Incidence, Risk and Prognosis of Parkinson Disease

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Incidence, Risk and Prognosis of Parkinson Disease

Incidentie, risico en prognose van de ziekte van Parkinson

Proefschrift

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1 Introduction

Parkinson disease (PD) is a progressive neurodegenerative disorder that mainly occurs at older ages and is clinically characterized by resting tremor, rigidity, bradykinesia and postural imbalance. These clinical manifestations of PD are caused by a selective degeneration of dopamine-producing neurons in the substantia nigra in the brain stem and the consequent dopamine shortage in the striatum.[1] Recent discoveries, in particular the identification of several gene mutations that cause familial forms of the disease, have led to new insights into the pathogenesis of PD. Of all PD cases, monogenetically inherited forms nevertheless make up only about 10%, and the majority therefore are sporadic. It is commonly thought that sporadic PD results from a complex interplay between various genetic susceptibility factors and environmental exposures, which induce neurodegeneration through various pathways, including dysfunction of mitochondria, oxidative stress and aberrant protein degradation.[2-5] However, the exact mechanisms underlying these processes are still not completely understood and therapies for PD are as yet only symptomatic.

Reliable estimates of the impact of PD in terms of incidence and prognosis in the general population are of importance in the light of the worldwide aging of populations. Well-designed epidemiological studies that quantify the problem of PD therefore are needed, but only a few large prospective population-based studies on PD have been conducted worldwide, and many of them were register-based. It is known from prevalence surveys that many PD patients, in particular those with mild symptoms, are missed when no direct screening is done, which may have led to underestimation of the incidence and biased estimates of the prognosis.[6] Prospectively designed epidemiological studies may also add to and strengthen the knowledge on pathogenesis and risk factors for PD, which is relevant for the development of better treatment. Much of the current knowledge on PD pathogenesis has been derived from animal models and genetic research on rare familial cases. Whereas animal models often focus on single risk factors and short-term effects, observational studies are well suited to study multiple potential risk factors and their interactions, which is of particular interest when studying complex multifactorial diseases like PD. Epidemiological studies with a long follow-up period probably also more accurately reflect the pathophysiological processes underlying PD, as these are thought to develop over a long period.

The objective of this thesis was to study incidence, prognosis and potential risk factors for PD in the general population. This was done within the context of the Rotterdam Study, a large prospective population-based cohort study in 7,983 participants aged 55 years and older.[7] Many potential risk factors were assessed at baseline and participants were followed with repeated in-person screening for PD

for up to 15 years. In this thesis, I will first review currently available epidemiological evidence on diagnosis, frequency, risk factors and prognosis of PD, with special attention to methodological issues (Chapter 2). Next, I describe the age- and sex-specific incidence of parkinsonism and PD in the general population (Chapter 3.1) and the relationship between subjective motor complaints in the absence of clinically obvious abnormalities at baseline and the risk to develop PD during follow-up (Chapter 3.2). Because recent evidence from animal models suggests that alterations in fat metabolism might be involved in PD pathogenesis,[8-10] I evaluated the association with PD risk of dietary intake of various types of fat (Chapter 4.1), as well as serum levels of total and HDL cholesterol (Chapter 4.2). Animal studies have furthermore indicated that high homocysteine levels might increase the risk of PD, as homocysteine administration induced mitochondrial dysfunction and increased oxidative stress.[11] Therefore, I investigated the influence on PD risk of the TT variant of the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism, which is associated with mild hyperhomocysteinemia (Chapter 5.1) and dietary intake of folate, vitamin B12 and vitamin B6, essential co-factors that are required to keep plasma homocysteine levels low (Chapter 5.2). The role of oxidative stress in PD was further explored by examining the relationship between serum levels of the antioxidant uric acid and the risk of PD (Chapter 5.3). Subsequently, I studied the prognosis of PD patients in terms of risk of dementia and mortality compared with participants without the disease (Chapter 6). In the final chapter, I aim to put our findings in a broader perspective (Chapter 7). I reflect on the relevance of our observations, discuss how they fit current knowledge on PD pathogenesis and give suggestions for future research.

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2 Epidemiology of Parkinson disease

Introduction

Parkinson disease (PD) is the second most common neurodegenerative disorder after Alzheimer disease and is expected to impose an increasing social and economic burden on societies with aging populations.[1] In recent years, the interest of the scientific community in PD has grown considerably, triggered by the discovery of several causative monogenetic mutations. However, these mutations likely explain only a small proportion of all PD patients and about 90% of cases are sporadic. Despite insights derived from genetic research, the exact pathogenetic mechanisms responsible for the selective dopaminergic cell loss underlying PD are still not entirely understood. Current thinking is that mitochondrial dysfunction, oxidative stress and protein mishandling play a central role in PD pathogenesis,[2-4] and that in sporadic PD these processes are induced by non-genetic factors, probably in interaction with susceptibility genes. Insight in non-genetic causes is needed to further the understanding of the pathogenesis of the disease and to develop effective therapeutic strategies. Large, well-designed, prospective population-based cohort studies are the only studies suited to examine the effects of multiple potential risk factors and their interactions, as well as effects that develop over a longer period.

In the past, numerous methodologically limited epidemiological studies on PD have been carried out, mostly small case-control or register-based studies and based on prevalent cases. Only in the last 5 to 7 years larger prospective studies have come to a stage where they have identified sufficient numbers of PD patients to examine incidence and potential risk factors of the disease. In this article, we will review what is presently known about the frequency, risk factors and prognosis of PD from epidemiological studies. Special attention will be given to methodological issues, as the usefulness of epidemiological data and interpretation of findings are largely dependent on the quality of the studies they were obtained from.

Diagnosis of PD in epidemiological research

A reliable and easily applicable clinical test or marker for PD is as yet not available, in spite of the development of promising sophisticated imaging techniques such as PET and SPECT scanning that might discriminate between PD patients and normal controls, or between PD and other parkinsonian syndromes.[5] Although potentially useful in a specialized setting, the value of these techniques in epidemiological research, in particular in low-risk cohorts, is limited given that they are costly and not yet widely available. Thus, PD diagnosis in epidemiological studies is still based on clinical manifestations. According to currently applied diagnostic criteria, the clinical syndrome of parkinsonism is characterized by resting tremor, bradykinesia,

rigidity, and postural imbalance and is diagnosed when at least two of these so-called cardinal signs are present.[6] A diagnosis of PD furthermore requires that parkinsonism is idiopathic, i.e. that potential causes of secondary parkinsonism (dementia, use of antipsychotic medication, vascular disease, head trauma, infections and other neurodegenerative diseases that involve the nigrostriatal system) are excluded. A good response of the symptoms to levodopa medication is often considered supportive for a diagnosis of PD, although not observed in all patients.[7]

One of the problems in epidemiological research on PD is that clinical criteria at best lead to a diagnosis of probable PD, while post mortem confirmation is required for a diagnosis of definite PD. As in epidemiological studies neuropathological material is usually not routinely collected, a certain degree of diagnostic uncertainty is inevitable.

Clinicopathological studies have shown that in less than 80% of the cases the clinical diagnosis of PD was confirmed at autopsy,[8] although the use of more strict clinical criteria in a specialist setting has been shown to improve diagnostic accuracy to up to 90%.[9, 10] The meaning of such pathological validation studies is nevertheless unclear, as universally accepted neuropathological criteria for PD are lacking.[6] Besides, post-mortem examination is only performed in a minority of patients and atypical cases are likely to be over-represented in pathology series.

Clinical diagnosis and differential diagnosis of PD may be difficult, especially in the early stages of the disease that are usually studied in prospective population-based research. In the elderly, frequently present conditions such as arthritis or neuropathy, that may resemble some of the cardinal signs or affect their presentation, may complicate diagnosis.[11] Furthermore, the clinical distinction between PD and other neurodegenerative diseases (dementia and disorders that involve the nigrostriatal system such as progressive supranuclear palsy (PSP), multiple system atrophy (MSA), dementia with Lewy bodies (DLB) and corticobasal degeneration (CBD)) can be hard because parkinsonian signs as well as non-motor manifestations, including cognitive, sensory, and autonomic disturbances, may occur in all these conditions. To distinguish PD from other neurodegenerative disorders the order in which motor and non-motor symptoms appear, as well as evaluation of disease progression and responsiveness to levodopa therapy are of importance. Most clinicians therefore consider a single visit not sufficient to make a definite diagnosis. In contrast, diagnosis of PD in epidemiological research is often based on a single assessment, increasing the possibility of misdiagnosis. A study design that enables long term follow-up of participants after an initial diagnosis is made, either through direct contact or medical records, is thus preferred over assessing disease status at one point in time.

Frequency

Methodological considerations

It has been noticed previously that results of studies on the prevalence and incidence of PD appear to vary according to applied methodology, which may complicate comparison across studies.[11-13] As in virtually all epidemiological studies diagnosis is based on clinical assessments, frequency estimates are affected by the set of diagnostic criteria that is employed to define PD. Not surprisingly, the use of stricter diagnostic criteria has been shown to yield lower frequency estimates.[11] Estimates of frequency measures are influenced even more by case-finding strategies. Studies performed in a clinical setting or those with record-based case finding methods fail to include patients who have not sought medical attention and thus underestimate the prevalence or incidence of PD in the general population. This has been illustrated by the results of several door-to-door prevalence surveys in which a considerable number of PD patients was detected that had not been diagnosed with the disease before.[12-16] Most incidence studies with in-person examination also yielded higher incidence rates than record-based studies and identified a noticeable amount of new, not previously recognized PD cases, who would not have been included in a record-based study.[17, 18]

Many authors present crude estimates of the prevalence or incidence for an entire population or a section of the population above a certain age. These are of little use, since they strongly depend upon the underlying age-distribution. Age-standardized rates are also of limited value, as differences in age distributions used for standardization may hamper comparison. In this review we therefore only summarize age-specific prevalence and incidence rates in the given figures.

Prevalence

Many epidemiological studies have been conducted in different settings worldwide to estimate the prevalence of PD in the general population. Crude prevalence estimates derived from these studies range between 55 and 900 per 100,000.[14-16, 19-26] In general, the prevalence of PD in industrialized countries is now estimated at 0.3% of the entire population and about 1% in those older than 60 years of age.[27] In figure 1, we summarize age-specific prevalence rates obtained from population-based surveys. PD clearly is an age-related disease, being rare before the age of 50 yet with prevalence estimates increasing with age,[12, 13, 15, 20-23, 26] up to 4% in the highest age groups. The importance of in-person examination for case-finding in epidemiological research is demonstrated by the high rate of newly diagnosed patients in prevalence studies with screening of all participants. The proportion of patients that were identified with PD who had never received this diagnosis before ranged from 24 to 42%.[12-14, 21-23]

Figure 1. *Prospective population-based prevalence studies on Parkinson disease; age-specific prevalence per 100 persons*

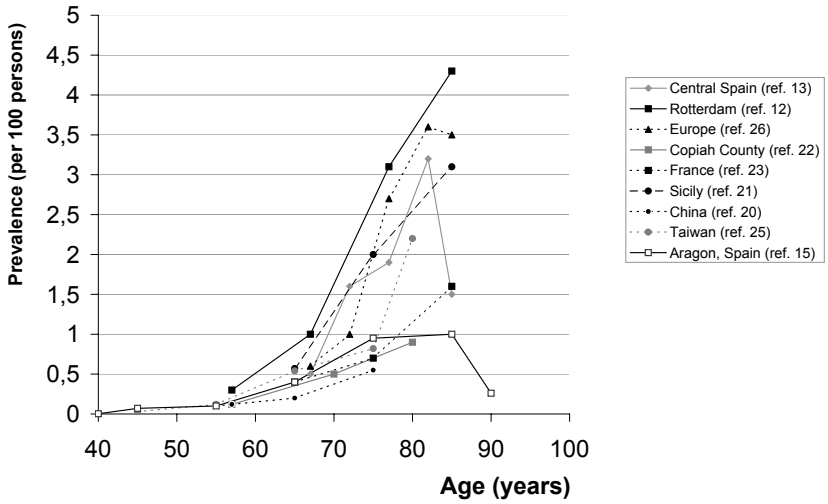
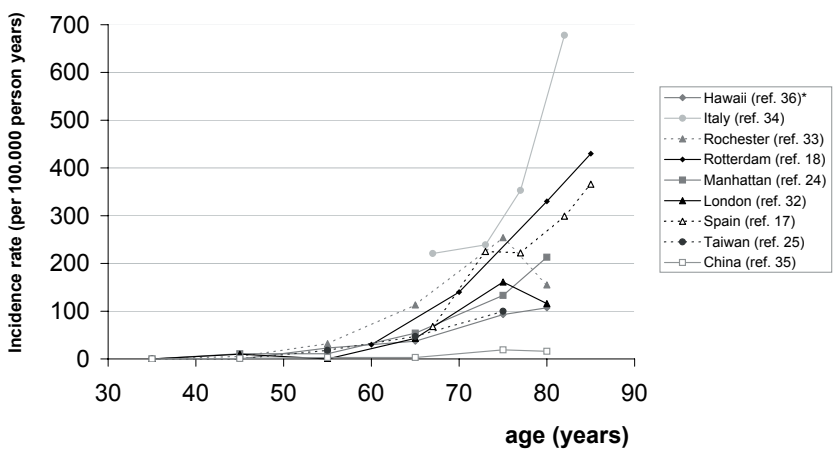


Figure 2. *Prospective population-based incidence studies on Parkinson disease; age-specific incidence rates per 100,000 person years*



*restricted to men

Some of the studies reported a higher prevalence of PD in men,[13, 15, 20, 24, 28] although in other studies estimates for men and women were not significantly different.[12, 14, 21, 23] Neuroprotective effects of estrogens have been suggested as a possible explanation for a higher risk of PD in men, but the role of estrogens in PD is still controversial.[29,30]

Incidence

Incidence rates may provide better tools to compare occurrence of PD, as they are theoretically not affected by differences in survival of patients. Given the low frequency of PD outside a clinical setting, incidence studies of PD in the general population require large cohorts and long follow-up periods, and incidence data therefore are relatively scarce compared to prevalence figures. Reported standardized incidences vary from 8 to18 per 100.000 person-years.[31] Figure 2 shows age-specific incidence rates, restricted to those from prospective population-based studies with either record-based or in-person case-finding.[17, 18, 24, 25, 32-36] Onset of PD is rarely observed before the age of 40 and a sharp increase of the incidence is seen after the age of 60. However, results on the highest age groups are conflicting. Some studies reported a drop in the incidence for the highest age groups,[36, 37] while others report that incidence rates continued to rise with age.[17, 18, 24, 34] The decline at higher ages has been suggested to be an artefact caused by increased diagnostic uncertainty due to presence of co-morbidity in the elderly and the fact that people at higher ages might not consult their physician for parkinsonian signs when they consider these part of the normal aging process.[26] Selective non-response or lost to follow-up in the highest age groups and impreciseness of the estimates due to low numbers may also have played a role.

Table 1. *Age adjusted male-to-female ratios with 95% confidence intervals for the incidence of PD, derived from prospective incidence studies*

Study (ref.)	PD cases / study population	Male-female ratio
Rochester[33]	138 / 53,885	1.6 (1.3 - 1.9)
China[35]	566 / 3,869,162	0.9 (0.6 - 1.4)
Manhattan[24]	83 / 213,000	1.6 (1.3 - 2.1)
Taiwan[25]	37 / 11,411	1.1 (0.5 - 2.7)
Central Spain[17]	30 / 5,160	2.6 (1.2 - 5.4)
Rotterdam[18]	67 / 6,566	1.5 (1.0 - 2.5)
Italy[34]	42 / 4,341	2.1 (1.1 - 4.1)

Most studies on the incidence of PD applied record-based case-finding methods, which is less costly and time-consuming. However, the value of in-person screening for epidemiological research again is proven by the proportion of newly diagnosed patients in studies that involved examination of all participants, ranging from 39% to 53%.[17, 18] As shown in figure 2, estimates of the incidence rates were higher for all age categories in studies that applied in-person screening methods.[17,18,34] Like in prevalence studies, several of the prospective studies found evidence for a higher incidence of PD in men.[17, 18, 24, 28, 33, 34, 37] Age-adjusted male-to-female ratios of prospective population-based incidence studies are presented in table 1.

Non-genetic risk factors

Methodological considerations

Many environmental risk factors have been hypothesized to be related to PD risk based on presumed underlying pathogenetic mechanisms of the disease. Much of the epidemiological research on risk factors has been conducted in the form of retrospective case-control studies, which are prone to several kinds of bias. Because potential risk factors are retrospectively assessed, recall bias may occur when PD patients are more likely to remember exposure to these factors than control subjects, due to increased awareness and better knowledge of the disease. Furthermore, controls are often not selected in an appropriate way, or ascertainment of PD is incomplete or inaccurate resulting in selected case-series. An important pitfall in case-control studies is the issue of reversed causality, i.e. the question whether observed differences are really causally related to PD or rather are a consequence of the disease. This latter issue holds particularly for studies on dietary factors, the majority of which is retrospectively designed. Dopamine shortage might affect food preferences,[38] and altered intake of certain nutrients in PD patients may thus be erroneously considered to have played an etiological role. Dietary habits are usually assessed by means of food frequency questionnaires. A certain amount of error and thus misclassification of intake is unavoidable when using such an instrument. Furthermore, intakes of many nutrients are highly correlated, which renders the identification of specific associations difficult. Another complicating issue in dietary research is the lack of knowledge about the critical time period or window of exposure during which patients are at risk to develop PD. It is therefore unclear whether it is early, late, or rather cumulative or average lifetime intake that is of importance when studying the risk of PD.

Prospective cohort studies, with exposure assessment before onset of PD, may overcome at least part of the problems that pertain to retrospective case-control

designs. However, cohort studies have their own limitations in that they are costly and less time-efficient, in particular those that apply in-person screening methods. Register-based studies, even when prospective, might still lead to biased results as PD patients outside medical care –most likely the ones with early or mild disease- are not included. Besides, the issue of reverse causality may play a role even in prospectively designed studies. Since the exact duration of the preclinical period in PD is unknown, some of the participants who seem disease-free at the start of the study may already suffer from changes due to presymptomatic dopaminergic degeneration, which might theoretically influence study results.

Exposure to pesticides and herbicides

The discovery in 1983 that several persons developed typical signs of PD after intravenous injection of drugs contaminated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and the subsequent finding that MPTP selectively damages dopaminergic cells in the substantia nigra[39, 40] led to the hypothesis that exposure to environmental toxins such as pesticides, herbicides and metals might be related to the risk of PD. Since then, many epidemiological studies have been conducted to examine the association between exposure to pesticides and herbicides, as well as hypothesized surrogate measures for exposure like farming, living in rural areas and well water drinking, and the risk of PD. The majority of these studies were retrospective case-control studies, and thus subject to most of the methodological limitations that were mentioned above. Evidence fairly consistently points towards a positive association between pesticide exposure and PD risk, although results were statistically significant in only half of the studies.[41] In a meta-analysis of 19 peer-reviewed studies on pesticide exposure and the risk of PD performed between 1989 and 1999, Priyadarshi et al found significant heterogeneity among studies and calculated a pooled odds ratio of 1.94 (95% CI 1.49 to 2.53),[42] which fits the results of more recently performed case-control studies.[43, 44] Findings on farming, rural living and well water drinking have been less consistent than those for occupational pesticide exposure and both strength and direction of observed associations show a wide variation across studies.[41] The relationship between (self-reported) pesticide exposure and plantation work and PD has only been examined prospectively in one large study among men. A significantly increased risk of PD was found among men who worked more than 10 years on a plantation, and a non-significant association for men exposed to pesticides.[45]

It is believed that pesticides cause parkinsonian features through inhibition of mitochondrial complex I, as MPTP as well as the herbicide paraquat and the plant-derived pesticide rotenone are selective complex-I inhibitors[46] and evidence for mitochondrial dysfunction has been obtained from post-mortem material.[2] Paraquat,

which has a chemical structure very similar to MPTP, has been shown to deplete dopamine in frogs[47] and rotenone was found to induce selective dopaminergic degeneration in the substantia nigra as well as clinical features of parkinsonism in rats.[48]

Smoking, coffee and alcohol consumption

Smoking is among the most studied risk factors for PD, and one of the few for which very consistent results were obtained. Numerous epidemiological studies have shown a reduced risk of PD among cigarette smokers. The vast majority of these were case-control studies, but some large prospective cohort studies have been carried out that confirmed their results.[49-51] In a large meta-analysis based on 44 case control studies and 4 cohort studies on the relationship between smoking and the risk of PD conducted in 20 countries between 1968 and 2001, a pooled relative risk of 0.59 was calculated for ever smokers, and a relative risk of 0.39 for current smokers. In pursuit of completeness, meta-analyses often apply rather broad eligibility criteria, and results might in part be driven by included studies of poor methodological quality. However, the pooled effect estimate for all case control studies in this meta-analysis was only modestly different from the association for all cohort studies.[52] Our overview in table 2 is restricted to population-based prospective studies on the association between smoking and PD. All of them showed a significant inverse association, with more or less similar effect estimates. The biological basis that might underlie this association is still poorly understood. Several mechanisms have been proposed to explain a potential neuroprotective effect of (components of) cigarette smoke. Cigarette smoke may stimulate dopamine release through nicotine, inhibit free radical damage through carbon monoxide and protect against neuronal damage by inhibition of monoamine oxidase B. It has also been hypothesized that PD patients are less prone to develop addictive behaviour such as smoking, given the role of dopaminergic pathways in reward mechanisms. [52, 53] An alternative theory states that PD patients have a characteristic cautious premorbid personality that would make them avoid novelty-seeking behaviours.[54, 55]

On the other hand, it has been suggested that the association might be due to several kinds of bias, including selective mortality of smokers among non-PD cases, inaccurate recording of PD diagnoses in smokers, and confounding by unknown factors.[52] These issues however are most likely to play a role in methodologically flawed studies. The consistency of findings across different study designs, including carefully conducted large prospective studies, provides evidence against bias as a sole explanation.

Table 2. *Smoking and the risk of PD, population-based prospective studies*

Study	Author (year)	Study size	PD cases	RR estimate	Category of comparison
Honolulu Asia Aging Study	Grandinetti[49](1994)	8,004 men	58	0.39 (0.22-0.70) 0.25 (0.14-0.46) 0.50 (0.28-0.87)	(ever vs never smoking) (current vs never smoking) (former vs never smoking)
Leisure World Cohort Study*	Paganini[50](2001)	13,979	395	0.42 (0.25-0.69) 0.92 (0.73-1.16)	(current vs never smoking) (former vs never smoking)
Nurses' Health Study	Hernan[51](2001)	121,700 women	153	0.59 (0.43-0.81) 0.4 (0.2-0.7) 0.7 (0.5-1.0)	(ever vs never smoking) (current vs never smoking) (former vs never smoking)
Health Professionals Follow-up Study	Hernan[51](2001)	51,529 men	146	0.49 (0.35-0.69) 0.3 (0.1-0.8) 0.5 (0.4-0.7)	(ever vs never smoking) (current vs never smoking) (former vs never smoking)
Rotterdam Study**		6,566	89	0.58 (0.34 - 0.98) 0.57 (0.29 - 1.13) 0.58 (0.33 - 1.02)	(ever vs never smoking) (current vs never smoking) (former vs never smoking)

** Nested case-control study **Unpublished results*

Table 3. *Coffee and alcohol consumption and the risk of PD, population-based prospective studies*

Study	Author (year)	Study size	PD cases	RR estimate	Category of comparison
Coffee:					
Honolulu Asia Aging Study	Ross[56] (2000)	8,004 men	102	0.45 (0.30-0.71)	(coffee vs non-coffee drinkers)
Leisure World Cohort Study*	Paganini[50] (2001)	13,979	395	0.64 (0.48-0.84)	(2 or more cups of coffee/day vs no coffee onsumption)
Nurses' Health Study	Ascherio[57] (2001)	121,700 women	153	0.8 (0.6-1.0)	(coffee vs non-coffee drinkers)
Health Professionals Follow-up Study	Ascherio[57] (2001)	51,529 men	146	0.7 (0.5-0.9)	(coffee vs non-coffee drinkers)
Framingham Study	Fink** (2001)	6,048	58	0.89 (0.49-1.63)	(coffee vs non-coffee drinkers)
Rotterdam Study***		5,289	72	0.82 (0.45-1.50)	(coffee vs non-coffee drinkers)
				0.91 (0.81-1.03)	(per cup of coffee/day)
Alcohol:					
Leisure World Cohort Study*	Paganini[50] (2001)	13,979	395	0.73 (0.56-0.96)	(2 or more drinks/day vs no alcohol consumption)
Nurses' Health Study	Hernan[62] (2003)	88,722 women	167	1.0 (0.4-2.2)	(highest vs lowest category)
Health Professionals Follow-up Study	Hernan[62] (2003)	47,367 men	248	0.6 (0.4-1.1)	(highest vs lowest category)
Rotterdam Study***		5,289	72	0.69 (0.40-1.17)	(alcohol vs non-alcohol drinkers)

** Nested case-control study ** Abstract *** Unpublished results*

A considerable number of studies also looked at coffee consumption in relation to PD risk. Results have been less convincing than those for smoking, but still fairly consistent. Again, findings from case-control studies have been confirmed in several large follow-up studies (see table 3).[50, 56, 57] A meta-analysis based on 8 case control studies and 5 cohort studies showed a significantly decreased PD risk for coffee drinkers (pooled RR 0.69) that was not attenuated when analyses were adjusted for smoking.[52] Because caffeine is an inhibitor of the adenosine A2 receptor and has been shown to improve motor deficits in a mouse model of PD, it is generally thought to be the responsible component.[56] Interestingly, in two cohort studies comprising only men a strong and significant inverse association was seen,[56, 57] while in a cohort of only women this association was weaker and only borderline significant.[57] Furthermore, in postmenopausal women from this latter cohort the effect of caffeine consumption on PD risk seemed dependent on the use of estrogen replacement therapy.[58] Based on the finding that estrogen is a competitive inhibitor of caffeine metabolism,[59] an interactive effect of caffeine and estrogens on PD risk has been suggested,[60] but as yet a clear explanation for these observations is lacking.

The relation between smoking and coffee consumption and PD, and the hypothesis that dopamine shortage in PD patients would make addictive behaviour less rewarding to them have led some researchers to examine the association between alcohol consumption and the risk of PD. Results of a number of case-control studies and some prospective cohort studies have not been very straightforward, with inverse associations in some studies,[50, 61] but no significant association in others (table 3).[62-65] As both caffeine and cigarette smoke, but not ethanol, have shown protective effects in animal studies, current thinking is more in favour of a biological mechanism for the inverse association with PD than a certain non-causal explanation.[53, 62]

Dietary factors

A broad range of food groups and specific nutrients has been investigated as potential risk factors that are either related to an increased or a decreased risk of PD. The majority of epidemiological research on dietary factors comprised case-control studies, and only a few prospective studies have been performed. The results that are summarized in tables 4-6 are restricted to those obtained from population-based, prospective cohort studies, as these are supposedly least affected by bias.

