Comorbidities in Parkinson's disease, cause or consequence?

Comorbiditeiten bij de ziekte van Parkinson, oorzaak of gevolg?
(met een samenvatting in het Nederlands)

Proefschrift

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General introduction and outline of the thesis

Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease. The prevalence is estimated to be about 1.8% for persons above the age of 65 years in Europe [1]. The pathophysiology of PD involves the progressive loss of dopamine-containing neurons of the substantia nigra pars compacta, leading to denervation of the nigrostriatal tract and significant reduction of dopamine at the striatal level. The resultant dopamine depletion leads to a number of typical motor features [2].

PD is characterized by motor symptoms (such as rigidity, bradykinesia) and a wide variety of non-motor symptoms (such as cognitive decline, depression), which makes PD a complex and incapacitating disease [3]. As a result, many PD patients carry a relatively heavy illness burden in the physical, mental and social dimensions of health-related quality of life compared with many other neurological or chronic conditions [4].

Age is the greatest risk factor for the development of PD. The prevalence and incidence increases nearly exponentially with age and peak after 80 years of age [5,6]. Currently, PD pathogenesis remains unclear, and the destruction of dopaminergic neurons in PD has been connected to a variety of factors. Genetic predisposing factors in combination with environmental factors are thought to be responsible for the cellular changes leading to progressive neurodegeneration in which mitochondrial dysfunction, oxidative mechanisms and neuro-inflammation are probably involved [7].

An ageing PD population

Along with increasing life expectancy of the population, incidence of age-associated morbidities increases as well [8], and morbidities more often occur together. Poly- or multi-morbidity is defined as the presence of two or more chronic or acute diseases in one person. Comorbidity may be related in several ways to the index disease. In complicating (or causal) comorbidity the additional chronic conditions are a consequence of the first chronic condition, for example polyneuropathy in patients with diabetes. Concurrent comorbid conditions are chronic diseases unrelated to the index condition, for example polyneuropathy in patients with PD [9]. In addition, the same common pathways that influence the process of aging, for example inflammation and senescence pathways, give rise to the onset of concomitant age-related diseases [10]. By understanding the common pathways of these age-related diseases and the nature of the complex relationships amongst them, insights can be gained into the impact of combinations of illnesses and symptoms on function and quality of life.

The association of PD with other clinical conditions is so far only poorly described. Some previous studies have described that patients with PD have a high rate of multi-morbidity, with up to 80% of patients with five or more chronic diseases [11-15]. A recent prospective study concluded that the total number of comorbidities at baseline were higher in PD patients than controls (4.4 ± 2.3 vs 5.2 ± 2.4 [p=0.001]) [16]. Diseases of the circulatory and endocrine system, and nutritional and metabolic diseases, like osteoporosis, diabetes and hypertension, were the most frequent in all groups [16]. Although the overall existence of concomitant diseases in PD is not well investigated, literature of one of the above-mentioned comorbidities alone and PD is more extensive studied [17-26]. Nevertheless, these studies yielded inconsistent results and the direction of the association is not clear, for example whether diabetes causes PD or the other way

around [27-29]. This can be mainly explained by the differences in study population and/or study design. In addition, these studies were small in size and most of them were observational, so no causal effect can immediately be inferred.

The multifactorial nature of age-associated comorbidity in PD

As written above, common pathways may contribute to concomitant diseases in PD, including some that potentially affect the process of ageing itself. This may include genetics and environmental and lifestyle factors. These factors may lead to common pathogenic mechanisms, for example inflammation and oxidative stress, which act potentially synergistic in causing both PD and concomitant diseases like osteoporosis, diabetes and hypertension (figure 1) [29-31]. For instance, age-related hormonal changes or lifestyle factors such as obesity, malnutrition and lack of physical activity may exacerbate inflammation and cellular damage leading to metabolic dysregulation. Metabolic dysfunction is a common process that may underlie a range of conditions. Examples include the progression of osteopenia to osteoporosis, insulin resistance to diabetes and hypertension to cardiovascular disease [32].

Another hypothesis, alongside common pathways, that explains the frequent occurrence of osteoporosis, diabetes and hypertension in patients with PD may the direct involved of these diseases in the pathological process leading to PD (complicating- (or causal) comorbidity). The current literature does not discriminate between these two hypotheses. In addition, inferring causality from observational data is problematic as it is not always clear which of the two associated variables is the cause and which the effect, or whether both are common effects of a third unobserved variable, or confounder. Consequently, understanding the association between

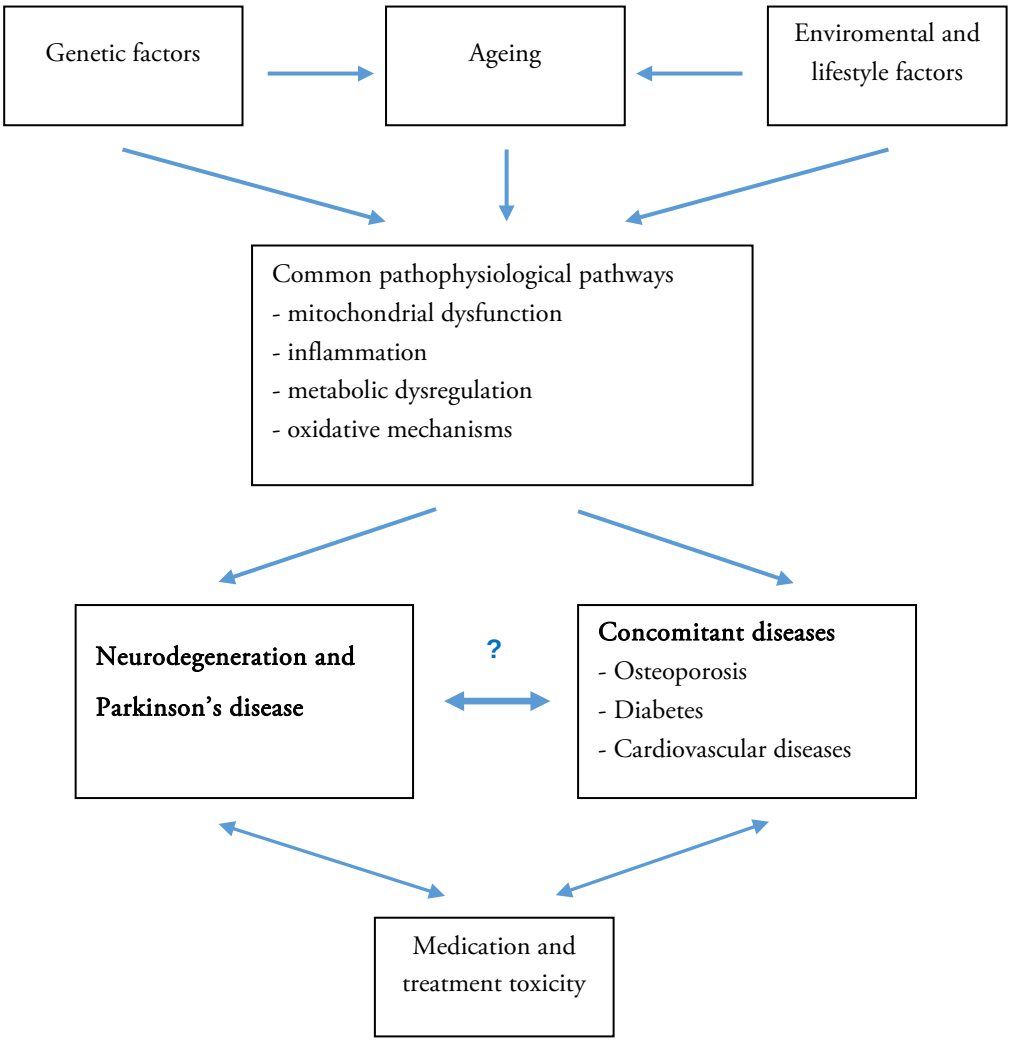
PD and osteoporosis, diabetes and hypertension requires further study, using methods that provide insights into the causal nature of observed associations and the (risk) factors contributing to these comorbidities in PD patients.

In conclusion, patients with PD may exhibit a high rate of concomitant diseases and several common pathways may contribute to this. In view of this observation the question arises which age-related and PD-related risk factors might be driving the increased rate of comorbidities and to what extent this association between these concomitant diseases is causal.

Objectives of this thesis

The aim of this thesis is to contribute to the body of knowledge of the (causal) association between PD and common concomitant diseases (osteoporosis, diabetes and hypertension) and PD and of factors contributing to these comorbidities in PD patients.

Figure 1 The multifactorial nature of age-associated comorbidities in PD



Outline of this thesis

In *chapter 2* the relation between osteoporosis and Parkinson's disease will be described. First, we give an overview of the clinical evidence, pathophysiology and treatment of osteoporosis in patients with PD (*chapter 2.1*). Second, the prevalence of osteoporosis and possible risk factors associated with bone loss in sedentary PD patients are investigated (*chapter 2.2*). In *chapter 3*, the association of diabetes and PD will be studied. In *chapter 3.1*, we use the principle of mendelian randomisation to investigate the causal nature of the relation of PD with T2D. In *chapter 3.2,* we investigate the role of genome wide significant loci of type 2 diabetes and the risk of PD. In *chapter 4*, cardiovascular diseases including hypertension and both osteoporosis and PD will be discussed. The effects of blood pressure parameters with the risk of PD will be investigated in a large prospective European cohort study in *chapter 4.1.* In *chapter 4.2* the relationship between atherosclerosis and bone mineral density, and the influence of insulin sensitivity and low-grade inflammation on this relationship in men is presented. In *chapter 5* the preceding chapters are summarized and discussed in the light of previous research, and future perspectives. A summary completes this thesis.

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2

Bone mineral density in Parkinson's disease

2.1

Parkinson's disease and osteoporosis

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Age Ageing. 2013 Mar;42(2):156-62

Abstract

Introduction

Patients with Parkinson's disease (PD) have a high risk of sustaining osteoporotic fractures as a result of falls and reduced bone mass. The objective of this study is to summarize the underlying pathophysiological mechanisms of bone loss in PD by reviewing the available literature.

Methods

A Medline search was performed for articles published between January 1975 and January 2011, using the keywords 'bone mineral density', 'bone loss', 'BMD', 'bone metabolism', 'osteoporosis', 'osteopenia', 'fractures', 'Parkinson's disease', and 'Parkinsonism'.

Results

Patients with PD have a lower bone mineral density (BMD) than age-matched controls. Bone loss in PD is multifactorial, resulting from immobility, decreased muscle strength, and low body weight. Vitamin D deficiency is also important, not only because it reduces BMD, but also because cell function in the substantia nigra depends on vitamin D. Lastly, hyperhomocysteinemia, an independent risk factor for osteoporosis, is common in PD, likely due to levodopa use, as well as vitamin B12 and folic acid deficiency. A few studies have demonstrated that treatment with bisphosphonates, active vitamin D and calcium can increase BMD in PD patients, thereby reducing the risk of fractures.

Conclusion

Bone loss in PD is multifactorial. It is clinically important because of the concomitant risk of fractures. Screening for osteoporosis should be considered more often, and therapeutic interventions should be initiated.

Introduction

Parkinson's disease (PD) is a common neurodegenerative disease characterized by both motor and non-motor symptoms. As a result, many PD patients are limited in their daily activities [1]. Compared with age-matched controls, PD patients have a significantly increased risk of fractures, mainly of the hip [2-7]. The consequences of such hip fractures in PD can be devastating, including decreased functionality, lengthy hospital stay, risk of nursing home admission and high mortality rates [8-11]. One explanation for the increases fracture risk in people with PD is falls, due to postural instability and gait disturbances. However, not all fractures in PD – and especially vertebral fractures – are related to falls [3, 7, 10]. The bone mineral density (BMD) of patients with PD is lower compared with healthy controls, thus worsening the fracture risk [3, 5, 7, 12]. However, it is unclear how many patients with PD experience bone loss. Published estimates of the prevalence of osteoporosis in PD vary considerable and the causes of bone loss, in particular, are not well described in literature. The aim of this study was to systematically review studies reporting bone loss in PD. In this review, we focus specifically on the pathophysiological mechanisms of bone loss, and treatment in patients with PD.

Literature search

A Medline search was performed for articles published between January 1975 and January 2011, using the keywords 'bone mineral density', 'bone loss', 'BMD', 'bone metabolism', 'fractures', 'Parkinson's disease', and 'parkinsonism'. Moreover, reference lists from the included studies were checked and author's names were searched for additional studies. All the articles were screened on the basis of their title, abstract, or both. Studies were included if participants had PD and the study evaluated risk factors for or interventions to prevent bone loss. Only studies in which dual energy X-ray absorptiometry of the hip or spine was used to measure BMD were included. Articles written in languages other than English, expert opinions, case reports, and articles of which the full text was not available were excluded.

Search results

This search yielded 403 studies. Twelve papers were considered eligible, using the above-mentioned criteria (figure 1). Three of those studies were prospective cohort studies, with a follow-up ranging from 1 to 6 years. The others were observational (mostly case control studies). Men and women were equally distributed and mean age varied from 60 to 78 years. Not all studies reported disease severity and duration, but when reported UPDRS varied from 25 to 33. Almost all patients had a Hoehn & Yahr stage >2, and mean disease duration varied from 2 to 6.5 years. Most studies did not take all relevant confounders (*e.g.* vitamin D concentration) into account. The characteristics of these studies are summarized in table 1.

Figure 1 Flow diagram of study selection process

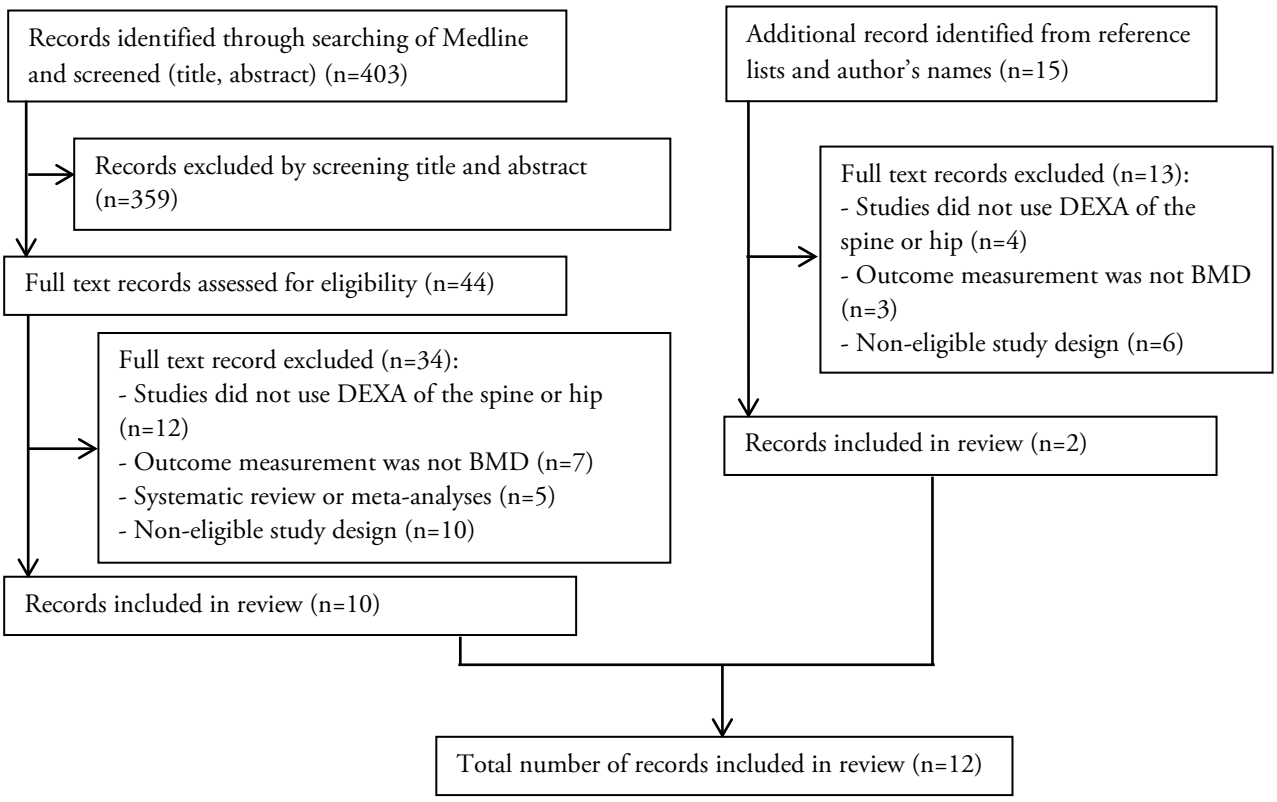


Table 1 Literature about BMD and M. Parkinson

Author	Date	Number of subjects	Outcome (BMD(DEXA))
Lam [23]	2010	108 pt; 216 co	Female, but not male, patients with PD have lower hip BMD.
Abou-Raya [14]	2009	82 pt; 82 co	BMD of all PD pt was significantly lowered. ($p<0.01$)
Song [16]	2009	107 pt;100 co	BMD was significantly lower in PD. ($p<0.001$)
Bezza [17]	2008	52 pt; 52 co	BMD at lumbar spine and hip was lower in PD. (spine 1.031 vs 1.175g/cm², $p<0.001$; hip 0.968 vs 1.054, $p=0.02$)
Fink [2]	2008	46 pt; 5891co	Total hip bone loss was greater in men with PD. (-1.08% vs. -0.36%, $p<0.001$)
Schneider [3]	2008	73 pt; 8032 co	Total hip BMD was 7.3% lower. ($p<0.01$); multivariate adjusted BMD 2.1% lower ($p=0.41$).
Kamanli [18]	2008	28 pt; 31co	In female patients hand BMD and right femoral neck BMD were significantly lower. ($p<0.05$)
Lörefalt [22]	2007	26 pt; 26 co	The BMD was lower at all sites in PD at year 1 and 2. ($p<0.05$)
Di Monaco [15]	2006	28 pt; 28 co	BMD was significantly lower in patients with trochanter fractures than in cervical fractures. ($p<0.028$)
Wood [20]	2005	105 pt	63% of the women were osteoporotic and 20% of men.
Fink [13]	2005	52 pt; 5943 co	PD was associated with lower BMD at the spine (-4.9%, $p=0.04$) and total hip (-5.3%, $p=0.07$)
Sato [7]	2001	115 pt (18 fracture, 86 non-fracture)	Elderly PD with low BMI, BMD and low vitamin D have increased risk of hip fracture, as do female patients with long postmenopausal intervals.
Taggart [19]	1995	51pt; 51co	BMD of lumbar spine was similar, total hip BMD was 10% lower ($p = 0.014$) and BMD of the neck of the femur 12% lower ($p<0.004$).
Kao [21]	1994	22 pt; 22 co	BMD was lower in PD.

Clinical evidence

Data from observational and case-control studies suggested an independent association between PD and lower BMD [13-22]. These data were confirmed by three longitudinal studies [2, 3, 21]. We will discuss these three latter studies in more detail next. Two studies investigated an annual loss of BMD in PD patients. Lörefalt *et al.* found significant reductions in total body, total hip and femoral neck BMD (3.9 vs 1.2%) in 26 PD patients compared with 26 controls. Low body weight and low physical activity were risk factors for low BMD in PD, whereas rigidity seemed to be protective, possible by increasing the mechanical load on bones. BMD, however, did not correlate with the severity of PD. An important limitation of this study is the small number of patients and controls [21].

In the Osteoporotic Fractures in Men Study, Fink *et al.* found a significantly ($p<0.001$) greater total hip bone loss of 1.1% compared with only 0.4% in community-dweling male patients (19 patients in 4,357 controls). investigated whether PD was independently associated with hip bone loss in 5937 community dwelling men. However, this study had several limitations: the number of men with PD was limited; PD was self-reported and the number of patients with follow-up data was low [2].

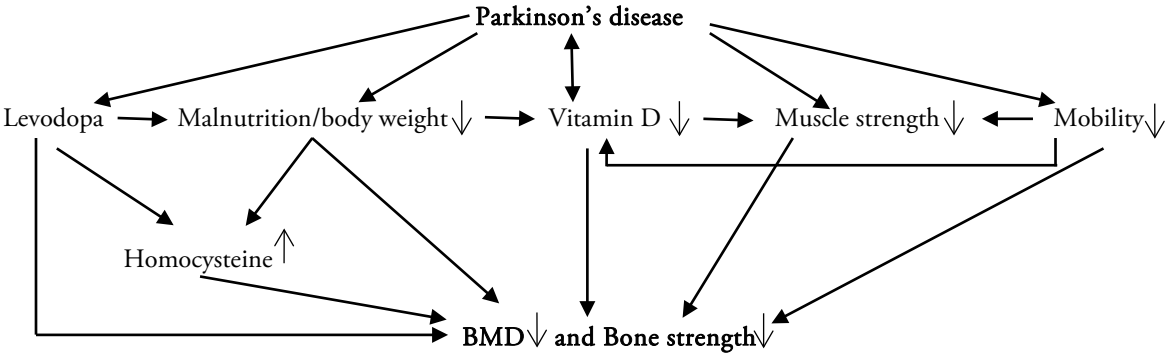
Schneider *et al.* investigated a cohort of community-dwelling women with and without PD for 6 years (73 patients and 8,032 controls). The authors found no significant difference in baseline BMD and in bone loss between the two groups after correcting for confounders. Body weight accounted for 60% of the difference in BMD. Because of the small number of patients at follow-up, the authors were unable to assess the association of PD with rate o change in hip BMD.

Besides the small proportion of patients, this study was als limited by the self-reporting of PD, so the duration and severity of the disease could not be taken into account [3].

Pathophysiology

Several factors might contribute to bone loss in PD (Figure 2). Most of these develop in the course of PD and affect or reinforce each other.

Figure 2 Factors influencing BMD and bone strength in Parkinson's disease



Physical activity and exercise

PD patients are less active compared with healthy controls [23]. Bone tissue is sensitive to its mechanical environment and is continuously stimulated by muscle contraction and weight-bearing movements, and responds to mechanical stress. Osteocytes and their dendritic connections are able to sense the fluid flow driven by stresses placed upon bone. In response to these stresses, osteocytes produce signaling molecules that stimulate bone remodeling by osteoclasts and osteoblasts [24, 25]. Subnormal mechanical stress as a result of immobilization leads to bone loss, with the rate of bone loss being influenced by the duration, intensity, and acuteness of immobilization [26]. There are indications that immobility is associated with bone

loss in PD, but research into this is limited. Only three studies investigated the association between physical performance/exercise and BMD in PD. The authors Lam and Fink found no association, Lorefalt, on the other hand, found that the amount of BMD in PD patients was directly correlated to physical activity [13, 21, 22]. Data on the association between BMD and the severity of PD are also conflicting. Only one prospective study mentioned severity. They found no correlation between BMD and severity of PD symptoms. However, besides the small number of patients, none of the patients were severely disabled [21]. In contrast, results of most observational studies have suggested significant association between disease severity and BMD. These studies also consisted of small numbers of patients. Most studies did not account for all potential confounders [14-18]. Although the evidence is scarce, it seems plausible that physical activity, which worsens as the disease progress, contributes to bone loss in PD.

