk associated with truncal vagotomy and elevated PD risk related to IBS highlight the importance of the gut-brain axis in the etiology of PD. Further studies on the potential mechanisms involving for instance intestinal inflammation and disturbance of gut microbiota are warranted; 3) we confirmed the association between poor olfaction and mortality and identified important mechanisms for this association through neurodegenerative disease such as PD and dementia as well as weight loss.

6.5 FUTURE PROSPECTIVES

Although the causes and underlying mechanism of PD remain unclear, special attention has been paid to Braak's hypothesis and the gut-brain connection. Further efforts are warranted to explore physiological conditions and factors that may affect the misfolding and aggregation of α-synuclein. Future research should be encouraged to elucidate the influence of viral and bacterial infections and related immune responses in the development PD. Differential abundances of gut microbes were observed in PD [31-33], and also in patients with RBD [156] that is a strong predictor of PD [157]. Prospective studies with reliable and repetitive measurements are essential to capture changes in the composition of microbiota in relation to PD risk. Moreover, human herpesviruses was recently linked to the etiology of AD [158], while the potential influence of viral infection on PD remains to be explored.

Studies on potential therapeutic roles of antibiotics, probiotics, anti-inflammatory drugs are also highly warranted. Lower PD risk has been linked to use of ibuprofen, but not other NSAIDs [63]. Intriguing results were reported very recently demonstrating the protective effect of anti-asthmatic drugs (i.e. beta2-adrenoreceptor stimulators)[159] and TNF-α inhibitors [138] on PD risk. Understanding the specific functions of pro-inflammatory molecules and the distribution of corresponding receptors may give us new perspectives in searching candidate targets for pharmaceutical treatments. Given the potential involvement of gut-brain-microbiota axis in PD etiology, future research are encouraged to explore the potential impact of antibiotics and probiotics on the etiology and development of PD.

Early detection of PD is particularly important given that once motor symptoms present, the majority of the dopaminergic neurons are already lost. Olfactory loss was included in the MDS

diagnostic criteria of prodromal PD [160] and results from **Study IV** also suggests the importance of olfaction tests. A recent study demonstrated that patterns of olfactory impairment may vary by different etiologies and that PD patients had poor performance in odor identification and discrimination but better performance on the odor threshold task [161]. Continued efforts are needed to optimize relevant tests and establish reliable biomarkers and criteria for early PD identification in population settings.

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