Antioxidants

The focus in nutritional epidemiology has been mainly on antioxidants, as oxidative stress is thought to be involved in the pathogenesis of PD[2] and antioxidants like vitamin E and vitamin C might protect cells against oxidative damage by neutralizing free radicals. Although intake of vitamin E or foods rich in vitamin E has been

associated with a significantly lower risk of PD in some case-control studies,[66, 67] this was confirmed in only one prospective cohort study,[68] whereas a prospective nested case-control study showed no significant association.[69] Clinical trials of vitamin E supplementation have shown no effect on primary end points such as the need to start levodopa therapy.[70] However, it should be pointed out that these trials were performed in clinically manifest PD patients, in whom a substantial proportion of the dopaminergic neurons have already degenerated, whereas neuroprotection from antioxidants is more likely to occur at very early, presymptomatic stages. Several studies examined the relation between vitamin C intake and PD risk, but no association was seen in most case-control studies[71-73] and one prospective study.[68]

Dietary iron

Several case-control studies have investigated the role of dietary iron, because iron may induce free radical formation and increased iron levels have been found in the substantia nigra of PD patients.[74] Two of these studies found a positive association between iron intake and PD,[72, 75] but two other observed no association.[71, 76] Results of prospective studies on the relation between dietary iron and the risk of PD have not been published thus far.

Fat and fatty acids

The relation between dietary fat and PD is as yet unclear. Most epidemiological studies focused mainly on intake of total fat and saturated fat, given that diets with high lipid content could theoretically increase the level of oxygen radicals through lipid peroxidation and thus affect the risk of PD.[77] Some case-control studies reported higher intakes of total fat and animal fat (a major source of saturated fat) in PD patients,[71, 72, 76] while one prospectively designed study showed no significant association[78] and another a significant inverse association between total fat intake and PD risk.[79] For total calorie intake a positive association was found in some case-control studies,[71, 72, 80] but could not be confirmed in any of the prospective studies.[78, 79, 81] Positive associations for dairy products and recently also for milk consumption have been observed in two prospective studies, although it remains unclear which is the responsible component.[82, 83]

Recently more attention has been given to unsaturated fatty acids, as these components of cell membranes are shown to have neuroprotective and anti-inflammatory properties and are precursors of endogenous cannabinoids, which modulate dopaminergic activity in the basal ganglia.[84] Within the Honolulu –Asia Aging study, a significantly reduced risk of PD was observed with higher intake of polyunsaturated fatty acids,[81] a finding that was recently confirmed in the Rotterdam Study.[79] In contrast, in the Health Professionals Follow-up Study and Nurses' Health Study only intake of arachidonic acid was associated with a lower risk of PD.[78]

Table 4. *Dietary factors and PD: antioxidant intake. Results from prospective, population-based epidemiological studies*

Study	Author (year)	Study size	PD cases	RR estimate	Category of comparison
Vitamin E:					
Health Professionals Follow-up Study	Zhang[68] (2002)	47,331 men	161	0.89 (0.58-1.34) (Total vitamin E intake)	(highest vs lowest quintile)
				0.65 (0.40-1.05) (Dietary intake only)	(highest vs lowest quintile)
Nurses' Health Study	Zhang[68] (2002)	76,890 women	210	0.58 (0.36-0.92) (Total vitamin E intake)	(highest vs lowest quintile)
				0.71 (0.44-1.14) (Dietary intake only)	(highest vs lowest quintile)
Honolulu Asia Aging Study	Morens[69] (1996)	8,006 men	84	0.83 (0.57-1.19)	(per ln of vitamin E intake)
Vitamin C:					
Health Professionals Follow-up Study	Zhang[68] (2002)	47,331 men	161	1.08 (0.66-1.78) (Total vitamin C intake)	(highest vs lowest quintile)
				1.55 (0.98-2.46) (Dietary intake only)	(highest vs lowest quintile)
Nurses' Health Study	Zhang[68] (2002)	76,890 women	210	0.88 (0.52-1.49) (Total vitamin C intake)	(highest vs lowest quintile)
				0.87 (0.54-1.42) (Dietary intake only)	(highest vs lowest quintile)
Beta-carotene:					
Health Professionals Follow-up Study and Nurses'Health Study	Zhang[68] (2002)	124,221 men and women	371	Pooled: 0.90 (0.63-1.30)	(highest vs lowest quintile)

RR: relative risk, ln: natural logarithm

Table 5. *Dietary factors and PD: intake of fat and fatty acids. Results from prospective, population-based epidemiological studies*

Study	Author (year)	Study size	PD cases	RR estimate	Category of comparison
Total calories:					
Health Professionals Follow-up Study	Chen[78] (2003)	47,331 men	191	1.11 (0.73-1.69)	(highest vs lowest quintile)
Nurses' Health Study	Chen[78] (2003)	88,563 women	168	0.91 (0.54-1.51)	(highest vs lowest quintile)
Honolulu Asia Aging Study	Abbott[81] (2003)	8,006 men	137	No association	(no RR estimate given)
Rotterdam Study	de Lau[79] (2005)	5,298	51	1.11 (0.81-1.50)	(per SD)
Total fat:					
Health Professionals Follow-up Study	Chen[78] (2003)	47,331 men	191	1.38 (0.87-2.18)	(highest vs lowest quintile)
Nurses' Health Study	Chen[78] (2003)	88,563 women	168	0.86 (0.52-1.43)	(highest vs lowest quintile)
Rotterdam Study	de Lau[79] (2005)	5,298	51	0.69 (0.52-0.91)	(per SD)
Saturated fat:					
Health Professionals Follow-up Study	Chen[78] (2003)	47,331 men	191	1.44 (0.92-2.25)	(highest vs lowest quintile)
Nurses' Health Study	Chen[78] (2003)	88,563 women	168	0.65 (0.37-1.16)	(highest vs lowest quintile)
Rotterdam Study	de Lau[79] (2005)	5,298	51	0.82 (0.61-1.10)	(per SD)
Animal fat:					
Health Professionals Follow-up Study	Chen[78] (2003)	47,331 men	191	1.42 (0.91-2.20)	(highest vs lowest quintile)
Nurses' Health Study	Chen[78] (2003)	88,563 women	168	0.65 (0.36-1.16)	(highest vs lowest quintile)

(continued on next page)

Table 5. *Dietary factors and PD: intake of fat and fatty acids.*
Results from prospective, population-based epidemiological studies (continued)

Study	Author (year)	Study size	PD cases	RR estimate	Category of comparison
Cholesterol:					
Health Professionals Follow-up Study	Chen[78] (2003)	47,331 men	191	1.10 (0.68-1.76)	(highest vs lowest quintile)
Nurses' Health Study	Chen[78] (2003)	88,563 women	168	0.79 (0.47-1.33)	(highest vs lowest quintile)
Honolulu Asia Aging Study	Abbott[81] (2003)	8,006 men	137	No association	(no RR estimate given)
Rotterdam Study	de Lau[79] (2005)	5,298	51	0.81 (0.59-1.10)	(per SD)
MUFA:					
Health Professionals Follow-up Study	Chen[78] (2003)	47,331 men	191	1.00 (0.63-1.61)	(highest vs lowest quintile)
Nurses' Health Study	Chen[78] (2003)	88,563 women	168	0.78 (0.45-1.35)	(highest vs lowest quintile)
Honolulu Asia Aging Study	Abbott[81] (2003)	8.006 men	137	No association	(no RR estimate given)
Rotterdam Study	de Lau[79] (2005)	5,298	51	0.68 (0.50-0.94)	(per SD)
PUFA:					
Health Professionals Follow-up Study	Chen[78] (2003)	47,331 men	191	0.82 (0.53-1.27)	(highest vs lowest quintile)
Nurses' Health Study	Chen[78] (2003)	88,563 women	168	0.92 (0.56-1.49)	(highest vs lowest quintile)
Honolulu Asia Aging Study	Abbott[81] (2003)	8,006 men	137	Signficant inverse ass.	(no RR estimate given)
Rotterdam Study	de Lau[79] (2005)	5,298	51	0.66 (0.46-0.96)	(per SD)

RR: relative risk, SD: standard deviation, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids

Table 6. *Dietary factors and PD: intake of vitamins involved in homocysteine metabolism and other nutrients. Results from prospective, population-based epidemiological studies*

Study	Author (year)	Study size	PD cases	RR estimate	Category of comparison
Folate:					
Health Professionals Follow-up Study	Chen[85] (2004)	47,341 men	248	1.1 (0.7-1.7)	(highest vs lowest quintile)
Nurses' Health Study	Chen[85] (2004)	88,716 women	167	1.4 (0.8-2.4)	(highest vs lowest quintile)
Rotterdam Study*		5,298	72	0.8 (0.4-1.5)	(highest vs lowest tertile)
Vitamin B12:					
Health Professionals Follow-up Study	Chen[85] (2004)	47,341 men	248	1.0 (0.7-1.4)	(highest vs lowest quintile)
Nurses' Health Study	Chen[85] (2004)	88,716 women	167	1.0 (0.7-1.4)	(highest vs lowest quintile)
Rotterdam Study*		5,298	72	1.1 (0.6-2.0)	(highest vs lowest tertile)
Vitamin B6:					
Health Professionals Follow-up Study	Chen[85] (2004)	47,341 men	248	1.0 (0.6-1.6)	(highest vs lowest quintile)
Nurses' Health Study	Chen[85] (2004)	88,716 women	167	1.1 (0.6-1.8)	(highest vs lowest quintile)
Rotterdam Study*		5,298	72	0.5 (0.2-1.0)	(highest vs lowest tertile)
Dairy products:					
Health Professionals Follow-up Study	Chen[82] (2002)	47,331 men	210	1.8 (1.2-2.8)	(highest vs lowest quartile)
Nurses' Health Study	Chen[82] (2002)	88,563 women	184	1.1 (0.7-1.7)	(highest vs lowest quartile)
Milk:					
Honolulu Asia Aging Study	Park[83] (2005)	7,504 men	128	2.3 (1.3-4.1)	(highest vs lowest group)

**Unpublished results*

Dietary factors related to homocysteine metabolism

Given the potential neurotoxic effects of homocysteine, intakes of nutrients that influence homocysteine levels (vitamin B6, vitamin B12 and folate) have been investigated in relation with PD. In one large prospective study no significant associations were observed,[85] whereas as yet unpublished results from the Rotterdam Study show a significantly decreased risk of PD with higher intake of vitamin B6 in the absence of an association for vitamin B12 and folate. This possibly indicates a neuroprotective effect of vitamin B6 that is unrelated to homocysteine metabolism, most likely through antioxidant capacities.[86]

Inflammation

The role of inflammation in the pathogenesis of PD is as yet unclear. Upregulation of cytokines was found in the brain and cerebrospinal fluid of PD patients and activated glial cells and reactive astrocytes have been observed in post-mortem material.[87, 88] However, it is uncertain whether this immune response is the cause or rather a consequence of neurodegeneration, as so far no prospective studies have been performed that investigated inflammatory markers in relation to PD. In one epidemiological study however, the use of non-steroid anti-inflammatory drugs (NSAIDs) was associated with a significantly decreased risk of PD, which may indicate a potential neuroprotective role of these drugs.[89]

Estrogens

The role of estrogen in PD is as yet unclear and still disputed. The higher prevalence and incidence figures for PD in various epidemiological studies have prompted the hypothesis that female sex hormones would somehow protect against neuronal cell death. Animal studies have provided evidence for beneficial effects of estrogens on PD. Estradiol was shown to increase dopamine synthesis and release in rats,[90, 91] and pretreatment with estradiol protected against MPTP-induced loss of dopaminergic neurons in mice.[92] Several studies suggest that estrogen may protect neurons via antioxidant effects, as estradiol was found to attenuate free radical formation.[93] In a small trial in postmenopausal women with PD, significant improvement of motor function was seen in women who received estrogens.[94] Case-control studies on the relationship between use of estrogens and PD risk show conflicting results,[95-97] as well as those regarding length of the reproductive period.[96, 98] Large observational studies that prospectively study the effect of estrogen levels or hormone therapy on the risk of PD therefore are needed, but no such study has been conducted so far.

Table 7. *Gene mutations identified in familial PD*

Gene/ Locus		Chromosome	Inheritance	Clinical features	Protein	Protein function
Alpha-synuclein [110,112]	(PARK 1)	4q21	AD	similar to IPD young onset, rapid progression	Alpha-synuclein	Lewy Body component
Parkin [110,112]	(PARK 2)	6q25.2-q27	AR	frequent dystonia, slow progression, no Lewy Bodies	Ubiquitin ligase	UPS component
UCHL-1 [110,112]	(PARK 5)	4p14	AD	similar to IPD	UCHL-1	UPS component
DJ-1 [110,112]	(PARK 7)	1p36	AR	young onset	DJ-1	Response to oxidative stress
PINK1 [112,113]	(PARK 6)	1p35-p36	AR	early onset, benign course levodopa-responsive	PTEN-induced kinase	Protection against mitochondrial dysfunction
LRRK2 [114]	(PARK 8)	12p11.2-q13.1	AD	similar to IPD	Dardarin	Unknown
NR4A2 [112,115]	(Nurr1)	2q22-q23	?*		Nuclear receptor	Differentiation / survival of dopaminergic neurons
PARK 3 [110]		2p13	AD	similar to IPD, typical Lewy Bodies		
PARK 4 [110]		4p16	AD	similar to IPD, plus dementia and dysautonomia young onset		
PARK 9 [112]		1p36	AR			
PARK 10 [110]		1p32	?	similar to IPD		

*AD: autosomal dominant, AR: autosomal recessive, IPD: idiopathic PD, UPS: ubiquitin proteasome system *Unclear whether causal or susceptibility gene*

PD and cancer

Epidemiological evidence suggests a reduced incidence of many common types of cancers in individuals with PD.[99-103] The initial hypothesis that this finding might have resulted from the inverse association between smoking and PD did not hold, since a reduced incidence has been described for both smoking-related and non-smoking-related cancers.[101, 104] An alternative hypothesis that was brought up recently states that a specific genetic background that can protect from cancer might also predispose an individual to neurodegeneration in PD, or vice versa.[104] Several of the genes that have been identified in familial PD were studied in cancer research before their link with PD was recognized. Some of the genes that cause familial PD (PINK1, UCHL-1, LRRK-2 and DJ-1) seem to have a peripheral role in the cell cycle and mutations in these genes might theoretically influence cancer risk. Although this theory is attractive as a potential explanation for the possible link between cancer and PD, it is still very preliminary. More research is needed to confirm the existence of such an association and to clarify much of the remaining questions.[104]

Genetic risk factors

Methodological considerations

Monogenetic causes do not appear to play a primary role in the majority of PD patients. Although in several studies a positive family history has been associated with an increased risk of PD,[105-107] in most cases a clear mode of inheritance could not be established. PD has therefore long been thought to be a purely sporadic disease, given that familial clustering might simply reflect the effect of shared environmental factors and does not necessarily imply a causal genetic pattern. A significant effect of genetic factors however was found in a study among almost 20,000 male twins, but predominantly in PD with onset before the age of 50 years.[108] Since 1997, several families have been identified with parkinsonism that displays a clear Mendelian mode of inheritance, and familial PD is now estimated to make up about 10% of all cases. Although methodological issues at first sight may not be a major concern in monogenetic disease, the discovery of genetic variants of PD has brought up some definition problems. First, one might wonder whether diseases with such a well-described cause as a single gene mutation are still to be classified as 'Parkinson disease', as according to current criteria this requires the parkinsonian syndrome to be idiopathic. While they are usually referred to as 'familial PD', it might also be argued that these diseases make up a distinct category of parkinsonian syndromes, especially because many of the familial forms display some atypical features like early onset, dystonia or occurrence of dementia. Second, a considerable variability of clinical manifestations has been found within families with the same gene mutation,

whereas persons with different genetic defects and different neuropathology may clinically be indistinguishable from each other.[109] These findings have led to the notion of a spectrum of neurodegenerative diseases that partially overlap, where current classification systems do not longer suffice.[110]

Sporadic cases of PD are generally thought to be the result of complex interactions between different genetic and environmental factors. Many epidemiological studies have tried to identify susceptibility genes that might contribute to PD risk, usually through a candidate gene approach in which polymorphisms of a gene potentially involved in PD are compared between patients and controls. A major issue is that of insufficient statistical power. Given the supposed multifactorial etiology underlying PD, the impact of each individual susceptibility gene is expected to be only modest and large numbers of case-control pairs are required to detect these small effects. Many of the epidemiological studies on susceptibility genes have found no effects, or weak associations that could not be reproduced in a different setting. Apart from the fact that many studies were underpowered, interpretation of results and comparison across studies is also complicated by methodological differences. Genetic association studies may differ with respect to methods and accuracy of PD diagnosis, the selection of control subjects, or choice of a specific polymorphism of the candidate gene under study. Besides, certain polymorphisms, especially those involved in toxin metabolism, might only increase PD risk in combination with particular environmental exposures or lifestyle factors, which are often not assessed.[111]

Causative genes

So far, six genes have been identified that cause monogenetically inherited PD, three with an autosomal dominant inheritance pattern (alpha-synuclein, UCHL-1 and LRRK-2) and three associated with autosomal recessive disease (parkin, DJ-1 and PINK1). In addition, one potential causative gene (NR4A2) and another four loci with as yet unknown genes are identified which are associated to familial forms of parkinsonism (PARK3, PARK4, PARK9, PARK10). As these genetic advances have been extensively reviewed previously[110, 112] we only summarize current genetic knowledge on monogenetic PD in an updated[113-115] table (table 7).

Susceptibility genes

A great number of potential susceptibility genes and their polymorphisms have been investigated in population-based association studies. Most of these were hypothesized to contribute to the risk of sporadic PD based on a biologically argued role in PD pathogenesis. The most frequently studied candidate genes are summarized in table 8 (grouped by supposed underlying pathogenetic mechanism) and include

Table 8. *Most frequently studied candidate genes for PD, grouped by supposed underlying pathogenetic mechanism*

Candidate gene	Abbreviation	Meta-analysis (ref.)
Dopaminergic metabolism		
Monoamine oxidase A / B [111]	(MAO-A)	NS[111]
	(MAO-B)	Sign[111]
Catechol-O-Methyltransferase [111]	(COMT)	NS[111]
Tyrosine hydroxylase [111]	(TH)	NA
Dopamine transporter [111]	(DAT)	NS[111]
Dopamine receptor 2, 4 [111]	(DRD2)	NS[111]
	(DRD4)	NS[111]
Xenobiotic metabolism		
Debrisoquine-4-hydroxylase [116,117]	(CYP2D6)	NS[116]
N-acetyltransferase 2 [111,117]	(NAT2)	Sign[111]
Glutathione transferases [111]	(GSTT1)	Sign[111]
	(GSTM1)	NS[111]
	(GSTP1)	NS[111]
	(GSTZ1)	NS[111]
Multidrug resistance [118]	(MDR-1)	NA
Mitochondrial metabolism		
tRNA-glu [111]		Sign[111]
ND2 [111]		NS[111]
Homocysteine metabolism		
Methylenetetrahydrofolate reductase [123,124]	(MTHFR)	NA
Lipoprotein-related		
Apolipoprotein E [117,119]	(APOE)	Sign[119]
Genes involved in other neurodegenerative diseases		
Tau-gene, H1 haplotype [121]		Sign[121]
Hormonal factors		
Estrogen receptor gene [122]	(ER)	NA

NS: not significant, NA: not applicable (not evaluated in meta-analysis)

genes involved in dopamine metabolism, mitochondrial metabolism, activation or detoxification of xenobiotics and exogenous toxins, other neurodegenerative diseases, familial PD and other putative relations, such as those to lipoproteins, hormonal factors and homocysteine metabolism.[111, 116-124] Unfortunately, many studies were methodologically flawed in terms of number of included subjects and inappropriate selection of controls. Most have shown either no or only small effects of these candidate genes, and often results have been contradicted or could not be replicated. Meta-analyses have been performed to allow pooling or aggregation of study results in order to increase statistical power. A meta-analysis of 84 association studies of 14 genes showed that polymorphisms in four genes (NAT2, MAOB, GST-T1, and tRNAGlu) were significantly associated with PD.[111] Another meta-analysis recently showed that the ε2 allele of the APOE gene was associated with an increased risk of PD[119] and a meta-analysis of seven case-control studies showed a significantly increased risk of PD in persons homozygous for the tau H1 haplotype.[121] The pathophysiologic significance of these polymorphisms is however not yet clear.

Prognosis

Methodological considerations

Several studies investigated the prognosis of PD patients with respect to life expectancy and the risk of developing dementia. Most of these studies have been performed on patient groups from specialized centers or have used medical records for case finding. Because patients with relatively mild symptoms probably are under-represented in these studies, results may be biased and not representative for the general population. Few prognostic studies on PD have used community- or population-based cohorts that most likely reflect a broader spectrum of disease severity and are expected to yield more accurate results. Besides, estimates of prognostic outcomes may be biased as in most studies a cohort of prevalent PD patients was followed, which means that these studies actually estimate prognosis after enrolment instead of prognosis after PD diagnosis. In several prospective epidemiological studies mortality rates of PD patients and controls were found to diverge more with increasing time since diagnosis.[125, 126] In case of a long delay between diagnosis and inclusion in the study this may therefore have led to overestimation of mortality risks and probably other aspects of prognosis.

Mortality

PD has been associated with a reduced life expectancy in most epidemiologic studies, only few of which were conducted before levodopa therapy became available.

In 1967, Hoehn and Yahr reported a standardized mortality ratio of 2.9, but their result was based on a clinical population.[127] The introduction of levodopa in 1969 initially seemed to return life expectancy to approximately normal.[128-130] However, the observed decline in mortality rates for PD patients was followed by a rise in the late 1970s and early 1980s, presumably because levodopa delayed the death of a cohort of elderly patients with on average 5 years, leading to a catch-up in mortality several years later.[131, 132] Although levodopa improved the quality of life in the majority of PD patients and probably prolonged life expectancy to some extent, most of the studies dating from after the introduction of levodopa still report a reduced survival in PD patients compared to persons without the disease, with mortality hazard ratios varying between 1.5 and 2.7 (see table 9).[36, 125, 126, 133-140]

Dementia, falls and institutionalization

One of the late non-motor manifestations of PD is the development of dementia, which affects about 25 to 40 % of patients with PD.[141] The main feature of this PD dementia, thought to be caused by spread of degeneration and Lewy Body formation to the cerebral cortex and limbic structures,[141] is impairment of executive functions. Besides, co-existing pathology of the Alzheimer type might also cause dementia in PD.[142] Epidemiological studies have reported relative risks of becoming demented for PD patients compared to those without the disease ranging between 1.7 and 5.9.[141, 143-146] Factors that seemed to influence the risk of dementia, although not consistently across studies, are age at onset of PD, disease duration or severity and APOE genotype.[120, 144, 146] Dementia seems in great part responsible for the reduced life expectancy of PD patients, as mortality risk was found to be only modestly increased in those who do not become demented.[146] Among other complications that were found to occur more often in PD patients were visits to a physician or the emergency-department, falling and nursing home residency.[138, 147]

Conclusions

In spite of the recent major advances in the genetics of PD, the vast majority of cases still are thought to be sporadic, resulting from interplay between several environmental factors and susceptibility genes. The role of environmental factors is best evaluated through large observational studies, preferably prospective cohort studies with a long period of follow-up, as these provide the opportunity to study long-term and potentially interactive effects. However, given the supposed multifactorial etiology of PD, each potential risk factor may be expected to contribute only modestly to PD risk and effects of risk factors may thus be difficult to demonstrate, especially when

Table 9. *Mortality hazard ratios for Parkinson disease*

Author (year)	Location (country)	Type of study	No. of PD cases	Prevalent / incident cases	Comparison category	Duration of follow-up	Mortality Hazard Ratio
Morens[36] (1996)	Honolulu (Hawai)	Population-based cohort study	92	incident	All other participants	29 yrs	2.5 (for ages 70-89)
Louis[136] (1997)	New York (US)	Hospital-based study	180	prevalent	controls	3.0 yrs	2.7 (1.7 - 4.4)
Hely[126] (1999)	Sidney (Australia)	Multicentre study	130	prevalent	SMR given	10 yrs	1.58 (1.21 - 2.02)
Berger[138] (2000)	Europe (5 countries)	5 population-based cohort studies	252	prevalent	All other participants	variable	2.3 (1.8 - 3.0)
Morgante[137] (2000)	Sicily (Italy)	Community-based survey	59	prevalent	118 controls	8 yrs	2.3 (1.6 - 3.39)
Guttman[135] (2001)	Ontario (Canada)	Administrative database	15,304	prevalent	Controls from database	6 yrs	2.5 (2.4 - 2.6)
Elbaz[125] (2003)	Olmsted (Min, US)	Register-based study	196	incident	185 controls	7.2 yrs	1.6 (1.2 - 2.14)
Fall[134] (2003)	Sweden	Community-based study	170	prevalent	150 controls	9.4 yrs	2.4 (1.9 - 3.0)
Herlofson[133] (2004)	Rogaland (Norway)	Community-based cohort study	245	prevalent	SMR given	8.7 yrs	1.52 (1.29 - 1.79)
Hughes[139] (2004)	Leeds (UK)	Hospital-based study	90	prevalent	50 controls	11 yrs	1.64 (1.21 - 2.23)
de Lau[140] (2005)	Rotterdam (NL)	Population-based cohort study	166	both	All other participants	6.9 yrs	1.83 (1.47 - 2.26)

studies are small and underpowered or prone to bias due to their study design. Although many epidemiological studies on PD have been performed, remarkably few of them were of sufficient methodological quality. It is therefore not surprising that for many of the studied risk factors conflicting results have been obtained or observations could not be replicated in well-designed studies. As yet, only few factors have been identified that have been consistently related to the risk of PD, the most convincing of which are older age and smoking habits. More evidence on environmental factors involved in PD must come from the larger prospective cohort studies that are now starting to reach sufficient amounts of follow-up time and PD patients to confirm earlier results and to detect potentially new risk factors for this devastating disease.

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3 Risk of Parkinson disease

3.1 Incidence of parkinsonism and Parkinson disease in a general population

Abstract

Background

Parkinson disease is the second most common neurodegenerative disease and the proportion of people suffering from parkinsonism and Parkinson disease is expected to increase as populations age. Few studies investigated the incidence of parkinsonism and Parkinson disease in the general population, and most of these were based on clinical data only.

Objective

To estimate the incidence of parkinsonism and Parkinson disease in a general population.

Methods

In the Rotterdam study, a prospective population-based cohort study in people aged 55 years and older, we assessed age- and sex-specific incidence rates of parkinsonism and Parkinson disease among 6,839 participants who were free of parkinsonism at baseline. Case-finding involved in-person screening at both baseline and two follow-up visits, and additional information was obtained through continuous monitoring of the cohort by computer linkage to general practitioners' and pharmacy records.

Results

After a mean follow-up period of 5.8 years, 132 subjects with incident parkinsonism were identified, of whom 67 (51%) had Parkinson disease. The incidence of both parkinsonism and Parkinson disease increased with age, with incidence rates for Parkinson disease rising from 0.3 per 1000 person years in subjects aged 55-65 years, to 4.4 per 1000 person years for those aged 85 years and older. The overall age-adjusted incidence rate of any parkinsonism was not different in men and women, but men seem to have a higher risk for Parkinson disease (male-to-female ratio 1.54; 95% CI 0.95 – 2.51).

Conclusion

Incidence rates for both parkinsonism and Parkinson disease were higher than those reported by most previous studies. This may be due to our intensive case-finding methods involving in-person screening.

Introduction

The proportion of people with Parkinson disease (PD) is expected to increase with the aging of worldwide populations and to cause substantial economic and social burdens on society.[1,2] Despite the increasing importance of PD, the exact incidence of the disease in the general population is still unknown. In contrast to prevalence figures, data on the incidence of parkinsonism and PD are scarce.[3] In all but three previous studies,[4-6] case finding was based on medical records. One of the limitations of these register-based studies is their failure to include patients who have not come to medical attention.[3,7] In several thorough population-based prevalence surveys on parkinsonism and PD, it was shown that underestimation of the prevalence by medical records may increase up to 52%, and increases with age.[8-10] We determined age- and sex-specific incidence rates of parkinsonism and PD within the Rotterdam Study, a prospective population-based cohort study of subjects aged ≥55 years. Search methods involved in-person screening and physical examination of participants at baseline and follow-up visits, and additional information was obtained by computer linkage to general practitioners' and pharmacy records.