Vitamin D deficiency

Vitamin D has a crucial role in bone metabolism, and a shortage of vitamin D is correlated with an increased risk of falls and fractures [27]. Vitamin D deficiency results in hypocalcemia and compensatory hyperparathyroidism, and an excess of parathyroid hormone causes bone resorption by stimulating osteoclast activity [26-28]. Vitamin D deficiency is common in PD and may be related to malnutrition, immobility and sunlight deprivation. The prevalence of vitamin D insufficiency is significantly higher in patients with PD compared to healthy controls or patients with Alzheimer disease, which suggests that there is a specific association between PD and vitamin D deficiency [29-31].

Vitamin D has an important role in the human brain. 1,25(OH)2D is synthesized in neurons and microglia by 1α-hydroxylase. Active vitamin D binds to vitamin D receptors (VDRs) and

regulates several genes involved in cell differentiation, proliferation, and apoptosis. VDRs are expressed throughout the brain, with the strongest expression of both 1α-hydroxylase and VDR being found in the (presumably dopaminergic) neurons of the substantia nigra [32]. Matkovits *et al.* showed that dopamine can induce VDR-mediated signaling in the absence of active vitamin D [33]. This supports the hypothesis that vitamin D has autocrine and paracrine functions in the nervous system. Vitamin D also seems to have neuroprotective actions, by inhibiting the synthesis of nitric oxide, by exerting direct antioxidant-like effects and anti-ischemic actions, and by modulating cytokine release [34, 35]. Vitamin D and PD are also linked at a gene level. Kim *et al.* found an association between PD and VDR gene polymorphisms, using genomic DNA extracted from peripheral blood from patients with PD and controls [36]. Several animal studies have shown that vitamin D is closely linked to genes involved in PD [36]. Newmark and Newmark even hypothesized that a chronically inadequate vitamin D intake may contribute to the pathogenesis of PD. They suggested that a continuous inadequate intake of vitamin D leads to a chronic loss of dopaminergic neurons in the brain [37]. A recent longitudinal study confirmed this hypothesis. Knekt *et al.* investigated a cohort of 3173 men and women free of PD in Finland with a follow up of 29 years and concluded that low vitamin D status predicted the development of PD (50 cases and 3,123 non-cases). However, this study had some weaknesses, such as a small number of cases, the single measurement of vitamin D, and the possibility of residual confounders due to the fact that risk factors for PD are not well known [38].

Muscle strength

Both vitamin D deficiency and decreased mobility reduce muscle strength (figure 2). Muscle strength has been negatively associated with BMD in various populations, and bone formation

and remodeling may be affected by local mechanical signals generated by muscle contraction [39-41]. Environmental influences (exercise, nutrition and vitamin D) as well as genetic factors influence this bone–muscle relationship [42, 43]. The isokinetic muscle strength of patients with PD is also reduced compared with age-matched controls, even in early disease stages, and declines further with disease progression. The specific cause of this weakness is not known [44]. One study reported lower extremity muscle strength (isometric hip flexion and knee extension) to be associated with hip BMD in women with PD (34 patients and and 30 controls), after correcting for several confounders [42]. Another study investigated the association between lumbar spine BMD and trunk muscle strength and rigidity and found trunk muscle strength, but not trunk rigidity, to be independently associated with lumbar spine BMD (43 patients and 29 controls) [45]. Both studies were limited by small sample sizes and possible selection bias.

Low body weight

Several studies have suggested that low body weight is a risk factor for low BMD in PD [17, 19, 22]. Schneider *et al.* found that weight accounted for 60% of the age-adjusted difference in hip BMD in 73 women with PD compared with 8,032 controls [3]. One explanation is the decreased mechanical load. In addition, a lower body fat content is associated with lower oestriol production in postmenopausal women, leading to a reduced BMD [46, 47]. Patients with PD are at high risk of poor nutrition for several reasons, such as impaired hand-mouth coordination, dysphagia, intestinal hypomotility, depression, cognitive deficits, and side effects of medication. At the same time, there is an increased energy requirement due to muscular rigidity and involuntary movements. In addition, malnutrition can lead to low levels of vitamin D, folic acid, and vitamin B12, with negative consequences on bone formation and strength [48,49].

Hyperhomocysteinemia

Hyperhomocysteinemia is an independent risk factor for osteoporotic fractures [50-52]. The catabolism of homocysteine depends on folic acid, vitamin B12, and vitamin B6, and thus folic acid and vitamin B12 deficiency can cause hyperhomocysteinemia. Homocysteine has a direct effect on bone by binding to extracellular collagen, which interferes with the formation of collagen cross-linking [53, 54]. In addition, *in vitro* studies have shown that homocysteine stimulates the differentiation of osteoclasts through increased generation of intracellular reactive oxygen species, and induces apoptosis of osteoblasts via the intracellular reactive oxygen species-mediated mitochondrial pathway and NF-κB activation in human bone marrow stromal cells [55-57]. The first mechanism results in poor bone quality and the second reduces BMD, both contributing to an increased fracture risk.

Hyperhomocysteinemia is common in PD and is associated with fracture risk and a low BMD [58-62]. In addition to vitamin B12 and folic acid deficiency, levodopa use may cause hyperhomocysteinemia. Levodopa and dopamine are methylated by catechol O-methyltransferase (COMT), with S-adenosylhomocysteine as methyl donor, to form S-adenohomocysteine. Since S-adenohomocysteine is rapidly converted to homocysteine, levodopa therapy can lead to hyperhomocysteinemia. Theoretically, inhibition of COMT should reduce levodopa-induced hyperhomocysteinemia, but the literature on this is contradictory. These discrepancies in the literature might be related to the different levels of vitamin B12 and folic acid in the included patients [63-68]. Two studies have shown that suppletion of vitamin B12 and folic acid decreases homocysteine levels in levodopa-treated patients with PD [69, 70]. Moreover, Lee *et al.* concluded that homocysteine-lowering therapy with folic acid and vitamin B12 prevents bone

loss in levodopa-treated patients [71]. Another recent study found that not levodopa use, but decreased levels of vitamin B12 and folic acid, cause hyperhomocysteinemia in PD [62].

Management and treatment

The paragraphs above indicate that a complex interaction between various factors can contribute to bone loss in patients with PD. Optimal management calls for careful assessment of all these factors, followed by tailored treatment where possible. Because scientific evidence concerning the treatment of osteoporosis in PD is scarce, we would like to recommend clinicians to treat PD patients according to the same principles that apply to non-parkinsonian patients with osteopenia or osteoporosis. Specific recommendations for treatment include: (i) lifestyle factors & exercise; (ii) dietary supplementation; and (iii) anti-osteoporotic medication.

The WHO developed the calculation tool FRAX to evaluate fracture risk of patients based on individual patient models that integrate clinical risk factors as well as BMD at the femoral neck. The risk factors of having 'PD' or 'falls related to PD' have however, not been quantified (sufficiently) in FRAX to give an accurate 10-year probability of fracture in these patient categories. The FRAX calculation tool can therefore not be recommended in calculating fracture risk in PD patients [72].

Lifestyle factors / exercise

Smoking and alcohol are well-known risk factors for osteoporosis, so patients should be advised to stop smoking and reduce alcohol consumption. Exercise is recognized as key modifiable lifestyle factor that is essential to the prevention and management of osteoporosis. Physical activity programs for maintaining BMD are based on a site-specific modifying effect, in addition to strengthening muscles and improving balance, thus reducing the overall risk of falls and fractures. The influence of exercise on BMD in PD is not well studied. The ParkFit study is currently being conducted. It researches whether a physical activity promotion program can increase physical activity levels in sedentary patients with PD [23].

Dietary supplementation

Sato *et al.* performed a randomized, double-blind, placebo-controlled trial of 1α-hydroxyvitamin D3 supplementation (1 μg/day) for 18 months in patients with PD (43 patients in both groups). After 18 months, the treatment group showed a smaller decrease in BMD (1.2% vs 6.7%; $p<0.00$) and a lower risk of non-vertebral fracture (18.6 vs 2.3%; OR 9.8, $p=0.003$) [73].
Lee *et al.* studied the effect of homocysteine-lowering therapy on preventing bone loss in patients with PD taking levodopa. Patients were randomly assigned to treatment (n=14) (folate 5 mg daily, mecobalamin 500 μg three times daily) or no treatment (n=13). Both groups took daily oral supplements of calcium (500 mg) and cholecalciferol (1000 IU). Follow-up was 12 months. The authors found that homocysteine-lowering therapy resulted in significantly greater improvements in BMD at the lumbar spine (4.4%), total femur (2.8%), and femur shaft (2.8%). Although this was a small trial and fracture reduction was not taken into account, it is an easy therapy with minor side effects [71].

Anti-osteoporotic medication

Only three studies focused on pharmacological treatment of osteoporosis in PD, all considering bisphosphonates. The role for selective oestrogen receptor modulators and strontium ranelate has not been evaluated in PD patients.

The first study, a 2-year, randomized, double-blind, placebo-controlled trial, studied the effect of risedronate in men with PD 9121 patients in both groups). Risedronate (2.5 mg) and ergocalciferol (1000 IU) daily were compared with ergocalciferol (1000 IU) and placebo. BMD increased 2.2% in the risedronate group and decreased 2.9% in the placebo group. Nine patients in the placebo group and three patients in the risedronate group sustained hip fractures. So, risedronate reduced the relative risk of a hip fracture by 0.33 (95% CI: 0.09-1.20) [74]. The same authors reported similar benefits in a study of elderly women with PD allocated to once weekly 17.5 mg risedronate and ergocalciferol compared with ergocalciferol and placebo (136 patiets in both groups) [75]. The third study investigated the effect of alendronate in a 2-year randomized, double-blind, placebo-controlled trial of elderly women with PD (144 patients in both groups). Patients were treated daily with alendronate (5 mg) or placebo, and both groups received ergocalciferol (1000 IU). BMD increased 1.3% in the intervention group and decreased 2.8% in the control group. Aledronate reduces the relative risk of hip fractures (14 versus 4 fractures) by 0.29 (95% CI: 0.10-0.85) [76]. A shortcoming of these studies was that BMD measurements were performed at the second metacarpal bone by computer X-ray densitometry and not by DEXA of the hip. Nevertheless, they found a decrease in the number of fractures. Altogether, bisphosphonates seem to be effective for osteoporosis in PD. No drug interaction occurs with levodopa or other medications used to treat PD and when a patient experience dysphagia bisphosphonates can be administrated intravenously.

Conclusion

Patients with PD have a lower BMD than age-matched controls. This reduced bone mass, in combination with frequent falls, explains the increased fracture risk of patients with PD. The BMD reduction in PD is multifactorial in origin, involving reduced mobility, vitamin D deficiency, hyperhomocysteinemia (caused by levodopa use, or vitamin B12 or folic acid deficiency), malnutrition/low body weight, and decreased muscle strength. All these factors are common in PD and act synergistically (figure 2). It is essential to monitor these factors in order to assess the risk of osteoporosis and, consequently, reduce the risk of developing fractures. Patients with PD are currently not routinely screened for osteoporosis [77], yet the high incidence of fractures in these patients, resulting in an increased morbidity and mortality, makes careful management necessary. An extensive risk assessment should be performed, including medication use, level of immobilization, muscle strength, and nutritional status. If a patient has several risk factors, then BMD should be measured with DEXA. If osteoporosis is present, treatment should be started with bisphosphonates, vitamin D supplementation, and an adequate intake of calcium. Osteoporosis in PD has not been extensively studied and further research is needed. Larger and more powerful studies should investigate the pathophysiology of osteoporosis in PD and ways to prevent bone loss and reduce the incidence of fractures.

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2.2

Bone mineral density and vitamin D status in Parkinson's disease

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Abstract

Introduction

Bone loss is more common in Parkinson's disease (PD) than in the general population. Several factors may be involved in the development of bone loss, including malnutrition, immobilization, low body mass index, decreased muscle strength, vitamin D deficiency and medication use. This study investigates the prevalence of osteoporosis and possible risk factors associated with bone loss in early stage PD.

Methods

In 186 PD patients (Hoehn and Yahr stage 1-2.5, mean age 64.1 years) bone mineral density (BMD) measurements were performed with DEXA. T- and Z-scores were calculated. Univariate linear regression analysis was performed to identify additional variables that contributed to BMD. 25-OH-vitamin D status of PD patients was compared with 802 controls (mean age 63.3 years) using linear regression analysis.

Results

Osteoporosis (11.8%) and osteopenia (41.4%) were common in PD patients. Mean Z-score of the hip was 0.24 (SD 0.93) and of the lumbar spine was 0.72 (SD 1.91). Female gender, low weight and low 25-OH-vitamine D were significantly correlated with BMD of the hip and lumbar spine. PD patients had significant lower 25-OH-vitamin D serum levels than controls (B= -10, p=0.000).

Conclusion

More than half of the patients with early stage PD had an abnormal BMD. Female gender, low weight, and low vitamin D concentration were associated with bone loss. Furthermore, vitamin D concentrations were reduced in PD patients. These results underscore the importance of proactive screening for bone loss and vitamin D deficiency, even in early stages of PD.

INTRODUCTION

Parkinson's disease (PD) is a common and incapacitating disorder affecting a sizeable proportion of the ageing community. Patients with PD have an increased risk of sustaining fractures [1,2] The main risk factors for fractures in PD are falls, due to underlying balance disorders and a decreased bone mineral density (BMD [3,4]. Bone loss appears to be more common in PD than in the general population. Several factors may be involved in the development of bone loss, including malnutrition, immobilisation, low body mass index (BMI), decreased muscle strength, vitamin D deficiency and certain medications [5]. Furthermore, osteoporosis is an important risk factor for fragility fractures, which are associated with increased morbidity. Therefore, osteoporosis screening might need special attention in this group of PD patients.

Although several studies of bone loss in PD have been conducted, most have focused on patients with advanced disease (Hoehn and Yahr 3 or more), also, the data about etiological factors are not entirely consistent [12,15,16]. This study evaluated the prevalence of osteopenia and osteoporosis in patients with early PD and also studied a number of risk factors known to be associated with bone loss.

METHODS

Subjects

PD patients

The study population consisted of subjects from the ParkFit study, a randomized controlled trial evaluating the effectiveness of a multifaceted behavioural change program to increase physical activity in PD patients. The rationale and study design have been described previously[6]. Patients participating in the ParkFit study were invited to also participate in the present study. Data collection took place between September 2008 and January 2010. Eligibility criteria were: (a) PD, according to the UK Brain Bank Criteria[7]; (b) age between 40 and 75 years; (c) sedentary lifestyle defined as: <3 times a week vigorous-intensity physical activity for <60 minutes; or <3 times a week moderate-intensity physical activity for <150 minutes); (d) Hoehn and Yahr ≤2.5. Exclusion criteria were: (a) unclear diagnosis (no gratifying and sustained response to dopaminergic therapy); (b) MMSE <24); (c) unable to complete Dutch questionnaires; (d) severe co-morbidity interfering with daily functioning; (e) daily institutionalized care; and (f) deep brain surgery. Informed consent was obtained before the first assessment. All subjects gave written informed consent prior to the study, as approved by the local Medical Ethical Committee. The present study has been approved by the appropriate ethics committee and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All subjects gave their informed consent prior to the inclusion in the study.

Measurements PD patients

Dual-Energy X-ray absorptiometry

Between 0-6 months after inclusion of the ParkFit study patients received a dual-energy X-ray absorptiometry (DEXA). BMD measurements were performed with DEXA using a Hologic QDR1000. Scanning was performed according to the instructions of the manufacturer. Quality assurance, including calibration was performed routinely every morning for DEXA, using the standard provided by the manufacturer. Quality assurance, including calibration was performed routinely every morning for DEXA, using the standard provided by the manufacturer. BMD was measured at the femoral neck, trochanteric and intertrochanteric regions and in the first four lumbar vertebrae. The difference between an individual's BMD and the mean for a reference population is expressed in standard deviation (SD) units. T- and Z-scores were calculated where the T-score is the SD of the individual BMD compared with the mean BMD score in a young healthy population, and the Z-score is the SD of the individual BMD compared to the mean BMD score of a similar sex-, age-, weight- and height-matched population. We used the WHO classification range to categorize subjects as normal ($T > -1$), osteopenic ($-2.5 < T \leq -1$), or osteoporotic ($T \leq -2.5$).

Physical activity

The level of physical activity was measured with a 7-day recall, based on an interview-based physical activity questionnaire , the LASA Physical Activity Questionnaire (LAPAQ)[10]. Patients were asked to list their daily amount of activity (frequency and duration), so total time spent on physical activity (in hours per week) could be calculated.

Isometric grip strength

Isometric grip strength (IGS) was measured using an adjustable hand held dynamometer (JAMAR dynamometer) at the non-dominant hand. The subjects were seated with their shoulder adducted and neutrally rotated. The dynamometer was held freely, without support. The elbow was flexed at 90^0 and care was taken that it did not touch the trunk. The forearm was in a neutral position, and the wrist was held between 0-30^0 dorsiflexion and between 0-15^0 ulnar deviation. The subjects were told to put maximal force on the dynamometer. The maximal value of two trials was noted in kilogram.

Body composition

Height and weight were measured in standing position without shoes. Body mass index (BMI) was calculated as the weight in kilograms divides by the square of heights in meters.

Other variables

A wide range of other variables was assessed: disease severity, (Hoehn and Yahr staging), motor section of the Unified Parkinson's Disease Rating Scale (UPDRS), duration of PD (date of diagnosis and date of first symptoms), mobility (Timed Up and Go test [TUG]) [11] and gait speed (participants were timed while walking 4 meters at their normal pace).

Participants were asked about current and past medication use. All participants were interviewed about their smoking and alcohol habits. The participants were asked to complete questionnaires about diet and sunlight exposure. The mean weakly dietary calcium and vitamin D intake was calculated for each participant. Furthermore, serum samples of both calcium and vitamin D were measured. Since low levels of testosterone have been shown to have a detrimental effect on bone

density in men, we analysed testosterone levels related to bone density. All patients were measured on dopaminergic medication.

25-hydroxy vitamin D3

25-hydroxy vitamin D (25(OH)D) levels were measured on the E170 modular (Roche Diagnostics, Mannheim, Germany) and compared with a reference group. The reference group consisted of 402 independent living women [8] and a 400 independently living men[9]. Vitamin D deficiency was defined as being a 25(OH)D of less than 50 nmol/l.

X-rays

X-ray radiographics of the spine and thoracal vertebrae were performed to asses vertebral compression fractures.

Statistical analysis

Bivariate associations were determined using the chi-square test for categorical variables and the unpaired *t*-test for continuous variables. Univariate linear regression analysis was performed to study the association between the several factors mentioned above and BMD. Age, gender, height and weight were included in the analysis, as these factors may influence BMD. Linear regression analysis was used to examine the 25(OH)D concentration between PD and controls after controlling for covariates age, gender, smoking and alcohol consumption. A significant level of 0.05 was set for all statistical tests.

RESULTS

Of the 586 PD patients included in the ParkFit study, 186 PD patients participated in the present study (table 2). Characteristics of the patients in the present study and PD patients in the ParkFit study are presented in table 1.

Bone mineral density in PD

The summed prevalence of osteoporosis and osteopenia was 53.2% (41.1%% osteopenia, 11.8% osteoporosis) in PD patients (table 3).The mean Z-score for the hip was 0.24, and for the lumbar spine 0.72.

Table 1 Characteristics of patients and controls

Variable	PD patients (n=186)	Control group (n=802)
Demographics		
Age	64.1 (7.7)	63.3 (8.9)
Men (%)	71%	50%
Weight (kg)	80 (13.1)	76.8 (13.9)
Height (cm)	173.8 (8.6)	170.9 (9.7)
BMI	27.2 (4)	26.2 (3.9)
Vitamin D		
25-OH-vitamine D nmol/l (mean,sd)	48.3 (20.2)	56.7 (22.9)
25-OH-vitamine D % insufficiency	56.2%	43.2%
PD characteristics		
Disease duration (years)	4.9 (4.2)	
UPDRS III	31 (9.0)	
HY 1 HY 1.5 HY 2 HY 2.5	2.1 2.1 83.2 9.9	
Physical activity		
Level of physical activity (hours/week)	12.2 (8-20.2)	
Regular (>3 time a week) going outside	97%	

Data reflect mean (SD), percentage or median (IQ-range). PD Parkinson's disease, BMI body mass index, UPDRS III unified Parkinson's disease rating scale part III, HY Hoehn and Yahr stage

Table 2 Characteristics of patients of the ParkFit study and subgroup

	Participants without DEXA (n=400)	Participant with DEXA (n=186)
Age	65.8 (7.7)	64.8 (7.5)
Gender (% men)	62.5% (n=250)	71% (n=132)
Weight (kg)	81.1 (15.4)	82.0 (13.0)
Height (cm)	171.0 (10.3)	173.8 (8.9)
BMI	27.7 (4.3)	27.2 (4.0)
Disease duration (years)	5.4 (4.7	4.9 (4.2)
UPDRS III	33.5 (11.0)	31.0 (9.0)
HY 1	1.8%	2.2%
HY 1.5	3.3 %	2.2%
HY 2	71.3%	85.5%
HY 2.5	16.3%	10.2%

Data reflect mean (SD), percentage or median (IQ-range). PD Parkinson's disease, BMI body mass index, UPDRS III unified Parkinson's disease rating scale part III, HY Hoehn and Yahr stage

Table 3 Bone mineral density in Parkinson's disease

BMD	PD patients
Osteoporosis/osteopenia (%)	98 (50.5%)
Normal bone mineral density	93 (49.5%)
BMD total hip (mean, sd) g /cm2	0.94 (0.1)
BMD lumbar spine (mean, sd) g/cm2	1.06 (0.2)
Z-score hip right (mean, sd)	0.25 (0.9)
Z-score hip left (mean, sd)	0.22 (0.9)
Z-score lumbar spine (mean, sd)	0.72 (1.9)

Data reflect mean (sd) or number (percentages %) BMD bone mineral density, PD Parkinson's disease

Determinants of BMD

Univariate regression analyses showed that female gender, low weight and low 25(OH)D were significantly correlated with BMD of both the hip and lumbar spine (table 4). Physical activity and isometric grip strength were also correlated with the BMD of the hip. No relationships between other factors and BMD were present. Multivariate regression analysis showed that the BMD of the hip and lumbar spine were related to female gender, low weight and low 25(OH)D (table 5).

Table 4 Univariate regression analysis BMD lumbar spine and hip total

Variable	BMD lumbar spine B (SE)	BMD hip total B (SE)
Age	0.001 (0.002); p=0.551	-0.001 (0.001); p=0.471
Weight	0.0006 (0.001); p= 0.000	0.004 (0.0001); p=0.000
Gender	-0.087 (0.033); p=0.009	-0.107 (0.020); p=0.000
25-OH-Vitamin-D	0.002 (0.001); p=0.013	0.001 (0.00); p=0.025
Physical activity	-0.013 (0.019); p=0.501	-0.031 (0.012); p=0.010
HY	-0.069 (0.056); p=0.216	-0.045 (0.035); p=0.202
TUG	0.004 (0.005); p=0.389	-0.005 (0.003); p=0.139
Isometric grip strength	0.001 (0.001); p=0.152	0.001 (0.000); p=0.001
Levodopa use	-0.075 (0.035); p=0.032	-0.014 (0.023); p=0.541
Homocystein	-0.001 (0.003); p=0.797	-0.002 (0.002); p=0.265
Testosterone	0.001 (0.003); p=0.781	0.000 (0.002); p=0.820

Data reflect regression coefficient (B), standard error (SE) and p-value (p).

Table 5 Multivariate regression analysis bone mineral density in Parkinson's disease

	Regression coefficients (SE); p-value	
Variable	BMD lumbar spine	BMD hip total
Age	-0.09 (0.03); p < 0.01*	-0.1 (0.02); p < 0.00*
Weight	0.01 (0.00); p < 0.00**	0.00 (0.00); p < 0.00**
Gender	0.002 (0.001); p < 0.01***	0.001 (0.00); p = 0.02***
25-OH-Vitamin-D	-	-0.021 (0.011); p = 0.063**
Isometric grip strength	-	0.0 0.001); p = 0.604**

** Corrected for age, ** corrected for age and gender, *** corrected for age, gender, height and weight*

25 (OH) D concentration PD versus controls

In PD patients 56,2% has a vitamin D deficiency (mean vitamin D concentration 48.3 nmol/l), compared 43.2% was vitamin D deficient (mean 56.7 nmol/l) (Table 1). 25(OH)D serum levels were significantly lower in PD compared to controls (differences = - 10.2 nmol/l, p < 0.000).
A higher portion of the samples were drawn in the winter to spring (when vitamin D levels are lower). The portion of samples drawn in the winter to spring were significantly lower than the portion drawn in the summer to fall (43.4 nmol/l vs. 58.8 nmol/l, p < 0.00), but these seasonal differences had no influence on the regression analyses we performed.

Testosterone levels

Regression analysis showed that testosterone levels have no significant relationship with BMD of the hip and lumbar spine (Hip: B= 0.000, p=0.820; Lumbar spine: B=0.001, p=0.781).

DISCUSSION

This study showed that over 50% of sedentary patients with early PD had an abnormal BMD. Specifically, 41.4% had osteopenia and 11.8% had osteoporosis. These findings are largely consistent with previous studies [4,3,12-15], although the prevalence observed here was lower compared to other studies, which may be explained by the lower H&Y stages in our cohort. Indeed, others have found a greater decrease in BMD in the subgroup with advanced disease in comparison with those with early disease[16]. A higher prevalence of bone loss in more advanced PD can be explained by further diminished activities of daily living, greater motor impairment, less sunlight exposure due to greater immobility and continuing weight loss, which all result in further lowering of vitamin D levels.

We should point out that the median Z-scores were very close to zero. Therefore, the prevalence of osteoporosis in PD may not be more common than in the general population. However, the absolute prevalence of osteopenia and osteoporosis is very high in this sample. Considering the morbidity and mortality related to hip fractures, especially in PD[17], it is important to be aware of the high prevalence of bone loss even in early stages of disease. Together with the high risk of falls (even in HY stage 2-2.5), bone loss is an important risk factor for fractures in PD patients. In our study, female gender, weight loss and low 25(OH)D levels were identified as significant risk factors associated with a lower BMD. Female gender and weight loss are well recognized risk

factors for bone loss in the general population and in patients with PD[5,14]. Weight loss is reported frequently in PD, also during early disease, and female patients are at higher risk [18,19].

To help prevent such fractures, preventive strategies are needed, including supplementation of vitamin D deficiency observed here and by others, or by promoting physical activities. Preventing or reverting weight loss might also help, as reduced body weight was an additional risk factor for bone loss. In addition, gait and balance training (and in particular treatment strategies aimed at reducing freezing of gait) may help to reduce falls.

The high incidence of vitamin D deficiency observed in the present study is remarkable. It has been suggested that vitamin D deficiency is caused by sunlight deprivation, and low vitamin D levels induces compensatory hyperparathyroidism, with further contributes to low BMD in patients with PD. However, in our study patients experienced sufficient sunlight exposure and adequate dietary intake of vitamin D. Furthermore, the results of this study demonstrated that PD patients had significant lower levels of vitamin D compared with controls. These findings are confirmed by earlier studies[20]. One study additionally showed that vitamin D deficiency was more common in PD compared to patients with Alzheimer disease[21]. Larger studies remain necessary to further investigate the association between vitamin D and bone loss. Pending further evidence, it is important to be alert of vitamin deficiency in PD and its possible effect on BMD and muscle strength. Lower muscle strengths itself has a negative effect on bone, also in PD[22,23], and is associated with an increased fall risk. We therefor recommend to consider routine measurement of vitamin D in older patients with PD, even in early stage of disease.

The present study is the largest series of PD patients who received bone densitometry measurements. The results of previous studies on the prevalence of osteoporosis are inconsistent

and not all studies used the same methods of assessment of BMD or the WHO definitions. A major advantage of our study is that many risk factors associated with bone loss were taken into account.

Our study also had several limitations. First, due to its cross-sectional nature, the associations observed here cannot be taken as definitive evidence of a causal relationship. Second, the lack of a control group for the BMD is a limitation. However, bone mineral density inherently has its own controls owing to the method of the statistical measurement of T and Z-scores. As such, a mean Z score of 0 would equate to an equivalent age-matched population. Finally, the present study was performed in a subgroup of patients selected from the ParkFit trial, an RCT that specifically selected sedentary PD patients, aiming to promote their levels of physical activity. The present findings can therefore not be extrapolated to all PD patients, but may apply only to this selected subpopulation.

We conclude that BMD is often affected, even in early PD. The lower BMD is mainly associated with vitamin D deficiency, lower body weight and female gender. These could be clinically important because of the concomitant risk of fractures in combination with an increased fall risk. We recommend that older patients with PD are evaluated for the risk of osteoporosis. Besides classical risk factors, vitamin D deficiency and weight loss should be addressed. In the case of osteoporosis, treatment with bisphosphonates could be considered, in combination with calcium and vitamin D supplementation.

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3

Diabetes and Parkinson's disease

3.1

A mendelian randomisation study of Parkinson's disease and type 2 diabetes: results from the EPIC-InterAct Study

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In preparation

Abstract

Objective

To estimate the unconfounded relation between Parkinson's disease (PD) and type 2 diabetes (T2D).

Methods

We used data from the European Prospective Investigation into Cancer and Nutrition (EPIC)-InterAct case-cohort study; comprising 9679 incident cases of T2D and a representative subcohort (n=12679) that also includes 589 of the incident cases, from eight European countries. A Mendelian Randomisation study was performed using a score based on genetic variants from 28 PD associated loci (PDGene database). We generated an instrumental variable estimate of the relationship of PD with T2DM by combining the SNP-PD associations, using the PDGene database, with the SNP-T2D associations from EPIC-InterAct-study, using the inverse variance weighted method. To booster power we combined our results for single SNPs with those from DIAGRAM and UKBiobank. Associations of PD on HbA1c and hsCRP were additionally examined.

Results

The mean (SD) age of the subcohort was 52 (9.6) years, and 39% were men. The genetic score was not associated with risk of T2D (pooled HR 0.98 (95%CI: 0.92-1.03) per SD higher genetic score). Instrumental variable analysis did not show a relationship between PD and T2D (OR 1.01, 95%CI: 0.96-1.05) in the EPIC-InterAct study. The meta-analysed data did also not reveal

a significant association (OR 1.01 (95%CI: 0.98-1.04)), using IV analysis. No association was found between the genetic score and Hba1c.

Conclusion

This study does not support a direct causal relation of PD with T2D.

Introduction

Parkinson's disease (PD) is a common neurodegenerative disease, affecting approximately 1.8% of the population over the age 65 (1). The prevalence of PD and comorbidities increase with age and therefore many patients with PD suffer from other diseases related to old age. About 0.3% - 2.4% of PD patients are additionally affected by diabetes mellitus (2-6). T2D and PD share multiple common pathophysiological mechanisms such as impaired mitochondrial metabolism, insulin resistance, therapies for glucose control, and other cardiovascular risk factors that often cluster with diabetes within the spectrum of metabolic syndrome (2,7). In particular, inflammation plays a critical role in the destruction of both pancreatic islet β-cells and dopaminergic neurons in the substantia nigra, making inflammation a possible common pathway for both diseases (2). As incidences of both PD and T2D rises, we believe that it is important to determine whether the diseases are causally related to each other.

Most of the studies investigating the association between PD and T2D are observational, and the direction and causality of this relationship is unclear (2-6). Inferring causality from observational data is problematic as it is not always clear which of two associated variables is the cause and which the effect, or whether both are common effects of a third unobserved variable, or confounder. A mendelian randomization (MR) approach uses genetic variants that index the exposure of interest to test for a causal relationship between exposure and outcome (8). Since genes can be thought of as randomized and fixed at conception, confounding factors will be equally distributed among different genotypes. As a consequence, MR analyses will be less prone to confounding than the directly observed association. Furthermore, it will be free of reverse causation since a phenotypic trait cannot cause genetic variation.

The most recent and largest genome wide association study (GWAS) of PD identified 28 independent genetic variants associated with for PD across 24 loci (9). Variants at these loci can be used as genetic instruments, to estimate the unconfounded effect of PD on the risk of T2D. The aim of the present study is to identify whether PD is causally related to the risk of T2D using genetic variant as instruments in an uni-directional MR analyses. In addition, associations of the genetic score with HbA1c and hsCRP (as a marker of chronic inflammation) will be examined.

Methods

Study population

The EPIC-InterAct study is a large, prospective case-cohort study involving 27,779 individuals from eight European countries (Denmark, France, Germany, Italy, the Netherlands, Spain, Sweden, and the United Kingdom), which is nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) [10]. The EPIC-InterAct study was designed to investigate the interplay between genetic and lifestyle factors and risk of T2D [11]. It comprises 12,403 incident cases of T2D and a representative subcohort (n=16,154) that also includes 778 of the 12,403 incident cases. The majority of study participants were aged 35 to 70 years and were recruited between 1991 and 2000, mainly from the general population. Exceptions were the French cohort, which included female members of a health insurance scheme for school and university employees, and the Spanish and Italian centres, which included blood donors. In addition, the Utrecht cohort (the Netherlands) and the Florence cohort (Italy) included women attending a breast cancer screening program. Most of the Oxford cohort (the UK) consisted of vegetarian and health-conscious volunteers. All participants gave written informed consent, and

the study was approved by the local ethics committee in the participating countries and the Internal Review Board of the International Agency for Research on Cancer. The full rationale and methods and detailed descriptions of the study populations of both EPIC-Europe and EPIC-InterAct have been reported elsewhere [10,11].

We excluded participants with missing information on alcohol intake (n=736) or BMI (n=197). We also excluded participants with missing genetic data (n=5077), leaving a total sample of 21769 subjects, consisting of a representative subcohort of n=12679, and 9679 incident cases of T2D, of whom 589 were also included in the subcohort.

Outcomes

Diabetes

Ascertainment and verification of incident diabetes has been described in detail elsewhere [10]. In short, incident diabetes cases were identified on the basis of self-report, linkage to primary care registers, secondary care registers, medication use and hospital admissions and mortality data. Identified cases were verified with further evidence, including individual medical record reviews. Participants were followed-up for occurrence of diabetes until 31st December 2007.

HbA1c and hsCRP

Nonfasting blood samples were taken at baseline. Laboratory measures were performed by the Stichting Huisartsen Laboratorium Groep (Etten-Leur, the Netherlands) on serum (except for participants in the Umeå center in Sweden, where only plasma samples were available) or erythrocyte samples that had been previously frozen in ultra–low-temperature freezers at 280°C or in liquid nitrogen. Cobas assays on a Roche HitachiModular P analyser were used to measure hs-CRP (particle enhanced immunoturbidimetric assay). Erythrocyte HbA1c was measured using Tosoh (HLC-723G8) ion exchange high-performance liquid chromatography on a Tosoh G8.

Genotyping

DNA was extracted from buffy coat from a citrated blood sample using standard procedures on an automated Autopure LS DNA extraction system (Qiagen, Hilden, Germany) with PUREGENE chemistry (Qiagen). Details on DNA extraction are described elsewhere (11). Participant were genotyped using the Illumina 660W-Quad BeadChip (n=9.035) or Illumina HumanCoreExome chip arrays (n=12.865) and harmonized, with imputation to the Haplotype Reference Consortium using IMPUTE v2.3.2. All Single nucleotide polymorphisms (SNPs) met quality control criteria for genotyping call rate (≥95%), or were well imputed (info≥0.99) (31-32).

Selection of known SNPs and genetic score

We selected common genetic variants that were associated with PD in the most recent meta-analysis from Nalls et al [8]. This includes data on 7893274 genetic variants in up to 13708 PD cases and 95282 controls. We limited the PD SNPs to those that were associated with PD in the analysis of all European ancestry individuals. In total, the following 28 SNPS previously

associated with PD were selected: rs35749011, rs114138760, rs823118, rs10797576, rs6430538, rs1474055, rs12637471, rs34311866, rs11724635, rs6812193, rs356182, rs34884217, rs7681154, rs9275326, rs13201101, rs199347, rs591323, rs117896735, rs329648, rs76904798, rs11060180, rs11158026, rs2414739, rs14235, rs17649553, rs11868035, rs12456492 and rs8118008 (or proxy rs58046886). Rs114138760 on locus PMVK had the strongest association with PD (OR 1.50 (95%CI: 1.27-1.77) per PD raising allele [PDGene database: www.pdgene.org]. None of the SNPs were in linkage disequilibrium with each other. The majority of SNPs were imputed, so quality metrics used are imputation quality and allele frequency. The imputation quality ranged from 0.87-0.99 for the Illumina 660W-Quad BeadChip and 0.76-0.99 for the Illumina HumanCoreExome chip (supplemental table 1). The Minor allele frequency ranged from 0.01 – 0.37 (supplemental table 1).

The alleles were code 0, 1, 2, according to the number of PD risk raising alleles. To take into account that the effect sizes of individual SNPs differ, we calculated a weighted genetic score, by weighting the individual SNPs by their effect on PD, using estimates from the previously published GWAS meta-analysis [8].

Covariates

Baseline information on lifestyle, diet and medical history were obtained from self-administered questionnaires. Weight and height were recorded by trained health professionals during a visit to a study centre. Presence of hypertension was based on self-reported diagnosis and / or use of medication.

Statistical analysis

Baseline characteristics were summarized as mean (SD) or percentages for the subcohort and incident T2D cases separately. The top two principal components that reflect EPIC-InterAct's genetic structure were estimated. SNPs were modelled per PD increasing allele (additive model). Associations of the PD related genetic score (per SD increase) and individual SNPs with incident T2D were examined with modified Cox regression using an estimation procedure for case-cohort design (Prentice -weighted method; Prentice RL, 1986), adjusted for study centre and sex, and the first two principle components to account for confounding by population stratification. We calculated country specific HRs, and used random-effects meta-analysis to calculate a pooled HR. Analyses were stratified by genetic platform (CoreExome and GWAS) and subsequently pooled. Age was used as underlying time variable, with age at recruitment as entry time, and exit time as the age at diagnosis of T2D, death, loss to follow up, or censoring at the end of follow-up, whichever came first.

We then generated instrumental variable estimates for the relationship of PD with T2DM by combining the SNP-PD associations from the PDGene database with the SNP-T2DM associations from InterAct using the inverse variance weighted method described by Burgess [13]. In a sensitivity analysis, we repeated the analysis excluding proxy SNPs.

To increase power for the analysis of the SNP's with incident diabetes, we further included data from DIAGRAM and UKBiobank. All studies participating in DIAGRAM included men and women of European descent. DIAGRAM data are publicy available at http://diagram-consortium.org/downloads.html. UK Biobank is a large prospective cohort study in the UK

established by the Medical Research Council (MRC) and the Wellcome Trust to enable approved researchers to investigate the role of genetic factors, environmental exposures and lifestyle in the causes of major diseases of late and middle age. A wide range of phenotypic data has been collected at recruitment and has recently been enhanced by the UK Biobank Genotyping Project (33).

Odds ratio's and 95% confidence intervals were extracted from DIAGRAM and UK Biobank and further meta-analyzed with the respective SNP's derived from EPIC-Interact. The number of type 2 diabetes cases included in this meta-analysis ranged from 17233 to 44799 per SNP, and the number of controls from 62157 to 235165 (supplemental table 2).

Associations of individual SNPs and the genetic score with potential confounders in the subcohort were explored to check the instrumental variable assumption. The associations of the genetic score with potential confounders were evaluated by linear regression for continuous and logistic regression for dichotomous confounders, adjusted for study centre, sex and the first 2 principle components. Analyses were stratified by genetic platform and subsequently pooled. In addition, we repeated the above mentioned IV -analysis of PD and diabetes with additional adjustment for potential confounders.

The associations of the PD genetic score (per SD increase) with HbA1c and hsCRP were examined with linear regression and associations were adjusted for study centre, sex, and age. Analyses were stratified by genetic platform and subsequently pooled. Both HbA1c and hsCRP were log-transformed because of their skewed distributions.

Results

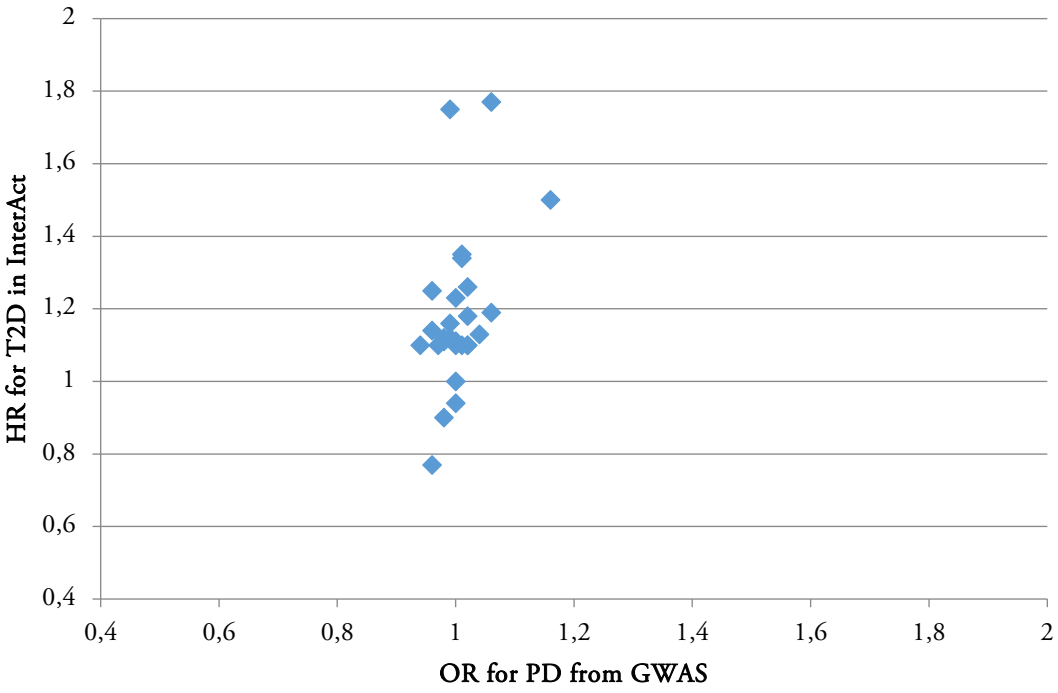
The mean (SD) age of the subcohort was 53 (9.2) years, and 39% were men. Baseline characteristics are shown in table 1 and supplemental table 3. In total, there were 9679 T2D cases, of which 589 were included in the subcohort.

Table 1 Baseline characteristics of subcohort participants and incident T2D cases in the EPIC-Interact study[1]

	Subcohort N=12679	Incident T2D cases N=9679
Age, years	53 (9.2)	56 (7.6)
Male (%)	39%	50%
BMI, kg/m²	26 (4.2)	30 (4.8)
Alcohol consumption, g/d, median (IQR)	14 (18.6)	15 (21.5)
Current smoker (%)	26	28
Genetic score	29.6 (3.5)	29.6 (3.4)
Incident T2D (%)	4.6	100

[1]N=12679 subcohort participants and 9679 incident type 2 diabetes cases; values are mean (SD), unless otherwise indicated.

Figure 1 HR for T2D from interact versus OR for PD per SNP

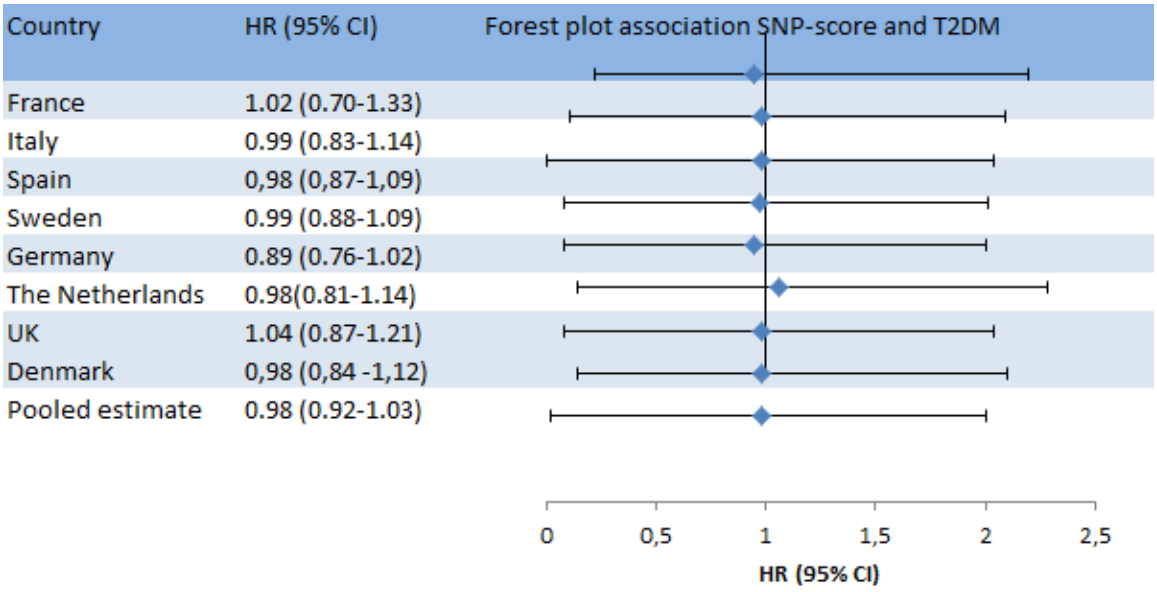


Associations of individual SNPs and genetic score with diabetes

The individual SNPs were generally not associated with risk of incident T2D in the EPIC-InterAct study, with the exception of rs114138760 (table 2). Figure 1 shows the relation between the HR for T2D from interact versus the OR for PD per SNP.