Methods

The Rotterdam Study

The Rotterdam Study is a prospective population-based cohort study on determinants of diseases in the elderly population.[11] All inhabitants of a suburb of Rotterdam, the Netherlands, aged ≥55 years were eligible. Of these, 7,983 subjects (response rate, 78%) agreed to participate. Baseline examinations took place between 1990 and 1993. All participants were interviewed at their homes and subsequently underwent extensive physical examination, which included a neurological screening, at the research center. Follow-up examinations took place in 1993 to 1994 and 1997 to 1999. The cohort also was continuously monitored for major disease outcomes and mortality through linkage of the study database to general practitioners' medical files. Informed consent was obtained from each participant, and the Medical Ethics Committee of the Erasmus Medical Center in Rotterdam approved the study.

Study population

At baseline, 6,969 participants underwent neurologic screening. Parkinsonism was diagnosed in 130 participants, 99 of whom had PD.[7] This resulted in a cohort of 6,839 subjects at risk to develop any kind of parkinsonism during the follow-up period. For the cohort at risk for PD, we further excluded 273 persons with dementia at baseline

because a diagnosis of dementia before the onset of parkinsonism precludes the diagnosis of PD.[7] Thus, the cohort at risk for PD consisted of 6,566 subjects free of parkinsonism and dementia at baseline.

Case-finding and diagnostic procedure

At baseline and follow-up evaluation, we used a two–phase design to identify subjects with parkinsonism or PD. In the first phase, all participants were screened at the research center for signs of parkinsonism (e.g., resting tremor, rigidity, bradykinesia, or impaired postural reflexes) in a standardized way. Those who screened positive received a structured clinical work-up, comprising the motor examination of the Unified PD Rating Scale (UPDRS)[12] and neurologic examination and history taking by a research physician specialized in neurologic disorders, to establish parkinsonism and to classify subtypes. Subjects who were suspected of having PD were invited for a further evaluation by a neurologist. In addition to the in-person follow-up evaluation, the cohort was continuously monitored for detection of new cases of parkinsonism through a surveillance system by computer linkage with the general practitioners' automated medical record systems.

Table 1. *Baseline characteristics of the study population*

	Cohort at risk for any parkinsonism, no (%)	Cohort at risk for PD, no (%)
N	6,839	6,566
Women	4,070 (59.5)	3,876 (59.0)
Age at baseline		
55 - 65 y	2,537 (37.1)	2,53 (38.5)
65 - 75 y	2,456 (35.9)	2,425 (36.9)
75 - 85 y	1,406 (20.6)	1,294 (19.7)
85 y and older	440 (6.4)	317 (4.8)
mean (SD)	69.4 (9.1)	68.8 (8.7)
Living in nursing home	518 (7.6)	336 (5.1)
Dementia	273 (4.0)	
History of stroke	194 (2.8)	155 (2.4)

PD : Parkinson disease, SD : standard deviation

Through this system, we were notified of incident cases of parkinsonism and had access to those subjects' medical records. If possible, a neurologist also examined these participants to confirm the diagnosis. We also used the information obtained from this surveillance system for subjects who could not be re-examined in person at follow-up evaluation (because of death, migration, disease, logistic reasons, or refusal). Furthermore, information on all participants who were prescribed antiparkinsonian drugs, identified by means of a computerized pharmacy database, was reviewed to check whether no cases had been missed.

Parkinsonism was diagnosed if two or more cardinal signs (e.g., resting tremor, rigidity, bradykinesia, or impaired postural reflexes) were present in a subject not taking antiparkinsonian drugs, or if in a person treated with antiparkinsonian medication, one or more signs, documented by medical history, had improved. PD was diagnosed if all causes of secondary parkinsonism, as well as dementia before the onset of parkinsonism, had been excluded.

The diagnostic criteria for the several subtypes of parkinsonism have been reported previously.[7] In short, parkinsonism resulting from other causes included 1) parkinsonism associated with dementia (with dementia onset clearly preceding the onset of parkinsonism); 2) drug-induced parkinsonism (use of neuroleptics or other antidopaminergic drugs in the 6 months preceding the onset of symptoms and without history of parkinsonism); 3) parkinsonism related to cerebrovascular disease (with a clear time relationship between cerebrovascular event and onset of atypical parkinsonism, preferably supported by neuroimaging, usually without tremor); 4) parkinsonism in multiple system atrophy or progressive supranuclear palsy; and 5) parkinsonism unspecified. Included in this latter category were subjects with more than one possible cause or with no clear time relationship between the possible cause and the onset of parkinsonism, as well as subjects in whom all other possible causes of parkinsonism could be excluded but who did not respond to antiparkinsonian drugs.

For the age of onset of PD, we took the age at midpoint between the examination in which parkinsonism first was identified and the preceding examination. An objective indication of the time when the first symptom actually appeared is hard to achieve for all patients in an equally adequate way because PD has an insidious onset. Given the relatively short screening intervals, we considered age between two subsequent examinations a good approximation of the actual age at onset.

Data analysis

We obtained age-specific incidence rates per 10-year age group by dividing the number of incident cases by the number of person-years at risk (calculated by adding each participant's contribution of follow-up time per age group). The 95% CIs were

based on the Poisson distribution. For participants who did not develop parkinsonism, the follow-up period ended at the second follow-up examination, at the date of invitation for the second follow-up examination (for nonresponders), or death. In case of incident parkinsonism, follow-up time ended at onset of parkinsonism or PD. Because subjects who developed dementia were considered no longer to be at risk for PD, follow-up time for incident PD, but not for parkinsonism, ended at onset of dementia.

Incidence rates of parkinsonism and PD per 10-year age group were calculated overall and for men and women separately. We transformed the age-specific incidence rates into cumulative incidences to estimate the risk of developing parkinsonism or PD between the age of 55 and 85 years, assuming no competing causes of death, for men and women. Age-adjusted male-to-female ratios for the incidence rates were calculated for each age category using Cox proportional hazards regression analysis.

Results

Table 1 shows the baseline characteristics of the cohort at risk for any parkinsonism and the cohort at risk for PD. Of the total cohort (n = 6,839), 1,364 subjects (19.9%) died during the follow-up period. Follow-up evaluation was virtually complete (99%) until December 31, 1999, with complete information during the first follow-up period and 67 persons lost to follow-up evaluation during the second follow-up period. After 39,879 person-years of follow-up evaluation (mean follow-up period, 5.8 years), 132 new cases of parkinsonism were identified. Of these, 67 subjects (51%) had PD, which makes PD the most common type of parkinsonism.

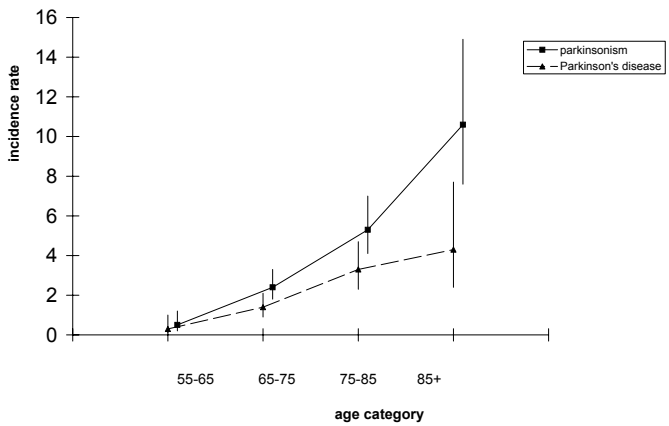

Figure 1.
Incidence rates (with 95% CIs) of parkinsonism and Parkinson disease per 1000 person years per age category

Fifty cases of PD were detected through the structured workup at the research center. Among participants who refused the follow-up visit or died during the interval, 17 cases of PD were identified through the continuous surveillance system by linkage to medical files. Of the incident PD cases, 26 had not been diagnosed with PD before (52% of those screened in person, 39% of all incident PD cases). Two cases that were diagnosed as PD during the first follow-up round were later revised when at the second follow-up evaluation no parkinsonian signs were observed while the participants were not using any antiparkinsonian medication. Besides PD, the other diagnoses were parkinsonism associated with dementia (18%), drug-induced parkinsonism (12%), cerebrovascular disease (5%), multiple system atrophy or progressive nuclear palsy (2%), and parkinsonism unspecified (12%).

In table 2, age- and sex-specific incidence rates and cumulative incidence, as well as age-adjusted male-to-female ratios, are shown for the cohort at risk for any parkinsonism. For the subjects at risk for PD, similar information is presented in table 3.The overall incidence rate was 3.3 per 1000 person-years (95% CI, 2.8 to 3.9) for any parkinsonism and 1.7 per 1000 person-years (95% CI, 1.4 to 2.2) for PD. The risk of developing any parkinsonism between the age of 55 and 85 years, assuming no competing causes of death, was 7.7% for women and 8.5% for men, and the risk of PD was 4.2% for women and 6.1% for men. As also shown in figure 1, incidence rates of parkinsonism and PD show a strong and consistent increase with age for men and women. Overall, men and women had identical risks for parkinsonism (age-adjusted male-to-female ratio 0.99; 95% CI, 0.68 to 1.44). However, the risk for PD was higher for men in all age categories except one (65 to 75 years), with the male-to-female ratio increasing with age (also shown in figure 2). The overall age-adjusted male-to-female ratio was 1.54, which was borderline significant (95% CI, 0.95 to 2.51).

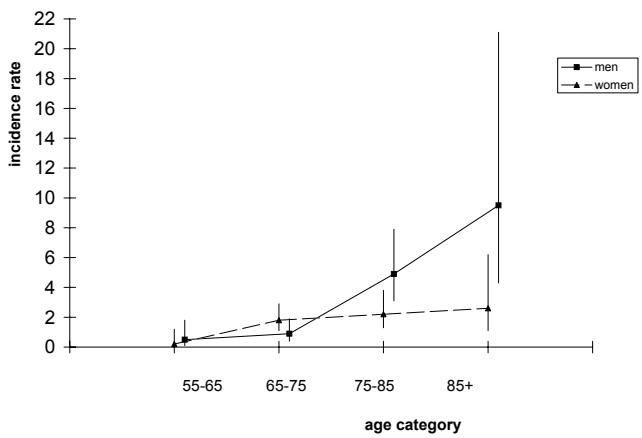

Figure 2.
Incidence rates (with 95% CIs) of Parkinson disease per 1000 person years per age category, in men and women

Table 2. *Incidence rates per 1000 person years (95 % CI) for any parkinsonism per 10-year age category*

	Men			Women			Overall				
Age group	Cases	Person years	Incidence rate (95% CI)	Cases	Person years	Incidence rate (95% CI)	Cases	Person years	Incidence rate (95% CI)	Male-to-female ratio (95% CI)	
55-65 y	3	4,356	0.7 (0.2-2.1)	2	6,017	0.3 (0.1-1.3)	5	10,374	0.5 (0.2-1.2)	2.08	(0.35-12.47)
65-75 y	10	7,104	1.4 (0.8-2.6)	29	9,101	3.2 (2.2-4.6)	39	16,206	2.4 (1.8-3.3)	0.38	(0.18-0.80)
75-85 y	25	3,660	6.8(4.6-10.1)	29	6,445	4.5 (3.1-6.5)	54	10,106	5.3 (4.1-7.0)	1.33	(0.77-2.31)
85+	9	744	12.1(9.3-23.3)	25	2,448	10.2(6.9-15.1)	34	3,193	10.6(7.6-14.9)	1.19	(0.50-2.85)
Total	47	15,865	8.5 %*	85	24,011	7.7 %*	132	39,878	7.9 %*	0.99†	(0.68-1.44)

** Cumulative incidence (risk of parkinsonism between age 55 and 85), assuming no competing causes of death.*
† Age-adjusted ratio.

Discussion

We assessed age- and sex-specific incidence rates of parkinsonism and PD in a large, prospective population-based cohort study. In men and women, we found that the incidence of parkinsonism and PD increased with age. The overall age-adjusted incidence rate of parkinsonism did not differ between men and women, but men seem to have a higher risk for PD.

Our study had several strengths. We followed a large cohort over a period of nearly six years, resulting in a large number of person-years of follow-up. At baseline and at follow-up evaluation, participants were screened in person and in a similar way, and if screened positive, received a similar diagnostic workup. Only a small proportion of the subjects who were suspected of parkinsonism during the screening did not undergo the second, diagnostic phase. For those, as well as for the persons who were screened at baseline but could not be screened at follow-up, we were able to achieve a complete follow-up evaluation during the first follow-up period and a virtually complete follow-up evaluation during the second follow-up period by using a population surveillance system. Our follow-up periods were relatively short. This reduced the proportion of subjects who could not be screened in person due to death, disease, or migration, and ensured a more complete case-ascertainment. It also allowed us in case of incident parkinsonism to take the midpoint between two subsequent examinations as a fairly accurate approximation of age at onset, which is usually hard to indicate exactly in a disease with an insidious onset like PD.

Furthermore, because of the continuous surveillance system, we had access to medical information concerning the period after symptom onset, providing data on response to medication and development of additional symptoms and thereby ensuring a more accurate diagnosis and subtyping of parkinsonism.

We found overall and age-specific incidence rates of parkinsonism and PD that are higher than most of the figures previously reported. Currently, there is no evidence suggesting that this can be explained by differences in environmental exposure or genetic makeup of the population of the Rotterdam Study, although we cannot completely exclude this possibility. We think that the high rates in our study are mainly the result of more intensive case finding methods. Most of the previously published studies were based on existing medical records.[13-17] Prevalence surveys have shown that a large proportion of patients with parkinsonism remain undiagnosed if search strategies only rely on medical records and no population screening is done.[3,7,9,10] As was reported by the Europarkinson group, this underestimation may vary from 11 to 52% and increases with age.[8] Only three previous studies on the incidence of PD involved in-person screening.[4-6] Two prospective population based studies in Italy

Table 3. *Incidence rates per 1000 person years (95% CI) for Parkinson disease per 10-year age category*

	Men			Women			Overall				
Age group	Cases	Person years	Incidence rate (95% CI)	Cases	Person years	Incidence rate (95% CI)	Cases	Person years	Incidence rate (95% CI)	Male-to-female ratio (95% CI)	
55-65 y	2	4,342	0.5 (0.1-1.8)	1	5,992	0.2 (0.0-1.2)	3	10,335	0.3 (0.1-1.0)	2.79	(0.25-30.75)
65-75 y	6	7,031	0.9 (0.4-1.9)	16	9,026	1.8 (1.1-2.9)	22	16,058	1.4 (0.9-2.1)	0.46	(0.18-1.16)
75-85 y	17	3,442	4.9 (3.1-7.9)	14	6,013	2.3 (1.4-3.9)	31	9,455	3.3 (2.3-4.7)	1.94	(0.96-3.94)
85 +	6	632	9.5 (4.3-21.1)	5	1,942	2.6 (1.1-6.2)	11	2,575	4.3 (2.4-7.7)	3.72	(1.13-12.19)
Total	31	15,447	6.1 %*	36	22,972	4.2 %*	67	38,422	4.9 %*	1.54†	(0.95-2.51)

** Cumulative incidence (risk of Parkinson disease between age 55 and 85), assuming no competing causes of death.*

† Age-adjusted ratio.

(ILSA) and Spain (NEDICES), both using a two-phase design, found incidence rates more or less similar to ours.[5,6] The third study, the Honolulu Heart Study (restricted to Hawaiian men of Japanese and Okinawan ancestry), showed lower figures.[4] However, this study had no in-person screening at baseline, and >20 years had elapsed between baseline and the follow up screening for PD. Therefore, subjects with early or mild parkinsonism who did not come to medical attention or were lost during the interval will not have been included in the incidence estimates.

Thirty-nine percent of the PD cases identified in our study had not been diagnosed with PD before and would thus not have been included as a PD case in a register-based study. In the NEDICES study, 53% of the cases with PD were newly identified through the screening process. This confirms the observation from the Europarkinson prevalence survey that the proportion of newly diagnosed patients in the Netherlands was relatively low compared with other European countries,[8] probably reflecting the low-threshold accessibility of Dutch medical services. Together with the increased awareness of parkinsonism and PD among general practitioners through participation in the Rotterdam Study, this could in part contribute to the relatively high number of cases observed in our study.

From pathology studies, it is known that there is always a certain amount of misdiagnosis of parkinsonism and PD.[18,19] Still, we do not find it likely that overdiagnosis of parkinsonism and PD has played a role in the high incidence figures because every participant was screened in person, and if possible, the diagnosis was confirmed by a neurologist applying strict diagnostic criteria. Moreover, we continued following participants after a diagnosis of parkinsonism or PD had been made. This enabled us to revise cases when additional symptoms appeared or participants did not respond to medication, thereby reducing the possibility of incorrectly diagnosing subjects with multiple system atrophy or dementia with Lewy bodies (DLB) as having PD.

We found that the incidence rates of parkinsonism and PD increased with age. No decrease in the incidence in the highest age groups was observed, as was previously reported.[4,16] A possible explanation is that the decrease in some of the earlier studies was an artificial one caused by selective nonresponse or loss to follow-up in the highest age groups. Furthermore, older people may not consult their physician for parkinsonian signs because these signs are often considered to be part of the normal aging process.[20] Therefore, parkinsonism in the oldest patients may be missed when case finding is based on medical records only.

We identified 26 subjects who developed parkinsonism after onset of dementia (20% of all incident parkinsonism). These participants might have DLB. DLB is suggested to be the second most common type of degenerative dementia in the elderly population and is clinically characterized by fluctuating cognitive impairment,

attentional deficits, psychiatric symptoms (especially visual hallucinations), and mild extrapyramidal features that usually develop after impairment of cognitive function.[21] Thus far, few population-based studies have been performed on the prevalence of DLB,[22] and incidence was studied only in the clinical setting of a memory and dementia assessment unit in Spain.[23] Although DLB was not specifically assessed in our study, our data might give some clues about the incidence of DLB in a general population. Assuming that subjects with parkinsonism associated with dementia all had DLB, the overall incidence rate in this cohort aged ≥55 years would be 0.7 per 1000 person-years (95% CI, 0.4 to 1.0).

Although only borderline significant, we found evidence for a higher incidence of PD in men. Previous studies show conflicting results on differences of the incidence of PD in men and women.[3] Several studies found a significant higher risk in men,[5,6,13,16] but a minority reported a female preponderance.[24,25] The exact cause of the possibly higher incidence of PD in men is still unknown, but it has been suggested that hormonal factors play a role in the etiology of PD and that estrogens may provide neuroprotective effects.[26-28]

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3.2 Subjective complaints precede Parkinson disease

Abstract

Background

Neuronal degeneration and dopamine loss in the preclinical phase of Parkinson disease may produce subtle complaints before clinically recognizable symptoms emerge.

Objective

To examine whether subjective complaints of stiffness, slowness, tremors, or postural imbalance in persons without clinical signs of parkinsonism are related to an increased risk of future Parkinson disease.

Methods

We recorded subjective complaints of stiffness, slowness of movement, tremors, and postural instability (falling or a feeling of imbalance) in a standardized interview in 6,038 non-demented participants in whom no parkinsonian signs were found on physical examination at baseline, and studied them prospectively for the occurrence of incident Parkinson disease. Participants were examined in-person both at baseline (1990-1993) and two follow-up visits (1993-1994 and 1997-1999) and the cohort was continuously monitored through computerized linkage of the study database to general practitioners' medical records.

Results

Participants who reported stiffness, tremors, or imbalance at baseline had a significantly increased risk to develop Parkinson disease during follow-up (hazard ratios (95% confidence intervals) for stiffness 2.11 (1.25 to 3.55), for tremor 2.09 (1.12 to 3.90) and for imbalance 3.47 (1.69 to 7.00)).

Conclusion

Subjective complaints of stiffness, tremors and imbalance are associated with an increased risk of future Parkinson disease and may reflect early effects of dopamine shortage, even when standard neurological testing cannot yet demonstrate any motor symptoms.

Introduction

Parkinson disease (PD) is caused by a selective degeneration of the dopaminergic neurons in the substantia nigra of the brain.[1] The typical clinical signs of PD (tremor, rigidity, bradykinesia, and postural instability) start to appear when degeneration and associated dopamine loss exceed 50%.[2,3] Manifest PD is thus preceded by a preclinical phase of several years, during which neuronal degeneration develops without motor symptoms being present yet.[4] Evidence suggests however that non-motor abnormalities may occur during this phase, such as olfactory dysfunction, personality disturbances, and depression.[3,5-8] More general non-specific symptoms have also been reported to predate the typical PD signs for several years.[9]
We hypothesized that moderate dopamine deficiency in preclinical PD might result in subtle subjective complaints specifically related to motor function, and we examined in a prospective population-based cohort study whether these complaints were associated with an increased risk of PD in the future.

Methods

The Rotterdam study

The Rotterdam study is a prospective, population-based cohort study on determinants of diseases in the elderly.[10] Of all inhabitants aged 55 years and older of a district of Rotterdam, 7,983 subjects (response rate 78%) agreed to participate. Both at baseline (1990-1993) and two follow-up rounds (1993-1994 and 1997-1999), all participants were interviewed and underwent extensive physical examination, including cognitive screening and screening for parkinsonian signs.[11-13] In addition, the cohort was continuously monitored for major disease outcomes and mortality through computerized linkage to general practitioners' medical files. Informed consent was obtained from each participant and the Medical Ethics Committee of the Erasmus Medical Center Rotterdam approved the study.

Subjective complaints assessed during the baseline interview

At baseline, all participants were interviewed at their homes by means of a standardized questionnaire. This questionnaire included a set of five symptom-specific questions that concern the four cardinal signs characteristic of PD. Participants were asked to indicate whether they ever experienced stiffness (rigidity), tremors of head, arms or legs (resting tremor), slowness of movement (bradykinesia), falling, or a feeling of imbalance (both related to postural imbalance). We assessed imbalance in a stepwise fashion by first asking participants whether they ever experienced dizziness, and if so,

to specify whether this concerned a near-fainting sensation (presyncope), spinning sensation (vertigo), a feeling of imbalance (disequilibrium), or the perception of lacking control over leg movement.

Assessment of Parkinson disease

Both at baseline and at follow-up, we used a two–phase design to identify subjects with PD.[12,13] All participants were screened for parkinsonian signs (rigidity, resting tremor, bradykinesia, and impaired postural reflexes) in a standardized way. Individuals who screened positive received a structural diagnostic workup comprising the Unified Parkinson's Disease Rating Scale[14] and neurological examination. Additional information obtained from the computerized surveillance system was reviewed by a panel of neurologists and research physicians. A neurologist examined persons who were suspected of having PD to confirm the diagnosis. Parkinsonism was diagnosed if at least two parkinsonian signs were present in a subject not taking antiparkinsonian drugs, or if at least one sign had improved after medication was started. PD was diagnosed when all causes of secondary parkinsonism (parkinsonism due to dementia, use of neuroleptics, cerebrovascular disease, multiple system atrophy, or progressive supranuclear palsy) could be excluded.

Table 1. *Baseline characteristics of the study population*

No.	6,038
Age, yr (SD)	68.5 (8.5)
Women	3,569 (59.1)
Subjective complaints:	
Stiffness	1,938 (32.1)
Tremor of arms, legs or head	636 (10.5)
Slowness of movement	1,262 (20.9)
Feeling of imbalance*	524 (10.7)
Falling	905 (15.0)
At least one complaint	3,146 (52.1)
At least two complaints	1,359 (22.5)

Values denote numbers (percentages), unless stated otherwise

** Assessed in 4,897 participants*

Study population

At baseline, 6,818 participants underwent neurological screening and provided information on subjective complaints. Of those, 116 subjects were diagnosed with any parkinsonism, including 89 PD cases.[12] To examine the relationship between baseline subjective complaints and the risk of incident PD, we excluded all participants diagnosed with any parkinsonism or dementia at baseline, as they could no longer fulfill the criteria for incident PD.[12] Because absence of parkinsonism does not preclude the presence of one cardinal sign, and to be maximally sure to evaluate subjects free of any parkinsonism, we only studied participants in whom none of the parkinsonian signs was found during the baseline neurological screening. This resulted in a study population at risk for PD of 6,038 persons free of parkinsonian signs and dementia at baseline.

Data analysis

Because the complaints that we studied specifically concerned the cardinal signs of PD, we first evaluated whether they were cross-sectionally related to the presence of PD at baseline. Odds ratios (ORs) for PD according to the presence of each complaint were calculated through binary logistic regression, adjusted for age and sex. To examine the association between baseline complaints in non-demented participants free of parkinsonian signs and the risk of future PD, we used Cox proportional hazards regression analysis to calculate Hazard ratios (HRs), adjusted for age and sex.

Results

Baseline characteristics of the study population are shown in table 1. The prevalence of self-reported complaints suggestive for parkinsonism appears considerably high in the elderly population, even in persons without any parkinsonian sign on physical examination. Over half of the participants (52.1%) reported at least one of the five complaints related to the typical features of PD. Stiffness was reported by almost one third of the study population, slowness by one fifth and tremors, imbalance, and falling each by over 10%.

Results of the cross-sectional analysis are shown in table 2. The vast majority (92.1%) of individuals diagnosed with PD at baseline had at least one complaint, and 75% reported at least two. As expected, reported stiffness, falling, and especially slowness of movement and tremors were strongly related to the presence of PD at baseline.

Table 3 shows the relation between reported complaints at baseline and the risk to develop PD during follow-up in non-demented participants who screened negative for any parkinsonian sign on routine examination. Follow-up information was

available for 99% of the participants, either through in-person re-examination or the continuous surveillance system. Complete in-person re-examination was performed in 80.6 % of the participants (84.6% of those still alive) in the first follow-up round and in 62 % (74.4 % of those still alive) in the second follow-up round. During a total of 35,429 person-years of follow-up (mean follow-up time, 5.8 years), 56 new cases of PD were identified. Of those, 43 cases were detected through the structured workup at the research center and 13 through the computerized surveillance system. Mean follow-up after disease onset of the incident cases was 4.3 (SD, 1.8) years.[11] Of the participants who developed PD during follow-up, 71.8% had reported at least one and 41.0% at least two of the complaints related to motor function at baseline. Complaints of stiffness at baseline were significantly associated with a more than two-fold increased risk of future PD (HR, 2.11; 95% confidence interval (CI), 1.25-3.55), as were reported tremors of arms, legs, or head (HR, 2.09; 95% CI, 1.12-3.90).

Table 2. *Association between reported complaints and the presence of PD at baseline, cross-sectionally. Odds ratios (95% CIs), adjusted for age and sex*

	OR (95% CI)
Stiffness	
No	1.00 (ref.)
Yes	2.43 (1.58-3.73)
Tremor of arms, legs or head	
No	1.00 (ref.)
Yes	13.61 (8.45-21.92)
Slowness of movement	
No	1.00 (ref.)
Yes	5.97 (3.74-9.52)
Feeling of imbalance	
No	1.00 (ref.)
Yes	1.27 (0.62-2.61)
Falling	
No	1.00 (ref.)
Yes	2.60 (1.66-4.08)

OR : odds ratio, CI : confidence interval

Interestingly, self-reported falling and slowness of movement, that were both significantly cross-sectionally related to the presence of PD at baseline, were not prospectively associated with an increased risk of future PD. A feeling of imbalance, on the other hand, showed a strong association with a future diagnosis of PD (HR, 3.47; 95% CI, 1.69-7.00), and no significant association with the presence of PD at baseline.