The PD associated genetic score was normally distributed in the study participants of the subcohort. The mean (SD) genetic score was 29.6 (3.5) in the subcohort and 29.6 (3.4) in diabetes cases. Figure 2 depicts the association of the PD genetic score with the hazard of diabetes in the EPIC-InterAct study by country and meta-analysed using random-effects model. The overall estimate did not indicate an effect on diabetes risk (HR 0.98 (95%CI: 0.92-1.03 per SD higher genetic score).

Figure 2 Forest plot of the association of PD related genetic score and T2D, per country and pooled



Association of genetic score with confounders

We detected a significant association of the genetic score with BMI and sex in the EPIC-InterAct subcohort. Other potential confounders of PD-T2D-relation did not show significant associations with the genetic score (supplemental table 4). Further analyses of the association between sex and genetic score by country revealed that the inverse association was mainly driven by the result in Sweden (supplemental table 5). Country-specific associations with BMI were significant or borderline significant in several countries (Supplemental table 3). Subsequently we analysed the association of BMI with each individual SNP. Only one SNP was significantly associated with BMI and two SNPs were borderline significant (Supplemental table 6).

Instrumental variable analysis of PD and T2D

There was no evidence for an association of PD and diabetes in IV analysis, with an OR of 1.01 (95%CI: 0.96-1.05). Excluding proxy SNPs did not change our findings (OR 1.01, 95%CI: 0.96-1.06). Excluding SNPs associated with BMI showed an OR of 1.00 (95%CI: 0.96-1.04).

Additional adjustment for BMI resulted on slightly stronger non-significant association (OR 1.04 (95%CI 1.00-1.08).

Because of the significant association between sex and genetic score in Sweden we repeated the analysis excluding Sweden, which did not change the results (OR 1.04, 95%CI: 0.99-1.08)).

We further meta-analyzed our EPIC-InterAct results for the single SNPs with those from DIAGRAM and UKBiobank. The summarized estimates did generally not indicate a clear significant association with T2D, with exception of four SNPs (table2). IV analysis using these meta-analyzed estimates did not indicate an effect on diabetes risk (OR 1.01 (95%CI: 0.98-1.04)).

Associations of genetic score with hsCRP and HbA1C

The genetic score was not associated with HbA1C (β -0.07, SE 0.05, p=0.16) and hsCRP levels (β -0.01, SE 0.01), p=0.33).

Table 2 Associations of individual pd related SNP's with incident type 2 diabetes

SNP	Chr	Nearest genes	Effect_ Allele	OR (95%CI) for PD[1]	HR (95%CI) for T2D[3]	Pooled OR (95% CI) for T2D[5]
rs35749011	1	GBA/SYT11	a	1.75 (1.56-1.96)	0.99 (0.88 - 1.12)	1.04 (0.93 - 1.15)
rs114138760	1	PMVK	c	1.50 (1.27-1.77)	**1.16 (1.01 - 1.36)**	0.99 (0.85 - 1.13)
rs823118	1	RAB7L1/NUCKS1	t	1.12 (1.09-1.15)	0.99 (0.94-1.03)	0.99 (0.96 - 1.02)
rs10797576	1	SIPA1L2	t	1.13 (1.09-1.18)	1.04 (0.98-1.10)	0.98 (0.94 - 1.02)
rs6430538	2	ACMSD/TMEM163	t	1.13 (1.11-1.18)	1.03 (0.98-1.08)	**1.03 (1.01 - 1.05)**
rs1474055	2	STK39	t	1.21 (1.16-1.27)	1.00 (0.96 - 1.05)	1.00 (0.95 - 1.05)
rs12637471	3	MCCC1	a	1.19(1.15-1.23)	0.93 (0.87-0.97)	0.98 (0.94 - 1.02)
rs34311866	4	TMEM175	t	1.26 (1.22-1.31)	1.02 (0.96-1.08)	0.97 (0.94 - 1.00)
rs11724635	4	BST1	a	1.12 (1.10-1.15)	0.98 (0.94-1.03)	0.98 (0.96 - 1.00)
rs6812193	4	FAM47E	t	1.09 (1.08-1.13)	1.04 (0.99-1.08)	1.01 (0.99 - 1.03)
rs356182	4	SNCA	a	1.34 (1.30-1.38)	1.01 (0.98 - 1.04)	1.02 (0.99 - 1.05)
rs34884217	4	TMEM175	a	1.35 (1.23-1.45)	1.01 (0.96 - 1.06)	1.02 (0.98 - 1.06)
rs7681154	4	SNCA	a	1.00 (0.97-1.03)	1.00 (0.97 - 1.03)	1.00 (0.97 - 1.03)
rs9275326	6	HLA-DQB1	t	1.25 (1.18-1.23)	0.96 (0.92 - 1.00)	**1.04 (1.00 - 1.08)**
rs13201101	6	C6orf10	t	1.18 (1.10-1.27)	1.02 (0.96 - 1.09)	1.04 (0.98 - 1.10)
rs199347	7	GPNMB	a	1.11 (1.08-1.14)	1.01 (0.99-1.04)	1.00 (0.98 - 1.02)
rs591323	8	FGF20 (intergenic)	a	1.09 (1.06-1.12)	0.97 (0.92-1.02)	1.01 (0.99 - 1.03)
rs117896735	10	INPP5F	a	1.77 (1.50-2.08)	1.06 (0.93 - 1.19)	0.99 (0.88 - 1.10)
rs329648	11	MIR4697	t	1.11 (1.07-1.14	0.99 (0.95-1.04)	0.97 (0.95 - 0.99)
rs76904798	12	LRRK2	t	1.16 (1.11-1.20)	0.99 (0.95 - 1.03)	0.99 (0.98 - 1.00)
rs11060180	12	CCDC62	a	1.09 (1.08-1.13)	0.98 (0.93-1.02)	**1.03 (1.01 - 1.05)**
rs11158026	14	GCH1	t	1.10 (1.06-1.13)	1.00 (0.97 - 1.03)	1.01 (0.98 - 1.04)
rs2414739	15	Intergenic	a	1.11 (1.09-1.14)	0.98 (0.93-1.03)	**1.02 (1.00 - 1.04)**
rs14235	16	BCKDK	a	1.10 (1.07-1.14)	0.97 (0.93-1.02)	1.00 (0.98 - 1.02)
rs17649553	17	MAPT	t	1.29 (1.25-1.33)	1.03 (0.98-1.08)	1.00 (0.96 - 1.04)
rs11868035	17	SREBF	a	1.06 (1.03-1.09)	0.99 (0.94-1.03)	1.01 (0.99 - 1.03)
rs12456492	18	RIT2	a	1.10 (1.07-1.14)	1.02 (0.98-1.07)	1.01 (0.99 - 1.03)
rs58046886	20	DDRGK1	a	1.11 (1.07-1.15)	1.00 (0.97 - 1.03)	0.98 (0.95 -1.01)

[1]obtained from PdGene database, [2] HR and 95%CI obtained from country-specific Prentice-weighted Cox regression models, with estimates combined across countries using random-effects meta-analysis, from InterAct [3]Pooled OR from Interact, DIAGRAM and UKBiobank.

Discussion

In this large European case-cohort study, instrumental variables reflecting PD did not show a significant association with the risk of developing type 2 diabetes. Further meta-analysing results with DIAGRAM and UKBiobank to bolster statistical power also did not indicate a meaningful association of PD and T2D. Hence our MR study does not suggest a substantial causal effect of PD on diabetes risk. We also found no statistically significant associations of the genetic sore with HbA1c and hsCRP in the EPIC-InterAct study.

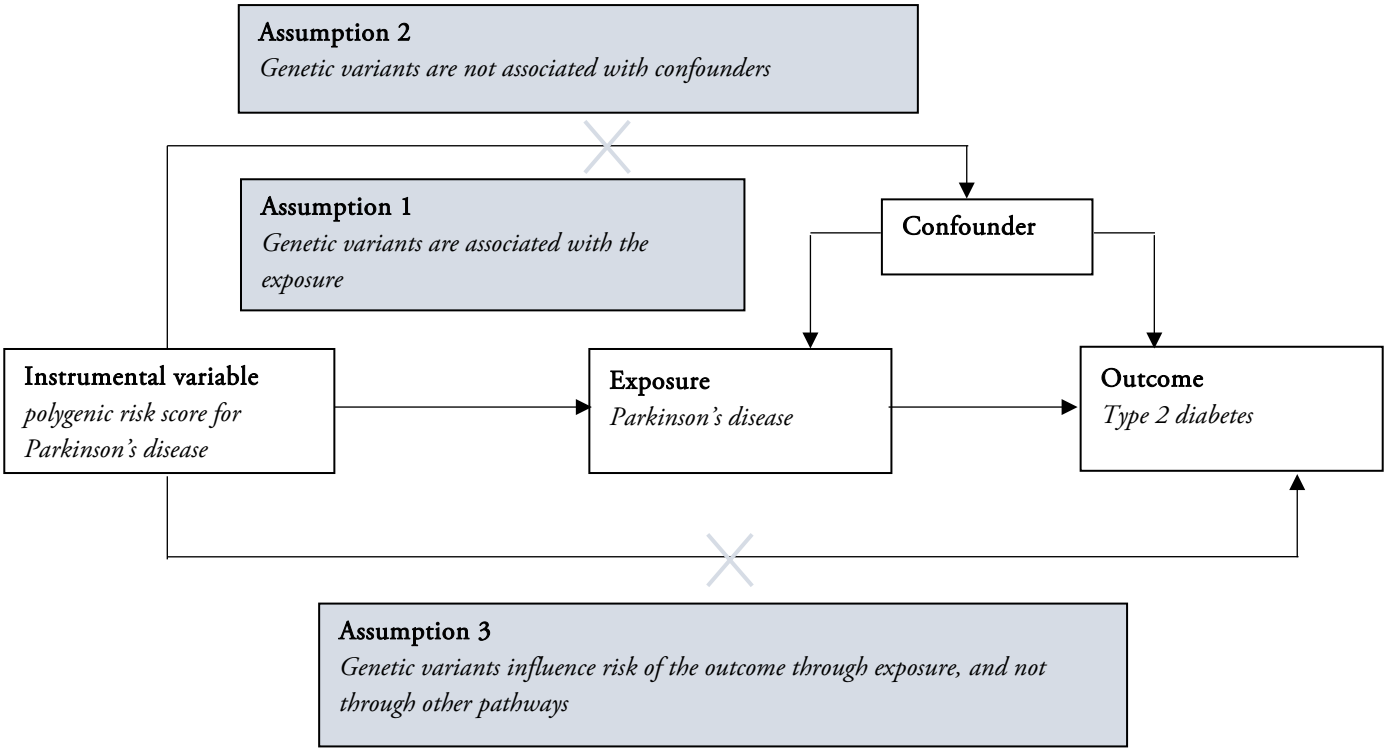
The major strengths of the present study include the prospective design, large sample size, heterogeneous European population and the MR approach. A limitation of our study is the uni-directional approach of our analysis.

MR is a valid approach to explore and estimate causal effects, assuming certain assumptions are met (figure 3). First, there has to be a strong association between the instrumental variable and risk factor of interest. All SNPs used in this study have previously been shown to be strongly associated with PD in large meta-analyses of GWAS. Moreover, we strengthened our instrumental tool by using a genetic score of multiple PD associated SNPs [13]. None of the SNPs were in linkage disequilibrium with each other, which justifies combining those SNPs.

Second, the instrumental variable must be independent of potential confounders of the observational relationship between PD and T2D (figure 3). We identified an association between sex and the genetic score, but out of the 8 countries, this was only significant in Sweden, suggesting it may be a chance finding. Nevertheless, we repeated the IV analysis excluding Sweden, which did not change our results. BMI was inversely associated with the genetic score in multiple countries. Because of this, we analysed the association between BMI and the individual

SNPs. Only one SNP was significantly associated with BMI and two SNPs were borderline significantly associated with BMI. After excluding these three SNPs in the IV analyses, the results were unaffected. We also adjusted for BMI in the IV analysis, but this did not substantially change our findings. In literature, we found no reported associations of these three SNPs with BMI. Consistently, a recent meta-analysis showed that BMI does not increase the risk of PD [21]. Nevertheless, there is clear evidence that PD patients generally have lower BMI compared with healthy controls, and some investigators found that BMI decline started 2-5 years prior to clinical diagnosis of PD [22,23]. We found no associations of the genetic score with other confounders.

Figure 3 Assumptions of a Mendelian Randomisation analysis



The third MR assumption is that the instrumental variable affects the outcome only through the risk factor of interest (figure 3). This assumption is untestable, and should be considered using information on the underlying biology. None of the SNPs used in this study were in linkage

disequilibrium with loci known to influence type 2 diabetes risk, which strengthens this assumption.

As far as we know this is the first study to investigate the potential causal relation of PD on T2D risk using MR. The results are consistent with a previous study comparing the prevalence and incidence of diabetes in patients with and without PD. In this large primary care–based observational study, T2D prevalence was closely similar between patients with or without PD. The risk of developing incident diabetes was lower for patients with PD, a finding that was limited to PD patients using levodopa [6]. Conversely, a clinical study has revealed a high incidence of glucose intolerance (>50%) in PD patients [14]. In contrast, survey data revealed that diabetes is established in 8–30% of patients with PD, consistently in excess of the prevalence found in non-PD individuals [5]. And last, in 2008 Scigliano showed a decreased risk of T2D in PD patients compared to controls, explained by the reduced autonomic activity induced by the illness [15].

The previously reported associations between PD and the risk of T2D might be explained by the following hypotheses. First, there might be an increased detection of hyperglycaemia through extensive medical contact/urine/blood tests among individuals with PD. Second, some drugs used to treat PD, such as levodopa, induce both hyperglycaemia and hyperinsulinaemia [16, 18]. Third, the direction of the association may be the other way around. In literature, there is growing evidence that T2D increases the risk of developing PD [19]. We recently explored whether we can find evidence for a causal link between T2D and PD by investigating whether SNPs for T2D are related to PD risk. Three T2D SNPs were nominally significantly associated with PD in the PDgene database. Additionally, investigated SNP-gene expression associations

(from the three significantly associated SNPs) provide some explanations for this association, among which inflammation [24]. In this study, we found no evidence of an association between PD and hsCRP levels, which may contradict this hypothesis. On the other hand, hsCRP may not be an appropriate systemic marker of inflammation to use because on the basis of MR studies it could be excluded as a causal mediator in cardiovascular diseases [20].

In conclusion, this study does not support the hypothesis that PD has a causal effect on the risk of developing T2D, suggesting that previously reported observational associations may be due to residual confounding. Further research is needed to examine if T2D has a causal effect on developing PD, a MR-approach would be preferable to determine the causality of this association.

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Supplemental tables:

Table 1 Imputation quality and allele frequency of SNPs included in the Mendelian Randomisation analyses of PD and type 2 diabetes

SNP	Imputation quality Illumina 660W-Quad BeadChip	Imputation quality Illumina HumanCoreExome chip	Minor allele frequency
rs35749011	0.98	0. 96	0.01
rs114138760	0.91	0.91	0.01
rs823118	0.99	0.99	0.35
rs10797576	0.99	0.99	0.13
rs6430538	0.98	0.76	0.37
rs1474055	0.99	0.999	0.13
rs12637471	0.99	0.999	0.21
rs34311866	0.98	0.99	0.18
rs11724635	0.99	0.99	0.36
rs6812193	NA	NA	0.34
rs356182	0.95	0.94	0.33
rs34884217	0.90	NA	0.11
rs7681154	0.99	0.99	0.37
rs9275326	0.99	0.99	0.11
rs13201101	0.99	0.99	0.05
rs199347	0.99	0.99	0.35
rs591323	0.99	0. 99	0.27
rs117896735	0.87	0.84	0.01
rs329648	0.99	0.99	0.34
rs76904798	0.99	0.99	0.13
rs11060180	NA	0.86	0.35
rs11158026	0.99	0.99	0.31
rs2414739	NA	NA	0.26
rs14235	0.99	0.99	0.33
rs17649553	0.99	0.99	0.23
rs11868035	NA	NA	0.29
rs12456492	0.99	NA	0.32
rs58046886	0.99	0.99	0.34

Table 2 Meta-analysed associations of PD associated SNPs with risk of type 2 diabetes, and numbers of cases and controls included in each analysis

SNP	OR (95% CI)	n-cases	n-controls
rs35749011	1.04 (0.93 - 1.15)	17323	144486
rs114138760	0.99 (0.85 - 1.13)	17323	144486
rs823118	0.99 (0.96 - 1.02)	17323	144486
rs10797576	0.98 (0.94 - 1.02)	17323	144486
rs6430538	1.03 (1.01 - 1.05)	29494	201348
rs1474055	1.00 (0.95 - 1.05)	17323	144486
rs12637471	0.98 (0.94 - 1.02)	17323	144486
rs34311866	0.97 (0.94 - 1.00)	17323	144486
rs11724635	0.98 (0.96 - 1.00)	26903	198296
rs6812193	1.01 (0.99 - 1.03)	26903	198296
rs356182	1.02 (0.99 - 1.05)	17323	144486
rs34884217	1.02 (0.98 - 1.06)	17323	144486
rs7681154	1.00 (0.97 - 1.03)	17323	144486
rs9275326	1.04 (1.00 - 1.08)	17323	144486
rs13201101	1.04 (0.98 - 1.10)	17323	144486
rs199347	1.00 (0.98 - 1.02)	26903	198296
rs591323	1.01 (0.99 - 1.03)	26903	198296
rs117896735	0.99 (0.88 - 1.10)	17323	144486
rs329648	0.97 (0.95 - 0.99)	29494	201348
rs76904798	0.99 (0.98 - 1.00)	17323	144486
rs11060180	1.03 (1.01 - 1.05)	22059	169651
rs11158026	1.01 (0.98 - 1.04)	17323	144486
rs2414739	1.02 (1.00 - 1.04)	26903	198296
rs14235	1.00 (0.98 - 1.02)	44799	235165
rs17649553	1.00 (0.96 - 1.04)	20748	62157
rs11868035	1.01 (0.99 - 1.03)	26903	198296
rs12456492	1.01 (0.99 - 1.03)	29494	201348
rs58046886	0.98 (0.95-1.01)	17323	144486

Table 3 Baseline characteristics by country in the subcohort

country	N	Incident DM2 N (%)	sex Male (%)	BMI, kg/m² Mean (SD)	Genetic score Mean (SD)
France	331	4 (1.2%)	0%	23 (3.8)	29.5 (3.6)
Italy	1461	40 (2.7%)	35%	27 (4.04)	29.7 (3.4)
Spain	2512	180 (7.2%)	37%	28 (4.2)	29.3 (3.4)
UK	1076	26 (2.4%)	39%	25 (3.8)	29.5 (3.5)
Netherlands	1175	32 (2.7%)	18%	25 (3.8)	29.7 (3.5)
Germany	1778	51 (2.9%)	41%	26 (4.03)	29.6 (3.6)
Sweden	2456	136 (5.5%)	43%	25 (4.0)	29.7 (3.4)
Denmark	1890	120 (6.3%)	53%	26 (3.9)	29.9 (3.5)

Table 4 Association of the weighted genetic score with potential confounders

Continuous traits	*B (SE); p*
BMI	β -0.01 (SE 0.002); p0.01
age	β 0.001 (SE 0.001); p0.48
Alcohol consumption	β 0.001 (SE 0.001); p0.80
Smoking	β -0.01 (SE 0.01); p0.74
Binary traits	*OR (95%CI)*
sex	0.97 (0.95-0.99)

β obtained from linear regression and OR obtained from logistic regression; estimates per SD higher genetic score.

Table 5 Association between PD genetic score and sex and BMI, by country and pooled

SEX	**Illumina 660**	**Illumina Core exome**	**Pooled GWAS and COREX**
By country	OR (95%CI)	OR (95%CI)	OR (95%CI)
1 France	-	-	-
2 Italy	0.96 (0.86-1.06)	0.97 (0.87-1.07)	0.97 (0.91-1.03)
3 Spain	0.92 (0.84-1.00)	1.01 (0.93-1.09)	0.96 (0.92-1.04)
4 UK	1.04 (0.92-1.16)	0.96 (0.86-1.06)	1.00 (0.92-1.08)
5 Netherlands	0.85 (0.63-1.07)	0.99 (0.85-1.13)	0.99 (0.96-1.02)
7 Germany	0.97 (0.87-1.07)	1.02 (0.94-1.10)	1.00 (0.94-1.06)
8 Sweden	0.94 (0.86-1.02)	0.94 (0.88-1.00)	0.94 (0.91-0.97)
9 Denmark	-	1.02 (0.96-1.08)	-
Pooled	0.95 (0.91-0.99)	0.99 (0.86-1.03)	0.97 (0.95-0.99)
BMI	**GWAS**	**COREX**	**Pooled GWAS and COREX**
By country		OR (95%CI)	OR (95%CI)
1 France	-0.02 (0.01) p=0.09	-0.03 (0.01) p=0.05	-0.02 (0.01) p=0.01
2 Italy	-0.01 (0.01) p= 0.19	-0.01 (0.00) p= 0.32	-0.01 (0.00) p=0.04
3 Spain	0.01 (0.01) p= 0.33	0.01 (0.00) p= 0.27	0.00 (0.00) p= 0.86
4 UK	0.01 (0.01) p= 0.32	0.00 (0.00) p=0.71	0.01 (0.01) p=0.31
5 Netherlands	0.00 (0.00) p=0.74	0.00 (0.01) p=0.60	0.00 (0.01) p= 0.55
7 Germany	-0.01 (0.01) p=0.38	-0.01 (0.01) p=0.08	-0.01 (0.00) p=0.06
8 Sweden	-0.00 (0.00) p=0.31	-0.01 (0.00) p=0.30	-0.01 (0.00) p=0.11
9 Denmark	-	-0.01 (0.00) p=0.09	-
Pooled	-0.01 (0.00) p=0.05	-0.01 (0.00) p=0.05	-0.01 (0.00) p=0.01

OR and 95% CI obtained from logistic regression analysis. Abbreviations: ß, linear regression coefficient; SE, standard error. Analyses were adjusted for study centre and stratified by genetic platform (GWAS and COREX) and subsequently pooled.

Table 6 Association between individual SNP and BMI in subcohort using linear regression

SNP	Chr	Nearest genes	Effect_Allele	B (SE) p
rs35749011	1	GBA/SYT11	G vs A	0.38 (0.22) p=0.08
rs114138760	1	PMVK	C vs G	-0.18 (0.29) p=0.52
rs823118	1	RAB7L1/NUCKS1	C vs T	-0.08 (0.05) p=0.12
rs10797576	1	SIPA1L2	T vs C	-0.03 (0.07) p=0.69
rs6430538	2	ACMSD/TMEM163	T vs C	-0.01 (0.06) p=0.90
rs1474055	2	STK39	C vs T	0.03 (0.07) p=0.66
rs12637471	3	MCCC1	A vs G	-0.05 (0.06) p=0.39
rs34311866	4	TMEM175	C vs T	-0.02 (0.11) p=0.86
rs11724635	4	BST1	C vs A	-0.10 (0.05) p=0.05
rs6812193	4	FAM47E	T vs C	0.00 (0.05) p=0.96
rs356182	4	SNCA	G vs A	0.01 (0.05) p=0.80
rs34884217	4	TMEM175	C vs A	0.08 (0.08) p=0.33
rs7681154	4	SNCA	A vs G	-0.02 (0.05) p=0.64
rs9275326	6	HLA-DQB1	T vs C	-0.02 (0.08) p=0.80
rs13201101	6	C6orf10	T vs C	-0.09 (0.12) p=0.45
rs199347	7	GPNMB	G vs A	0.14 (0.05) p=0.01
rs591323	8	FGF20 (intergenic)	A vs G	0.05 (0.06) p=0.42
rs117896735	10	INPP5F	A vs G	-0.27 (0.23) p=0.25
rs329648	11	MIR4697	T vs C	0.02 (0.05) p=0.70
rs76904798	12	LRRK2	T vs C	-0.03 (0.07) p=0.66
rs11060180	12	CCDC62	G vs A	0.01 (0.05) p=0.87
rs11158026	14	GCH1	T vs C	0.10 (0.05) p=0.06
rs2414739	15	Intergenic	G vs A	0.05 (0.06) p=0.41
rs14235	16	BCKDK	A vs G	-0.06 (0.05) p=0.27
rs17649553	17	MAPT	T vs C	-0.01 (0.05) p=0.86
rs11868035	17	SREBF	A vs G	-0.01 (0.05) p=0.87
rs12456492	18	RIT2	G vs A	-0.01 (0.05) p=0.80
rs8118008	20	DDRGK1	A vs G	0.02 (0.05) p=0.72

Abbreviations: ß, linear regression coefficient; SE, standard error.
Analyses were adjusted for study centre and sex and stratified by genetic platform and subsequently pooled.

3.2

Are type 2 diabetes and Parkinson's disease causally related?

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Y.T. van der Schouw

Submitted

Abstract

Introduction

Recent experimental and clinical studies showed that type 2 diabetes (T2D) and Parkinson's disease (PD) may be related and share several dysregulated pathways. We aimed to investigate whether genome-wide significant SNPs for T2D are related to PD risk, and also look at the SNP-gene associations.

Methods

First we used data from genome wide association studies (GWAS) for significant SNPs for T2D. Second, we looked-up these selected T2D SNPs in PDGene database for a significant association with PD risk. Third, the T2D SNPs that were associated with PD at a nominal significance level of $p<0.05$ were searched against the GENEVAR (GENe Expression Variation) database, a collected database of expression quantitative trait locus (eQTL) results, available via Sanger Institute.

Results

For T2D 105 GWAS studies were retrieved from the Hugenavigator database of which 14 were included in our study. From these studies 84 SNP's were extracted. Three T2D SNPs were nominally significantly associated with PD in the PDgene database; rs10244051, located on chr 7:15063833, rs896854 located on chr 8:95960511 and rs7480010 located on chr 11:42216718.

Searching the Sanger Institute GENAVAR database for these three SNP's revealed several SNP-gene associations.

Conclusion

Three T2D related SNP's were marginal significantly associated with PD risk. Additionally, investigated SNP-gene expression associations provide some explanations for this association, namely inflammation, mitochondrial dysfunction, insulin sensitivity and oxidative stress.

Introduction

In aging people Parkinson's disease (PD) is the second most common neurodegenerative disease afflicting about 1% of people over 65 years old and 4-5% of people over 85 years [1]. These late onset, sporadic forms of PD are thought to have a complex aetiology, influenced by lifestyle and environmental factors in addition to variants in numerous genes. Type 2 diabetes (T2D) has been associated with chronic neurodegeneration and based on cohort studies, about 0.3% - 2.4% of PD patients are additionally affected by diabetes mellitus [2-8]. The association between the two diseases has been investigated in several previous studies, summarized in two recent meta-analyses, with inconsistent findings [9-10].

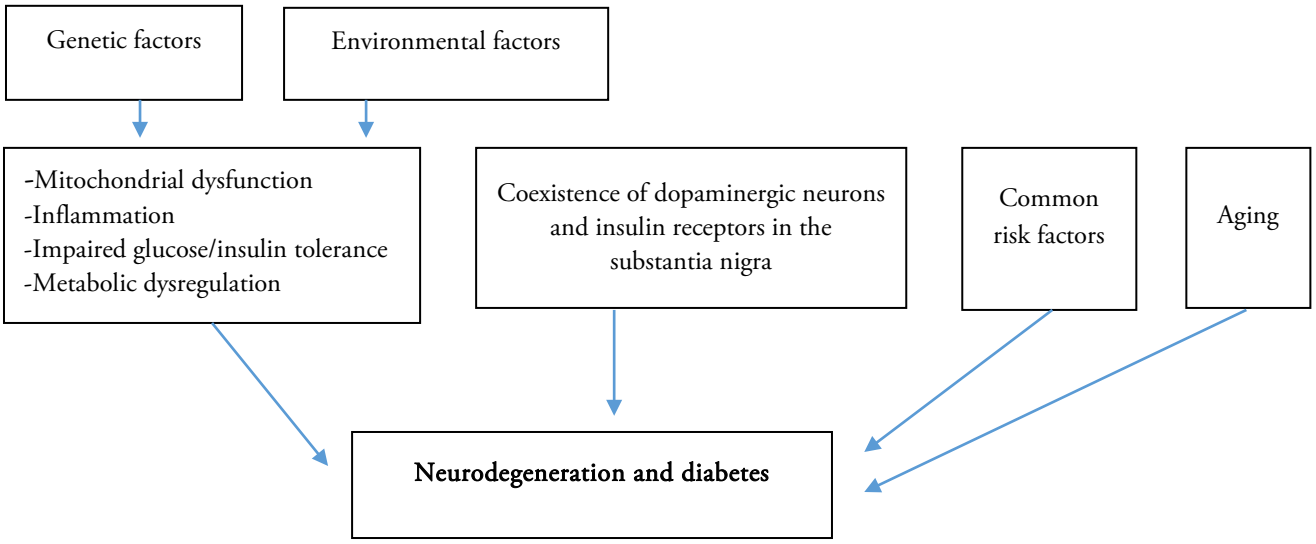
T2D and PD share multiple common pathophysiological mechanisms (figure 1). In particular, inflammation plays a critical role in the destruction of both pancreatic islet β-cells and dopaminergic neurons in the substantia nigra [11]. Other suggested pathogenic pathways are impaired mitochondrial metabolism, abnormal insulin production, insulin resistance, therapies for glucose control, and other cardiovascular risk factors that often cluster with diabetes within the spectrum of metabolic syndrome [11]. Moreover, insulin and dopamine may exert reciprocal regulation between PD and diabetes [12]. However, not all previous studies have adequately accounted for potential confounding factors.

In addition, inferring causality from observational data is problematic as it is not always clear which of two associated variables is the cause and which the effect, or whether both are common effects of a third unobserved variable, or confounder. Consequently, understanding the association between T2D and PD requires further study, using methods that provide insights into the causal nature of observed associations. If type 2 diabetes is directly involved in the pathological process leading to PD, then inherited variation changing T2D risk should affect PD

risk in the direction and magnitude predicted by associations with T2D. A recent study by Chung et al. investigated in 500 Asian PD patients whether genome-wide significant loci of T2DM were associated with PD. They did not find evidence that these loci of T2DM play no role in the risk of PD. They did not study SNP-gene, gene-gene or gene-environmental interactions [13].

In this study, we aim to explore whether we can find evidence for a causal link between T2D and PD in Caucasians by investigating whether causal SNPs for T2D are related to PD risk, and also look at the SNP-gene associations.

Figure 1 Conceptual model of candidate pathways contributing to both neurodegeneration and diabetes



Methods

We used data from genome wide association studies (GWAS). For this purpose, the electronic database Hugenavigator version 2.0, GWAS Integrator (Yu et al., 2008; http://hugenavigator.net/) was searched for GWAS studies in the trait T2D (until March 2014). Articles were selected if they reported original data about testing for SNP main effect on T2D risk. A SNP was included if the SNP-T2D association was found to be replicated in one independent studies. SNP's associated with T2D in a population other than European, white or Caucasian were excluded. Several types of articles were excluded: reviews or editorials, nonhuman studies (cell culture or animal studies), and pharmacogenetics studies for anti-diabetic medication. In addition, studies that did not report sufficient data for effect estimates of the genetic associations were also excluded. From the remaining articles genome-wide significant SNPs were selected.

Second, we contacted PDGene database for a look-up of the selected genome-wide significant T2D SNPs in PDGene (http://www.pdgene.org). The PDGene database provides exact OR and p-values for the top 10,000 most significant SNPs found to be associated with PD. For all other SNPs, only direction of effect and binned p-value are provided. Binned p value signifies that they are <0.05 but larger or equal to $1x10^{-4}$ [14].

Third, the T2D SNPs that were associated with PD at a nominal significance level of p<0.05 were searched against the GENEVAR (GENe Expression Variation) database, a collected database of expression quantitative trait locus (eQTL) results, available via Sanger Institute. Genevar is a platform of databases and web services designed for data integration, analysis and visualization of SNP-gene associations in eQTL studies (http://www.sanger.ac.uk/resources/software/ genevar/) (31). Genevar provides spearman's correlation coefficient and p-value for analysis between observed SNP-gene associations in a 2-Mb region surrounding the SNP in different tissue types/populations (Lymphoblastoid cell lines from eight HapMap3 populations, lymphoid, skin, and adipose tissues derived from healthy female twin pairs and fibroblast, lymfoid and T-cell lines derived from umbilical cords of 75 Geneva GenCord individuals).

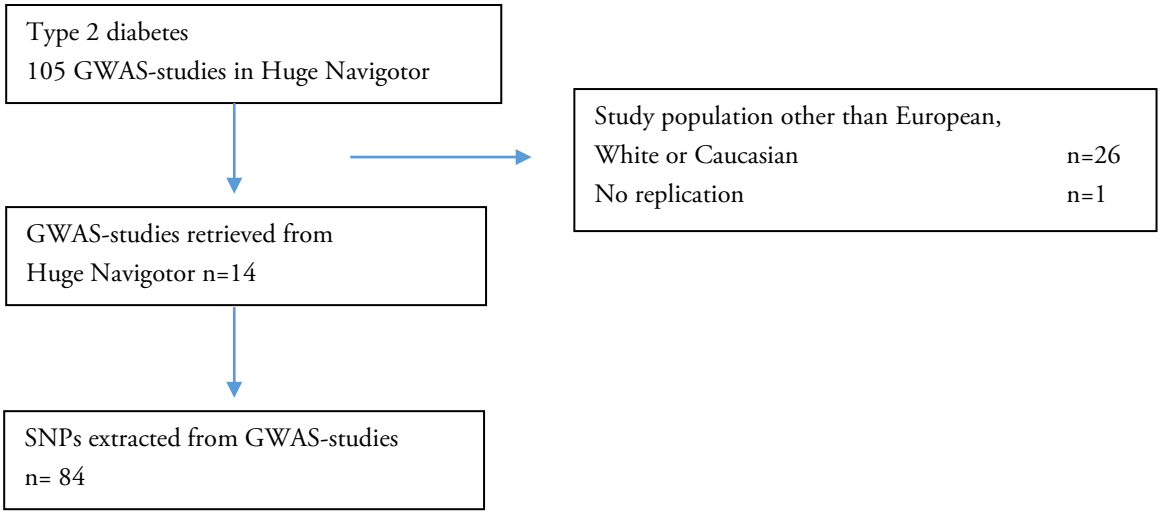
Results

For T2D (trait type 2 diabetes) 105 GWAS studies were retrieved from the Hugenavigator database of which 14 were included in our study (flowchart figure 2). From these studies 84 SNP's (rs-numbers) were extracted (table 1).

Table 1 SNP's retrieved from GWAS hits for T2D

SNP	Chromosome	Nearest gene	SNP	Chromosome	Nearest gene	SNP	Chromosome	Nearest gene
RS10923931	1	NOTCH2	RS1535435	6	AHI1	RS3740878	11	EXT2
RS2641348	1	ADAM30	RS9494266	6	LOC441171	RS11037909	11	EXT2
RS10490072	2	BCL11A	RS9472138	6	VEGFA	RS1113132	11	EXT2
RS243021	2	BCL11A	RS864745	7	JAZF1	RS9300039	11	-
RS6712932	2	-	RS972283	7	KLF14	RS1387153	11	MTNR1B
RS7578587	2	THADA	RS10244051	7	DGKB	RS10830963	11	MTNR1B
RS2943641	2	IRS1	RS4607517	7	GCK	RS294364	11	IRS1
RS7578326	2	IRS1	RS1990458	7	GCK	RS10770141	11	TH/INS
RS780094	2	GCKR	Rs13266634	8	SLC30A8	RS7961581	12	TSPAN8/LGR5
RS7593730	2	RBMS1	RS896854	8	TP53INP1	RS1153188	12	DCD
RS4402960	3	IGF2BP2	RS10811661	9	CDKN2B	RS1531343	12	HMGA2
RS1470579	3	IGF2BP2	RS564398	9	CDKN2B	RS17179453	12	HMGA2
RS1570579	3	IGF2BP2	RS13292136	9	CHCHD9	RS7957197	12	HNF1A
RS1801282	3	PPARG	Rs7903146	10	TCF7L2	RS11634397	15	ZFAND6
RS7649970	3	PPARG	RS7901695	10	TCF7L2	RS2903265	15	ZFAND6
RS12255372	3	PPARG	RS12255372	10	TCF7L2	RS8042680	15	PRC1
RS4607103	3	ADAMTS9	RS7910485	10	STK32C	rs8050136	16	FTO
RS9860730	3	ADAMTS9	Rs1111875	10	HHEX	RS11642841	16	FTO
RS17036101	3	SYN2/PPARG	RS7923837	10	HHEX	RS4925115	17	SREBF1
RS4689388	4	WFS1	RS5015480	10	HHEX	RS4430796	17	HNF1B
RS4688985	4	WFS1	RS12779790	10	CDC123/CAMK1D	RS58996864	18	BCL2
RS10010131	4	WFS1	RS231362	11	KCNQ1	RS19471596	19	GATAD2A
RS4457053	5	ZBED3	RS163184	11	KCNQ1	RS200801	21	-
RS10946398	6	CDKAL1	RS2237892	11	KCNQ1	RS158081	21	-
RS9368222	6	CDKAL1	RS1552224	11	CENTD2	RS2254434	21	-
RS7754840	6	CDKAL1	RS5215	11	KCNJ11	RS757110	-	ABCC8
Rs7756992	6	CDKAL1	RS5219	11	KCNJ11	Rs7593730	-	RBMS1/ITGB6
RS4712523	6	CDKAL1	RS7480010	11	LOC387761	RS5945326	-	DUSP9

Figure 2 Flowchart of SNP selection in T2D



T2D SNP look-up in PDgene database

Three T2D SNPs were nominally significantly associated with PD in the PDgene database; rs10244051, located on chr 7:15063833, rs896854 located on chr 8:95960511 and rs7480010 located on chr 11:42216718 (table 2). Associations of the remaining SNPs with PD were all non-significant.

Sanger Institute GENEVAR database analysis

Searching the Sanger Institute GENAVAR database for rs10244051 revealed correlations with the following genes DGKB, AGMO (TMEM 195), and MEOX2. Observed SNP-gene associations in a 2-MB region surrounding rs896854 were: TP53INP1, RBM35A, GEM, DPY19L4, C8orf38, PLEKHF2, CCNE2, KIAA1429, RAD54B, and CDH17. No SNP-gene associations were found for rs7480010 in the GENAVAR database. LOC387761 is the nearest gene to this SNP.

Table 2 The three significant SNP's and association with PD

SNP	Chr	Position	N_dataset	Allele_contrast	OR_direction	Binned_p-value
Rs10244051	7	15063833	13	T vs G	>1	<0.05
Rs 896854	8	95960511	13	T vs C	>1	<0.05
Rs7180010	11	12216718	13	A vs G	< 1	<0.05

Discussion

No T2D SNPs reached the genome-wide significance level in the PDGene GWAS meta-analysis, but three T2D related SNPs were found to be nominally significantly associated with PD. To study the association of these SNPs with gene expression we used the Sanger Institute GENEVAR database and identified thirteen eQTL loci surrounding these SNP's. We will describe those loci and their potential involvement in the observed association between T2D and PD, classified per SNP, below.

First, The DGKB-gene encodes for diacylglycerol kinase (DGK) beta, a protein that regulates the intracellular concentration of diacylglycerol. Nine mammalian isotypes have been identified and most isotypes show high expression in the brain, often in distinct brain regions, suggesting that each individual isotype has a unique function. The various DGK isotypes are located on different chromosomes. Two of the three DGK isotypes located on chromosome 11 have tissue expression both in the pancreas and brain. This finding suggests a potential link between T2D and PD, as DGK potentially affects a number of biological events including cell growth, neuronal transmission, and cytoskeleton remodeling [15,16].

Rs10244051, located on chr 7:15063833, is intergenic between LOC100128217 and the AGMO-gene [17, 18]. The protein encoded by the AGMO gene is a tetrahydrobiopterin- and

iron-dependent enzyme that cleaves the ether bond of alkylglycerols. Sequence comparisons distinguish this protein as forming a third, distinct class of tetrahydrobiopterin-dependent enzymes. Tetrahydrobiopterin is a naturally occurring essential cofactor of the three aromatic amino acid hydroxylase enzymes, used in the degradation of amino acid phenylalanine and in the biosynthesis of the neurotransmitters serotonin (5-hydroxytryptamine, 5-HT), melatonin, dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline), and is a cofactor for the production of nitric oxide (NO) by the nitric oxide synthases. Nitric oxide is a signalling molecule that plays a key role in the pathogenesis of inflammation, which is one of the candidate pathways connecting T2D and PD (figure 1).

The protein encoded by MEOX2-gene is a transcription factor that has been identified as master regulator of vascular cell differentiation and remodeling [19]. Mice that lack one *Meox2* allele demonstrate reduced cerebral vascular density and brain hypoperfusion resulting in neurodegenerative changes [20].

Second, rs896854 located on chr 8:95960511. The TP53INP1 gene encodes for tumor protein p53-inducible nuclear protein. This is an anti-proliferative and pro-apoptotic protein involved in cell stress response which acts as a dual regulator of transcription and autophagy. It acts as an antioxidant and plays a major role in p53/TP53-driven oxidative stress response [21]. Oxidative stress causing mitochondrial dysfunction is one of the assumed common pathways connecting T2D to PD [11].

The RBM35A-gene (also known as ESPR1-gene) encodes epithelial splicing regulatory protein. This is a mRNA splicing factor that regulates the formation of epithelial cell-specific isoforms and specifically regulates the expression of FGFR2-IIIb, an epithelial cell-specific isoform of FGFR2.

In the brain, the FGFR2-IIIb receptor is expressed by astrocytes and microglia. Stimulation of the receptor enhances the secretion of inflammatory cytokines, leading to neuroinflammation [22]. Both epidemiological and genetic studies support a role of neuroinflammation in the pathophysiology of PD [23]. Furthermore, FGFR1-IIIb expression is high in pancreatic epithelium enriched in progenitor cells [24].

C8orf38 or NDUFAF6 gene encodes NADH dehydrogenase (ubiquinone) complex I. Complex I is the first enzyme of the mitochondrial electron transport chain. Mutations in the subunits of complex I can cause mitochondrial dysfunction [25]. Mitochondrial dysfunction is another possible molecular pathway linking T2D to PD (figure 1). In fact, the inhibition of complex I has been shown to cause the production of peroxides and a decrease in proteasome activity, which may lead to Parkinson's disease [26] and mutations of NADH dehydrogenase (complex I) subunit 1 have been reported to be associated with non-insulin-dependent diabetes [27].

GTP-binding protein GEM, encoded by the GEM gene, plays a role as a regulatory protein in the receptor mediated signal transduction. Mice deficient in GEM-GTPase show abnormal glucose stimulated insulin secretion due to defects in beta-cel calcium handling [28]. Since insulin resistance or deficiency may lead to neurodegeneration in PD, this may connect both diseases.

No functional explanation was found for DPY19L4, PLEKHF2, RAD54B, and CDH17

Third, rs7480010 located on chr 11:42216718. No genes were found in the GENEVAR database. Further literature search connected rs7480010 tot LOC387761-gene. One study showed that SNP rs7480010 (LOC387761) can contribute to a failure in insulin secretion and increase BMI in diabetic patients [29]. Rs7480010 is contributing to diabetes susceptibility primarily through effects on insulin secretion or through homeostatic regulation of the balance of

insulin secretion and insulin sensitivity. However, no strong evidence for insulin sensitivity is found. Insulin sensitivity itself can lead to weakening of neuroprotective signalling by these molecules and contribute to the onset of neurodegenerative disorders [30].

Conclusion

In this study three T2D related SNP's were marginal significantly associated with PD. Additionally investigated SNP-gene expression associations provide some explanations for this association, namely inflammation, mitochondrial dysfunction, insulin sensitivity and oxidative stress. These convergent molecular pathways may be part of the explanations why diabetic patients tend to show a higher future risk for PD.

Acknowledgements

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4

Cardiovascular diseases in Parkinson's disease and osteoporosis

4.1

Blood pressure and risk of Parkinson's disease in a pan-European study

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In preparation

Abstract

Introduction

Cardiovascular risk factors have been associated with Parkinson disease (PD), but data on blood pressure and PD are conflicting. It has been suggested that this associations is more profound in women. We sought to examine the association of blood pressure and hypertension with the risk of PD among men and women in a pan-European study.

Methods

We used data from the NeuroEPIC4PD cohort, comprising 220.494 men and women aged 52 ± 10 years at baseline. Cox proportional hazard models were used to estimate the hazard ratio's (HR) and 95% CI of various blood pressure (BP) parameters in relation to PD onset, adjusted for confounders. Analyses were repeated in men and women separately.