Comment

In this population-based study, a considerable proportion of the elderly experienced stiffness, slowness, tremors, falling, or a feeling of imbalance. Even among persons without any parkinsonian signs on clinical examination, over half of participants reported at least one of these complaints. Furthermore, persons who reported stiffness, tremors, or a feeling of imbalance at baseline had an increased risk to develop PD during follow-up. One of the major strengths of this study is its prospective design; complaints were assessed while future disease status was unknown and therefore recall bias is not an issue. Moreover, we used in-person screening of participants instead of register-based methods to assess parkinsonism. This limits the possibility that at baseline relatively early or mild cases of PD who had not sought medical attention yet were incorrectly diagnosed as not having PD. Besides, we restricted our study population to those participants in whom no abnormalities were found on physical examination specifically aimed at detecting parkinsonian signs. We also consider incorrect diagnoses during follow-up unlikely. Follow-up was almost complete, we applied strict diagnostic criteria for PD, and continued to follow participants after a diagnosis had been made, which enabled us to revise diagnoses on the basis of additional information if necessary.[12] Unfortunately, we could not perform in-person re-examinations in all of the participants due to death, refusal, or inability to visit the research center because of disease or handicaps. One might thus argue that incident PD cases may have been missed, probably especially those with the postural instability / gait disorder-dominant form of PD, which is more difficult to diagnose without standardized screening. However, since the majority of participants underwent direct examination and the computerized surveillance system provided virtually complete coverage for those who could not be seen, we think this possibility is limited and will not have affected our results substantially. Limitations of the baseline questionnaire include absence of questions on the duration of reported complaints, which precludes potentially interesting subanalyses, and the fact that both limb and head tremor were assessed in one question. Because head tremor usually is not considered typical for PD, a question about limb tremor only would probably have yielded more PD-specific results.

Our findings support the notion that clinically manifest PD is preceded by a preclinical phase that is not entirely asymptomatic. Subjective complaints related to motor function might indicate a very early phase of not yet diagnosable PD, during which dopamine loss is not sufficient to produce overt typical PD symptoms, but may result in subtle signs that are very mild or only intermittently present and therefore not likely to be detected in routine screening or examination. In our study, falling was related cross-sectionally, but not prospectively to the risk of PD, while for a subjective feeling of imbalance the opposite held true. Perceived imbalance may be a very early symptom that progresses to overt postural instability and an increased risk of falling later in the course of the disease. In the same way, a subjective feeling of stiffness may precede clinically detectable rigidity and patients may experience occasional tremors long before clinical examination confirms their perception.

Table 3. *Subjective complaints related to motor function and the risk of incident PD. Hazard ratios (95% CIs), adjusted for age and sex*

	HR (95% CI)
Stiffness	
No	1.00 (ref.)
Yes	2.11 (1.25-3.55)
Tremor of arms, legs or head	
No	1.00 (ref.)
Yes	2.09 (1.12-3.90)
Slowness of movement	
No	1.00 (ref.)
Yes	1.49 (0.84-2.65)
Feeling of imbalance	
No	1.00 (ref.)
Yes	3.47 (1.69-7.00)
Falling	
No	1.00 (ref.)
Yes	0.61 (0.27-1.36)

HR : hazard ratio, CI : confidence interval

It has been observed that prior to developing clinically manifest PD, many patients experience a range of non-specific symptoms, such as depression, fatigue, anxiety, or pain.[8,9] Similar findings have been observed in prospective studies of Alzheimer disease (AD) or cerebral small vessel disease, which showed that subjective memory complaints in persons without objective cognitive impairment were associated with an increased risk of developing dementia or more white matter lesions.[15-18] Both PD and AD are characterized by a phase of neuronal degeneration and loss of function before the appearance of typical symptoms,[4] and apparently such a preclinical phase also exists in slowly progressive vascular disease. Researchers have shown particular interest in possible markers of preclinical disease, since putative neuroprotective agents would ideally be administered as early in the degenerative process as possible and preferably before clinical symptoms appear.[19,20] Several potential biomarkers for presymptomatic PD are now being investigated, such as loss of the dopamine transporter detected by PET imaging, subtle abnormalities on psychological testing, olfactory dysfunction, and biochemical markers in serum or CSF.[5,20,21] A questionnaire on complaints related to motor function is in itself probably of limited use as a preclinical marker. In spite of the strong associations between self-reported complaints and the risk of future PD, the specificity will be low, given the high proportion of elderly reporting these complaints. Combined with the relatively low prevalence and incidence of PD in the general population[12,22] this will result in a low positive predictive value (defined as the proportion of persons with a positive test that will actually develop the disease). As a matter of fact, in our data each of the subjective complaints had a positive predictive value of no higher than 1 to 2.5%, which is clearly insufficient for a questionnaire to be used alone as a marker for preclinical disease. It is, however, generally believed that a single test will unlikely fulfil all criteria for the ideal biomarker and that presumably a stepwise approach with a simple and inexpensive initial screening test is required.[4,20] A questionnaire on subjective complaints might qualify for being part of such a first step.

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4 Risk factors: cholesterol and lipids

4.1 Dietary fatty acids and the risk of Parkinson disease

Abstract

Background

Unsaturated fatty acids are important constituents of neuronal cell membranes and have neuroprotective, antioxidant, and anti-inflammatory properties.

Objective

To examine whether a high intake of unsaturated fatty acids is associated with a lower risk of Parkinson disease.

Methods

In the Rotterdam Study, a prospective population-based cohort study of people aged ≥ 55 years, we evaluated the association between intake of unsaturated fatty acids and the risk of incident Parkinson disease among 5,289 subjects who were free of dementia and parkinsonism and underwent complete dietary assessment at baseline. Parkinson disease was assessed through repeated in-person examination and the cohort was continuously monitored by computer linkage to medical records. We analyzed the data using Cox proportional hazards regression models.

Results

After a mean follow up of 6.0 years, we identified 51 participants with incident Parkinson disease. Intakes of total fat, monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) were significantly associated with a lower risk of Parkinson disease (adjusted Hazard Ratios per standard deviation increase of energy-adjusted intake of total fat, 0.69 (95% confidence interval (CI) 0.52 to 0.91), of MUFAs, 0.68 (0.50 to 0.94), and of PUFAs, 0.66 (0.46 to 0.96)). No associations were found for dietary saturated fat, cholesterol, or trans fat.

Conclusion

These findings suggest that high intake of unsaturated fatty acids might protect against Parkinson disease.

Introduction

Parkinson disease (PD) is the second most common neurodegenerative disorder, caused by a selective degeneration of dopaminergic cells in the substantia nigra. The exact mechanism underlying this process is still unclear, but oxidative stress, mitochondrial dysfunction, and inflammation are thought to play a major role.[1-3] There are several reasons why dietary intake of unsaturated fatty acids might influence the risk of neurodegenerative diseases and in particular PD. First, polyunsaturated fatty acids (PUFAs) have anti-inflammatory and neuroprotective properties,[4-7] and monounsaturated fatty acids (MUFAs) are thought to reduce oxidative stress.[8,9] Second, the fatty acid composition of cell membranes is affected by diet. In infants and young animals, dietary deficiencies in MUFAs and PUFAs reportedly lead to poorer brain function.[5,6,10] Third, PUFAs are precursors for endogenous cannabinoids, which play a role in the control of movement by modulating dopaminergic activity in the basal ganglia.[11,12] Finally, evidence is increasing that genes involved in lipid metabolism may also regulate toxicity of alpha-synuclein, the major component of the inclusion bodies found in the brains of patients with PD.[13]

Previous epidemiologic studies on the association between dietary fat intake and the risk of PD have shown inconsistent results.[14-21] Most of these studies were case-control studies or investigated only total fat intake. We hypothesized that higher dietary intake of unsaturated fatty acids might lower the risk of PD and examined this association prospectively within the population-based Rotterdam Study.

Methods

The Rotterdam Study

The Rotterdam Study is a large prospective cohort study on determinants of diseases in the elderly.[22] All inhabitants aged ≥ 55 years of a district of Rotterdam were eligible. Of these, 7,983 subjects (response rate 78%) agreed to participate. Baseline examinations took place between 1990 and 1993. All participants were interviewed and subsequently underwent extensive physical examination, which included a neurologic screening, at the research center. Follow-up examinations took place in 1993 to 1994 and 1997 to 1999. In addition, the cohort was continuously monitored for major disease outcomes and mortality through linkage of the study database to general practitioners' medical files. Informed consent was obtained from each participant, and the Medical Ethics Committee of the Erasmus Medical CenterRotterdam approved the study.

Assessment of PD

A two-stage protocol was used at baseline and both follow-up examinations to identify subjects with PD.[23] At the research center, all participants were screened for symptoms of parkinsonism by study physicians. Individuals who screened positive received a structural diagnostic work-up using the Unified Parkinson's Disease Rating Scale to establish and further classify parkinsonism. A neurologist examined persons who were suspected of having PD to confirm the diagnosis. PD was diagnosed if two or more cardinal signs (resting tremor, rigidity, bradykinesia, or impaired postural reflexes) were present in a subject not taking antiparkinsonian drugs or if at least one sign had improved after starting antiparkinsonian medication and all causes of secondary parkinsonism had been excluded. In addition, the cohort was continuously monitored for new cases of parkinsonism by linkage to general practitioners' and other medical records. Information obtained from this surveillance system was reviewed by a panel of neurologists and research physicians to establish parkinsonism or PD. Because of the relatively short screening intervals, the midpoint between age at last screening and age at time of diagnosis was considered a good approximation of age at onset.

Dietary assessment

Dietary intake was assessed in two stages at baseline. During the home interview, participants received a checklist to indicate all foods and drinks they had consumed at least twice a month during the preceding year. The checklist also contained questions on use of supplements and prescribed diets. Subsequently, at the research center, a trained dietician interviewed the participants on the basis of the completed checklist. An extensive, validated semiquantitative food-frequency questionnaire was used to quantify the amounts of food and drink intake, and these data were converted to energy intake and nutrient intake using the computerized Dutch Food Composition Table.[24] For this study, we used data on intake of total energy (in kilocalories per day, including energy from alcohol), total fat, saturated fatty acids, trans fatty acids, cholesterol, MUFAs, PUFAs (subdivided into the omega-3 PUFAs alpha-linolenic acid, docosahexaenoic acid and eicosapentaenoic acid (together denoted as n3- PUFAs) and the omega-6 PUFAs linoleic acid and arachidonic acid (together denoted as n6-PUFAs)), carbohydrates, dairy products, alcohol (all calculated in grams per day), vitamin E (in milligrams per day), and coffee (expressed in number of cups per day).

Total fat intake consists of intake of saturated and unsaturated fatty acids and cholesterol. Over 99% of the energy from dietary fat is provided by fatty acids. Dietary cholesterol is found only in food from animal sources, including meat, eggs, milk, and butter. Main sources of saturated fat are dairy products and meat. MUFAs are

present in sunflower oil, peanut oil, and olive oil and hence relatively abundant in the so-called Mediterranean diet. PUFAs are found in vegetable oils (n6-PUFAs) and fish and marine animals (n3-PUFAs). MUFAs and PUFAs together make up the category of cis-unsaturated fatty acids. Trans-fat is industrially produced by adding hydrogen to unsaturated fatty acids and can be found in margarine and snack foods.

Other variables

The baseline interview contained questions about smoking habits (categorized as never, former, and current smoking) and medication use. Drugs were classified according to their Anatomic-Therapeutical-Chemical code.[25] APOE genotyping was performed as described previously on coded genomic DNA samples drawn at the baseline visit.[26] In case of discrepancies, APOE genotyping was repeated.

Study sample

At baseline, 6,969 participants underwent screening for PD. Parkinsonism was identified in 130 participants, of whom 99 had PD.[27] This resulted in 6,839 subjects free of parkinsonism at baseline. The food frequency questionnaire was not administered to participants with dementia at baseline because they might give unreliable information on dietary habits. Nursing home residents did not receive a food frequency questionnaire either because their current diet may not reflect dietary habits in the past. Persons with incomplete data or inconsistencies in their reported daily intake were excluded. The study sample thus consisted of 5,289 independently living participants with normal cognition and free of parkinsonism at baseline.

Data analysis

Because of the high correlation between fat intake and total energy intake, energy-adjusted intake of fat and fatty acids was calculated by means of the residual method.[28] We used Cox proportional hazards regression analyses to evaluate the associations between energy-adjusted intake of various types of fat and the risk of PD. Subjects who became demented were censored at age at onset of dementia, because subjects with dementia were considered to be no longer at risk for PD.[27] Persons with incident parkinsonism but not PD were also censored at age at onset of parkinsonism for the same reason.

First, we performed analyses with intake of fat expressed in tertiles to check whether the relation between dietary fat and the risk of PD showed gross deviations from linearity. As this appeared not to be the case, analyses were then performed using a linear term for fat intake, with regression coefficients expressed per standard deviation (SD) of energy-adjusted intake.

Table 1. *Baseline characteristics of the study population*

Characteristic			
Age (yr), mean (SD)	67.6 (7.7)		
Women, no (%)	3,122 (59.0)		
Cigarette smoking:			
Current, no (%)	1,225 (23.2)		
Former, no (%)	2,252 (42.6)		
Never, no (%)	1,781 (33.7)		
Use of lipid lowering medication at baseline, no (%)	137 (2.6)		
APOE genotype:			
ε3ε3, no(%)	2,962 (56.0)		
At least one ε2 allele, no (%)†	700 (13.2)		
At least one ε4 allele, no (%)†	1,268 (24.0)		
Intake, median per tertile (grams/day)	Tertile 1	Tertile 2	Tertile 3
Total energy, kcal/day	1507,3	1922,9	2426,6
Total carbohydrates	155,9	205,6	268,8
Total fat	55,1	77,4	105,1
Saturated fatty acids	23,4	32,6	44,6
Cholesterol	0,15	0,22	0,32
Unsaturated trans fatty acids	1,6	2,4	3,7
Unsaturated cis fatty acids	27,5	39,7	55,3
MUFA	16,4	23	31,4
PUFA	9,5	16,4	24,9
n-3 PUFA	0,8	1,2	1,9
Alpha-linolenic acid	0,6	1,0	1,7
Docosahexaenoic acid	0,01	0,05	0,15
Eicosapentaenoic acid	0,01	0,03	0,11
n-6 PUFA	8,3	15,0	23,3
Linoleic acid	8,2	14,8	23,1
Arachidonic acid	0,06	0,10	0,14
Dairy products	166,9	361,10	602,1
Vitamin E	8,1	12,90	19,3
Coffee, cups/day	1,0	4,0	6,0

SD : standard deviation, APOE : Apolipoprotein E,

MUFA : monounsaturated fatty acids, PUFA : polyunsaturated fatty acids

† *Exclusion of participants with ε2ε4 genotype*

Models were initially adjusted for age and sex. Additional adjustments were made for cigarette smoking, as smoking is reportedly associated with a lower risk of PD[29] and might be related to dietary habits as well. Because previous studies have indicated a possible inverse relationship between dietary intake of vitamin E, a fat-soluble vitamin, and the risk of PD,[30] models were also adjusted for daily vitamin E intake. We also considered consumption of dairy products, alcohol, and coffee to be potential confounders and therefore repeated analyses with these variables included in the models.[29,31,32] APOE genotype is known to influence lipid metabolism and thus might modify the relation between fat intake and PD risk.[33,34] Therefore, we additionally adjusted for APOE genotype (ε3ε3, ε4+ and ε2+ [ε2ε4 excluded]). Because of low numbers, it was not possible to perform separate analyses in these three strata of APOE genotype. Because the association between fat intake and risk of PD might be disturbed by the use of lipid-lowering medication, all analyses were repeated after exclusion of subjects who used lipid-lowering medication at baseline (n=137).
It has been suggested that the dopamine deficiency in early PD may cause a change in food preferences, leading to a shift toward higher carbohydrate intake at the expense of dietary fat.[35] Therefore, to check whether the associations that we found could be attributed to the consequence of preclinical PD at baseline, we adjusted all models for total energy-adjusted carbohydrate intake. Furthermore, we performed separate analyses using only incident cases identified at the first follow-up round (mean follow-up 2.1 years; short follow-up) and those identified at the second follow-up round (mean follow-up since baseline 6.0 years; long follow-up). For this latter analysis, we excluded all person-time until first follow-up and all persons who had developed PD or dementia within the first follow-up interval.

Results

Baseline characteristics of the study sample are shown in table 1. Follow-up information was available on 6,778 individuals (99%), through either complete re-examination at the research center or the continuous surveillance system. After a mean follow-up of 6.0 years, 77 subjects with incident parkinsonism were identified, of whom 51 had incident PD.
Hazard ratios (HRs) with 95 % confidence intervals (CIs) for the association between energy-adjusted intake of various types of dietary fat and the risk of PD are presented in table 2. Total energy intake itself was not associated with risk of PD in our study (adjusted HR 1.24, 95% CI 0.87 to 1.77). Intakes of total fat, MUFAs, and n3-PUFAs were significantly associated with a lower risk of PD (age- and sex-adjusted HR per SD of energy-adjusted intake for total fat 0.67 [95% CI 0.51 to 0.89], for MUFAs 0.67 [95% CI 0.49 to 0.91], and for n3-PUFAs 0.66 [95% CI 0.46 to 0.94]). After additional

adjustments for smoking and vitamin E intake, a significantly decreased risk of PD was also observed for intake of n6-PUFAs (HR 0.69, 95% CI 0.47 to 1.00). Of the n3-PUFA subtypes, only alpha-linolenic acid was significantly associated with a lower risk of PD (HR 0.65, 95% CI 0.45 to 0.95), whereas of the n6-PUFAs, only linoleic acid seemed protective (HR 0.79, 95% CI 0.59 to 1.06). No significant association was found between intake of saturated fatty acids, cholesterol, or trans-fatty acids and the risk of PD. None of the results changed when we adjusted the analyses for intake of total carbohydrates, coffee, dairy products, or alcohol or when we excluded participants who used lipid-lowering medication at baseline. Additional adjustment for APOE genotype did not change any of these associations. Results were not different for men and women.

Table 2. *Dietary fat and the risk of Parkinson disease. Hazard ratios (95% CIs) per standard deviation of energy-adjusted intake (grams/day)*

	SD	Adjusted for age and sex HR (95% CI)	Additionally adjusted for smoking and intake of vitamin E HR (95% CI)
Total fat	13.2	0.67 (0.51 - 0.89)	0.69 (0.52 - 0.91)
Saturated fatty acids	7.2	0.82 (0.61 - 1.10)	0.82 (0.61 - 1.10)
Cholesterol	0.06	0.81 (0.61 - 1.08)	0.81 (0.59 - 1.10)
Unsaturated trans fatty acids	1.0	0.87 (0.64 - 1.18)	0.84 (0.61 - 1.17)
Unsaturated cis fatty acids	9.7	0.67 (0.51 - 0.90)	0.64 (0.47 - 0.87)
MUFA	5.4	0.67 (0.49 - 0.91)	0.68 (0.50 - 0.94)
PUFA	6.7	0.77 (0.58 - 1.03)	0.66 (0.46 - 0.96)
n-3 PUFA	0.6	0.66 (0.46 - 0.94)	0.68 (0.48 - 0.97)
Alpha-linolenic acid	0.6	0.65 (0.45 - 0.95)	0.64 (0.44 - 0.92)
Docosahexaenoic acid	0.1	1.02 (0.79 - 1.31)	1.03 (0.45 - 2.38)
Eicosapentaenoic acid	0.09	1.02 (0.81 - 1.29)	1.03 (0.81 - 1.31)
n-6 PUFA	6.6	0.79 (0.59 - 1.06)	0.69 (0.47 - 1.00)
Linoleic acid	6.6	0.79 (0.59 - 1.06)	0.68 (0.47 – 1.00)
Arachidonic acid	0.04	0.96 (0.72 - 1.28)	0.96 (0.72 - 1.27)

SD : standard deviation, HR : hazard ratio, CI : confidence interval,
MUFA : monounsaturated fatty acids, PUFA : polyunsaturated fatty acids

Table 3 shows the results of the analyses stratified on duration of follow-up. HRs for fat intake derived after short follow-up (mean 2.1 years) did not differ from those derived after longer follow-up (mean 6.0 years). Results were similar after adjusting for smoking, coffee consumption, and intake of dairy products, vitamin E, and total carbohydrates. In contrast, we found a positive association between total carbohydrate intake and risk of PD for participants who developed PD within a few years since baseline, but no association for those who developed PD after a longer period.

Discussion

In this large, prospective population-based study, we found that a higher dietary intake of unsaturated fatty acids was associated with a decreased risk of PD.

An important feature of our study is the fact that dietary habits were assessed before onset of PD. Most previous studies on the relationship between dietary fat and PD used a retrospective case-control design, which makes it difficult to determine whether differences in fat intake play a causal role in PD or rather are a consequence of the disease. Dopamine shortage in PD patients might influence food preferences and eating behavior and thereby change dietary habits.[35] We considered that in the current study, bias might have occurred owing to the presence of preclinical disease at the time of baseline assessments. The length of the preclinical period in PD is unknown. Yet we considered that if persons changed their dietary habits because of preclinical PD, these changes would be more outspoken the shorter the interval between dietary assessment and diagnosis of PD. We therefore separately analyzed the data of the first and those of the second follow up round. Total carbohydrate intake tended to be positively associated with the risk of PD but, indeed, only when follow-up was short, probably reflecting a changed food preference in presymptomatic disease. In contrast, the point estimates for the associations for fatty acid intake with risk of PD were very similar for short and longer follow up, although owing to the smaller number of PD cases in each substrata, the confidence intervals became wider and included 1. Furthermore, adjusting for total carbohydrate intake did not change the results. Together, these findings make it unlikely that the observed associations actually resulted from a shift toward more carbohydrate consumption at the expense of fat intake in early PD.

Single dietary assessment by means of a food frequency questionnaire may lead to misclassification of nutrient intake. However, because future disease status was unknown at baseline, this misclassification, if any, will most likely have occurred to the same extent in participants with and without PD, causing dilution of the results and thus underestimation of the association.

Our extensive case finding procedures, almost complete follow-up, and strict diagnostic criteria ensure an optimal case ascertainment and limit the possibility of misclassification of PD. Moreover, we continued following participants after a diagnosis of PD had been made, which enabled us to revise cases on the basis of additional information. Although we cannot completely rule out the possibility of residual confounding, it is unlikely that our results can be entirely explained by confounding by other factors, since adjusting for several potential confounders did not essentially change any of the results.

Table 3. *Hazard ratios (95% CIs) of Parkinson disease per standard deviation of energy-adjusted intake of fat and carbohydrates (grams/day), adjusted for age and sex*

	Overall (n=51) HR (95% CI)	Short follow-up (n=25) HR (95% CI)	Longer follow-up (n=26) HR (95% CI)
Total fat	0.67 (0.51 - 0.89)	0.70 (0.48 - 1.04)	0.74 (0.50 - 1.09)
Saturated fatty acids	0.82 (0.61 - 1.10)	0.83 (0.54 - 1.26)	0.85 (0.57 - 1.27)
Cholesterol	0.81 (0.61 - 1.08)	0.81 (0.53 - 1.23)	0.86 (0.58 - 1.28)
Unsaturated trans fatty acids	0.87 (0.64 - 1.18)	0.85 (0.54 - 1.35)	0.87 (0.57 - 1.33)
Unsaturated cis fatty acids	0.67 (0.51 - 0.90)	0.73 (0.48 - 1.10)	0.73 (0.49 - 1.09)
MUFA	0.67 (0.49 - 0.91)	0.71 (0.45 - 1.09)	0.72 (0.47 - 1.10)
PUFA	0.77 (0.58 - 1.03)	0.82 (0.54 - 1.25)	0.82 (0.55 - 1.23)
n-3 PUFA	0.66 (0.46 - 0.94)	0.79 (0.50 - 1.25)	0.60 (0.36 - 1.01)
Alpha linolenic acid	0.64 (0.44 - 0.92)	0.69 (0.41 - 1.14)	0.65 (0.39 - 1.09)
Docosahexaenoic acid	1.03 (0.45 - 2.38)	1.13 (0.94 - 1.36)	0.74 (0.41 - 1.33)
Eicosapentaenoic acid	1.02 (0.81 - 1.29)	1.13 (0.94 - 1.35)	0.72 (0.40 - 1.30)
n-6 PUFA	0.79 (0.59 - 1.06)	0.83 (0.55 - 1.27)	0.85 (0.57 - 1.27)
Linoleic acid	0.79 (0.59 - 1.06)	0.83 (0.55 - 1.27)	0.85 (0.57 - 1.27)
Arachidonic acid	0.96 (0.72 - 1.27)	1.07 (0.73 - 1.57)	0.88 (0.58 - 1.33)
Carbohydrates	1.29 (0.98 - 1.70)	1.41 (0.97 - 2.06)	0.98 (0.67 - 1.45)

SD = standard deviation, HR = hazard ratio, CI = confidence interval, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids

Evidence from several research areas underlines the importance of unsaturated fatty acids for neuronal cell function. Dietary intake affects fatty acid composition of the cell membrane, which determines membrane fluidity and function of membrane transporters and enzymes.[5,6] Unsaturated fatty acids may be protective against the pathogenetic processes of oxidative damage and inflammation supposedly involved in PD.[1-3] Both MUFAs and PUFAs are shown to have anti-inflammatory and immune-modulating properties[6,7,36] and might protect against oxidative stress.[5,6,8] PUFAs are shown to inhibit neuronal apoptosis,4 which is thought to play a role in the pathogenesis of PD.[37] Furthermore, PUFAs (mainly the n6 type) are precursors for endocannabinoids, endogenous ligands that bind to a specific receptor (CB1) that is particularly abundant in the basal ganglia.[38] The endocannabinoid system modulates dopaminergic activity in the basal ganglia, thereby playing an important role in the control of movement.[11,12] Animal studies have shown that endocannabinoid levels can be modified by the amount PUFAs in the diet.[39] Interestingly, several recent findings suggest a role of lipid metabolism in the pathogenesis of PD. A genomic screen in yeast recently showed that genes that enhanced toxicity of alpha-synuclein, the major component of the pathologic inclusions found in PD, clustered in the processes of lipid metabolism.[13] Besides, in vitro studies showed that the formation of the potentially cytotoxic alpha-synuclein oligomers is regulated by fatty acids.[40]

Epidemiologic studies indicate that high dietary intake of both PUFAs and MUFAs might protect against cognitive decline and Alzheimer disease,[41-46] although the results from the Rotterdam Study could not be confirmed at longer follow-up.[47] Previous epidemiologic studies on the relation between dietary fat and PD show inconsistent results. Most of these were retrospective case-control studies focusing mainly on intake of total fat and saturated fat. Three case-control studies reported higher intakes of total fat and animal fat (a major source of saturated fat) in PD patients,[15,16,18] whereas results from two other case-control studies and the prospectively designed Health Professionals Follow-up Study and the Nurses' Health Study showed no significant associations between dietary total fat or animal fat and the risk of PD.[14,17,19] Few studies focused on unsaturated fat in relation to PD. Within the Honolulu –Asia Aging study, a prospective cohort study among men of Japanese ancestry, a significantly reduced risk of PD was observed with higher intake of PUFAs, which is in line with our observations.[21] In the Health Professionals Follow-up Study and the Nurses' Health Study, no significant association between intake of unsaturated fat and the risk of PD was found, although the effect estimates suggested a slightly lower risk of PD with higher intake of PUFAs. Of the several PUFA subtypes, only arachidonic acid was significantly associated with a lower PD risk.[14] However, isocaloric replacement of polyunsaturated fat with saturated fat was associated with a significantly increased risk of PD in the men in the Health Professionals Follow-up Study, which may again

fit our observation of a possible protective effect of polyunsaturated fat. Both the Health Professionals Follow-up Study and the Nurses' Health Study had longer follow-up and repeated instead of single dietary assessments and therefore probably less misclassification with regard to life-time cumulative dietary exposure than the Rotterdam Study. On the other hand, case ascertainment was based on self-reported medical diagnoses of PD and did not involve in-person screening, which makes it likely that a larger proportion of PD patients was missed or misdiagnosed in those studies. Possible explanations for the discrepant findings are that the larger diagnostic misclassification in the US studies may have limited the power to find a significant effect or that it is later-life dietary exposure rather than average life-time intake that is related to the occurrence of PD.

The association we observed for n3-PUFA intake seemed to be driven by the subtype of alpha-linolenic acid, which makes up 88% of all n3-PUFA intake. Linoleic acid accounts for 99% of all n6-PUFA intake and drove the association for total n6-PUFA. Intakes of other subtypes were very low and not related to the risk of PD. We found an inverse association of total fat intake with PD risk, which was very similar to the one for cis-unsaturated fatty acids (MUFA and PUFA together). This association may have been partly driven by the association between cis-unsaturated fatty acids and PD, as more than half of total fat intake consists of cis-unsaturated fatty acids. However, we also saw a trend for an inverse association between saturated fatty acids and cholesterol and the risk of PD. It is unclear whether these latter associations were due to chance or resulted from some as yet unexplained mechanism. Additional prospective studies and a longer follow-up period are needed to confirm our results.

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4.2 Serum cholesterol levels and the risk of Parkinson disease

Abstract

Background

Alterations in lipid and cholesterol metabolism are likely involved in the pathogenesis of neurodegenerative diseases, including Parkinson disease. Moreover, serum cholesterol is closely correlated with serum concentration of coenzyme Q10, a powerful antioxidant and mitochondrial electron acceptor that has shown beneficial effects in animal studies and trials on Parkinson disease.

Objective

To examine whether serum levels of total and HDL-cholesterol are related to the risk of Parkinson disease.

Methods

In the population-based Rotterdam study, we prospectively examined the association between serum levels of total and HDL cholesterol and the risk of Parkinson disease among 6,465 subjects aged ≥55 years. Parkinson disease was assessed through repeated in-person examination and continuous monitoring by computer linkage to medical records. We analyzed the data using Cox' proportional hazards regression models.

Results

After a mean follow up of 9.4 years, we identified 87 participants with incident Parkinson disease. Higher baseline levels of total cholesterol were associated with a significantly decreased risk of Parkinson disease (age and sex adjusted hazard ratio per mmol increase in serum cholesterol 0.77 (95% confidence interval 0.64-0.94)), with clear evidence for a dose-effect relationship. Further analyses showed that the association was restricted to women and remained unchanged after adjusting for multiple potential confounders. No association was observed between HDL cholesterol and risk of Parkinson disease.

Conclusion

Total cholesterol levels are inversely associated with the risk of Parkinson disease in women. The strong correlation -especially in women- between serum levels of cholesterol and the antioxidant coenzyme Q10 might provide a clue to explain these findings.

Introduction

Parkinson disease (PD), the second most common neurodegenerative disorder, is pathologically characterized by degeneration of dopamine-producing cells in the substantia nigra of the brain and the presence of intracellular inclusions called Lewy Bodies.[1] The exact mechanism that underlies the selective dopaminergic cell death in PD is as yet unknown, but mitochondrial dysfunction, oxidative stress and protein mishandling are thought to play a major role.[2-4]

Cholesterol is a major component of neuronal cell membranes and synapses and essential for maintaining their structure and function.[5] Evidence is increasing that changes in metabolism of cholesterol and other lipids are involved in neurodegeneration. Although most evidence originates from research in Alzheimer disease (AD),[5-13] several recent findings also suggest a role of lipid metabolism in PD pathogenesis. Results of in vitro studies suggest an association between lipids and the localization and structure of the alpha-synuclein protein, the major component of the pathological Lewy Bodies found in PD brains. Alpha-synuclein specifically binds to cholesterol-enriched domains of the neuronal cell membrane[14] and alpha-synuclein toxicity seems to be mediated by fatty acids and by genes involved in lipid metabolism.[15-18] Lower serum levels of total cholesterol have been described in patients with PD compared with controls.[6,19] Moreover, serum cholesterol is the most important determinant of serum levels of coenzyme Q10, a powerful antioxidant and electron acceptor for mitochondrial complex I and II.[20] Because mitochondrial complex I dysfunction is thought to play a key role in PD pathogenesis, the effects of oral supplementation of coenzyme Q10 have been examined in animal models and an initial trial in PD patients, with promising results.[20-22]

To evaluate a potential role of cholesterol in PD, we examined the relationship between serum levels of total and HDL cholesterol and the risk of PD prospectively among 6,465 participants of the population-based Rotterdam Study.

Methods

The Rotterdam Study

The Rotterdam Study is a prospective population-based cohort study on determinants of diseases in the elderly.[23] Of all inhabitants aged ≥55 years of a district of Rotterdam, 7,983 subjects (response rate 78%) agreed to participate and gave their informed consent. At baseline (1990-1993) and three follow-up rounds (1993-1994, 1997-1999 and 2002-2004), participants were interviewed at their homes and subsequently underwent extensive physical examination and venipuncture at the research center.

In addition, the cohort was continuously monitored for major disease outcomes and mortality through computerized linkage of the study database to general practitioners' medical files. The Medical Ethics Committee of the Erasmus Medical Center Rotterdam approved the study.

Assessment of Parkinson disease

Both at baseline and at follow-up, we used a two–phase design to identify subjects with PD.[24,25] All participants were screened at the research center for cardinal signs of parkinsonism (resting tremor, rigidity, bradykinesia, or impaired postural reflexes) in a standardized way. Individuals who screened positive received a structural diagnostic workup comprising the Unified Parkinson's Disease Rating Scale[26] and neurological examination to establish and further classify parkinsonism. Information obtained from the computerized surveillance system was reviewed by a panel of neurologists and research physicians. A neurologist examined persons who were suspected of having PD to confirm the diagnosis. PD was diagnosed if two or more cardinal signs were present in a subject not taking antiparkinsonian drugs, or if at least one sign had improved after starting antiparkinsonian medication and all causes of secondary parkinsonism (parkinsonism associated with dementia, drug-induced parkinsonism, parkinsonism related to cerebrovascular disease, and parkinsonism in multiple system atrophy or progressive supranuclear palsy) had been excluded.

Assessment of serum levels of total and HDL cholesterol

Serum concentrations of total cholesterol and high-density lipoprotein were determined within 2 weeks after venipuncture by an automated enzymatic procedure in non-fasting blood samples drawn at the baseline visit to the research center.[27] Non-HDL cholesterol was computed by subtracting HDL cholesterol from total cholesterol and was used to calculate the ratio of non-HDL to HDL cholesterol.

Assessment of covariates

Smoking habits and use of medication, including lipid-lowering agents, were assessed during the baseline interview, which also contained a semiquantitative food frequency questionnaire (SFFQ). Ever use of lipid-lowering agents during the study period was recorded by computerized linkage to the automated pharmacies serving the study area. Drugs were classified according to their Anatomic-Therapeutical-Chemical (ATC) code.[28] Dietary intakes of vitamin E and coffee were assessed by means of the SFFQ as described in detail elsewhere.[29,30] Height and weight were measured to calculate body mass index (BMI, kg/m^2) and Apolipoprotein E (APOE) genotyping was performed on coded genomic DNA samples as described previously.[31]

Study population

At baseline, 6,969 participants underwent complete neurological screening. We excluded participants diagnosed with parkinsonism (n=130) or dementia (n=273) at baseline, resulting in a cohort at risk to develop PD during follow-up of 6,566 persons. In 6,465 of those, serum total and HDL cholesterol measurements were available

Table 1. *Baseline characteristics of the study population*

Mean (SD)	Overall	Men	Women
N (%)	6,465	2,654 (41.1)	3,811 (58.9)
Age (years)	69.0 (8.6)	67.8 (8.0)	69.3 (9.0)
Total cholesterol (mmol/l)	6.64 (1.23)	6.33 (1.18)	6.85 (1.22)
HDL-cholesterol (mmol/l)	1.35 (0.37)	1.21 (0.33)	1.44 (0.37)
Non-HDL/HDL ratio (unit)	5.25 (1.63)	5.50 (1.62)	5.07 (1.61)
Smoking			
Current, no (%)	1,500 (23.2)	783 (29.5)	712 (18.7)
Former, no (%)	2,728 (42.2)	1,651(62.2)	1,075 (28.2)
Never, no (%)	2,237 (34.6)	220 (8.3)	2,024 (53.1)
Coffee consumption, cups/day	3.3 (2.2)	3.6 (2.4)	3.1 (2.0)
Vitamin E intake, g/day	13.8 (6.2)	15.4 (6.7)	12.8 (5.6)
APOE genotype*			
ε3ε3, no(%)	3,639 (60.3)	1,537 (61.4)	2,102 (59.5)
At least one ε2 allele, no (%)	841 (13.9)	311 (12.4)	530 (15.0)
At least one ε4 allele, no (%)	1,555 (25.8)	657 (26.2)	898 (25.4)
BMI, kg/m2	26.3 (3.7)	25.7 (3.0)	26.8 (4.1)
Use of lipid-lowering medication			
At baseline, no(%)	160 (2.5)	73 (2.8)	87 (2.3)
Ever use, no (%)	1,008 (15.6)	431 (16.2)	577 (15.1)

SD : standard deviation, BMI : body mass index
** Genotyping performed in 6,035 participants*

Table 2. *Serum total cholesterol and the risk of PD. HR (95% CI), adjusted for age and sex*

Overall			Men			Women		
	HR	(95% CI)		HR	(95% CI)		HR	(95% CI)
Per 1.0 mmol	0.77	(0.64 - 0.94)	Per 1.0 mmol	1.01	(0.78 - 1.30)	Per 1.0 mmol	0.59	(0.45 - 0.78)
Quartiles:			Quartiles:			Quartiles:		
< 5.9	1.00	(ref.)	<5.6	1.00	(ref.)	< 6.1	1.00	(ref.)
5.9 - 6.6	0.82	(0.48 - 1.41)	5.6 - 6.3	0.60	(0.24 - 1.50)	6.1 - 6.8	0.58	(0.27 - 1.24)
6.6 - 7.4	0.55	(0.30 - 1.02)	6.3 - 7.1	1.39	(0.67 - 2.89)	6.8 - 7.6	0.46	(0.20 - 1.05)
> 7.4	0.55	(0.30 - 1.04)	> 7.1	0.86	(0.36 - 2.02)	> 7.6	0.16	(0.05 - 0.55)

PD ; Parkinson disease, HR : hazard ratio, CI : confidence interval

Data analysis

We used Cox proportional hazards regression analysis to evaluate the association of baseline serum total cholesterol, HDL cholesterol, and the ratio of non-HDL to HDL cholesterol with the risk of incident PD. Cholesterol levels were analyzed both as continuous variables and in quartiles of the distribution. Analyses were initially adjusted for age and sex. Additional adjustments were made for smoking habits (classified as former, current, or never smoking), vitamin E intake (in milligrams/day), and coffee consumption (expressed in number of cups/day), because all these factors have been related to a decreased risk of PD[32-34] and probably are associated with concentrations of total and HDL cholesterol.[35] Analyses were further adjusted for BMI, baseline and ever use of cholesterol-lowering medication, and APOE genotype (categorized into ε3ε3,ε4+ and ε2+ [ε2ε4 excluded]), as APOE genotype is an important determinant of plasma cholesterol[5] and the ε2 allele is associated with an increased risk of PD.[36,37]

All analyses were repeated after exclusion of participants who reported use of lipid-lowering medication at baseline. We performed separate analyses for men and women, as mean baseline serum levels for men and women were significantly different. Analyses were furthermore stratified by smoking status, coffee consumption, APOE genotype, high or low vitamin E intake, and use of lipid-lowering medication.

Results

Follow-up information was available on 99% of the participants, either through complete in-person re-examination or the continuous monitoring system. During a total of 60,709 person-years of follow-up (mean follow-up time, 9.4 years), 87 new cases of PD were detected.

Baseline characteristics of the study population are shown in table 1. Table 2 shows hazard ratios (HRs) with 95% confidence intervals (CIs) for PD according to serum total cholesterol, overall and for men and women separately. Overall, higher levels of serum total cholesterol were associated with a significantly decreased risk of PD, with analyses in quartiles showing a clear linear relationship. Stratified analyses showed a strong association in women, but no association in men. None of the other stratified analyses showed different results across strata. Results for HDL levels are shown in table 3. No obvious association was seen in men. In women there was a slight positive association, but without clear evidence for linearity. Results for the non-HDL to HDL ratio were very similar to those for total cholesterol (table 4).

Table 3. *Serum HDL cholesterol and the risk of PD. HR (95% CI), adjusted for age and sex*

Overall			Men			Women		
	HR (95% CI)			HR (95% CI)			HR (95% CI)	
Per 0.1 mmol	1.04	(0.99 - 1.09)	Per 0.1 mmol	1.01	(0.92 - 1.10)	Per 0.1 mmol	1.06	(1.01 - 1.12)
Quartiles:			Quartiles:			Quartiles:		
< 1.1	1.00	(ref.)	< 1.0	1.00	(ref.)	< 1.2	1.00	(ref.)
1.1 - 1.3	1.36	(0.73 - 2.56)	1.0 - 1.2	1.31	(0.53 - 3.29)	1.2 - 1.4	1.36	(0.52 - 3.57)
1.3 - 1.6	1.04	(0.54 - 2.00)	1.2 - 1.4	1.35	(0.52 - 3.42)	1.4 - 1.7	0.64	(0.21 - 1.90)
> 1.6	1.81	(0.96 - 3.42)	> 1.4	1.38	(0.56 - 3.43)	> 1.7	2.69	(1.11 - 6.49)

PD : Parkinson disease, HR : hazard ratio, CI : confidence interval

Additional adjustments for smoking, dietary vitamin E, coffee consumption, BMI, APOE genotype, and baseline or ever use of lipid-lowering drugs did not change any of the results; neither did exclusion of participants who used lipid-lowering medication at baseline.

Discussion

In this prospective, population-based cohort study with a large number of person-years of follow-up, we found that serum total cholesterol and the ratio of non-HDL to HDL cholesterol were inversely related to the risk of PD. This association appeared restricted to women. As serum cholesterol levels were measured before the clinical onset of PD, the observed associations are unlikely to have resulted from consequences of the disease itself. We furthermore consider it improbable that the associations may be explained by the effect of smoking habits, coffee consumption, APOE genotype, BMI, use of lipid-lowering drugs, or vitamin E intake on PD risk, since results remained virtually unchanged after adjusting for all these potential confounders. Potential misdiagnosis is limited because of the extensive case finding procedures, strict diagnostic criteria, and almost complete follow-up.

Evidence is accumulating that alterations in metabolism of cholesterol and other lipids are involved in the pathogenesis of neurodegenerative diseases.[5-8] A positive association between serum cholesterol and the risk of AD has been observed in some epidemiological studies,[9,10,38] and a decreased risk of AD was found in users of cholesterol-lowering statins,[11] although cholesterol-independent effects of statins on cerebral circulation and inflammation might in part be responsible for this finding.[7] Abnormalities in cholesterol metabolism have been described in brains of AD patients compared with control subjects[12] and evidence suggests that cholesterol influences production and probably clearance and aggregation of the amyloid-beta peptide, the principal component of the extracellular plaques that are considered a pathological hallmark of AD.[7,8,13,39]

Several lines of evidence also indicate a role of lipid metabolism in the pathogenesis of PD, which offers one possible explanation for our findings. Decreased cholesterol synthesis was observed in skin fibroblasts from patients with PD[40] and lower levels of total cholesterol have been described in PD patients compared with controls.[6,19] Localization and structure of the alpha-synuclein protein, which in aggregated form is a major component of the pathological inclusion bodies found in the brains of PD patients,[16] have been linked to lipids and cholesterol. In vitro studies have shown that alpha-synuclein is closely associated to lipid rafts, cholesterol-enriched domains in the cell membrane that are involved in protein activity and cell signaling.[14]

Table 4. *Non-HDL to HDL ratio and the risk of PD. HR (95% CI), adjusted for age and sex*

Overall			Men			Women		
	HR (95% CI)			HR (95% CI)			HR (95% CI)	
Per unit	0.80	(0.69 - 0.94)	Per unit	0.95	(0.79 - 1.16)	Per unit	0.62	(0.48 - 0.81)
Quartiles:			Quartiles:			Quartiles:		
< 4.1	1.00	(ref.)	< 4.4	1.00	(ref.)	< 3.9	1.00	(ref.)
4.1 - 5.0	0.64	(0.37 - 1.12)	4.4 - 5.3	1.65	(0.75 - 3.63)	3.9 - 4.8	0.45	(0.21 - 1.00)
5.0 - 6.1	0.59	(0.33 - 1.03)	5.3 - 6.4	1.21	(0.51 - 2.87)	4.8 - 5.8	0.40	(0.18 - 0.92)
> 6.1	0.45	(0.24 - 0.84)	> 6.4	1.08	(0.44 - 2.68)	> 5.8	0.25	(0.09 - 0.66)

PD : Parkinson disease, HR : hazard ratio, CI : confidence interval

Oligomerization of alpha-synuclein, which might be the first step in the formation of the insoluble aggregates, is regulated by fatty acids,[16-18] and genes that modified alpha-synuclein toxicity in yeast were found to be mostly involved in processes of lipid metabolism.[15]

We previously found a significant inverse association between dietary intake of total fat and unsaturated fatty acids and the risk of PD in the prospective Rotterdam Study. Our results also suggested an inverse association between dietary cholesterol and PD risk, although they did not reach statistical significance.[30] A decreased risk of PD with higher intake of polyunsaturated fatty acids was observed in another prospective cohort study as well.[41] Furthermore, results from a recent meta-analysis showed that carriers of the APOE ε2 allele, which is associated with lower plasma levels of total cholesterol,[5] have an increased risk of PD.[36,37] The latter finding is particularly interesting because for AD, the ε2 allele is considered protective, whereas the APOE ε4 allele (associated with higher total cholesterol levels) is an important genetic risk factor.[42] This parallels the contrast between our finding of an inverse association between total cholesterol and PD risk and the previously reported positive association between total cholesterol and AD risk.

The intact blood–brain barrier, however, is impermeable to cholesterol-transporting lipoproteins and most brain cholesterol is synthesized in situ.[7] It is therefore unclear to what extent serum cholesterol levels correspond with brain levels and whether they accurately reflect changes in cholesterol metabolism in the central nervous system. An alternative hypothesis to explain our findings is provided by the strong correlation between serum cholesterol and serum concentration of coenzyme Q10, a molecule present in all body cells that acts as both an essential electron acceptor for complex I and II in the mitochondrial respiratory chain and as a powerful endogenous antioxidant.[20] Serum cholesterol is the most important determinant of coenzyme Q10 levels, as both substances derive from the same biosynthetic pathway and virtually all coenzyme Q10 in plasma is incorporated in lipoproteins, primarily LDL.[43-45] Because oxidative stress and mitochondrial complex I dysfunction are thought to play a key role in PD pathogenesis[2-4] and reduced coenzyme Q10 levels have been found in mitochondria from PD patients,[46] there is growing interest in the use of coenzyme Q_{10} as a potential treatment for PD.[20,47] In animal models, oral administration of coenzyme Q10 increased brain concentrations and attenuated 3-NP-induced lesions[20] and MPTP-induced striatal dopamine depletion.[21] In a multicenter placebo-controlled randomized phase II trial, treatment with coenzyme Q10 significantly reduced the worsening of PD.[22]

Unfortunately, we were not able to directly test the hypothesis that our results might reflect an inverse association between coenzyme Q10 concentration and PD risk, because we can no longer measure levels of coenzyme Q10 in baseline serum

from the Rotterdam Study, due to instability of the samples after a long storage period.[48] Another issue is why the observed association was restricted to women. In a study conducted to investigate determinants of coenzyme Q10 levels, it was found that 18.4% of the variation in coenzyme Q10 levels in men is explained by total cholesterol, against 30.7% in women.[44] Mean levels of coenzyme Q10 were found to be significantly higher in men, even after adjusting for several covariates, including serum cholesterol.[44] The lack of an association between cholesterol levels and PD risk in men might thus be due to a weaker correlation between cholesterol and coenzyme Q10 levels.
We think our results are potentially relevant and call for further research on the relationship between cholesterol, coenzyme Q10 and the risk of PD.

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5 Risk factors : homocysteine metabolism and oxidative stress

5.1 MTHFR C677T genotype and the risk of Parkinson disease

Abstract

Background

Elevated plasma levels of homocysteine might accelerate the selective dopaminergic cell death underlying Parkinson disease, through direct neurotoxic effects. The TT genotype of the C677T polymorphism of the methylenetetrahydrofolate reductase (MTHFR) gene is associated with reduced enzymatic activity, resulting in mild hyperhomocysteinemia.

Objective

To investigate whether the MTHFR TT genotype is associated with an increased risk of Parkinson disease.

Methods

In the Rotterdam Study, a prospective population-based cohort study of people aged ≥ 55 years, we evaluated the association between MTHFR C677T genotype and the risk of Parkinson disease in 5,920 subjects who were free of dementia and parkinsonism at baseline. MTHFR genotyping was performed on baseline blood samples and incident Parkinson disease was assessed through repeated in-person examination and continuous monitoring of the cohort by computer linkage to medical records. We analyzed the data using Cox proportional hazards regression models.

Results

After a mean follow up of 5.8 years, we identified 65 participants with incident Parkinson disease. TT genotype, compared to CC and CT genotypes, was associated with an increased risk of Parkinson disease, which was borderline significant (relative risk (RR) 1.74, 95% confidence interval (CI), 0.91 to 3.32, p=0.09). Analyses stratified on smoking status showed a strong and significant increase in risk of Parkinson disease associated with the TT genotype in smokers (RR, 3.74; 95% CI, 1.78 to 7.85), and no association in non-smokers (RR, 0.28; 95% CI, 0.04 to 2.07).

Conclusion

Our findings support the hypothesis that homocysteine plays a role in the pathogenesis of Parkinson disease.

Introduction

Oxidative stress and mitochondrial dysfunction are thought to be involved in the selective dopaminergic cell death in Parkinson disease (PD).[1,2] Increasing evidence suggests that high plasma levels of homocysteine might contribute to these processes through direct neurotoxic effects. In animal models of PD, brain injections of homocysteine exacerbated 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced motor dysfunction and loss of dopaminergic neurons. Homocysteine has also been observed to cause DNA strand breaks and to enhance oxidative stress, mitochondrial dysfunction, and apoptosis induced by rotenone and iron in cultured human dopaminergic cells.[2,3]

Increased plasma levels of homocysteine have been found in patients with PD, although mainly in those receiving L-dopa therapy.[4-6] Therefore, it is unclear whether this increase precedes PD or is caused by the L-dopa itself.

Methylenetetrahydrofolate reductase (MTHFR) is a folate-dependent enzyme that catalyzes remethylation of homocysteine. Individuals homozygous for the C677T polymorphism of the MTHFR gene (the TT genotype) display reduced enzymatic activity, resulting in mild hyperhomocysteinemia.[7-9] We hypothesized that through increasing plasma homocysteine, the TT genotype might be associated with a greater risk for PD, and studied this association within the population-based Rotterdam Study.

Materials and methods

The Rotterdam Study is a prospective, population-based cohort study among 7,983 individuals aged 55 years or older in a district of Rotterdam, the Netherlands.[10] Both at baseline (1990 to 1993) and two follow-up rounds (1993 to 1994 and 1997 to 1999), all participants underwent interviewing, extensive physical examination, and venipuncture. We used a two-phase design to identify subjects with PD.[11] Participants were screened for parkinsonian signs (resting tremor, rigidity, bradykinesia, or impaired postural reflexes) in a standardized way. Individuals who screened positive received a structural diagnostic workup comprising the Unified Parkinson's Disease Rating Scale[12] and neurological examination. In addition, the cohort was continuously monitored through linkage to general practitioners' medical files. All available information was reviewed by a multidisciplinary panel to make a final diagnosis. PD was diagnosed if two or more cardinal signs were present in a participant not taking antiparkinsonian drugs, or if at least one sign had improved after medication was started, and when all causes of secondary parkinsonism (dementia, use of neuroleptics,

cerebrovascular disease, multiple system atrophy, or progressive supranuclear palsy) could be excluded.

The C677T polymorphism was genotyped on baseline DNA samples, using the Taqman allelic discrimination assay (Applied Biosystems, CA, USA). Forward and reverse primer were 5'-CCTCAAAGAAAAGCTGCGTGATG and 5"-GCACTTGAAGGAGAAGGTGTCT, respectively, and the probes were VIC-ATGAAATCGACTCCCGC and FAM-ATGAAATCGGCTCCCGC. Reactions were performed on the Taqman Prism 7900HT in 384 wells format. At baseline, we assessed smoking status (classified as ever [current or former] or never smoking) and measured intima-media thickness of the carotid arteries by ultrasonography according to a protocol that was described previously.[13] Homocysteine was measured only in a small random subset of the study population (n=652).

Baseline neurological screening was performed in 6,969 participants. We excluded participants diagnosed with any parkinsonism (n=130) or dementia (n=273) at baseline, resulting in a cohort at risk for development of PD during follow-up of 6,566 individuals. Of those, MTHFR genotyping was performed in 5,920 individuals. Because hyperhomocysteinemic effects have only been described for the TT genotype without any allele dosage effect,[7,9] we used a recessive model with the CC and CT genotype together as a reference group to analyze the effect of the TT genotype on the risk for incident PD.

Table 1. *Baseline characteristics of the study population*

Characteristic	Entire cohort	Incident PD cases	Subjects without PD
No. of subjects	5,920	65	5,855
Women, no (%)	3,452 (58.3)	35 (53.8)	3,417 (58.4)
Age, yr (SD)	68.7 (8.6)	74.9 (7.2)	68.7 (8.6)
Smokers, no (%)	3,872 (65.4)	34 (52.3)	3,841 (65.6)
Nonsmokers, no (%)	2,048 (34.6)	31 (47.7)	2,014 (34.4)
Genotype			
CC, no (%)	2,680 (45.3)	27 (41.5)	2,653 (45.3)
CT, no (%)	2,635 (44.5)	27 (41.5)	2,608 (44.5)
TT, no (%)	605 (10.2)	11 (16.9)	594 (10.1)

PD : Parkinson disease, SD : Standard deviation

Analyses were preformed with Cox' proportional hazards regression analysis, adjusted for age and sex. We also adjusted for intima-media thickness as a measure of atherosclerosis to evaluate whether vascular mechanisms could explain the results. Because smoking influences homocysteine levels[14] and is associated with a decreased risk of PD,[15] analyses were both adjusted for and stratified on smoking status. We tested for statistical interaction between TT genotype and smoking by adding a multiplicative interaction term to the model.

Results

Follow-up information was available on 99% of the participants, either through in-person examination or the computerized monitoring system. During a total of 34,512 person-years of follow-up (mean, 5.8 years), 65 new cases of PD were detected. MTHFR genotype distribution of the study population was in Hardy-Weinberg equilibrium and similar across age categories. Of participants who remained free of PD, 10.1 % appeared homozygous for the T allele compared with 16.9% of the incident PD cases (table 1).

Table 2 shows the relative risk (RR) estimates with 95 % confidence interval (CI) of development of PD according to MTHFR genotype. The TT genotype, compared with CC and CT genotypes, was associated with an increased risk of PD, which was borderline significant (RR, 1.74; 95% CI, 0.91 to 3.32; p=0.09). Adjusting for intima-media thickness and smoking did not change the results substantially. However, stratified analyses showed a strong and significant increase in risk for PD associated with the TT genotype in smokers (RR, 3.74; 95 % CI, 1.78 to 7.85), but no association was found in nonsmokers (RR, 0.28; 95 % CI, 0.04 to 2.07). The multiplicative interaction term was highly significant (p=0.021).