Results

In total, 734 incident PD cases were identified during follow-up. Overall, no associations between any of the BP parameters and PD risk was found in the whole cohort. In women, measured blood pressure above 140/90 mmHg (HR 1.51, 95%CI 1.11-2.05), diastolic blood pressure ≥ 90 mmHG (HR 1.45, 95% CI 1.00-2.11) and composite hypertension (HR 1.38, 95% CI 1.03-1.84) were significantly associated with the risk of developing PD. In men, no significant associations were found. The interaction with sex was statistically significant for composite HT.

Conclusion

Our data indicate that high blood pressure is a risk factor for PD in women, but not in men. Future studies should replicate the findings and elucidate underlying mechanisms.

Introduction

Idiopathic Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease and its incidence rises steeply with age. The median age of onset is 60 years, and the mean duration of the disease from diagnosis to death is 15 years [1]. Despite the fact that the role of environmental factors is increasingly recognized, surprisingly few risk/protective factors have been firmly established so far. In the PRIAMO study, a cross-sectional in patients with PD performed in Italy, hypertension was the most frequently reported concomitant, non-neurological disorder accounting for 41.5% of the subjects [2]. Several population-based studies suggested that cardiovascular risk factors, such as diabetes mellitus and high cholesterol, are associated with PD [3,4]. Thus far, the association between blood pressure (BP) and PD remains unclear, which can be mainly explained by the differences in study population and/or study design [5-9]. A large population-based prospective study of Finish men and women reported that in women high BP and hypertension were associated with a 60% increased risk of PD compared to normal BP, no association was found in men [5].

The aim of this study is to investigate whether BP, self-reported hypertension and self-reported use of antihypertensive drugs is related to the risk of PD among men and women. For this purpose, we used data from the NeuroEPIC4PD study, a large, prospective pan-European cohort [10].

Methods

Design and study population

The NeuroEPIC4PD study included 220.494 individuals recruited from the general population in Sweden (Umea and Malmo), UK (Cambridge), Netherlands (Utrecht), Germany (Heidelberg), Spain (Navarra, San Sebastian, and Murcia), Italy (Turin, Varese, Florence and Naples[11], and Greece, residing in defined geographical areas between 1992 and 2002 within the EPIC study . The Naples and Utrecht cohorts were restricted to women, whereas all other cohorts involved both sexes. The EPIC study was approved by the ethical committee of the International Agency for Research on Cancer and by the ethics committees of each participating centre; all participants signed an informed consent. Recruitment procedures and more details have been described elsewhere in detail [10].

Case ascertainment

A total of 881 PD cases were identified in 13 EPIC centers; 147 prevalent cases and 734 incident cases. The present analysis has been conducted on a total sample of 219.926 subjects, including 734 incident PD cases. Procedures for PD case ascertainment in the EPIC cohort has been described elsewhere [11].

Blood pressure measurements

For the blood pressure measurements, uniform procedures were recommended but no standard method or common type of instrument was introduced (12). Blood pressure measurements were obtained using a variety of devices and methods. Details about this procedure and differences between countries and centres are described elsewhere (13). Systolic and diastolic blood pressure

recordings were made only in Italy, Germany, Greece and in subsets of participants in three of five centres in Spain (comprising 14% of Spanish participants), Sweden, the Netherlands and in UK centres, in about 62% of baseline participants. In most centres, the measurements were taken in duplicate, from the right arm, in a sitting position, using either a mercury manometer or an oscillometric device [13-17], and the mean of the duplicates was used in the analyses.

We defined several blood pressure parameters for analysis with PD risk. We studied measured systolic (SBP) as well as diastolic blood pressure (DBP) separately. We categorized participants as having measured hypertension (mHT) when the measured SBP was ≥ 140 and/or DBP ≥ 90 mmHg, following the guidelines of the Seventh Report of Joint National Committee on Prevention, Detection, Evaluation, and treatment of High Blood Pressure [18]. We also defined systolic hypertension (sHT) when SBP was ≥140, regardless of DBP, and diastolic hypertension (dHT) when DBP ≥ 90 mmHg, regardless of SBP. We further analyzed self-reported hypertension (SRHT) and self-reported treatment for hypertension (SRTHT). Last, we combined mHT, sHT, dHT, SRHT and SRTHT into one composite hypertension parameter (cHT). We defined cHT as present when either one of the above-mentioned BP variables was positive.

Assessment of Anthropometric, Lifestyle, and Dietary Exposures

Baseline information on medical history (including self-reported diagnosis of hypertension and/or use of antihypertensive medication) were obtained from self-administered questionnaires. With participants not wearing shoes, weight was measured to the nearest 0.1 kg and height was measured—dependent on the study centre—to the nearest 0.1, 0.5, or 1.0 cm. BMI was calculated as weight in kilograms divided by height in metres squared (kg/m2). Lifestyle questionnaires were used to obtain information on education, smoking status, and physical

activity level. Information on alcohol and coffee intake was collected at baseline using validated country/centre-specific dietary questionnaires [13,19].

Statistical Methods

Information on blood pressure variables at recruitment was missing for 71.513 subjects regarding systolic blood pressure, 71.530 subjects regarding diastolic blood pressure, and 55.437 subjects regarding reported hypertension and/or antihypertensive drug use. The missing data were imputed through multiple imputation, using 10 iterations and predictive mean matching in in SPSS Statistics (IBM), version 21. Analyses were performed in the imputed datasets, and results were pooled using Rubin's rule (27).

Baseline characteristics were presented as mean (SD) or percentages. Cox proportional hazard models, using follow-up time as underlying time axis, stratified by center, were used to estimate the hazard ratio's (HR) and 95% CI of BP variables in relation to PD onset. In model 1 we adjusted for sex, age, in model 2 additional adjustments for lifestyle factors (BMI, smoking, alcohol consumption, education, coffee use and physical activity) were made and in model 3 additional adjustments for comorbidities (hyperlipidemia and diabetes) were made. SBP and DBP analyses were additionally adjusted for antihypertensive use. We rescaled the continuous blood pressure variables to increases by 10 mmHg instead of 1 mmHg. In addition, analyses were repeated in men and women separately. We tested statistical interaction of sex by simultaneously including sex, the BP variable and their cross-product term in the same model.

We conducted a sensitivity analysis comparing complete cases with imputed data for the composite HT endpoint in the overall population (because a complete case sensitivity analysis for most determinants was not really possible because of the great amount of missing data).

Because in literature there may be differences to onset of PD at different age (9), analyses were additionally stratified by age of onset (< 70 versus ≥ 70 years). We tested statistical interaction of these age-groups by simultaneously including the categorical age variable, the BP variable and their cross-product term in the same model.

Results

Demographic characteristics at baseline are shown in table 1. The mean age at recruitment was 52 (SD 10) years and 62% were female. In total, there were 71,658 hypertensive subjects (cHT). In the hypertensive subjects 0.4% developed PD as compared to 0.2% in the normotensive subjects (table 2). Baseline characteristics by sex are shown in table 3. Mean age of onset of PD was 68.8 years and mean time until diagnosis was 7.3 years.

Table 1 Baseline characteristics (n= 219.926)

	All (N=219.926)	PD incident cases (N=734)
	Mean (SD)	**Mean (SD)**
Age at recruitment (y)	52 (10)	61 (9)
SBP (mmHg)	131 (19)	137 (19)
DBP (mmHg)	81 (11)	83 (11)
BMI (kg/m$^{2)}$	26 (4)	27 (4)
Alcohol consumption (g/d)	12 (18)	11 (16)
Coffee use (g/d)	284 (286)	227 (254)
	Percentage	**Percentage**
Sex (% women)	62	49
mHT	24	28
SHT	20	25
DHT	14	17
SRHT	18	23
SRTHT	11	13
cHT	33	29
Smoking status (% never smoked)	42	44
Physical activity index (% inactive)	24	34
Hyperlipidemia	16	15
Diabetes	3	6
Education level (% > secondary school)	14	9

Abbreviations: systolic blood pressure (SBP), diastolic blood pressure (DBP), self-reported hypertension (SRHT), self-reported treatment of hypertension (SRTHT), measured hypertension (mHT).

Table 2 Demographic characteristics for subjects with and without hypertension (cHT)

	HT (N=71.658)	No-HT (N=115.961)
Incident Parkinson disease (n)	286 (0.4%)	278 (0.2%)
	Mean (SD)	**Mean (SD)**
Age at recruitment (y)	56 (9)	50 (10)
SBP (mmHg)	147 (17)	119 (11)
DBP (mmHg)	89 (10)	75 (7)
BMI ($kg/m^{2)}$	28 (5)	26 (4)
Alcohol consumption (g/d)	12 (19)	12 (18)
Coffee use (g/d)	272 (278)	291 (291)
	Percentage	**Percentage**
Sex (% women)	60	64
Smoking status (% never smoked)	51	47
Physical activity index (% inactive)	33	23
Hyperlipidemia	24	15
Diabetes	6	2
Education level (% > secondary school)	12	26

Abbreviations: systolic blood pressure (SBP), diastolic blood pressure (DBP).

None of the BP parameters was significantly associated with the risk of PD in the whole sample (table 4). We performed sex-stratified analysis to verify whether the association of BP parameters with PD varied by sex. In women, measured blood pressure above 140/90 mmHg (HR 1.51, 95%CI 1.11-2.05), diastolic blood pressure ≥ 90 mmHg (HR 1.45, 95% CI 1.00-2.11) and composite hypertension (HR 1.38, 95% CI 1.03-1.84) were significantly associated with the risk of developing PD (table 5). In men, there was no significant association of any of the BP parameters with PD (table 5).

The interaction of BP parameters with sex was statistically significant for cHT (p_interaction = 0.03), and borderline significant interaction terms was found for DBP (p_interaction = 0.07) and dHT (p_interaction = 0.08). No significance was found for SBP (p_interaction = 0.17), mHT (p_interaction = 0.13), sHT (p_interaction = 0.17), SRHT (p_interaction = 0.57) and SRTHT (p_interaction = 0.27) (table 5).

Table 3 Baseline characteristics by sex

	Men (N=83.047)	Women (N=136.879)
Incident Parkinson disease (n)	378	356
	Mean (SD)	**Mean (SD)**
Age at recruitment (y)	52 (10)	52 (10)
SBP (mmHg)	132 (17)	129 (20)
DBP (mmHg)	83 (11)	80 (11)
BMI (kg/m$^{2)}$	27 (4)	26 (5)
Alcohol consumption (g/d)	20 (24)	7 (11)
Coffee use (g/d)	288 (294)	281 (282)
	Percentage	**Percentage**
mHT	25	23
SHT	20	20
DHT	17	13
SRHT	19	18
SRTHT	10	11
cHT	35	31
Smoking status (% never smoked)	29	50
Physical activity index (% inactive)	19	27
Hyperlipidemia	19	14
Diabetes	4	3
Education level (% > secondary school)	16	13

Abbreviations: systolic blood pressure (SBP), diastolic blood pressure (DBP), self-reported hypertension (SRHT), measured hypertension (mHT), composite hypertension (cHT).

HRs for the associations of BP parameters with PD risk were not statistically different between age of onset of PD (< 70 versus ≥ 70 years) in the whole sample or for men. In women with age of diagnosis ≥ 70 HRs for several BP parameters seemed higher than for women aged < 70 years, e.g. for cHT HR 1.53, 95%CI 1.18-1.98. However, none of the differences were statistically significant, nor was the interaction term (table 6).

The sensitivity analysis for cHT in the cases with complete data showed no difference with the results from imputed data: HR_{CC} 1.02 (0.80-1.30) vs HR_{imp} 1.03 (0.86-1.24).

Table 4 Hazard ratio's (HR) and 95% confidence intervals from cox regression models investigating hypertension variables in relation to PD onset

	Model 1	Model 2	Model 3
	HR (95% C.I.)	HR (95% C.I.)	HR (95% C.I.)
SBP	0.99 (0.94-1.05)	1.00 (0.94-1.06)	1.00 (0.94-1.06)
DBP	1.00 (0.88-1.10)	1.01 (0.89-1.13)	1.04 (0.95-1.14)
mHT	1.07 (0.67-1.32)	1.11 (0.89-1.38)	1.13 (0.90-1.42)
SHT	1.00 (0.82-1.23)	1.04 (0.83-1.29)	1.07 (0.85-1.33)
DHT	1.10 (0.90-1.34)	1.11 (0.83-1.47)	1.13 (0.85-1.50)
SRHT	1.15 (0.89-1.47)	1.13 (0.88-1.46)	1.11 (0.85-1.43)
SRTH	1.12 (0.84-1.49)	1.13 (0.85-1.51)	1.13 (0.84-1.53)
cHT	1.01 (0.84-1.20)	1.02 (0.86-1.22)	1.03 (0.86-1.24)

Model 1 adjusted for sex, age, model 2 is additionally adjusted the for lifestyle factors BMI, smoking, alcohol consumption, education, coffee use and physical activity, and model 3 is additionally adjusted for comorbidities hyperlipidemia and diabetes. SBP and DBP analyses were additionally adjusted for antihypertensive use. Abbreviations: systolic blood pressure (SBP), diastolic blood pressure (DBP), measured hypertension (mHT), systolic hypertension (SHT), diastolic hypertension (DHT), self-reported hypertension (SRHT), self-reported treatment of hypertension (SRTHT), composite hypertension (cHT).

Table 6 Hazard ratio's (HR) and 95% confidence intervals from cox regression models investigating hypertension variables in relation to PD onset stratified by age of onset

Women	Age <70 (N=361)	Age ≥70 (N=373)	p-value for age-interaction
	HR (95% C.I.)	HR (95% C.I.)	
SBP	1.06 (0.91-1.23	1.04 (0.91-1.17)	0.77
DBP	1.01 (0.79-1.29)	1.02 (0.81-1.19)	0.86
mHT	1.08 (0.66-1.77)	1.56 (0.95-2.54)	0.49
SHT	1.38 (0.89-2.16)	1.44 (0.89-2.34)	0.37
DHT	1.22 (0.62-2.42)	1.47 (0.84-2.55)	0.72
SRHT	1.30 (0.82-2.07)	1.38 (0.73-2.61)	0.68
SRTH	1.05 (0.55-2.02)	1.37 (0.71-2.66)	0.31
cHT	1.11 (0.72-1.72)	1.53 (1.18-1.98)	0.31

Model 3 adjusted for sex, age, BMI, smoking, alcohol consumption, physical activity, hyperlipidemia, education, diabetes and coffee use. MHT, SBP, DBP, sHT and dHT analyses were additionally adjusted for antihypertensive use. Abbreviations: systolic blood pressure (SBP), diastolic blood pressure (DBP), measured hypertension (mHT), systolic hypertension (SHT), diastolic hypertension (DHT), self-reported hypertension (SRHT), self-reported treatment of hypertension (SRTHT), composite hypertension (cHT).

Table 5 Hazard ratio's and 95% confidence intervals from cox regression models investigating hypertension variables in relation to PD onset by sex

	Men (N=83.047)			Women (N=136.879)			
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3	p-value for sex-interaction (model 3)
SBP	0.94 (0.86-1.02)	0.95 (0.87-1.03)	0.96 (0.88-1.00)	1.07 (0.99-1.16)	1.07 (0.99-1.17)	1.08 (0.99-1.18)	0.17
DBP	0.92 (0.80-1.05)	0.93 (0.81-1.09)	0.95 (0.82-1.10)	1.10 (0.95-1.28)	1.12 (0.96-1.30)	1.13 (0.97-1.32)	0.07
mHT	0.87 (0.67-1.16)	0.93 (0.70-1.25)	0.83 (0.65-1.07)	1.49 (1.13-1.98)	1.51 (1.12-2.04)	1.51 (1.11-2.05)	0.13
sHT	0.82 (0.62-1.09)	0.87 (0.65-1.16)	0.96 (0.72-1.27)	1.34 (1.01-1.79)	1.36 (0.99-1.86)	1.37 (0.99-1.89)	0.17
dHT	0.89 (0.63-1.26)	0.93 (0.66-1.32)	1.03 (0.73-1.44)	1.43 (1.00-2.05)	1.45 (1.00-2.10)	1.45 (1.00-2.11)	0.08
SRHT	1.02 (0.72-1.43)	1.01 (0.70-1.45)	0.94 (0.70-1.27)	1.47 (1.08-1.99)	1.45 (1.05-1.98)	1.35 (0.98-1.87)	0.57
SRTHT	0.97 (0.64-1.47)	0.98 (0.65-1.45)	0.85 (0.58-1.25)	1.35 (0.90-2.02)	1.37 (0.92-2.05)	1.36 (0.89-2.05)	0.27
cHT	0.81 (0.64-1.03)	0.85 (0.66-1.08)	0.85 (0.67-1.07)	1.43 (1.10-1.87)	1.41 (1.09-1.83)	1.38 (1.03-1.84)	0.03

Model 1 adjusted for sex, age, model 2 additional adjustments for lifestyle factors and model 3 additional adjustments for comorbidities. SBP and DBP analyses were additionally adjusted for antihypertensive use.

Abbreviations: systolic blood pressure (SBP), diastolic blood pressure (DBP), measured hypertension (mHT), systolic hypertension (SHT), diastolic hypertension (DHT), self-reported hypertension (SRHT), self-reported treatment of hypertension (SRTHT), composite hypertension (cHT).

Discussion

In this large, well-established prospective European cohort study we found that, in women, measured hypertension (HR 1.51, 95%CI 1.11-2.05), diastolic hypertension (HR 1.45, 95% CI 1.00-2.11) and composite hypertension (HR 1.38, 95% CI 1.03-1.84) were significantly associated with the risk of developing PD. In men, however, none of the BP parameters was associated with PD.

The major strengths of our study include the prospective design, the large cohort of men and women, and the validated outcome ascertainment [11]. The large sample had sufficient statistical power for subgroup analyses. The main limitation of this study, however, is the large amount of missing data on information of hypertension status at recruitment. We dealt with this issue through multiple imputation.

Our results are in line with another prospective study based on 7 surveys of representative samples of the general population in Finland. A total number of 59 540 participants (age 25 to 74 years; 51.8% women) who were free of PD and stroke at baseline were prospectively followed, to identify incident PD cases. This study showed, in women, compared with normotensive subjects, high-normal BP and hypertension are associated with a 60% increased risk of PD [5]. These results were not seen in men.

Other studies are not in line with our study, but only one of them had a prospective design. The analysis of the Nurses' Health Study and the Health Professionals Follow-up Study investigated the association between self-reported HT and PD and found no association (RR=0.96, 95%CI 0.80-1.15) [7]. A case-control study conducted in Japan by Miyake et al. found a significant inverse association between HT and PD (HR 0.43, 95%CI 0.29-0.64), mean age 68.5 years [6].

Another case-control study in Italy found that high systolic BP was independently related to a reduced risk of PD, mean age 58,1 years old [8]. This inverse association found in case-control studies may be explained by the use of prevalent PD cases and the ambiguous exposure time in case-control studies, knowing that BP frequently declines with progression of PD because of autonomic dysfunction [20], leading to reverse causation.

An observation in previous studies is that the effect of blood pressure parameters on PD differs between the age of onset of PD. Schelp et al. suggested that hypertension is a risk factor for PD in subjects < 65 years and a protective factor in subjects ≥ 65 (9). Qiu et al found an elevated systolic blood pressure among women aged ≥55 years or an increased diastolic blood pressure among women aged <55 years were significantly or marginally associated with an increased HR of PD (5). In our study mean age of onset of PD was 68.8 years and mean time until diagnosis was 7.3 years. Stratification by age of onset of 70 years did not reveal any significant associations between BP parameters and the risk of PD, except that in women ≥ 70 years cHT was associated with the risk of PD (HR 1.53, 95%CI 1.18-1.98). We could not reveal other age specific associations, but this may be partly explained by small number of incident cases per age group (361 vs 373).

The sex-specific association with PD has been reported for other cardiovascular risk factors as well, in particular for hypercholesterolemia [7,21]. In addition, women have an increased lifetime risk of stroke compared to men, largely due to a steep increase in stroke incidence in older postmenopausal women. Evidence has implicated genetic and epigenetic factors, differential activation of cell-death programs, cell-cell signalling pathways, and systemic immune responses as contributors to sex differences in ischemic stroke [22]. Additionally, women have higher rates of

hypertension when they age [23]. There are some pathophysiological mechanisms linking hypertensive status to PD [24]. For example, inflammation and oxidative stress have been involved in dopaminergic neuron degeneration [25]. Additionally, renine-angiotensin system (RAS) may also link hypertension to PD. Brain RAS over activity can lead to dopaminergic degeneration. Interestingly, oestrogen depletion increases RAS activity and neuroinflammation, which may lead to dopaminergic degeneration [26]. This last phenomenon may explain the sex specific association in combination with higher rates of hypertension in postmenopausal women.

In conclusion, the present study showed that, in women, mHT, dHT, and cHT are associated with an increased risk of PD. Our results warrant further research in the mechanisms involved. In particular, the use of Mendelian randomisation could provide compelling final evidence whether the association between BP and PD is causal.

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4.2

Links between atherosclerosis and osteoporosis in middle aged and elderly men

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Abstract

Introduction

Although the incidences of osteoporosis and atherosclerosis increase with age, there is growing evidence that the coincidental occurrence of both diseases may be independent of age. In general, studies in men are scarce and results are inconsistent. The objective of this study was to investigate the relationship between atherosclerosis and bone mineral density, and the influence of insulin sensitivity and low grade inflammation on this relationship in 332 men without CVD.

Methods

Aortic Pulse wave velocity (PWV), augmentation index (AIX) and measurements of carotid intima media thickness (CIMT) were assessed. BMD measurements were performed with dual-X-ray absorptiometry (DEXA), subcutaneous fat by ultrasonography. Serum concentrations of lipids, hsCRP, glucose and insulin were measured. Insulin sensitivity was calculated by use of the quantitative insulin sensitivity (QUICKI). We used multivariate linear regression models to examine the association of hsCRP, insulin sensitivity, PWV, Aix, CIMT with BMD.

Results

A higher CIMT was significantly associated with higher BMD after multivariate adjustment (ß 99.7; p=0.02). Further adjustment for weight attenuated the estimates towards non-significant. No association was found between PWV or AIX and BMD. Lower insulin sensitivity was associated with higher BMD (ß -645.1; p<0.01). After adjustment for weight this association was no longer significant. A similar effect was seen for the association between hsCRP and BMD.

Conclusion

In this population of healthy, non-obese, men without a history of cardiovascular disease the

positively association between cardiovascular parameters and BMD was mainly explained by weight, suggesting that in this population weight plays a protective role in the development of osteoporosis.