Unfortunately, the low number of incident PD cases with available homocysteine measurements (n=4) did not allow us to examine the association between plasma homocysteine and the risk for PD directly. Levels of homocysteine, however, appeared to be greater in individuals homozygous for the T allele compared with those with the CC or CT genotype (mean level in TT group = 16.9 µmol/L, mean level in CC/CT group = 15.0 µmol/L, p=0.07).

Discussion

In this prospective, population-based cohort study, we found that the hyperhomocysteinemic MTHFR 677 TT genotype was associated with an increased risk for PD, particularly in smokers. Methodological strengths of our study are its population-based and prospective nature, extensive case-finding procedures, and

virtually complete follow-up with a large number of person-years. Unfortunately, the number of incident PD cases was limited, which resulted in rather wide CIs and precluded potentially interesting subanalyses, for example, in strata of number of pack-years smoked.

Homocysteine is increasingly discussed to be involved in the pathogenesis of neurodegenerative diseases through direct neurotoxic effects. It may promote cell death through stimulation of N-methyl-D-aspartate receptors or by inducing DNA strand breaks.[3,16] In a mouse model of PD, brain injections of homocysteine exacerbated 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced motor dysfunction, dopamine depletion, and loss of dopaminergic neurons. Furthermore, in cultured human dopaminergic cells, homocysteine enhanced oxidative stress, mitochondrial dysfunction, and apoptosis induced by rotenone and iron.[2] Several studies have reported increased plasma homocysteine levels in PD patients; however, this occurred mainly in patients receiving L-dopa therapy. Because the relation between homocysteine levels and PD has not been examined prospectively thus far, it is still unclear whether hyperhomocysteinemia precedes the onset of PD or rather is a result of treatment.[5,6] The MTHFR 677 TT polymorphism codes for a MTHFR variant with reduced enzymatic activity, resulting in increased plasma homocysteine levels, and might therefore increase the risk for PD.

Table 2. *Risk of Parkinson disease according to MTHFR genotype, stratified by smoking status (relative risks with 95 % confidence intervals)*

Genotype	Overall	Smokers	Nonsmokers	
CC / CT	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	p-interaction
TT[a]	1.74 (0.91 - 3.32)	3.74 (1.78 - 7.85)	0.28 (0.04 - 2.07)	0.021
TT[b]	1.92 (0.93 – 3.97)	3.92 (1.69 – 9.09)	0.39 (0.05 – 2.86)	0.044

[a] *Adjusted for age and sex*

[b] *Additionally adjusted for IMT and (only the overall analysis) for smoking status*

Smoking is also reported to increase homocysteine levels, and previous studies showed an effect of TT genotype on plasma homocysteine that was especially pronounced in smokers, probably because both factors affect remethylation of homocysteine to methionine.[8,14] This might explain our observation that the association between the MTHFR 677 TT genotype and PD was restricted to smokers. Our findings support the hypothesis that homocysteine metabolism is involved in the pathogenesis of PD.

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5.2 Dietary folate, vitamin B12, and vitamin B6 and the risk of Parkinson disease

Abstract

Background

Elevated homocysteine levels might accelerate the selective dopaminergic cell death underlying Parkinson disease, through direct neurotoxic effects. Higher dietary intakes of folate, vitamin B12, and vitamin B6 (essential co-factors in homocysteine metabolism) might decrease the risk of Parkinson disease through lowering of plasma homocysteine. Besides, evidence suggests neuroprotective effects of vitamin B6 independent from its role in homocysteine metabolism, most likely through antioxidant activities.

Objective

To determine whether high dietary intakes of folate, vitamin B12, and vitamin B6 are related to the risk of Parkinson disease.

Methods

In the Rotterdam Study, a prospective, population-based cohort study of people aged ≥55 years, we evaluated the association between intake of folate, vitamin B12, and vitamin B6 and the risk of incident Parkinson disease among 5,289 participants who were free of dementia and parkinsonism and underwent complete dietary assessment at baseline. Parkinson disease was assessed through repeated in-person examination and continuous monitoring by computer linkage to medical records. Data were analyzed using Cox proportional hazards regression analysis.

Results

After a mean follow-up of 9.7 years, we identified 72 participants with incident Parkinson disease. Higher dietary intake of vitamin B6 was associated with a significantly decreased risk of Parkinson disease (Hazard Ratio per standard deviation, 0.69 (95% confidence interval, 0.50-0.96), for highest versus lowest tertile, 0.46 (0.22-0.96)), while no association was observed for dietary folate and vitamin B12.

Conclusion

These findings suggest that vitamin B6 may decrease the risk of Parkinson disease, either through lowering homocysteine levels, or through anti-oxidative effects.

Introduction

Parkinson disease (PD), a progressive neurodegenerative disorder which is clinically characterized by resting tremor, rigidity, bradykinesia, and postural imbalance, is caused by selective degeneration of dopaminergic neurons in the substantia nigra of the brain stem. The exact mechanism that underlies this process is as yet unclear, but oxidative stress is generally thought to play a prominent role.[1,2]

High homocysteine levels might enhance dopaminergic degeneration through direct neurotoxic effects. In 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) animal models of PD, brain injections of homocysteine exacerbated dopaminergic cell loss and motor dysfunction.[3] Homocysteine was furthermore found to cause DNA strand breaks and increase rotenone-induced oxidative stress and apoptosis in cultured human dopaminergic neurons.[3,4] Some studies have reported elevated homocysteine levels in PD patients, but due to their retrospective design it is unclear whether this increase preceded the disease or rather was a result of treatment with levodopa.[5-7] Prospective studies on homocysteine levels and PD risk have not been published thus far, but our group previously found an increased risk of PD in carriers of the hyperhomocysteinemic MTHFR 677 TT genotype.[8]

Homocysteine levels are kept low by remethylation to methionine, which requires folate and vitamin B12, and by conversion to cysteine, for which vitamin B6 is an essential co-factor.[9] Plasma homocysteine reportedly is inversely related to both plasma levels and dietary intakes of folate, vitamin B12, and vitamin B6.[10-13] Vitamin B6 (pyridoxine) might furthermore influence PD risk through antioxidant effects and through its role in dopamine synthesis.[14-19]

We hypothesized that higher intakes of folate, vitamin B12, and vitamin B6 might be associated with a decreased risk of PD, and studied this association prospectively in 5,289 participants of the population-based Rotterdam Study.

Subjects and methods

The Rotterdam Study

The Rotterdam Study is a prospective, population-based cohort study among 7,983 persons aged ≥55 years in a district of Rotterdam, the Netherlands.[20] At baseline (1990-1993), all participants were interviewed and subsequently underwent extensive physical examination, which included a neurologic screening. Follow-up examinations according to the same protocol took place in 1993-1994, 1997-1999, and 2002-2004. In addition, the cohort was continuously monitored for major disease outcomes and mortality through computerized linkage of the study database to general practitioners'

medical files. Informed consent was obtained from each participant, and the Medical Ethics Committee of the Erasmus Medical Center Rotterdam approved the study.

Assessment of PD

In all four examination rounds, we used a similar two–phase design to identify subjects with PD.[21] Participants were screened for cardinal signs of parkinsonism (resting tremor, rigidity, bradykinesia, or impaired postural reflexes) in a standardized way. Persons in whom at least one of these signs was observed were considered screen-positive and received a structural diagnostic workup, comprising the Unified Parkinson's Disease Rating Scale[22] and neurologic examination. Besides, a panel of neurologists and research physicians reviewed the information that was obtained from the computerized surveillance system to detect possible new cases of PD. PD was diagnosed if two or more cardinal signs were present in a participant not taking antiparkinsonian drugs, or if at least one sign had improved through medication, and when all causes of secondary parkinsonism (dementia, use of neuroleptics, cerebrovascular disease, multiple system atrophy, or progressive supranuclear palsy) could be excluded.

Assessment of dietary variables and covariates

At baseline, dietary intake was assessed through an interview by a trained dietician, using an extensive, validated semiquantitative food-frequency questionnaire (SFFQ).[23] The amounts of food and drink intake indicated on the SFFQ were converted to energy intake and nutrient intake by means of the computerized Dutch Food Composition Table. For the present study, we used data on dietary intake of folate (in µg/d), vitamin B12 (in µg/d), vitamin B6 (in mg/d), alcohol (in g/d), coffee (number of cups/d), and total energy (in kcal/d), as well as data on use of B-vitamin and folate supplements.

At baseline, we assessed smoking status during the interview (classified as ever [current or former] or never smoking) and measured intima-media thickness (IMT) of the carotid arteries by ultrasonography as described elsewhere.[24]

Study population

Baseline neurologic screening was performed in 6,969 participants, of whom 130 were diagnosed with any parkinsonism, resulting in a cohort at risk to develop PD during follow-up of 6,839 persons. The SFFQ was not administered to participants with dementia at baseline (because they might give unreliable dietary information), and nursing home residents (as their current diet may not reflect dietary habits in the past). Participants with incomplete or inconsistent dietary data were also excluded from the analyses. The study sample for the present study thus comprised 5,289

independently living participants free of dementia and parkinsonism at baseline, for whom complete dietary data were available.

Data analysis

The relation between dietary folate, vitamin B12, and vitamin B6 and the risk of incident PD was analyzed with Cox proportional hazards regression analysis, adjusted for age, sex, and total energy intake, per standard deviation and expressed in tertiles of intake.

Because smoking reportedly influences both homocysteine levels[25] and PD risk,[26] analyses were both adjusted for and stratified by smoking status. Additional adjustments were made for intake of alcohol and coffee, being determinants of plasma homocysteine,[25] and for IMT as a measure of atherosclerosis, to evaluate whether vascular mechanisms could explain the results. We re-analyzed the data after exclusion of users of B-vitamin and folate supplements (n=675), and after adding supplement users to the highest tertile of intake, and examined the effect of supplement use itself on PD risk. We tested for statistical interaction between dietary intake and smoking status by adding multiplicative interaction terms to the model. All analyses were performed using SPSS software, version 12.0.

Table 1. *Baseline characteristics of the study population*

Characteristic	Mean (SD)
N	5,289
Age, yrs	67.7 (7.7)
Intake:	
Total energy, kcal/day	1,975.2 (504.2)
Vitamin B12, micrograms/day	5.3 (4.5)
Vitamin B6, milligrams/day	1.63 (0.4)
Folate, micrograms/day	218.7 (77.8)
Alcohol, g/day	10.4 (15.2)
Coffee, no. of cups/day	3.3 (2.2)
Use of vitamin B/folate supplements, no (%)	675 (12.7)
Ever smokers, no (%)	3,495 (66.1)
Intima-media thickness, mm	0.79 (0.15)

SD: standard deviation

Results

Follow-up information was available on 99% of the participants, either through in-person examination or the computerized monitoring system. During a total of 53,262 person-years of follow-up (mean, 9.7 years), 72 new cases of PD were identified. Of those, 44 cases were detected through the structured workup at the research center and 28 through the computerized surveillance system.

In table 1, baseline characteristics of the study population are presented. Table 2 shows the hazard ratios (HRs) with 95% confidence intervals (CIs) for the association between dietary folate, vitamin B12, and vitamin B6 and the risk of PD, per standard deviation and in tertiles of intake. Higher intake of vitamin B6 was associated with a significantly decreased risk of PD (HR per SD, 0.69 (95%CI, 0.50-0.96), for highest versus lowest tertile 0.46 (0.22-0.96)), with evidence for a dose-effect relationship (p-value for trend over tertiles, 0.048). No association with PD risk was seen for dietary vitamin B12 and folate.

Results were not substantially different after adjusting for smoking, alcohol intake, coffee consumption, and IMT, or when supplement users were excluded or added to the highest tertile. Supplement use itself was not associated with the risk of PD (data not shown). Adding dietary folate, vitamin B12, and vitamin B6 simultaneously to the model for mutual adjustments did not change the effect estimates for vitamin B6 and B12, but attenuated the HR for folate intake to 1.04 (95%CI, 0.64-1.70).

Stratified analyses (see table 3) showed a significant inverse association between vitamin B6 intake and PD risk in smokers (HR per SD, 0.53; 95%CI, 0.35-0.81), but no association in non-smokers (HR, 0.99; 95%CI 0.58-1.68). The multiplicative interaction term containing vitamin B6 intake and smoking was significant (p=0.036). Results for vitamin B12 and folate intake were not different for smokers and non-smokers.

Discussion

In this prospective, population-based cohort study we found that higher intake of vitamin B6, but not vitamin B12 or folate, was associated with a significantly decreased risk of PD, particularly in smokers. Methodological strengths of this study include the extensive case-finding procedures and virtually complete follow-up with a large number of person-years. Moreover, the prospective design with dietary assessments before onset of PD limits the possibility that changes in intake as a consequence of the disease influenced the results. Among the constraints of our study are the limited statistical power to perform stratified analyses, and the possibility of misclassification of nutrient intake inherent to a food frequency questionnaire.

Table 2. *Dietary folate, vitamin B12, and vitamin B6 and the risk of PD.*
Hazard Ratios, adjusted for age, sex, and total energy intake, with 95 % confidence intervals

Folate intake	HR (95% CI)	Vitamin B12 intake	HR (95% CI)	Vitamin B6 intake	HR (95% CI)
Per SD	0.80 (0.57 - 1.13)	Per SD	0.88 (0.63 - 1.23)	Per SD	0.69 (0.50 - 0.96)
Tertiles:		Tertiles:		Tertiles:	
< 185.1 µg/d	1.00 (ref.)	< 3.48 µg/d	1.00 (ref.)	< 1.43 mg/d	1.00 (ref.)
185.1 - 230.9 µg/d	1.40 (0.80 - 2.46)	3.48 - 5.18 µg/d	0.91 (0.50 - 1.65)	1.43 - 1.74 mg/d	0.98 (0.56 - 1.71)
> 230.9 µg/d	0.75 (0.37 - 1.49)	> 5.18 µg/d	1.11 (0.61 - 2.01)	> 1.74 mg/d	0.46 (0.22 - 0.96)
	p-trend 0.431		p-trend 0.707		p-trend 0.048

PD: Parkinson disease, HR: hazard ratio, CI: confidence interval, SD: standard deviation

Table 3. *Dietary vitamin B6 and the risk of PD, in smokers and non-smokers*
Hazard Ratios, adjusted for age, sex, and total energy intake, with 95% confidence intervals

	Overall HR (95% CI)	Ever smokers HR (95% CI)	Never smokers HR (95% CI)	p-interaction
Per SD	0.69 (0.50 - 0.96)	0.53 (0.35 - 0.81)	0.99 (0.58 - 1.68)	0.036
Tertiles:				
< 1.43 mg/d	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	
1.43 - 1.74 mg/d	0.98 (0.56 - 1.71)	0.98 (0.49 - 1.99)	0.85 (0.34 - 2.14)	
> 1.74 mg/d	0.46 (0.22 - 0.96)	0.26 (0.10 - 0.70)	0.97 (0.32 - 2.94)	

PD: Parkinson disease, HR: hazard ratio, CI: confidence interval, SD: standard deviation

Future disease status though was unknown at baseline, and dietary misclassification –if any- therefore most likely did not differ between participants with and without incident PD, causing at most an underestimation of the association. A certain degree of uncertainty regarding disease status is unavoidable in epidemiologic studies on PD, as in the absence of an unambiguous test for the disease the diagnosis is still based on clinical criteria. However, by applying extensive case-finding methods and strict diagnostic criteria, and through the availability of additional and long-term data from the computerized follow-up system, we think that diagnostic inaccuracy was reduced as much as possible.

Considering the potential neurotoxicity of homocysteine,[3,4,8] we hypothesized that higher intakes of folate and vitamins B12 and B6 might decrease the risk of PD by lowering homocysteine levels. We found, indeed, a significant inverse association for vitamin B6, but no significant association for folate and vitamin B12. This may point towards an additional mechanism underlying the association for vitamin B6 intake. Several lines of evidence suggest neuroprotective properties of vitamin B6 through antioxidant capacities, in addition to lowering plasma homocysteine.[14-19] As oxidative stress is thought to play a prominent role in PD pathogenesis,[2] higher vitamin B6 intake may thus reduce PD risk through antioxidant effects. In rats, oxidative stress was induced by vitamin B6 deficiency[18] and prevented by vitamin B6 supplementatio n.[14]Antioxidant activities of vitamin B6 were also observed in yeast cells, monocytes and blood plasma.[15,16,19] Furthermore, vitamin B6 is a cofactor in dopamine synthesis and is required to convert homocysteine to cysteine, which in turn is the rate-limiting forerunner in the synthesis of glutathione.[19] Reduced levels of glutathione, a major antioxidant, have been found in dopaminergic neurons of PD patients.[2]

The relation between dietary folate, vitamin B12, and vitamin B6 and the risk of PD has only been examined in one study previously, in which no significant associations were observed for either of these vitamins.[27] In this US-based prospective study among health professionals, with repeated dietary assessments and case ascertainment based on self-reported diagnoses, median intakes of folate (men 388 µg/d, women 277 µg/d), vitamin B12 (men 10.4 µg/d, women 6.8 µg/d), and vitamin B6 (men 5.6 mg/d, women 2.3 mg/d) were considerably higher than the corresponding median intakes in the Rotterdam Study (folate: men 223 µg/d, women 197 µg/d; vitamin B12: men 4.7 µg/d, women 3.9 µg/d; vitamin B6: men 1.8 mg/d, women 1.5 mg/d), probably due to a raised health-consciousness among the US health professionals, together with the 1998 FDA requirement to fortify grain products with folate in the US.[28] These differences in intake may explain the discrepant findings for vitamin B6 intake, as adequate intake in the US-based study probably precluded demonstrating associations with the risk of PD.

The inverse relation between vitamin B6 intake and PD risk in our study appeared only present in smokers. A potential explanation for this finding is the increased free radical load and associated oxidative stress in smokers[29] and thus a stronger expected antioxidant effect of vitamin B6. Alternatively, putative neuroprotective effects of cigarette smoke components[26] might reinforce the beneficial effects of vitamin B6 intake on PD risk.

In conclusion, our findings suggest that higher dietary intake of vitamin B6 may decrease the risk of PD, either through lowering homocysteine levels, or through its antioxidant properties.

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5.3 Serum levels of uric acid and the risk of Parkinson disease

Abstract

Background

Oxidative stress is thought to play a key role in the pathogenesis of Parkinson disease. Uric acid might protect against Parkinson disease through anti-oxidant and iron-chelating properties.

Objective

To examine whether higher serum levels of uric acid are associated with a lower risk of Parkinson disease.

Methods

In the Rotterdam Study, a prospective population-based cohort study of people aged ≥55 years, we evaluated the association between serum levels of uric acid and the risk of incident Parkinson disease among 4,695 participants. Parkinson disease was assessed through repeated in-person examination and the cohort was continuously monitored by computer linkage to medical records. We analyzed the data using Cox proportional hazards regression models.

Results

After a mean follow up of 9.4 years, we identified 68 participants with incident Parkinson disease. Higher serum levels of uric acid were associated with a significantly decreased risk of Parkinson disease (adjusted hazard ratio per standard deviation increase, 0.70; 95% confidence interval, 0.50-0.98), with strong evidence for a dose-effect relationship (p-value for trend over quartiles 0.035).

Conclusion

Our findings support the hypothesis that oxidative stress contributes to the risk of Parkinson disease and suggest a potential protective effect of the natural antioxidant and free radical scavenger uric acid.

Introduction

The exact cause of the selective dopaminergic cell death that underlies Parkinson disease (PD) is still unknown, but oxidative stress and mitochondrial dysfunction are generally thought to play a prominent role.[1] Uric acid is an important natural antioxidant that may reduce oxidative stress through its actions as a scavenger of free radicals and iron-chelator.[2-5] Uric acid was found to suppress oxidative stress and prevent dopaminergic cell death in animal models of PD,[6] and reduced levels of uric acid have been observed in the substantia nigra of PD patients compared with controls.[7,8] In the only prospective study to our knowledge that evaluated the relationship between uric acid concentrations and the risk of PD thus far, a significantly lower PD risk was found for men with uric acid concentrations above the median.[9]

We hypothesized that through antioxidant effects, higher concentrations of uric acid might protect against PD and studied this association prospectively in the population-based Rotterdam Study.

Subjects and methods

The Rotterdam Study is a prospective population-based cohort study among 7,983 persons aged 55 years or older in a district of Rotterdam, the Netherlands.[10] Both at baseline (1990-1993) and three follow-up rounds (1993-1994, 1997-1999, and 2002-2004), all participants were interviewed and underwent extensive physical examination and venipuncture. We used a two–phase design to identify subjects with PD.[11] Participants were screened for parkinsonian signs (resting tremor, rigidity, bradykinesia, or impaired postural reflexes) in a standardized way. Individuals who screened positive received a structural diagnostic workup comprising the Unified Parkinson's Disease Rating Scale (UPDRS)[12] and neurological examination. In addition, the cohort was continuously monitored through computerized linkage to general practitioners' medical files. PD was diagnosed if two or more parkinsonian signs were present in a person not taking antiparkinsonian drugs, or if at least one sign had improved after medication was started, and when all causes of secondary parkinsonism (dementia, use of neuroleptics, cerebrovascular disease, multiple system atrophy, or progressive supranuclear palsy) could be excluded.

Uric acid activity was determined in serum obtained from nonfasting blood within 1 week after baseline venipuncture by an enzymatic method using a Kone Diagnostica reagent kit and a Kone autoanalyzer.[13] At baseline, smoking status (classified as current, former, or never smoking), medication use, consumption of dairy products, and alcohol intake (g/day) were assessed during the interview, and height and weight

***Table 1**. Baseline characteristics of the study population and relationship of characteristics with serum uric acid levels*

Characteristic (n=4,695)		Difference in serum uric acid level (µmol/l) (95% CI), adjusted for age and sex[a]			P-value
Serum uric acid, µmol/l, mean (SD)	323.2 (81.2)				
Quartile 1, range	107.0 - 267.8				
Quartile 2, range	267.8 - 310.4				
Quartile 3, range	310.4 - 374.0				
Quartile 4, range	> 374.0				
Women, no (%)	2,856 (60.8)	(women vs men)	-50.00	(-54.54 to -45.62)[b]	< 0.000
Age (yrs), mean (SD)	69.5 (8.6)	(per SD increase)	10.00	(7.69 to 12.32)	< 0.000
Smoking:					
Current, no (%)	1,131 (24.1)				
Former, no (%)	1,939 (41.3)				
Never, no (%)	1,625 (34.6)	(ever vs never smoking)	1.53	(-3.77 to 6.83)	0.570
Intake:					
Alcohol, (g/day), mean (SD)	10.4 (15.5)	(per SD increase)	7.33	(4.86 to 9.80)	< 0.000
Dairy products, (g/day), mean (SD)	396.2 (259.8)	(per SD increase)	-5.20	(-7.45 to -2.94)	< 0.000
BMI (kg/m2), mean (SD)	26.4 (3.8)	(per SD increase)	22.53	(20.38 to 24.69)	< 0.000
Use of anti-gout medication at baseline, no (%)	25 (0.7)	(use vs no use)	23.83	(-7.97 to 55.64)	0.14

SD = Standard deviation, BMI = Body Mass Index
[a]linear regression coefficients with 95 % confidence intervals, [b]only adjusted for age

were measured to calculate body mass index (BMI, kg/m^2).
Baseline neurological screening was performed in 6,969 participants. We excluded participants diagnosed with any parkinsonism (n=130) or dementia (n=273) at baseline, and participants who visited the research center after December 31, 1992 (n=1871), because by that time uric acid measurements were stopped due to financial constraints. The study population thus consisted of 4,695 participants free of parkinsonism and dementia at baseline with serum uric acid levels available.

The relation between serum uric acid levels and the risk of incident PD was analyzed by means of Cox proportional hazards regression analysis, with serum uric acid as a continuous variable (expressed per standard deviation increase) as well as in quartiles of its distribution. Analyses were initially adjusted for age and sex. We additionally adjusted for smoking habits, alcohol intake, consumption of dairy products, and BMI, because these variables reportedly affect serum uric acid levels and might be associated with PD risk, and therefore were considered potential confounders.[14-19] Analyses were repeated after stratification on sex and smoking status and after exclusion of participants who used uric acid-lowering (anti-gout) medication at baseline.

Results

Follow-up was virtually complete (> 98%), through either in-person examination or the computerized monitoring system. During a total of 44,121 person-years of follow-up (mean, 9.4 years), 68 new cases of PD were detected. Baseline characteristics of the study population, as well as regression coefficients with 95% confidence intervals (CIs) for the relation between potential confounders and serum uric acid levels, are presented in Table 1.
Table 2 shows the hazard ratios (HRs) with 95% CIs of developing PD according to serum uric acid concentration. Higher serum uric acid levels seemed associated with a lower risk of PD in the age- and sex-adjusted analyses (HR per standard deviation increase 0.82; 95% CI, 0.63-1.08). This association was significant after additional adjustments for smoking, BMI, consumption of dairy products, and alcohol intake (adjusted HR per standard deviation increase 0.71; 95% CI, 0.51-0.98), with evidence for a dose-effect relationship (adjusted HR for highest compared with lowest quartile 0.42 (95% CI, 0.18-0.96), p-value for trend over quartiles 0.040). The strengthening of the association was almost exclusively caused by the adjustment for BMI, being the potential confounder that appeared most strongly associated with uric acid levels (see Table 1).

Results were not significantly different for men and women, or for smokers and nonsmokers. Exclusion of participants who used anti-gout preparations at baseline (n=25) did not change the results either (data not shown).

Discussion

In this large, population-based cohort study we found that higher serum levels of uric acid were associated with a significantly decreased risk of PD. Bias is unlikely to explain our findings, given the prospective nature of our study, extensive case ascertainment, and nearly complete follow-up. Besides, the results show evidence for a dose-effect relationship and were not attenuated after adjusting for several potential confounders. Uric acid measurements were stopped before all patients had visited the research center. However, as participants were invited in random order, we do not think this affected our results.

Uric acid is an important antioxidant and scavenger of free radicals.[2,4,5] Because oxidative stress is thought to play a key role in the pathogenesis of PD,[1] uric acid might exert protective effects against PD through its antioxidant capacities. Furthermore, uric acid has a strong ability to bind iron,[3] which may contribute to oxidative damage in PD by enhancing generation of reactive oxygen species.[20]

Table 2. *Serum uric acid and the risk of Parkinson disease. Hazard Ratios (HRs) with 95 % confidence intervals (CIs), per standard deviation (SD) and in quartiles*

	Model 1 [a]		Model 2 [b]	
	HR	(95% CI)	HR	(95% CI)
Per SD	0.82	(0.63 - 1.08)	0.71	(0.51 - 0.98)
Quartiles:				
1	1.00	(ref.)	1.00	(ref.)
2	0.75	(0.39 - 1.45)	0.68	(0.34 - 1.39)
3	0.70	(0.36 - 1.35)	0.59	(0.28 - 1.25)
4	0.50	(0.24 - 1.03)	0.42	(0.18 - 0.96)
	p-trend 0.065		*p*-trend 0.040	

[a] *Adjusted for age and sex*
[b] *Additionally adjusted for smoking, alcohol intake, consumption of dairy products, and BMI*

Two studies on postmortem material have shown reduced levels of uric acid in the substantia nigra of PD patients compared with controls.[7,8] In a mouse model of PD, treatment with uric acid suppressed oxidative stress and prevented death of dopaminergic cells caused by administration of homocysteine or iron.[6]

As yet, the relationship between serum uric acid and PD has been examined prospectively in only one study, which was restricted to men. Serum uric acid levels in the Honolulu Heart Study were measured in 1965 in 7,968 middle-aged men, who were followed up until 1994 for incident PD. Participants with baseline uric acid concentrations above the median had a significantly lower risk to develop PD during follow-up, which is in line with our findings.[9] Although the possibility that our findings are due to residual confounding or confounding by unmeasured factors cannot be completely ruled out, we think these results support the hypothesis that oxidative stress is involved in the pathogenesis of PD and that uric acid might reduce the risk of PD via antioxidant and iron-chelating properties.