Introduction

Osteoporosis and atherosclerosis are chronic systemic diseases and major public health problems worldwide. They are both important causes of morbidity and mortality in aging men. Although the incidences of osteoporosis and atherosclerosis increase with age, there is growing evidence that the coincidental occurrence of both diseases may be independent of age [1]. In healthy individuals, the relationship between atherosclerosis and bone mass has not been extensively studied. Most studies are conducted in postmenopausal women or subjects with cardiovascular disease, while only few studies studied the relationship between atherosclerosis and bone mineral density (BMD) in men with inconsistent results [2-5]. On the other hand, it is widely recognized that lifestyle measures and measures aimed at reducing adverse effects on bone of drugs and diseases have to be recommended throughout life in everyone [6].

The current evidence indicates that individuals with prevalent (sub)clinical cardiovascular disease (CVD) are at increased risk for bone loss and subsequent fractures [7]. Measurement of the intimal media thickness (IMT) in the carotid arteries is a non-invasive, sensitive and reproducible technique for identifying subclinical cardiovascular disease. In postmenopausal women, most of the studies reported an association of increased IMT with low bone density. In the one study that included men, findings were in line with the results in women [2].

Arterial wall calcification would be expected to increase arterial stiffness. Pulse wave velocity (PWV) and augmentation index (Aix) are measurements of arterial stiffness and both predictors of adverse cardiovascular events [8-9]. Bone loss is associated with increased arterial stiffness in postmenopausal females and hemodialysis patients [10-12]. Few studies have addressed the question of an association between osteoporosis and arterial stiffness in healthy men [13-14].

Findings were not consistent, which may be due to the use of suboptimal measures of arterial stiffness and BMD [15].

Atherosclerosis and osteoporosis could be linked through common risk factors or a shared pathophysiological mechanism. Possible candidates of linking factors are low grade inflammation insulin sensitivity. Markers of inflammation, such as high sensitivity C-reactive protein (hsCRP) have been associated with cardiovascular mortality in both sexes [15] and levels of hsCRP were associated with lower BMD [16-17]. In patient with type 1 diabetes BMD is lower than in non-diabetic subjects. In patients with type 2 diabetes bone mineral density is similar to or higher than in non-diabetic subjects [18].

The aim of this study is to investigate the relationship between atherosclerosis measured by subclinical CVD (carotid IMT) and arterial stiffness (PWV and AIX) and BMD in independently living men aged 40 to 80 years. In addition, we studied the role of insulin sensitivity, low grade inflammation and body composition on these relationships.

Methods

Study population

For the present study data were used from a cross-sectional study among 400 independently living men aged 40-80 years. A random sample of 400 men was included in this study by sampling 100 men in each decade of age. Recruitment procedures have been described elsewhere in detail [19]. The study was approved by the institutional review board of the University Medical Center Utrecht and written informed consent was obtained from all participants before enrolment in the study. Data collection took place between March 2001 and April 2002. For the present analysis, men with a history of CVD were excluded (n=68), leaving 332 men for analysis.

Cardiovascular risk factors

Height and weight were measured in standing position without shoes. Body mass index (BMI) was calculated as the weight in kilograms divides by the square of heights in meters.

Blood pressure was measured in the morning after 10 min rest, twice on the right upper arm (sitting position) with a semi-automated device (Dinamap 8100; Critikon Inc.,Tampa, Finland). Mean systolic and diastolic blood pressure was calculated as the average of the two measurements of systolic and diastolic blood pressure was used for analysis and further calculation.

Fasting venous blood samples were obtained. Automatic enzymatic procedure was used to determine serum total cholesterol (Synchron LX Systems; Beckman Coulter, USA). High density lipoprotein (HDL) cholesterol and triglycerides were measured similarly. Low-density-lipoprotein (LDL) cholesterol was calculated using the Friedewald formula [19]. Fasting blood glucose was determined by using a reagent-strip glucose oxidase method using a GlucoTouch reflectometer (LifeScan, Inc., Benelux). Fasting insulin levels were measured using an IMMULITE 2000

Analyzer (DPC, USA). To assess insulin sensitivity, the quantitative insulin sensitivity check index (QUICKI) was calculated, which has a high correlation with insulin sensitivity measured with the glucose clamp technique. QUICKI index can be determined from fasting insulin and glucose values according to the equation: QUICKI index = 1/[log (I0) + log (G0)], in which I0 is fasting insulin (mIU/liter) and G0 is fasting glucose (mg/dl = mmol/liter x 18.182) [21]. High sensitivity C-reactive protein (hc-CRP) was measured using a Behring Nefelmeter II (Dade Behring, Liederbach, Germany). The lower limit of detection was 0.175 mg/l and the inter assay variation was 2.4%. Hs-CRP levels > 10mg/l can be taken as evidence of active inflammation processes (e.g. infection, trauma), therefore subjects with hs-CRP >10 mg/l were excluded. 25OHD was determined using an automated assay for the quantitative determination of 25-hydroxyvitamin D (IDSiSYS, UK), with 100% 25OHD3 and 25OHD2 cospecificity. Assay range was 13.75–350 nmol/L and sensitivity 13.75 nmol/L.

Smoking was estimated from self-report and categorized in current, former and never smokers. Alcohol intake was estimated using a validated food frequency questionnaire [22-23]. A medical doctor obtained information about the prevalence of disease and medication use from a specified medical history. Fasting venous blood samples were drawn and analysed.

Hypertension was defined as systolic blood pressure ≥140mmHg and/or diastolic blood pressure ≥90mmHg and/or use of anti-hypertensive medication. Diabetes mellitus was defined as treatment with insulin or oral hypoglycemic agents and/or fasting plasma venous glucose >6.9 mmol/l.

Hyperlipidemia was defined as treatment with lipid lowering medication and/or serum total cholesterol >6.5 mmol/l. Presence of CVD was based on a positive answer in the questionnaire

on history of coronary artery disease, peripheral artery disease or stroke. Physical activity was assessed using the validated Voorrips questionnaire for the elderly [24].

Assessment of functional and structural measures of the vessel wall

As structural measure of the vessel wall we used carotid intima-media thickness (CIMT) as a measure of the extent of subclinical atherosclerosis. Ultrasonography of both the left and right carotid artery was performed using a 7.5MHz linear array transducer (Acuson Aspen, Mountain View, CA, USA). Details of the procedure for CIMT measurement have been published previously [25].

As functional measures of the vessel wall, we used measures of vascular stiffness; carotid-femoral pulse wave velocity (PWV) and aortic Augmentation Index (AIx). The PWV was determined noninvasively using a SphygmoCor device (PWV Medical, Sydney, Australia), which allowed an online pulse wave recording and automatic calculation of PWV using transducers (Millar SPT 301 pressure transducer; Milar Instruments, Sydney, Australia). Details of the procedure for aortic PWV measurement have been published previously [26].

The aortic AIx was derived by applantation tonometry of the radial artery and was calculated as the difference between the first and second systolic peaks of the ascending aortic waveform expressed as a percentage of the central pulse pressure (the difference between central systolic and diastolic pressure divided by pulse pressure). The details of this method have been described elsewhere [27].

Assessment of bone mineral density

BMD measurements were performed with Dual-Energy X-ray absorptiometry (DEXA) using a Hologic QDR1000 (Hologic Europe, Zaventem, Belgium). Scanning was performed according to the instructions of the manufacturer. Quality assurance, including calibration was performed routinely every morning for DEXA, using the standard provided by the manufacturer. Quality assurance, including calibration was performed routinely every morning for DEXA, using the standard provided by the manufacturer. In the analyses performed we used total body BMD.

Data analysis

Distributions of anthropometric measures, lifestyle characteristics, and laboratory measurements were expressed as mean and standard deviation for continuous variables and frequency and percentage for categorical variables.

Linear regression analysis was performed to estimate the strength of the associations between the cardiovascular parameters and BMD and described as the linear regression coefficient (ß) and its standard error (SE). Assumptions for linear regression including linearity of the relationship between dependent and independent variables and normality of the variables was checked in advance and violations were fixed by transformations or excluding outliers. Two extreme values in PWV (2.75 and 30.51 m/s) were excluded, since they were considered biologically implausible. Hs-CRP levels > 10 mg/l can be taken as evidence of active inflammatory processes (for example, trauma, infection), therefore subjects with hsCRP >10 mg/l were excluded from the analysis (n=17). Both hsCRP and PWV were log-transformed because of their skewed distributions. Crude models were adjusted in five steps. First, we adjusted for age. Second, we added the confounders that are necessary to take into account (alcohol consumption, smoking, physical

performance and height). To elucidate whether and to what extent the associations might be explained by intermediate factors, further analyses also adjusted separately for weight. The level of statistical significance was set at $p<0.05$ in all analyses. Analyses will be performed with the SPSS statistical software package, version 15.0 (SPSS Inc, Chicago, III).

Results

Table 1 Baseline characteristics

	Mean ± SD
Age (years)	59 ± 11
Body mass index (kg/m²)	26 ± 3
Bone mineral density (mg/cm2)	1147 ± 93
Smoking (pack years)	14.4 ± 19.4
Alcohol consumption (units/week)	13.3 ± 13.6
Physical activity (Voorrips score)	9.2 ± 1.7
Fasting glucose (mmol/l)	5.9 ± 1.4
Vitamin D (nmol/l)	61.9 ± 25.6
IMT (mm)	0.81 ± 0.15
Pulse wave velocity (m/s)	9.2 ± 2.8
Aix (%)	23.9 ± 10.3
hsCRP (mg/ml)	2.64 ± 5.9
Insulin sensitivity (QUICKI)	0.35 ± 0.04

Table 1 provides the characteristics of the study population. The median age of the total study group was 59 years (range 40-80).

A higher CIMT was statistically significantly associated with higher BMD after adjustment for

age, smoking, alcohol, physical activity, vitamin D and height (ß 99.7; p=0.02). Further adjustment for weight attenuated the estimates towards non-significant (table 2).

We found no association between PWV or AIx and BMD (table 2). Linear regression analyses showed that lower insulin sensitivity was associated with higher BMD (ß-645.1; p0.00). After adjustment for weight this association was no longer significant (table 3). A similar effect was seen for the association between hsCRP and BMD (table 3).

Table 2 Associations between cardiovascular parameters and BMD

Variable	Model 1		Model 2		Model 3		Model 4	
	ß (SE)	p	ß (SE)	p	ß (SE)	p	ß (SE)	p
PWV	-20.1 (21.0))	0.3	27.6 (27.2)	0.3	39.5 (27.6)	0.2	11.2 (25.9)	0.3
Aix	-0.2 (0.5)	0.8	0.2 (0.5)	0.7	0.9 (0.5)	0.1	0.4 (0.5)	0.4
CIMT	-14.6 (35)	0.7	98.8 (44.9)	0.03	108.6(44.6)	0.02	56.5 (38.6)	0.1

Abbreviations: ß, linear regression coefficient; SE, standard error and p, p-value. Model 1: crude model; Model 2: adjusted for age; Model 3: multivariable adjusted for age, smoking, alcohol, height and physical activity; Model 4: model 3 additionally adjusted for weight.

Table 3 Associations between insulin sensitivity, hsCRP and BMD

Variable	Model 1		Model 2		Model 3		Model 4	
	ß (SE)	p	ß (SE)	p	ß (SE)	p	ß (SE)	p
Insulin sensitivity	-441.9 (142.2)	0.00	-573.1 (142.7)	0.00	-645.1 (141.1)	0.00	127.7 (149.9)	0.4
hsCRP	9.6 (5.3)	0.07	14.5 (5.4)	0.01	16.8 (5.4)	0.00	6.6 (4.8)	0.18

Abbreviations: ß, linear regression coefficient; SE, standard error and p, p-value. Model 1: crude model; Model 2: adjusted for age; Model 3: multivariable adjusted for age, smoking, alcohol, height and physical activity; Model 4: model 3 additionally adjusted for weight.

Discussion

This population-based cross-sectional study of middle-aged and elderly men, without a history of CVD, showed that CIMT, serum levels of hsCRP and insulin sensitivity were related to BMD. Adjustment for weight attenuated the association between CIMT, insulin sensitivity and hsCRP and BMD, suggesting that apart from a direct effect on bone CIMT and insulin sensitivity might be particularly associated with weight, which in turn is related to BMD. Arterial stiffness, measured by PWV and Aix, was not associated with BMD in this population. To the best of our knowledge, this cross-sectional study is the first to investigate the association between low BMD and atherosclerosis in a large sample of middle-aged and elderly men without history of cardiovascular disease.

To appreciate these findings some issues need to be addressed. First, the interpretability of the results may be restricted by several factors inherent to the cross-sectional design and does not provide direct evidence of cause and effect. Second, we studied total BMD, while in clinical practice femoral and lumbar BMD are used. On the other hand, in studies evaluating the association between low bone mass and vascular calcification, an independent association was shown between BMD at cortical sites, as represented by low hip BMD and vascular calcification, while studies measuring BMD at predominantly trabecular sites, namely spinal sites, failed to demonstrate an association. This may be related to the fact that the trabecular bone compartment is more metabolically active than the cortical compartment due to its larger surface area and exposure.

In the present study, subclinical atherosclerosis measured by CIMT was related to higher BMD, an effect that was no longer significant after adjustment for weight. Schaffer et al found similar

results in young and middle-aged (<60) men and women, whereas in older individuals, BMD was inversely associated with CIMT [2]. Other studies relating the degree of atherosclerosis and bone mineral density have shown variable results (no association or inversely associated), perhaps because populations, methods, and the anatomical sites chosen to assess osteoporosis and atherosclerosis vary dramatically between and among studies. Most studies included post-menopausal women or older men with cardiovascular disease or DM [2, 28-30].

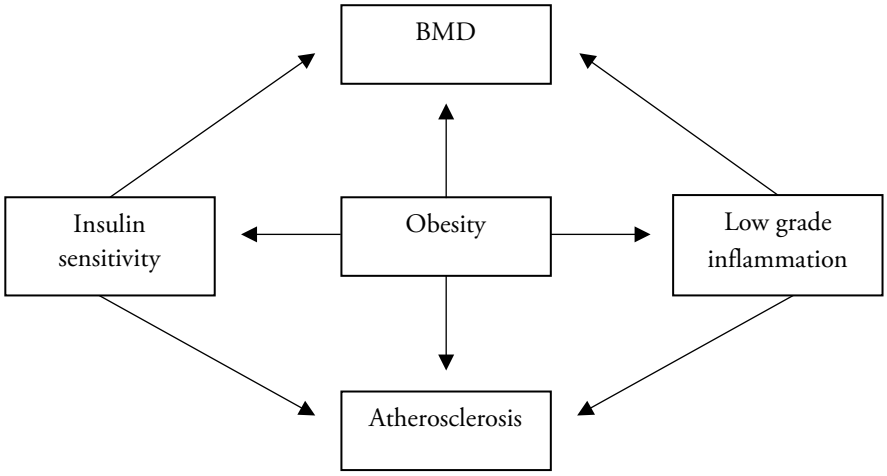
We found no association of Aix and PWV with BMD. Previous literature regarding the association between PWV and Aix is inconsistent and only few studies included men [31-34]. A link between osteoporosis and PWV in men has been recently suggested in a clinical study in Japanese men. This study found a correlation between brachial ankle PWV and ultrasound detected osteoporosis [14]. Another study, in healthy men >60 years found no correlation between osteoporosis and PWV [13]. Aix and bone loss is scarcely studied. One study found that Aix was increased in osteoporotic postmenopausal women free of cardiovascular disease and risk factors.

The association we found between insulin sensitivity and BMD is in line with several observational studies [35-37]. After adjustment weight, the association was no longer significant. Complex pathophysiological mechanisms associated with insulin resistance and hyperglycemia are involved in deleterious effects on osteoblast function and bone formation [19]. Higher levels of hsCRP are associated with increased fracture risk, although previous studies on CRP and BMD have yielded conflicting results and evidence supporting an association in men has been limited. Most studies finding an association between low grade inflammation and BMD are conducted in

patients with manifest cardiovascular disease [38-41]. In our study, excluding subjects with pre-existing cardiovascular disease, we found no association between hsCRP and BMD, after adjusting for weight. These contrasting results may be explained by the fact that in our healthy study population inflammation levels are negligible in comparison with patients with manifest cardiovascular disease and therefore not affect BMD.

As written above and shown in table 2 and 3 after adjustment for weight the significant associations we found were no longer significant, suggesting that the relation between subclinical atherosclerosis (measured by CIMT), hsCRP ad insulin sensitivity and BMD, in our healthy study population, was partly explained by weight. Interestingly, obesity is considered to have opposites effects on bone and arterial aging. It is well known that overweight and obesity increase arterial stiffness, these effects being partially related to increased blood pressure in overweight subjects, but also to the several other mechanisms such as metabolic disturbances, which can also increase arterial stiffness. Body weight and obesity have been reported to increase BMD due to purely mechanical loading reasons.

Figure 1 Associations obesity and BMD and atherosclerosis



Mechanical loading appears to stimulate bone formation by decreasing apoptosis and increasing proliferation and differentiation of both osteoblasts and osteocytes by an activation of the intracellular signalling Wnt/β-catenin. Body fat mass, a component of body weight and one of the most important indices of obesity, may have beneficial effects on bone through several molecular and hormonal pathways (adipocytokine). It has also been reported that excessive fat mass may promote osteoporosis probably due to the presence of associated metabolic disorders (insulin sensitivity) and low-grade inflammation. So, the interaction between body weight and BMD is very complex and both positively and inversely associations are defined (figure 1). We conclude that the negative effects of weight on bone are mostly attribute to excessive fat mass and its associated low grade inflammation and metabolic disorders. Since our study population consisted of healthy mostly non-obese men (only 39 subjects (11%) were obese), this explains the protective effect of weight on BMD in this population.

In line with this, Benetos et al studied the individual effects of lean and fat mass on bone mineral density and arterial stiffness in elderly men. Bone and arterial aging were differently influenced by

lean and fat mass. Aortic stiffness increased with fat mass and presence of associated metabolic disorders, but was not influenced by lean mass. On the other hand, bone mineral density was positively and strongly correlated with lean mass, whereas fat mass and associated metabolic disorders had no effect [13].

In summary, in this population of healthy middle aged and elderly men without a history of cardiovascular disease the positively association between cardiovascular parameters and BMD was mainly explained by weight. Suggesting that in non-obese men without cardiovascular disease weight plays a protective role in the development of osteoporosis.

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5

General discussion

General Discussion

Parkinson's disease (PD) is a common neurodegenerative disease, affecting approximately 1.8% of the population over the age of 65 [1]. The prevalence of PD and comorbidities increase with age and therefore many patients with PD suffer from other diseases related to old age. The presence of comorbid disease has consequences for disability, substantially adding to disease burden [2]. In this thesis, we have shown that comorbidities like osteoporosis, T2D and hypertension are common in PD. The high prevalence of these conditions may be explained by several reasons. First, the risk of developing most chronic diseases increases progressively with age. Second, we proved that T2D and hypertension are both risk factors for developing PD (complicating- (or causal-) comorbidity). And third, we showed that T2D, hypertension and PD share inflammation as an important common pathway, thus explaining the clustering of comorbidities in PD (concurrent comorbidity). We also showed that besides inflammation other disease-specific risk factors connect osteoporosis and PD, i.e. hyperhomocysteinemia, BMI, and vitamin D, whereas insulin sensitivity and oxidative stress connectT2D and PD, and the renine-angiotensine system and oxidative stress link hypertension and PD. In this final chapter, the results of this thesis will be placed in broader perspective, including methodological issues. Implications of these findings will be discussed, including some directions for future research

Methodological considerations

Both in this thesis, and in the related literature, we encountered various methodological problems. These methodological issues are in general related to the study population or the study design.

Related to the study population

To date PD has been investigated in relatively small cohorts [3-13] (with the numbers of PD cases ranging from 41 [11] to 656 [12]) or using self-reported diagnosis only [13]. Self-reports to identify PD might have led to diagnostic and reporting errors, particularly underreports since some PD patients might not report their diagnosis on follow-up questionnaire.

Large established prospective population studies offer an important opportunity for investigating the role of risk factors in rarer diseases such as PD, with relatively small additional effort for ascertaining cases. In chapter 4.1 we used data from the European Prospective Investigation into Cancer and Nutrition cohort (EPIC), that implemented an extensive procedure for ascertaining and validating 734 PD cases. In brief, the case ascertainment and validation strategy followed two of the three step approach: 1) Phase I: record linkage with local sources of information (i.e. drug registries, hospital records) for identifying possible cases; 2) Phase II: Clinical record retrieval and collection of clinical data for PD case identification; 3) Phase III: across centre validation [14]. A cohort study like this, with large sample size (N=220494) and well characterised and validated case definition, has a high power for detecting small associations for a relatively rare condition and is essential for investigating the suggested associations for PD.

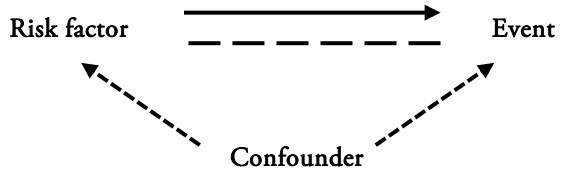
Related to study design

Most of the studies investigating the association between PD and other diseases are observational in nature and are based on databases using different case definitions for exposure and outcome, and it is therefore often a challenge to compare their results. These databases are mostly limited by residual confounding (the datasets were not primarily designed to study the association and many of the potentially relevant confounders are missing or incomplete), or detection bias (patients seek medical aid when visiting their neurologist, and other medical conditions are more

likely to be diagnosed). However, observational studies can aid in generating a hypothesis and establishing an association between exposure and outcome. There are three types of observational studies, including cohort studies, case-control studies, and cross-sectional studies [16]. In this thesis, we used three cross-sectional studies (chapter 2.2, 3.2, 4.2) and two prospective cohort studies (chapter 3.1 and 4.1). Methodological problems in cross-sectional include the examination of data on outcome and exposure at one particular time point, so no causal association can be established. In cohort studies exposure is identified before the outcome, so cohort studies are better able to assess causality, but still confounding cannot be totally excluded. Disadvantage is the need for a large sample size and the potentially long follow-up duration of the study design, especially in rare diseases like PD. Therefore case-control studies can be helpful, but evaluating exposure status is a methodological issue in case-control studies, where recall bias can play a role.

So, inferring causality from observational data is problematic as it is not always clear which of two associated variables is the cause and which the effect, or whether both are common effects of a third unobserved variable or confounder (figure 1). Another problem in observational studies is reverse causation, which refers to a direction of cause-and-effect contrary to a common presumption (figure 1). Reverse causation for example may occur in a classical case-control study when patients changed their behavior as a result of the PD diagnosis.

Figure 1 Relation between risk factor and event in observational studies. The effect of confounding is indicated in small stripes and of reverse causation in larger stripes



Consequently, for understanding whether the association between comorbidities and PD are causal we should use methods that provide insights into the causal nature of observed associations. If a concomitant disease is directly involved in the pathological process leading to PD, then inherited variation changing the risk of that disease should affect PD risk in the direction and magnitude predicted by associations with that disease.