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6 Prognosis of Parkinson disease: risk of dementia and mortality

Abstract

Background

Most prognostic studies on Parkinson disease have been hospital-based or applied register-based case-finding methods. Potential underrepresentation of mild cases may have given biased results.

Objective

To evaluate whether Parkinson disease is associated with an increased risk of dementia and death.

Methods

In the Rotterdam Study, a prospective population-based cohort study of people aged ≥55 years, we compared the risk of incident dementia and death of patients with Parkinson disease and participants without Parkinson disease. The study sample comprised 6,969 persons, including 99 prevalent and 67 incident cases of Parkinson disease. Parkinson disease and dementia were assessed through repeated in-person examination at baseline (1990-1993) and two follow-up visits (1993-1994 and 1997-1999). Computerized linkage to medical and municipality records provided additional information on disease outcomes and mortality. We analyzed the data using Cox proportional hazards regression models.

Results

Patients with Parkinson disease had an increased risk of dementia (hazard ratio (HR), 2.8; 95% confidence interval (CI), 1.8-4.4), which was especially pronounced in participants carrying at least one APOE ε2 allele (HR, 13.5; 95% CI, 4.5-40.6). Parkinson disease was associated with an increased mortality risk (HR, 1.8; 95% CI, 1.5-2.3). The association consistently diminished when analyses were sequentially restricted to patients with shorter disease duration and after adjustment for the occurrence of dementia.

Conclusion

Especially PD patients who carry an APOE ε2 allele have an increased risk of developing dementia. Increased mortality risk in Parkinson disease is dependent on disease duration and only modest in the absence of dementia.

Introduction

The prevalence of Parkinson disease (PD), the second most common neurodegenerative disorder, is expected to increase as populations worldwide age. Insight into the prognosis is therefore desirable. PD has been associated with an increased risk of developing dementia and a reduced life expectancy. However, most prognostic studies have been hospital-based, yielding results that are not representative of the general population. Our group previously showed that, even in population-based studies, a considerable proportion of cases of PD remain undiagnosed when case finding relies on medical records only and no population screening is done.[1] The potential under-representation of relatively mild cases in register-based studies might result in overestimating the risk of dementia or mortality. Another issue is whether the prognosis of patients with PD varies with apolipoprotein E (APOE) genotype, because previous studies have shown conflicting results.[2-6]

In a prospective population-based cohort study involving in-person examination of all participants, we evaluated the prognosis of PD with respect to dementia and mortality, studying both prevalent cases identified at baseline and incident cases diagnosed during follow-up. We furthermore investigated to what extent reduced survival in patients with PD is due to their higher risk of dementia, and whether APOE genotype influences prognosis.

Methods

The Rotterdam Study

The Rotterdam Study is a prospective population-based cohort study among 7,983 subjects aged 55 years and older. At baseline (1990-1993) and two follow-up visits (1993-1994 and 1997-1999), participants were interviewed and underwent extensive physical examination. In addition, the cohort was continuously monitored for major disease outcomes and mortality through computerized linkage to general practitioners' medical files. All participants gave their informed consent, and the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam, the Netherlands approved the study.

Assessment of Parkinson disease and dementia

At baseline and follow-up, we used a two-stage protocol to identify subjects with PD and a three-stage protocol to assess dementia, both of which have been described extensively elsewhere.[1,7-9] Briefly, all participants were screened for symptoms of parkinsonism, and those who screened positive received a structural diagnostic workup using the Unified Parkinson's Disease Rating Scale. Persons suspected of having PD

were examined by a neurologist. PD was diagnosed if at least two parkinsonian signs were present or if at least one sign had improved through medication and all causes of secondary parkinsonism had been excluded. Age at diagnosis of PD and Hoehn and Yahr scale score for disease severity were assessed in the diagnostic workup and verified from medical records if possible.
Cognitive screening of all participants was performed with the Mini-Mental State Examination and Geriatric Mental State schedule. Subjects in whom screening was positive were examined with the Cambridge Examination of Mental Disorders in the Elderly. If the result of this examination was inconclusive, a neuropsychologist performed further examination and, if possible, magnetic resonance imaging was done. Final diagnosis was made according to Diagnostic and Statistical Manual of Mental Disorders, Revised Third Edition criteria.[10] The computerized surveillance system continuously provided additional information on both PD and dementia.

Assessment of mortality and covariates
Mortality until January 1, 2000, was assessed through continuous monitoring of the municipal address files and computerized reports from general practitioners on the deaths of participants. Information on highest attained educational level, smoking habits, medication use, and living situation was obtained during the baseline interview. APOE genotyping was performed on baseline samples.[2]

Study population
At baseline, 6,969 participants underwent neurologic screening. PD was diagnosed in 99 participants (prevalent PD). During follow-up, another 67 patients were identified (incident PD). All 6,969 participants were followed up to study mortality risk.
To examine the risk of incident dementia, we excluded participants with incomplete baseline cognitive screening (n=6) and those diagnosed as having dementia at baseline (n=312). The resulting study sample of 6,651 nondemented participants comprised 72 prevalent and 67 incident PD cases.

Data analysis
Hazard ratios (HRs) for incident dementia and mortality were computed by means of Cox proportional hazards regression analysis allowing for delayed entry, with age as the time scale and PD as a time-dependent covariate. Models were initially adjusted for age and sex. Potential confounders we additionally adjusted for were smoking (ever versus never), nursing home residency, antiparkinsonian medication use, and educational level (primary education only versus more than primary education). Because of the wide range of disease duration of prevalent PD cases at the time of inclusion in the study, we performed separate analyses for cases with disease

duration of 5 years or more and less than 5 years. Within the latter group, we further looked separately into those with less than 2 years duration (hence including incident PD cases) and incident PD cases only. To evaluate whether and to what extent reduced survival in patients with PD is explained by an increased risk of dementia, we adjusted for occurrence of dementia in a time-dependent fashion. All analyses were stratified on APOE genotype (ε3ε3, ε4+, and ε2+; ε2ε4 excluded) to examine potential modifying effects on prognosis.

Table 1. *Baseline characteristics of the study population*

Characteristic	Free of PD at baseline* (n = 6,870)	Prevalent PD at baseline (n = 99)	Incident PD during follow-up (n = 67)
Age at baseline, mean (SD), y	69.4 (9.1)	78.3 (8.2)	74.6 (7.2)
Women, no (%)	4,092 (59.6)	64 (64.6)	36 (53.7)
Ever smoking, no (%)	4,373 (63.7)	50 (50.5)	34 (50.7)
APOE genotype, no (%)±			
ε2+	833 (13.8)	16 (21.1)	10 (14.9)
ε3 ε3	3,813 (59.9)	45 (59.2)	35 (59.7)
ε4+	1,673 (26.3)	15 (19.7)	19 (25.4)
Primary education only, no (%)	2,624 (38.2)	44 (44.4)	25 (37.3)
Nursing home residency, no (%)	533 (7.8)	43 (43.4)	8 (11.9)
Antiparkinsonian medication use, no (%)	9 (0.1)	52 (52.5)	0 (0.0)
Dementia at baseline, no (%)	290 (4.2)	22 (22.2)	NA
No cognitive testing at baseline, no (%)	1 (0.01)	5 (5.1)	NA
Age at onset of PD, mean (SD), y	NA	71.2 (9.9)	77.5 (7.1)
Duration of disease, mean (SD), y	NA	5.7 (5.4)	NA
Hoehn and Yahr scale score, mean (SD)	NA	2.3 (1.2)	1.8 (1.0)

PD: Parkinson disease, SD: standard deviation, APOE: Apolipoprotein E gene, NA: not applicable

**Includes people who developed PD during follow-up (incident PD)*

±Available for 6,445 participants

Median survival from diagnosis was calculated with the Kaplan-Meier method. All analyses were performed with SAS software (version 8.2; SAS Institute Inc, Cary, NC).

Results

Table 1 displays baseline characteristics of the study population. Follow-up was virtually complete (99%) until January 1, 2000. The total follow-up time was 48,606 person-years (overall mean, 6.9 years; mean of incident PD cases after disease onset, 4.3 years). The mean Hoehn and Yahr scale score of patients with prevalent PD (2.3) and especially of patients with incident PD (1.8) was relatively low compared with previous studies.

Risk of dementia

At baseline, 22% of the participants with PD and 4% of those without PD were diagnosed as having dementia. Demented patients with PD were significantly older than those without dementia. Of the cohort free of dementia at baseline, 21 (15.1%) of the 139 patients with PD and 318 (4.9%) of the 6,512 participants without PD developed dementia during follow-up. The presence of PD was associated with a significantly increased risk of dementia (HR, 2.80; 95% CI, 1.79 - 4.38; table 2). Results were similar after additional adjustments and for subgroups of disease duration at baseline.

Table 2. *PD and the risk of incident dementia, in strata of APOE genotype; Hazard ratios (HRs) with 95% confidence intervals (CIs)*

Model	Overall HR (95% CI)	APOE ε2+ HR (95% CI)	APOE ε3ε3 HR (95% CI)	APOE ε4+ HR (95% CI)	P -value interaction
1	2.80 (1.79-4.38)	13.46 (4.46-40.64)	1.74 (0.77-3.97)	6.27 (3.07-12.82)	0.000
2	2.82 (1.80-4.42)	14.26 (4.68-43.50)	1.82 (0.80-4.16)	6.49 (3.17-13.31)	0.024

Model 1: adjusted for age and sex
Model 2 : adjusted for age, sex, and educational level

However, restricting analyses to only incident PD cases resulted in a higher estimate (HR, 4.74; 95% CI, 2.49 - 9.02). Disease duration did not seem to affect dementia risk within PD cases (HR increase per year, 0.96; 95% CI, 0.84-1.09). The association of PD with incident dementia was more pronounced in participants with at least one APOE ε4 allele (HR, 6.27; 95% CI, 3.07 - 12.82), and especially in those carrying at least one APOE ε2 allele (HR, 13.46; 95% CI, 4.46-40.64), compared with ε3ε3 carriers.

Mortality risk

During follow-up, 90 (54.2%) of the 166 patients with PD and 1,623 (23.9%) of the 6,803 participants without PD died. Median survival since diagnosis of PD was 9.1 years (95% CI, 7.4 - 10.9 years). Overall, PD was associated with a significantly increased mortality risk (HR, 1.83; 95% CI, 1.47 - 2.26; table 3). However, HRs consistently decreased when the analyses were sequentially restricted to patients in whom PD was diagnosed more recently. Additional adjustments did not substantially change the results, but adjusting for occurrence of dementia yielded lower mortality HRs. The effect of PD on survival was not different for men and women, or by strata of APOE genotype (data not shown). Within PD cases, mortality risk was influenced by disease duration (HR increase per year, 1.03; 95% CI, 0.99 - 1.07) and by occurrence of dementia (HR, 2.85; 95% CI, 1.77 - 4.62).

Comment

The strengths of this study are its population-based nature, size, and almost complete follow-up. In addition, thorough case ascertainment for PD and dementia was ensured through in-person instead of record-based screening methods. The use of strict diagnostic criteria enhanced diagnostic accuracy, and continuous monitoring of participants after diagnosis enabled us to revise diagnoses on the basis of additional information. Furthermore, because we followed up prevalent as well as incident PD cases, we could evaluate the effect of disease duration on prognosis and potential bias in prevalent cohorts.

An increased risk of dementia associated with PD has repeatedly been reported, with relative risks varying from 1.7-5.9.[11-14] Our estimate of a 2.8-times increased risk is relatively low. A possible explanation is the low average disease severity in our study, which resulted from our screening methods, through which we identified a large number of previously unrecognized patients with mild PD.[1,7] Moreover, we consider it likely that PD patients who agreed to participate at baseline had fewer cognitive complaints and thus a lower risk of future dementia than nonresponders, which may have led us to underestimate the risk for prevalent cases. The HR for incident cases was notably higher (4.7), despite lower disease severity, and presumably reflects the

Table 3. *PD and mortality risk, according to disease duration at time of enrollment; hazard ratios with 95% confidence intervals*

		Disease duration*			
	All cases (n = 166)	>5 years (n = 52)	<5 years (n = 114)	<2 years (n = 87)	Incident cases only (n = 67)
Age at enrollment (SD), y	76.8 (7.9)	78.9 (8.1)	75.8 (7.7)	74.8 (7.8)	74.6 (7.2)
Hoehn & Yahr scale score (SD)±	2.1 (1.2)	2.4 (1.3)	2.0 (1.1)	1.8 (1.0)	1.8 (1.0)
Model 1, HR (95% CI)**	1.83 (1.47-2.26)	2.52 (1.81-3.51)	1.53 (1.16-2.01)	1.37 (0.98-1.89)	1.29 (0.87-1.92)
Model 2, HR (95% CI)**	1.57 (1.27-1.95)	2.11 (1.52-2.94)	1.36 (1.03-1.79)	1.27 (0.92-1.76)	1.27 (0.85-1.89)

PD : Parkinson disease, SD : standard deviation, HR : hazard ratio, CI : confidence interval

** Categories >5yrs and <5yrs are mutually exclusive; categories <2yrs and incident cases are subgroups of the <5yrs category*
± At time of study-entry (prevalent cases) or diagnosis (incident cases)

*** Model 1 was adjusted for age and sex; model 2 was adjusted for age, sex, and occurrence of dementia (time-dependent)*

actual situation more accurately._

We found that the effect of PD on dementia risk was more pronounced in participants carrying at least one APOE ε4 allele and remarkably strong in those carrying at least one APOE ε2 allele. Apolipoprotein E, coded for by the APOE gene, is a polymorphic protein abundant in the brain that is involved in lipid transport, immunoregulation, and modulation of cell growth.[15] For Alzheimer disease, the APOE ε4 allele is an established risk factor, whereas the ε2 allele is considered protective.[15] In contrast, a recent meta-analysis[3] confirmed the repeatedly observed association of the ε2 allele with an increased risk of PD, while results for the ε4 allele were inconsistent.[2-5] A significant positive association with occurrence of dementia in patients with PD has been observed for both the ε4 allele and the ε2 allele,[2,6] which suggests that the APOE gene might modify the risk of dementia associated with PD. However, the exact mechanism by which APOE genotype or apolipoprotein E isoforms influence the risk and course of PD is still unclear.

The overall mortality HR of 1.8 we observed is in line with figures from other studies, ranging from 1.5-2.7.[16-24] We found that the mortality HR was higher for patients with longer disease duration and relatively low for newly diagnosed incident cases. This fits previous observations[20,25] that mortality rates in patients with PD were not increased compared with those in controls in the first years of follow-up and differed more as time since diagnosis increased. Apart from the effect of aging, disease duration thus seems to influence mortality risk in PD, and differences in the composition of study populations with respect to mean and range of duration of PD may lead to different estimates of mortality risk. From studies in prevalent cohorts, in fact, only prognosis after enrollment can be derived, which is different from prognosis after diagnosis of PD, especially in case of a long delay between diagnosis and inclusion in the study. Since we observed that mean Hoehn and Yahr scale scores were consistently higher in categories of longer disease duration, the effect of disease duration on mortality risk might reflect the effect of disease severity. Independent effects of disease severity on mortality have been described previously.[14] To correctly evaluate prognosis after PD diagnosis, prospective studies of incident cases are required. These are difficult, given the low incidence rate of PD, and were only conducted twice previously.[21,25] Both studies found a mortality HR somewhat higher than our figure for incident cases. This may be accounted for by the relatively high proportion of patients with mild PD and the fact that follow-up of incident PD cases was relatively short in our study.

Although both PD and dementia have separately been associated with increased mortality, few studies have investigated to what extent dementia contributes to the observed shorter survival in patients with PD.[14,19,24] We tried to evaluate this by adjusting in a time-dependent fashion for the occurrence of dementia, which led to

lower estimates of the mortality HRs in all strata of disease duration. This suggests that part of the reduced life expectancy of patients with PD can be ascribed to their increased risk of becoming demented. In fact, mortality risk is only slightly increased in the absence of dementia.

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7 General discussion

The aim of this thesis was to assess the impact of Parkinson disease (PD) in the general population in terms of frequency and prognosis, and to identify potential risk factors for the disease. The studies we conducted were all embedded in the Rotterdam Study, a large prospective population-based cohort study in participants aged 55 years and older. In this chapter, I will briefly summarize the main findings and try to place them in a broader perspective. First, I will consider some methodological issues relevant for epidemiological research on PD in general and the Rotterdam Study in particular. I will furthermore discuss how our findings fit into current knowledge and models for PD pathogenesis. Finally, I will reflect on the relevance and potential implications of our observations and discuss directions for future research.

Main findings

Incidence of PD

In the Rotterdam Study the incidence of PD consistently increased with age, and seemed to be higher in men (chapter 3.1). The incidence rates in our study were higher than those reported by most previous studies, most likely due to our intensive case-finding methods. A considerable proportion of the PD cases identified in our study had not been diagnosed with PD before, and hence would not have been included in a register-based study.

Subjective complaints precede onset of clinically manifest PD

At baseline, more than half of the non-demented participants in whom no parkinsonian signs were observed on physical examination nevertheless reported at least one complaint suggestive for parkinsonism (i.e. stiffness, occasional tremors, slowness of movement, falling or a feeling of imbalance). In these participants we found that subjective complaints about stiffness, tremors or imbalance at baseline were associated with an increased risk to develop PD during follow-up (chapter 3.2). Given the high proportion of elderly reporting these complaints and the relatively low frequency of PD in the general population, the specificity and positive predictive value of such complaints will be insufficient to use them as a sole screening instrument for preclinical disease. However, in a stepwise approach to detect early PD a questionnaire on subjective complaints might be an inexpensive initial test.

Risk factors: role of cholesterol and other lipids

We prospectively studied the association between dietary intake of various types of fat, as well as serum levels of total and HDL cholesterol and the risk of PD. People with higher intake of unsaturated fatty acids, main elements of neuronal

cell membranes with neuroprotective and antioxidant properties, had a significantly decreased risk of incident PD (chapter 4.1). This association remained unchanged after adjustments for several potential confounders and after exclusion of PD cases that were diagnosed relatively shortly after baseline dietary assessments. The results presented in chapter 4.1 were based on follow-up until January 1, 2000 and although they also suggested an inverse association between dietary cholesterol intake and PD risk, this did not reach statistical significance. Further analyses after we had completed an additional number of on average 3.4 years of follow-up indeed showed that higher dietary intake of cholesterol was related to a significantly lower risk of PD, in particular in women. This fitted the observation that higher baseline levels of total cholesterol were associated with a significantly lower risk of PD in a dose-effect manner (chapter 4.2), although only in women. Again, adjusting for several potential confounders did not change the results.

Risk factors: homocysteine and oxidative stress

Homocysteine may contribute to neuronal cell death in PD through neurotoxic effects, probably by increasing oxidative stress. We therefore investigated the effect on PD risk of the TT variant of the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism, which is associated with mild hyperhomocysteinemia, and of dietary intake of folate, vitamin B12 and vitamin B6, essential co-factors that are required to keep plasma homocysteine levels low. The TT genotype was associated with an increased risk of PD, particularly in smokers (chapter 5.1). This is in accordance with reported synergistic effects of smoking and TT genotype on plasma homocysteine levels. Higher dietary intake of vitamin B6, but not of folate or vitamin B12, was associated with a significantly decreased risk of PD (chapter 5.2). This observation might be explained by the antioxidant properties of vitamin B6, in addition to its homocysteine-lowering effects, although its function as a co-factor for dopamine synthesis might also play a role. We also prospectively examined the relationship between serum levels of the antioxidant uric acid and PD risk. Higher levels of uric acid were associated with a significantly lower risk of PD (chapter 5.3). A dose-effect relationship was found and the association did not change after adjusting for potential confounders.

Prognosis: risk of dementia and mortality

We observed that patients with PD had a significantly increased risk of developing dementia and a reduced life expectancy compared with participants without PD (chapter 6). The risk of dementia was more pronounced in participants carrying at least one APOE ε4 allele, and especially in those carrying at least one APOE ε2 allele, compared with ε3ε3 carriers. Increased mortality risk was more prominent in

PD patients with longer disease duration and was attenuated after adjustment for the occurrence of dementia, which suggests that an increased risk of dementia is partly responsible for the reduced survival in PD.

Methodological considerations

Most of the methodological issues related to case finding, diagnosis and potential sources of bias in epidemiological research on PD in general have already been extensively discussed in chapter 2. Methodological strengths of the Rotterdam Study (the large number of participants, prospective design and in-person examination of all subjects) by which many of these problems have at least partly been overcome are described in the previous chapters. However, even carefully designed and conducted epidemiological studies on PD have methodological limitations that should be borne in mind when interpreting the results.

An almost inevitable limitation is the inaccuracy inherent to PD diagnosis in an epidemiological setting. As long as a reliable test or marker for PD suited for large-scale population-based research (i.e. widely available, inexpensive and minimally invasive) is not available, diagnosis will depend on clinical criteria. According to current consensus criteria, a definite diagnosis requires confirmation in post-mortem material.[1] Therefore, unless neuropathological material is routinely collected, at best a diagnosis of 'probable PD' is obtained, which from clinicopathological studies is estimated to be correct in only less than 80% of the cases.[2-4] It should, however, be noted that post-mortem tissue is usually obtained at the end-stages of the illness, and one might dispute whether observed changes really reflect the processes at the time of diagnosis.

In the majority of epidemiological studies on PD, including ours, examination of post-mortem material is not part of the standard data collection and a certain amount of misclassification of disease status is thus unavoidable. We have tried to reduce diagnostic inaccuracy as much as possible by applying extensive case-finding methods and strict clinical diagnostic criteria for PD. Besides, long-term follow-up of participants was assured through a continuous computerized surveillance system. This enabled us to evaluate disease course and progression, response to levodopa medication and appearance of additional cognitive, autonomic or other non-motor symptoms, which are important for PD diagnosis and cannot be appreciated when disease is assessed at one point in time only.

A second issue concerns the preclinical period in PD. As was described in chapter 3.2, neuronal degeneration is already ongoing for at least several years before PD becomes clinically manifest.[5,6] As the duration of this preclinical phase is not known, early dopamine deficiency might theoretically exert its effects in persons seemingly unaffected at baseline. Even in prospective studies like ours, changes resulting from early disease processes may thus be mistaken for causally related risk factors.
However, this form of bias is less likely to be of influence as time between assessment of risk factors and diagnosis of PD increases, and will thus be of limited concern in studies with longer duration of follow-up like the Rotterdam Study. Moreover, the design with multiple follow-up rounds allows for separate analyses of patients diagnosed at different intervals from baseline assessments, to evaluate potential influence of preclinical disease at baseline (as described in chapter 4.1 on dietary fat intake).

How do our findings fit current knowledge on PD pathogenesis?

It is as yet unclear which mechanisms underlie the selective dopaminergic cell death in PD. Until less than a decade ago, most insights into the pathogenesis of PD were derived from post-mortem studies, epidemiological research and experimental animal models. The discovery of the first causative gene mutation responsible for familial PD in 1997 and subsequent identification of several other causative genes gave new impulses to PD research and greatly enhanced knowledge on pathogenetic mechanisms that may underlie both familial and sporadic forms of the disease.[7] Current notion is that sporadic PD is a multifactorial disease, resulting from complex interactions of several environmental and genetic factors.[7-10] Although PD seems to have many different causes, including gene mutations for hereditary PD and factors involved in sporadic cases, these appear to converge on one or a few common molecular mechanisms that ultimately lead to dopaminergic cell death.[8]

It is commonly believed that there is sufficient evidence for a role of oxidative stress in PD pathogenesis, mainly on the basis of post-mortem observations of increased oxidative stress markers in the substantia nigra of PD patients.[8,11,12] Oxidative stress results from damage by free radicals, also known as reactive oxygen species (ROS), which are highly reactive molecules that may damage cell structures, resulting in functional alteration of proteins, lipids and DNA. Much research has focused on potential sources of oxidative stress, i.e. sources of increased formation of ROS or impaired defense against ROS. Dysfunction of complex I of the mitochondrial

respiratory chain seems to be one of the major sources of free radical formation that is specifically involved in PD pathogenesis.[8] Decreased activity of this enzyme complex (NADH:ubiquinone oxidoreductase) has been described in the substantia nigra and platelets from PD patients.[13,14] Furthermore, three selective inhibitors of complex I have been found to induce selective dopaminergic cell death as well as clinical features resembling PD: the synthetic drug contaminant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), the herbicide paraquat and the widely used insecticide rotenone.[15] Impairment of complex I induces oxidative stress, but oxidative stress itself may also lead to impaired complex I function, which makes it difficult to determine which one is the primary process.[11] Recent research on the biological function of gene products of DJ-1 and PINK1, both linked to familial PD, has further contributed to the notion that mitochondrial dysfunction and increased oxidative stress might be central to PD pathogenesis. The DJ-1 protein seems to be involved in the cell's response to oxidative stress,[16] and the PINK1 protein, a mitochondrial protein kinase, may protect the cell against mitochondrial dysfunction.[17]

Several of our findings are in line with the concept of oxidative stress as an important molecular mechanism underlying PD. The observation that the incidence of PD sharply rises with age is consistent with this idea, as increasing age is known to be accompanied by enhanced oxidative stress.[11] In animal models, oxidative stress has been described as a result of homocysteine administration.[18] The observed association between the hyperhomocysteinemic MTHFR 677 TT genotype and an increased risk of PD is therefore also in agreement with the oxidative stress hypothesis. Other findings in favor of this hypothesis are the observations that several substances known to have antioxidant (i.e. oxidative stress-lowering) properties were associated with a decreased risk of PD in our studies. These include dietary unsaturated fatty acids, dietary vitamin B6 and serum levels of uric acid. We also found an inverse association between serum levels of cholesterol and PD risk. Although highly speculative, a potential hypothesis to explain this finding is provided by the strong correlation between cholesterol level and serum concentration of coenzyme Q10, a powerful anti-oxidant and mitochondrial complex I acceptor.[19,20] Through a link with coenzyme Q10, our observation might theoretically support not only the concept of oxidative stress in PD pathogenesis, but also more specifically a role of complex I dysfunction.

The first mutation that was identified for familial PD (alpha-synuclein, PARK1)[21] is associated with the formation of an abnormal, aggregated form of the alpha-synuclein protein. Alpha-synuclein was subsequently found to be the main component of the pathological Lewy Bodies that are seen in familial as well as sporadic PD,[22] which

gave rise to the idea that impaired folding and degradation of proteins may contribute to PD pathogenesis.[23] This notion is supported by evidence of malfunctioning of the pathway responsible for the elimination of abnormal cellular proteins, the ubiquitin proteasomal system (UPS), in post-mortem substantia nigra from PD patients.[24] Inhibition of the UPS was furthermore found to induce formation of inclusion bodies and selective loss of dopaminergic neurons in cultured neurons and rat brain.[25] Two of the genetic mutations for familial PD (parkin and UCHL-1) are directly associated with the UPS. Parkin is an E3 ubiquitin-protein ligase that ubiquinates target proteins before they are degraded,[26] and UCHL-1 is an enzyme of the UPS that can hydrolyze peptide-ubiquitin bonds and recycle ubiquitin monomers for re-use.[27] Mutations in these genes are hypothesized to induce accumulation of damaged or misfolded proteins through malfunction of the UPS.[28]

None of our findings directly relates to misfolded proteins or dysfunction of the UPS, but according to preliminary evidence from laboratory studies, aggregation of alpha-synuclein is probably linked to fat metabolism. Fatty acids seem to regulate alpha-synuclein oligomerization, which might precede the formation of insoluble aggregates[29,30] and genes that affect alpha-synuclein toxicity in yeast were found to be involved in lipid metabolism.[31] We found that both dietary intake of unsaturated fatty acids and serum cholesterol levels were inversely related to the risk of PD, and that APOE genotype seems to modify the risk of dementia associated with PD. These observations suggest a role of fat metabolism in PD pathogenesis, which might act through processes of protein aggregation.