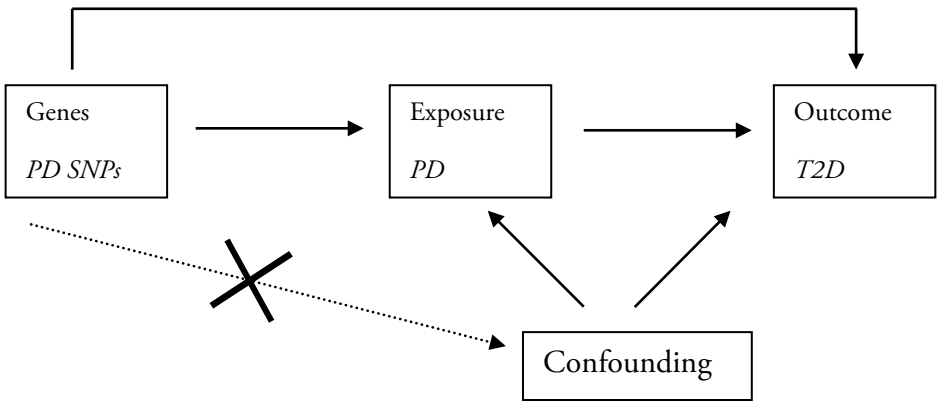
The emergence of robust genome-wide association studies (GWAS) promises to explain the genetic aetiology of the common sporadic forms of complex diseases. GWAS should also be an unbiased generator of intermediate factors that are relevant in the disease pathogenesis. Furthermore, the interpretation of the GWAS and the translation of the genetic findings into functional mechanisms that are biological important can lead to a better understanding of disease pathogenesis [17].

In chapter 3.2 we used genome-wide significant SNPs for type 2 diabetes (T2D) and investigated if they were related to PD risk. In this study three T2D related SNP's were significantly associated with PD. Additionally investigated SNP-gene expression associations provided some explanations for these associations, namely inflammation, mitochondrial dysfunction, insulin sensitivity and oxidative stress. These convergent molecular pathways may be part of the explanations why diabetic patients tend to show a higher future risk for PD. This positive association between

T2D and PD is in line with findings from several previous studies [18-23]. These findings are summarized in two recent meta-analyses, with inconsistent findings [24-25]. The different results in these meta-analyses may again be explained by differences in study design. The first meta-analysis, which only included cohort studies, found a positive association and the second meta-analysis, including only case-control studies, found a negative association. As stated above, the use of cohort studies may be preferable because of evidently causal hypothesis verification.

Another way to investigate whether associations of risk factors with disease outcomes are causal, is the concept of Mendelian randomization (MR), using a genetic variant as instrumental variable (IV) [26]. Figure 2 illustrates this concept. MR studies are a valid way to explore evidence for causality, given that certain assumptions are met. First, there has to be a strong association between the instrumental variable and risk factor of interest. Second, the instrumental variable must be independent of potential confounders of the observational relationship between exposure and outcome. Third, the instrumental variable affects the outcome only through the risk factor of interest. Figure 2 illustrates the concept of MR.

Figure 2 Diagram illustrating causal relationships between genetic variants, putative causal trait (exposure), putative effect trait (outcome) and confounders



In chapter 3.1 we used the concept of Mendelian Randomisation (MR), using a genetic variant as instrumental variable (IV), to investigate the causal nature of the relation of PD with T2D (figure 2, italic). We used a genetic score based on genetic variants with 28 PD associated loci (PDGene database). We then generated an instrumental variable estimates for the relation of PD with T2DM by combining the SNP-PD associations, using the above mentions genetic variants, with the pooled SNP-T2DM associations from InterAct and DIAGRAM using the inverse variance weighted method. In this study we found no evidence of a causal effect of PD on the risk of T2D. No other MR studies have been performed investigating this association. Previous observational studies exploring the risk of developing T2D in patients with PD yielded inconsistent results, varying from an increased [27] to decreased risk [28].

Comorbidities in PD: a causal factor for disease

The studied comorbidities in this thesis could be associated with the risk of PD in several ways. To disentangle whether these comorbidities are causally related to PD and their possible roles in the causal chain will be successively discussed in view of the above mentioned paragraph, the results of this thesis, and evidence from the literature. Results are summarized in figure 3.

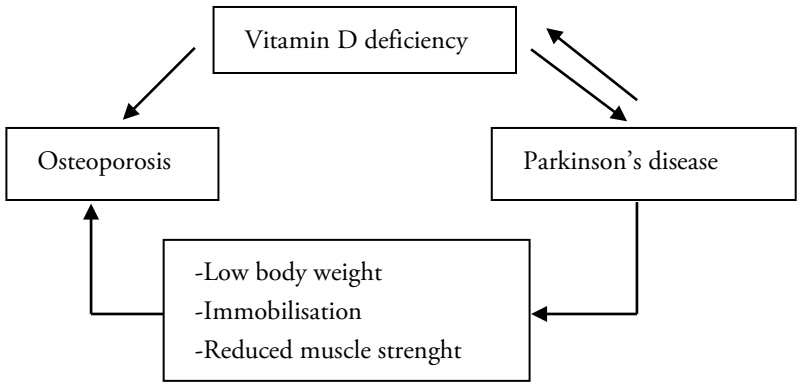
Osteoporosis and PD

Until now, there is no evidence for a causal relation between osteoporosis and PD (figure 3.1). We showed that there is an indication for a complex interaction between various risk factors, like vitamin D deficiency, that can contribute to bone loss in PD as shown in chapter 2.1 and 2.2. Optimal management of osteoporosis in PD patients calls for an extensive risk assessment of all of these factors, including medication use, level of immobilization, muscle strength and nutritional status. The high incidence of vitamin D deficiency, which is consistent with the literature [46], is

particularly interesting, taken into account the early stage of disease, sufficient sunlight exposure and adequate dietary intake. It is suggested in literature that vitamin D plays an important role in the pathogenesis of PD itself [47]. The underlying mechanism suggests that persistent vitamin D deficiency may lead to chronic loss of dopaminergic neurons in the brain. However, the evidence of this association is limited to a few cross-sectional studies and only one longitudinal study [48]. Until now, it is not clearly understood whether vitamin D deficiency is influencing the pathogenesis of PD or that PD itself leads to vitamin D deficiency. Larger studies are needed to investigate the association between vitamin D and bone loss and because of causality issues bidirectional MR studies may be a study design of choice. Nevertheless, it is important to be alert of vitamin D deficiency in PD and its possible effect on BMD and muscle strength. So, we suggest regular vitamin D measurements in patients with PD. Pending further evidence, it would appear prudent to perform regular measurements of vitamin D in older PD patients, starting early on in the course of their disease.

Figure 3 Diagram illustrating possible association between comorbidities and PD. 1. Osteoporosis and PD. 2. T2D and PD. 3. HT and PD

1.



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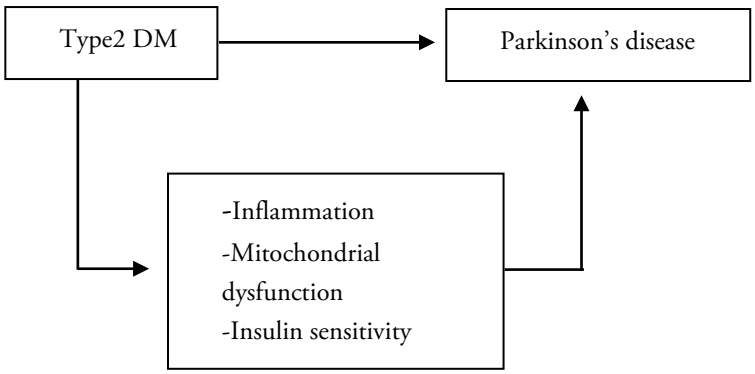

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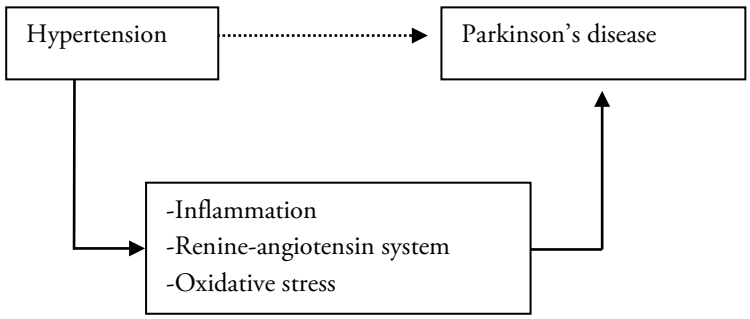


T2D and PD

In chapter 3.1, a MR study does not support the hypothesis that PD has a causal effect on the risk of developing T2D. The observed association of PD and T2D in observational studies is potentially the other way around (figure 3.2). The results shown in chapter 3.2 are in line with this hypothesis, supported by growing evidence in literature [29], but to confirm these results a MR should be performed. Both impairment of insulin action and high glucose might cause dopamine neuron depletion. Furthermore, subclinical chronic inflammation may precede the development of diabetes, and dopamine neuron depletion may be caused by inflammation and oxidative stress [30]. In our study, we found no evidence of an association between PD and hsCRP levels, which may contradict this hypothesis (chapter 3.1). On the other hand, hsCRP may not be an appropriate systemic marker of inflammation to use. On the basis of MR studies hsCRP could be excluded as a causal mediator in cardiovascular diseases [31], whereas IL6 was shown to be causally related to cardiovascular disease [49-50].

HT and PD

The results in chapter 4.1 indicate that high blood pressure is a risk factor for PD in women, but not in men (figure 3.3). These results are in line with another prospective study based on 7 surveys of representative samples of the general population in Finland [32]. There are some pathophysiological mechanisms linking hypertensive status to PD [33]. For example, inflammation and oxidative stress have been involved in dopaminergic neuron degeneration [34]. Additionally, renine-angiotensin system (RAS) may also link hypertension to PD. Brain RAS over activity can lead to dopaminergic degeneration. Interestingly, oestrogen depletion increases RAS activity and neuroinflammation, which may lead to dopaminergic degeneration [35]. This last

phenomenon may explain the sex-specific association in combination with higher rates of hypertension in postmenopausal women. Our results warrant further research in the mechanisms involved. In particular, the use of Mendelian randomisation could provide compelling final evidence if the association between BP and PD is causal.

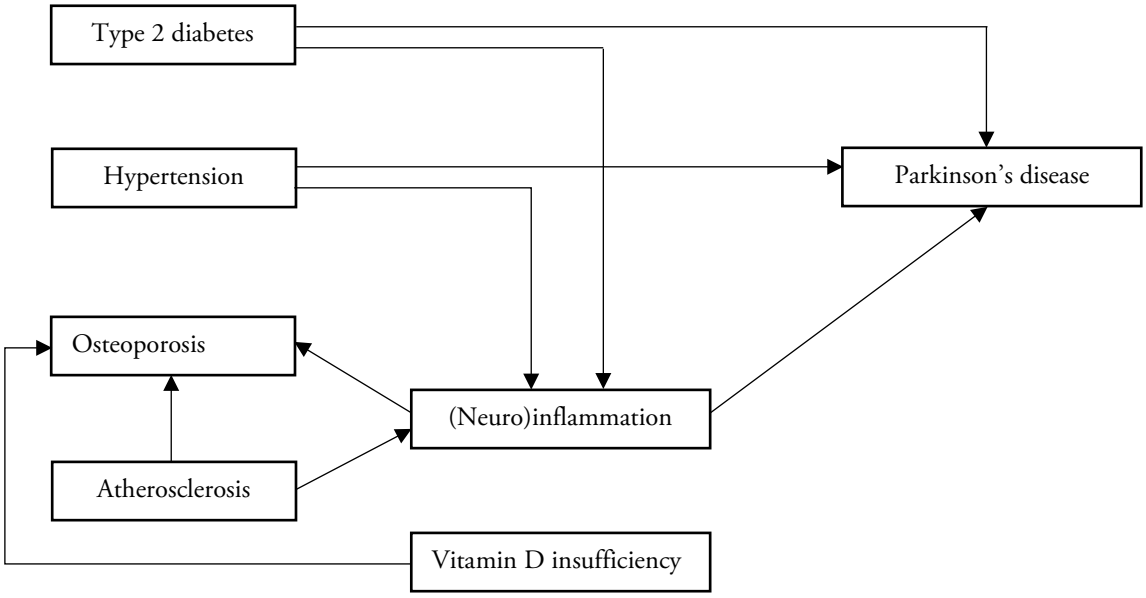
Causal factor and pathophysiological mechanism: inflammation

As stated above/previously stated, the high prevalence of the chronic conditions in PD in this thesis may be partly explained as the consequence of one of these chronic conditions (causal). Another part may be explained by the fact that these chronic diseases share more than just age as an etiologic risk factor. Several lines of evidence support a role of (neuro) inflammation in the causal chain of the investigated concomitant diseases in this thesis and PD (figure 4). As shown in chapter 2.1, chronic inflammation may connect PD and osteoporosis. We concluded the same in chapter 3.2 for T2D and PD, in chapter 4.1 for BP and PD and in chapter 4.2 for cardiovascular risk factors and BMD.

What makes inflammation such an important and possible connecting factor in these chronic conditions? First, age-related changes in the immune system result in elevated levels of catabolic cytokines contributing to a chronic inflammatory state [36, 37,38]. Besides advanced aging, hypertension, diabetes and atherosclerosis could promote neuro-inflammation, not only establishing the condition as a central pathophysiological mechanism, but also constantly fuelling it [39] Subtle, but continuous neuro-inflammation has been connected to PD. The exact role of inflammation in PD is yet not clear. Nevertheless, a significant increase in the level of inflammatory markers in the substantia nigra and cerebrospinal fluid of PD patients has been observed (40). It has been proposed that this neuro-inflammation may be the cause of, rather

than a response to, the observed neuronal loss [40,41]. In our study, we found no evidence of an association between PD and hsCRP levels, which may contradict this hypothesis (chapter 3.1). On the other hand, hsCRP may not be an appropriate systemic marker of inflammation to use, because on the basis of MR studies it could be excluded as a causal mediator in cardiovascular diseases [31]. Of special interest is the connection between vitamin D and neuro-inflammation in PD. As concluded in chapter 2.2 vitamin insufficiency was common in patients with PD, even in early stage of the disease. We suggested in chapter 2.1 and 2.2 a possible pathophysiological role for vitamin D in PD. Vitamin D has potent immunomodulatory activities, but the role in neuro-inflammation remain unclear. A recent study observed that vitamin D treatment produced significant neuroprotection of dopaminergic neurons in a PD animal model [42].

Figure 4 Role of (neuro) inflammation in the causal chain of concomitant diseases and PD



Future directions

In this thesis, we have shown that comorbidities like osteoporosis, T2D and hypertension are common in PD. The high prevalence of these conditions may be partly causal and partly because of age and shared risk factors. These findings have made us question the relevance of a disease management model in the care of patients with PD. Several authors have discussed the conflicting relevance of multiple conditions in one patient and disease-specific guidelines [43,44]. Although disease-specific guidelines are usually evidence-based, this does not mean that they are applicable in all situations. In studies on which disease-specific guidelines are based, patients with multiple conditions are usually excluded. In addition, disease specific guidelines often do not take into consideration the simultaneous occurrence of other diseases. Therefore, the value of disease specific guidelines decreases in proportion to the increase in multiple morbidities. Poly-morbidity requires an integrated patient-oriented approach rather than a fragmented disease-oriented

approach. Guideline development must systematically approach the most common disease combinations and, in case of an absence of evidence, outline high-priority research questions. Such an approach is needed not only in guidelines, but also in studies investigating patients with PD. As, at present, chronic illness contributes 60% of the global burden of disease, which by the year 2020 will increase to 80%, such a focus in healthcare research is of ultimate importance [45]. By understanding the common pathways of chronic diseases and the nature of the complex relationships amongst them, insights can be gained into the impact of combinations of illnesses and symptoms on function and quality of life. It is increasingly clear that chronic disease research must be approached holistically rather than on a disease-by-disease basis.

Hence, further research is needed to examine biological pathways and systems (including causality) for understanding specific combinations of comorbidities in PD. Systematic understanding of the molecular mechanisms underlying complex and potentially interacting chronic diseases in PD can improve strategies for treatment and prevention of PD. Taking advantage of the vast amount of genetic data from the GWAS, these GWAS data provides unprecedented opportunities to identify genes and genomic regions associated with complex traits in human populations, including disease risk. At the same time, clinical trials should ascertain comorbid diseases and enrol multimorbid persons to study applicable interventions. In particular, data coming from large prospective cohort studies, with reliable case ascertainment strategies, can shed light on the potential mechanisms of action of factors known to be associated with PD. Using modern advanced epidemiological techniques, such as Mendelian randomisation, can provide insight in causality. In particular, the use of Mendelian randomisation for providing compelling final evidence on the causal association between inflammation (IL6), vitamin D, hypertension and diabetes on PD will answer some of the remaining question in this thesis

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6

Summary

Parkinson's disease (PD) is one of the most common neurodegenerative diseases. Ageing is a major risk factor for PD; due to the growing elderly population, the prevalence will increase sharply in the next decades. In this ageing population, concomitant diseases are common and have a negative effect on the quality of life in patient with PD. Whether these concomitant diseases are causally related to PD or a consequence of several (age-related) common pathways is not clear.

In this thesis, we studied the (causal) association between PD and osteoporosis, diabetes and hypertension and factors contributing to these comorbidities in PD patients. This chapter summarizes the main findings of this thesis.

In Chapter 2, the association between bone loss and PD is described. In Chapter 2.2 we performed a narrative literature review on bone loss is in PD. Patients with PD have a lower BMD than age-matched controls. This reduced bone mass, in combination with frequent falls, explains the increased fracture risk of patients with PD. The BMD reduction in PD is multifactorial in origin, involving reduced mobility, vitamin D deficiency, hyperhomocysteinemia (caused by levodopa use, or vitamin B12 and/or folic acid deficiency), malnutrition/low body weight, and decreased muscle strength. All these factors are common in PD and act synergistically. In Chapter 2.2, the prevalence of osteoporosis in sedentary PD patients was investigated in 186 PD patients in an early stage of the disease (Hoehn and Yahr stage 1 to 2.5) . Furthermore, the possible risk factors that are associated with bone loss in PD sedentary patients were studied. The prevalence of osteoporosis and osteopenia was 53.2% in the study patients with an early stage of PD. These results were consistent with previous studies about osteoporosis in PD. Female gender, weight loss and low vitamin D levels were identified as significant risk factors for decreased BMD. Furthermore, vitamin D concentrations were significantly lower in

comparison with sex and age- matched controls without PD. The high incidence (56.2%) of vitamin D deficiency in early stage of PD is prominent (Chapter 2.2). These findings are consistent with other studies. Until now, the influence of vitamin D deficiency on the pathogenesis of PD or the possibility that PD itself leads to vitamin D deficiency (as a result of malnutrition and less sunlight exposure as a consequence of reduced mobilisation) is not clearly understood.

In chapter 3 the association of type 2 diabetes (T2D) and PD is studied. In chapter 3.1, we used data of the European Prospective Investigation into Cancer and Nutrition (EPIC)-InterAct case-cohort study, comprising 9679 incident cases of T2D and a representative subcohort (n=12679) that also includes 589 of the incident cases, from eight European countries. A Mendelian Randomisation study was performed using a score based on genetic variants with 28 PD associated loci (PDGene database). We generated instrumental variable estimates for the relation of PD with T2DM by combining the SNP-PD associations, using the PDGene database, with the pooled SNP-T2DM associations from InterAct and DIAGRAM using the inverse variance weighted method. Instrumental variable analysis did not show a relation between PD and T2D (OR 1.01, 95%CI: 0.96-1.07). Adjustment for potential confounders did not change these findings. In conclusion, our study did not support a direct causal association of PD on the risk of T2D. The major strengths of this study include the prospective design, large sample size, heterogeneous European population and the MR approach. In chapter 3.2, we searched for evidence for a causal link between T2D and PD using genome-wide association studies (GWAS). If T2D is directly involved in the pathological process leading to PD, then inherited variation changing T2D risk should affect PD risk in the direction and magnitude predicted by associations with T2D. In this study, we aim to explore whether we can find evidence for a causal link

between T2D and PD by investigating whether SNPs for T2D are related to PD risk. For T2D (trait type 2 diabetes) 105 GWAS studies were retrieved from the Hugenavigator database of which 14 were included in our study. From these studies 84 SNP's (rs-numbers) were extracted. Three T2D SNPs were nominally significantly associated with PD in the PDgene database. Additionally, investigated SNP-gene expression associations (from the three significantly associated SNPs) provide some explanations for this association, namely inflammation, mitochondrial dysfunction, insulin sensitivity and oxidative stress. These convergent molecular pathways may be part of the explanations why diabetic patients tend to show a higher future risk for PD.

In chapter 4, cardiovascular diseases including hypertension and both osteoporosis and PD is discussed. In chapter 4.1 we used data from the NeuroEPIC4PD cohort, comprising 220494 men and women aged 52 ± 10 years at baseline. In total, 734 incident PD cases occurred during follow-up. In women, measured blood pressure above 140/90 (HR 1.34, 95%CI 1.00-1.82), systolic blood pressure ≥ 140 (HR 1.34, 95% CI 1.00-1.82), diastolic blood pressure ≥ 90 (HR 1.51, 95% CI 1.07-2.15), self-reported treatment of hypertension (1.61, 95% CI 1.02-2.56) and composite hypertension (HR1.44, 95% CI 1.10-1.88) were significantly associated with the risk of developing PD. In men, no significant association was found. So, in conclusion, our data indicate that high blood pressure is a risk factor for PD in women. These results are in line with another prospective study based on 7 surveys of representative samples of the general population in Finland. The major strengths of our study include the prospective design, the large cohort of men and women, and the validated outcome ascertainment. In chapter 4.2 the relationship between atherosclerosis and bone mineral density, and the influence of insulin sensitivity and low

grade inflammation on this relationship in men is presented. In this population of healthy, non-obese, middle aged and elderly men without a history of cardiovascular disease the positively association between cardiovascular parameters and BMD was mainly explained by weight, suggesting that in non-obese men without cardiovascular disease weight plays a protective role in the development of osteoporosis.

The main results of the above studies are reviewed and discussed in chapter 5 of this thesis. We discussed methodological problems related to the study population, study design and causality issues. The methods used in chapter 3.1 (MR-concept), 3.2 (using GWAS data) and 4.1 (using large cohort studies with reliable case ascertainment strategies) can provide evidence on the possible causal association between concomitant diseases and PD. They showed evidence for a causal relation between T2D and hypertension in PD. In addition, several common pathways, especially inflammation, may explain the clustering of these diseases in PD. However, many questions in this discussion still need to be addressed and await further research.