It is still not completely understood why the systemic processes of mitochondrial dysfunction, oxidative stress and impairment of the UPS selectively affect the substantia nigra in PD patients. The brain depends mostly on mitochondrial energy supply, which is associated with the production of reactive oxygen species and an increased sensitivity to mitochondrial dysfunction.[32] A possible explanation that applies more specifically to the substantia nigra is the suggestion that neuromelanin, a dark brown pigment present in the cells of the substantia nigra, is responsible for the selective susceptibility of these dopaminergic cells.[33] Besides, products of dopamine metabolism are thought to increase the vulnerability of dopaminergic cells by inducing additional oxidative stress.[34]

Another unsolved issue is the inverse association between smoking and the risk of PD, which is one of the most robust findings in many epidemiological studies. Because of the consistency across different study designs, including several large prospective studies, and because the effects of smoking appear to be dose-dependent, these

findings are commonly considered to indicate a true association.[35] In the Rotterdam Study, we observed a hazard ratio for PD of 0.58 for ever compared to never smokers, which is in line with the effects sizes obtained in other studies (see chapter 2). Our finding that smoking modified the relationship between MTHFR genotype and PD as well as the association of vitamin B6 intake and PD also provides evidence that smoking somehow affects the pathological processes underlying the disease. An explanatory biological mechanism for the effect of smoking is however still lacking. Beneficial effects of cigarette smoke that have been hypothesized include stimulation of dopamine release, inhibition of monoamine oxidase B, upregulation of nicotinic receptors by nicotine and inhibition of free radical damage through carbon monoxide,[35] but as yet no link with either oxidative stress, mitochondrial dysfunction or UPS impairment has been established. On the other hand, the inverse association between smoking and PD might also be considered an epiphenomenon and does not necessarily indicate a causal relation.

Implications of our findings

We observed that the incidence of PD increases with age and that PD patients have an increased risk to develop dementia and a reduced life expectancy compared to people without the disease. These findings on the impact of PD in the general population are of importance for health care planning, notably in the light of the worldwide aging of populations. PD may be expected to become more prevalent and to impose an increasing socio-economic burden on societies. It may furthermore be concluded that an increased level of awareness of PD is needed in clinical practice, given that over one third of the patients who fulfilled the clinical criteria for PD in our study had never received this diagnosis before. Parkinsonian symptoms apparently are often considered normal phenomena of physiological aging instead of potential disease manifestations, both by patients and physicians. The presence of co-morbidity, in particular in the elderly, may further complicate PD diagnosis. Familiarity with PD and its clinical appearance should enhance timely recognition of symptoms suggestive for parkinsonism. This could prevent that symptomatic treatment, which may give substantial symptom relief, is being withheld. Even in the absence of obvious parkinsonian signs on physical examination, subjective complaints of stiffness, tremors or imbalance deserve careful attention. Our observation that persons reporting such complaints had an increased risk to develop PD during follow-up suggests that preclinical dopamine deficiency may induce subtle signs before onset of typical motor symptoms. This notion may also be relevant to the detection and diagnosis of presymptomatic PD, which will become more urgent once

preventive therapies are available. The assessment of subjective complaints might be a simple and inexpensive first step of a series of tests meant to identify patients in the early stages of neurodegeneration.

It would be premature to draw conclusions regarding treatment or prevention of PD from the results of our studies on potential risk factors for the disease. Before any recommendations can be given on dietary intake, measurement of serum levels or genetic screening, our data need confirmation in other studies. The implications of our findings rather lie in their contribution to current knowledge on the pathofysiological processes that underlie the selective dopaminergic cell death in PD. As was discussed previously, most of these findings are in line with the idea that oxidative stress is implicated in PD pathogenesis. Much of the evidence for a role of oxidative stress is derived from animal models, post-mortem studies and genetic research and mainly concerns biochemical or neuropathological characteristics in individuals or animals already affected by PD. This may complicate the distinction between causal mechanisms on the one hand and reactive phenomena secondary to the disease process on the other. Prospectively designed epidemiological studies like the Rotterdam Study, in which potential risk factors are assessed before onset of disease, may help to draw conclusions regarding causal mechanisms. Observational studies in large cohorts are well suited to test laboratory findings in the general population. Integration of lessons learned form experimental studies, genetics and epidemiology will eventually help to identity the common pathways involved in PD.

Recommendations for future research

Despite all the recent advances that have led to new and crucial pathofysiological insights, PD is still an incurable disease. Therapies for PD are as yet only symptomatic and especially chronic medication use is accompanied by considerable side effects. A major challenge for the next years is therefore to further unravel the mechanisms responsible for dopaminergic cell death, in order to stimulate the development of treatments that may slow down, stop or preferably prevent the disease. The various pathways that have been implicated in PD pathogenesis provide targets for putative neuroprotective therapies. Many candidate drugs have been identified and tested in small-scale preliminary trials, including the antioxidants vitamin E and coenzyme Q10, MAO-B inhibitor deprenyl and several dopamine agonists that have been demonstrated to protect dopaminergic neurons and to inhibit apoptosis in laboratory studies.[36] Although evidence has been found for neuroprotection from coenzyme Q10 in the form of slowing of disease progression, preventive or curative effects have not yet been observed for any of the investigated agents.[37]

Several of our findings deserve further exploration, as they might provide clues to PD pathogenesis. This holds in particular for the putative link between fat metabolism and PD. The significance of the inverse association between serum total cholesterol and the risk of PD is as yet unclear, but could be further elucidated in epidemiological studies with long term follow-up and repeated cholesterol measurements, which would provide an opportunity to see whether cholesterol levels vary with disease progression or severity. More insights could come from evaluating the effect on PD risk of polymorphisms of genes involved in transport and metabolism of cholesterol, such as the hepatic lipase gene, cholesterol ester transfer protein (CETP) and apolipoprotein E (APOE) genes,[38,39] or a potential modifying effect of these polymorphisms on the effect of other risk factors for PD. At the start of the Rotterdam Study in the early 1990s, use of lipid lowering drugs was not yet as widespread among elderly people as it is nowadays. Therefore, low numbers precluded analyses on the association between statin use and PD risk. This will, however, become less of a problem now that participants are still followed and both the number of persons with a prescription for lipid-lowering medication and the number of incident PD cases are expected to increase. The potential link with coenzyme Q10 is of particular interest given its role in mitochondrial complex I and antioxidant effects, and merits evaluation in a large prospective study with coenzyme Q10 measurements before onset of PD.

The intriguing observation that smoking seems to protect against PD has been the subject of many research projects, but underlying mechanisms have not yet been found. Other findings from epidemiological research that need to be clarified include the higher incidence of PD in men and the increased risk of PD in carriers of the APOE ε2 allele. Thus far, potentially interesting interactions and subgroup effects that might provide more insight could not be evaluated in many cohort studies, because of low numbers of PD cases. Due to the relatively low incidence of PD (at least compared to other major disease outcomes like dementia and cardiovascular illness), large cohorts and long follow-up periods are needed to identify a sufficient number of patients to allow for more sophisticated analyses. As was mentioned before, follow-up is still ongoing and more PD patients are expected to be identified, especially since participants are getting older.

Besides longer follow-up and larger numbers, the implementation of new techniques in epidemiological studies may open up new possibilities to evaluate the role of earlier hypothesized but not yet proven risk factors in PD. MRI scanning of the brain in large population-based studies might for example shed a light on the hypothesis that iron metabolism is involved in PD pathogenesis. Iron is thought to contribute to neuronal damage in PD through enhanced generation of reactive oxygen species

and an increase in oxidative stress.[40] Increased iron levels have been found post mortem in the substantia nigra of PD patients and a positive association between dietary iron intake and PD risk has been observed.[40,41] Prospective studies with iron measurements on MRI before onset of PD have not been conducted thus far, but might be valuable for research on the role of iron in PD pathogenesis.

Not only pathogenetic insight, but also the diagnosis of PD should be improved, as inaccuracy inherent to the clinical diagnosis of PD is a problem in both research and clinical practice. An easily applicable and reliable test or marker for PD would greatly improve diagnostic accuracy. Candidate biomarkers for PD that have been investigated so far are characteristics of functional neuroimaging techniques such as PET and SPECT scanning[42] and markers of oxidative stress and mitochondrial dysfunction in brain tissue, cerebrospinal fluid and blood such as malonaldehyde, superoxide radicals, the coenzyme Q10 redox ratio, 8-hydroxy-2'-deoxyguanosine from oxidized DNA and 8-hydroxyguanosine from RNA oxidation.[43] However, functional imaging is still expensive and not yet widely available, and none of the biochemical markers was proven specific enough to be useful as a diagnostic marker in clinical practice.[44]

Biomarkers could also be used to diagnose PD in very early or even preclinical stages. It has been shown that typical motor symptoms of PD only appear after more than half of the dopaminergic neurons have degenerated,[45,46] implying that the processes leading to cell death have already been set into motion long before a clinical diagnosis can be made. It is of particular interest to identify means to detect patients during this preclinical period, because disease-modifying and preventive therapies, including neural cell transplantation, likely are most beneficial when applied as early in the pathogenetic process as possible.[6,47] Prospective population-based epidemiological studies are particularly well suited to identify markers of preclinical disease, as biochemical and clinical characteristics are assessed when all participants are still without symptoms, which enables comparison between future patients and persons who remain disease-free during follow-up. Proteomics, a methodology that studies and compares peptide profiles in various tissues, is likely to make an increasingly important contribution to this kind of research, given the recent interest in the processes of protein misfolding and degradation supposedly involved in PD pathogenesis. It should finally be pointed out that considering the complex and multifactorial etiology of PD, it is probably more useful to combine potential biomarkers, gene polymorphisms, clinical characteristics and other risk factors to construct models that may predict future risk of disease. Again, the study design of population-based prospective cohort studies provides an optimal setting to accomplish this challenge.

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8 Summary / Samenvatting

Summary

Parkinson disease (PD) is the second most common neurodegenerative disorder, and is clinically characterized by resting tremor, rigidity, bradykinesia and postural imbalance. These typical motor symptoms result from a selective degeneration of dopamine-producing neurons in the substantia nigra in the brain stem. Despite intensive research in the last decades, the pathogenetic mechanisms responsible for this process are still not completely understood and therapies for PD are as yet only symptomatic. Current thinking is that in the majority of cases, PD is a multifactorial disease that results from interactions between several genetic and non-genetic risk factors. Since 1997, several gene mutations have been identified that cause familial forms of the disease with a clear Mendelian mode of inheritance, but monogenetically determined PD is thought to make up only 10% of all cases. Although there seem to be many different causes for PD, they converge on several common molecular mechanisms that ultimately lead to dopaminergic cell death. These include dysfunction of mitochondria, oxidative stress and impaired function of the ubiquitin-proteasome system, which is responsible for the elimination of misfolded or damaged proteins. More insight in these pathogenetic pathways is needed to acquire a better understanding of the disease and to develop effective therapeutic interventions.

The aim of this thesis was to assess the impact of PD in the general population in terms of frequency and prognosis, and to identify potential risk factors for the disease. The studies we conducted were all embedded in the Rotterdam Study, a large prospective population-based cohort study in 7,983 participants aged 55 years and older, with assessment of many potential risk factors at baseline and repeated in-person screening for PD.

Chapter 1 is a brief introduction to the work presented in this thesis. In **Chapter 2**, I discuss currently available evidence on diagnosis, frequency, risk factors and prognosis of PD from an epidemiological point of view. Numerous epidemiological studies on PD have been carried out, but many of them were hampered by inferior study designs, low numbers of included subjects or inadequate case ascertainment or –definition. We therefore paid special attention to methodological issues and the influence of study design on the usefulness of epidemiological data and interpretation of findings.

In **Chapter 3.1**, I describe the results of our study on the incidence of parkinsonism and PD. The incidence of both parkinsonism and PD consistently increased with age. Incidence rates in our study were higher than those reported by most previous studies, most likely due to our intensive case-finding methods. Over one third of the incident PD cases that we identified had not been diagnosed with PD before. The incidence

of PD seemed higher in men, possibly as a result of neuroprotective effects that have been found for estrogens. These findings suggest a substantial underdiagnosis of PD in the general population and emphasize the importance of direct examination of all participants in epidemiological studies on PD.

In **Chapter 3.2**, I evaluate the relationship between subjective motor complaints at baseline in participants free of dementia and without clinically obvious parkinsonian signs, and the risk to develop PD during follow-up. Subjective complaints of tremors, stiffness, slowness, a feeling of imbalance or falling were reported by more than half of the participants at baseline, although no parkinsonian signs were observed on physical examination. We found that subjective complaints about stiffness, tremors or imbalance at baseline were associated with an increased risk to develop PD during follow-up. Our results suggest that early dopamine deficiency may induce subtle signs before onset of typical motor symptoms, and that a questionnaire on subjective complaints could add to the earlier recognition of PD.

Chapter 4.1 addresses the relationship between dietary intake of various types of fat and the risk of PD. People with higher intake of unsaturated fatty acids at baseline had a significantly decreased risk of incident PD. Given the reported antioxidant properties of polyunsaturated fatty acids, this is in line with the hypothesis that oxidative stress plays a role in dopaminergic cell death in PD. On the other hand, recent evidence suggests that alterations in fat metabolism might be involved in PD pathogenesis. In **Chapter 4.2**, I describe the association between serum levels of total and HDL cholesterol and PD risk. Higher baseline levels of total cholesterol were associated with a significantly lower risk of PD in a dose-effect manner, although only in women. Possible explanations for this association include the link with fat metabolism or the correlation between cholesterol and the strong antioxidant and electron acceptor for mitochondrial complex I, coenzyme Q10.

Evidence from animal studies suggests that high levels of homocysteine may contribute to neuronal cell death in PD, probably by increasing oxidative stress. In **Chapter 5.1**, I therefore explore the association between the TT variant of the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism, which is associated with mild hyperhomocysteinemia, and the risk of PD. The TT genotype was associated with an increased risk of PD, particularly in smokers, which fits reported synergistic effects of smoking and TT genotype on plasma homocysteine levels. In **Chapter 5.2** we investigated the effect on PD risk of dietary intake of folate, vitamin B12 and vitamin B6, essential co-factors that are required to keep plasma homocysteine levels low. Higher dietary intake of vitamin B6, but not of folate or vitamin B12, was associated with a significantly decreased risk of PD. These findings may be explained by the antioxidant properties that have been reported for vitamin B6 (in addition to its homocysteine-lowering effects), or its role in dopamine synthesis. To further explore

the role of oxidative stress in PD, we studied the relationship between serum levels of the antioxidant uric acid and the risk of PD. In **Chapter 5.3** I report our finding that higher levels of uric acid were associated with a significantly lower risk of PD, with a clear dose-effect relationship.

Chapter 6 describes the prognosis of PD patients in terms of risk of dementia and mortality. Patients with PD had a significantly increased risk of developing dementia and a reduced life expectancy compared to participants without PD. The risk of dementia was especially pronounced in participants carrying at least one APOE ε2 allele. Although modifying effects of APOE genotype on the prevalence of PD and the risk of dementia associated with PD have been described previously, a biological explanation is as yet lacking. Increased mortality risk was more prominent in PD patients with longer disease duration and was attenuated after adjustment for the occurrence of dementia, which suggests that the increased risk of dementia is partly responsible for the reduced survival in PD patients.

In **Chapter 7** an attempt is made to place our findings in a broader perspective. I discuss how our findings fit into current knowledge and models for PD pathogenesis, reflect on the relevance and potential implications of our observations and discuss directions for future research.

Samenvatting

De ziekte van Parkinson is na de ziekte van Alzheimer de meest voorkomende neurodegeneratieve aandoening. Kenmerkende verschijnselen voor de ziekte van Parkinson zijn rusttremor, rigiditeit, bradykinesie en gestoorde houdingsreflexen. Deze symptomen zijn het gevolg van het afsterven van dopamine-producerende zenuwcellen in de substantia nigra in de hersenstam. Ondanks recente wetenschappelijke doorbraken zijn de precieze oorzaken en onderliggende pathogenetische mechanismen van de ziekte nog grotendeels onbekend en is er tot op heden geen preventieve of curatieve therapie voorhanden.
Sinds 1997 zijn enkele genmutaties ontdekt die verantwoordelijk zijn voor familiaire vormen van de ziekte van Parkinson. Het aandeel van deze monogenetisch bepaalde varianten wordt echter geschat op slechts 10% van alle ziektegevallen. In de overige gevallen wordt de zieke beschouwd als een multifactoriële aandoening die het gevolg is van wisselwerking tussen diverse genetische en niet-genetische risicofactoren. Hoewel er sprake lijkt van vele en zeer diverse potentiële risicofactoren, zijn deze waarschijnlijk terug te voeren op een beperkt aantal moleculaire mechanismen die uiteindelijk leiden tot dopaminerge celdood. Als belangrijkste mechanismen worden momenteel gezien mitochondriële disfunctie, oxidatieve schade en stoornissen in het ubiquitine-proteasoom systeem, een systeem verantwoordelijk voor afbraak en eliminatie van afwijkende of beschadigde eiwitten uit lichaamscellen. Meer inzicht in deze mechanismen is vereist om de ziekte van Parkinson te doorgronden en werkzame therapieën te ontwikkelen.

Het doel van het onderzoek dat is beschreven in dit proefschrift was het vaststellen van de frequentie en prognose van de ziekte van Parkinson in de algemene bevolking en het identificeren van mogelijke risicofactoren voor de ziekte. Alle studies maakten deel uit van het Erasmus Rotterdam Gezondheid en Ouderen (ERGO) onderzoek, een groot prospectief bevolkingsonderzoek naar frequentie en oorzaken van chronische ziekten bij ouderen. Tussen 1990 en 1993 werden 7983 personen van 55 jaar en ouder uitgebreid onderzocht en geïnterviewd, waarbij gegevens werden vastgelegd over diverse mogelijke risicofactoren. In de jaren die daarop volgden werd van alle deelnemers onder meer bijgehouden of ze de ziekte van Parkinson ontwikkelden. Hiertoe werden de deelnemers opnieuw lichamelijk onderzocht (in 1993-1994, 1997-1999 en 2002-2004) en werden medische dossiers en medicatiegegevens geraadpleegd via een continue geautomatiseerde koppeling met bestanden van huisartsen en apotheken.
Na een korte inleiding in **hoofdstuk 1** geven we in **hoofdstuk 2** een uitgebreid overzicht van de tot nu toe gepubliceerde resultaten van epidemiologisch onderzoek naar de

diagnose, frequentie, risicofactoren en prognose van de ziekte van Parkinson. Hoewel er wereldwijd vele epidemiologische studies zijn uitgevoerd, was een aanzienlijk deel hiervan wat betreft studie-opzet, uitvoering en omvang niet optimaal. In dit hoofdstuk besteden we bij het bespreken van deze studies dan ook vooral aandacht aan de methodologische kwaliteit, omdat deze van invloed is op de bruikbaarheid en de interpretatie van de verkregen resultaten.

Hoofdstuk 3.1 beschrijft ons onderzoek naar de incidentie van parkinsonisme en de ziekte van Parkinson in de algemene bevolking. Voor zowel parkinsonisme als de ziekte van Parkinson zagen we een duidelijke stijging van de incidentie met toenemende leeftijd. De ziekte van Parkinson leek iets vaker voor te komen bij mannen dan bij vrouwen, hoewel het verschil net niet statistisch significant was. De incidentie van de ziekte van Parkinson lag in onze studie hoger dan in veel van de eerder gepubliceerde onderzoeken. Dit is vermoedelijk het gevolg van de intensieve manier waarop in het ERGO onderzoek naar aanwijzingen voor het bestaan van de ziekte van Parkinson wordt gezocht. Door niet alleen gebruik te maken van informatie in medische dossiers, maar de deelnemers ook zo veel mogelijk persoonlijk te onderzoeken werd een aanzienlijk aantal patiënten ontdekt bij wie de diagnose ziekte van Parkinson nog niet eerder was gesteld, hetgeen doet vermoeden dat deze aandoening lang niet altijd herkend wordt. Daarnaast benadrukt deze observatie het belang van direct lichamelijk onderzoek van alle deelnemers in epidemiologische onderzoeken naar de ziekte van Parkinson .

In **hoofdstuk 3.2** beschrijven we de relatie tussen subjectieve motorische klachten en het risico om later de ziekte van Parkinson te ontwikkelen. Van de deelnemers die bij het lichamelijk onderzoek aan het begin van de studie geen parkinsonistische verschijnselen vertoonden, rapporteerde meer dan de helft desondanks subjectieve klachten van stijfheid, trillen, traagheid, een onvast gevoel of vallen. We vonden dat subjectieve klachten van stijfheid, trillen of een onvast gevoel bij aanvang van de studie geassocieerd waren met een significant verhoogd risico om later in het onderzoek te ziekte van Parkinson te ontwikkelen. Dit kan betekenen dat een tekort aan dopamine vroeg in het ziekteproces al subtiele verschijnselen kan geven, voordat de voor de ziekte zo kenmerkende motorische symptomen optreden. Een vragenlijst over dergelijke subjectieve klachten zou dan ook mogelijk een bijdrage kunnen leveren aan het opsporen van de ziekte van Parkinson in een vroeger stadium.

In **hoofdstuk 4.1** onderzoeken we het verband tussen consumptie van verschillende typen vetzuren (gemeten via een voedingsvragenlijst) en het risico op de ziekte van Parkinson. Een hogere inname van onverzadigde vetzuren bleek geassocieerd met een significant lager risico op de ziekte van Parkinson. Gezien de anti-oxidant werking van onverzadigde vetzuren ondersteunt deze bevinding de hypothese dat oxidatieve schade een rol speelt bij het afsterven van dopaminerge zenuwcellen. Daarnaast

suggereren recente literatuurgegevens dat veranderingen in vetmetabolisme een rol kunnen spelen bij de pathogenese van neurodegeneratieve aandoeningen. In **hoofdstuk 4.2** bekijken we daarom de relatie tussen het cholesterol (totaal cholesterol en HDL cholesterol) gehalte in het bloed en het risico op de ziekte van Parkinson. Hogere spiegels van totaal cholesterol bleken gerelateerd aan een significant verlaagd risico op de ziekte van Parkinson, echter alleen bij vrouwen. Een duidelijke verklaring voor deze observatie ontbreekt nog, maar mogelijk speelt de correlatie tussen cholesterol en concentratie in het bloed van co-enzym Q10 een rol. Co-enzym Q10 is een sterke anti-oxidant en elektronenacceptor voor het mitochondriële complex I, en zou volgens recente theorieën een gunstige invloed kunnen hebben op ziekten waarbij de functie van mitochondriën is gestoord.
Dieronderzoeken hebben aangetoond dat hoge concentraties van homocysteine het proces van dopaminerge celdood kunnen bespoedigen, mogelijk door een toename van oxidatieve schade. Om deze reden onderzochten we het effect op het risico op de ziekte van Parkinson van de TT variant van het methylenetetrahydrofolate reductase (MTHFR) C677T polymorfisme. Personen met deze genvariant hebben vaak een licht verhoogde homocysteineconcentratie in het bloed. In **hoofdstuk 5.1** beschrijven we dat deelnemers met het TT genotype, met name rokers, een significant verhoogd risico hadden op de ziekte van Parkinson. Dit past bij eerder beschreven synergistische effecten van roken en het TT genotype op de homocysteine concentratie. In **hoofdstuk 5.2** beschrijven we de relatie tussen inname via de voeding van foliumzuur, vitamine B12 en vitamine B6 –vitaminen die nodig zijn om het homocysteine gehalte in het bloed laag te houden- en het risico op de ziekte van Parkinson. Een hogere consumptie van vitamine B6, maar niet van foliumzuur of vitamine B12, was gerelateerd aan een verlaagd risico op de ziekte van Parkinson. Dit wordt mogelijk verklaard doordat vitamine B6 niet alleen een rol speelt in het metabolisme van homocysteine, maar ook bij de synthese van dopamine, en daarnaast anti-oxidatieve eigenschappen vertoont. Om de mogelijke invloed van oxidatieve schade verder te onderzoeken, keken we vervolgens naar de invloed van de concentratie van urinezuur in het bloed. In **hoofdstuk 5.3** presenteren we onze bevinding dat een hogere concentratie van urinezuur (een sterke anti-oxidant) gepaard gaat met een lager risico op de ziekte van Parkinson.
Hoofdstuk 6 behandelt de prognose van patiënten met de ziekte van Parkinson. Deelnemers met de ziekte van Parkinson hadden een significant groter risico om dement te worden en een significant verkorte levensverwachting vergeleken met deelnemers zonder de ziekte. Met name patiënten met het APOE ε2 allel hadden een sterk verhoogd risico op het ontwikkelen van dementie. De biologische verklaring voor deze bevinding is nog onduidelijk, hoewel een mogelijke invloed van het APOE genotype op de ziekte van Parkinson en dementie bij de ziekte van Parkinson eerder

beschreven is. Een verhoogde mortaliteit werd met name gezien onder patiënten die al langere tijd leden aan de ziekte van Parkinson en was minder uitgesproken na correctie voor het optreden van dementie, hetgeen suggereert dat de verkorte levensverwachting van patiënten met de ziekte van Parkinson gedeeltelijk is toe te schrijven aan een verhoogd risico om dement te worden.
Tot slot probeer ik in **hoofdstuk 7** onze observaties in een breder perspectief te plaatsen. Ik bespreek hoe onze bevindingen aansluiten bij de huidige inzichten omtrent de pathogenese van de ziekte van Parkinson en besteed aandacht aan mogelijke implicaties voor de huidige praktijk en toekomstig onderzoek.

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About the author

Lonneke de Lau was born on September 17th, 1973 in Middelburg, the Netherlands. In 1991, she graduated from secondary school (Strabrecht College, Geldrop) and started her medical training at the University of Maastricht. From 1994 to 1997 she studied cultural anthropology and Spanish at the Radboud University Nijmegen. In 1996 she participated in a research project on microglial reactivity in rat brains at the Universitat Autònoma de Barcelona. After she graduated from medical school in 1999, she provided medical care to refugees from the Kosovo war in a center in Arnhem for 9 months and spent 3 months working as a primary care physician in a rural clinic in Las Palomas (Queretaro), Mexico. Subsequently, she worked as a resident in Neurology at the OLVG hospital in Amsterdam (2001) and the Erasmus Medical Center in Rotterdam (first half of 2002). In August 2002 she started the work described in this thesis in the Neuroepidemiology group of the Department of Epidemiology and Biostatistics (prof. dr. M.M.B.Breteler) in collaboration with the department of Neurology (prof. dr. P.J. Koudstaal) of the Erasmus Medical Center. In 2004 she received a Master of Science degree in Clinical Epidemiology at the Netherlands Institute of Health Sciences (NIHES). She will start her residency in Neurology at the Erasmus Medical Center (prof. dr. P.A.E. Sillevis Smitt) after her thesis defense on February 1st, 2006.