Impaired olfactory ability in patients with Parkinson’s disease: The importance of olfactory testing in order to improve diagnostic accuracy

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Abstract

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Problem: Parkinson’s disease (PD) is the second most common neurodegenerative disease. Its symptoms are complex and the amount of differential diagnoses is high. This in turn, leads to a low accuracy of PD diagnosis and to a misdiagnosis rate of up to 30%. This high rate causes immense burden not only to the patients but also to the society and economy. In order to alleviate these burdens and to reduce costs a means for early and reliable diagnosis of PD is needed. A frequent, robust, and early symptom in PD is decreased olfactory ability. The potential of olfactory testing and the options of assessment have yet to be determined.

Methods: The ParkCHIP study, a cross sectional study with case-control design takes aim at the development of a biomarker chip for the detection of PD. We utilized results of the ParkCHIP study to address the questions outlined above. Overall, the ParkCHIP study group existed of 211 PD patients and 148 healthy controls (HC). Within this thesis, participants were classified into different study groups. 148 PD patients and the same amount of HC were defined as ParkCHIP I study group. For Park CHIP I, we investigated the feasibility of the 16 Sniffin’ sticks odor identification test as a supportive diagnostic tool to improve the accuracy of PD diagnosis and its applicability as a point-of-care test. We used random forest classifier to determine the best subset of odors, which yields reliable classification into PD patients and HC. We qualified the value of self-assessment of olfactory function versus Sniffin’ stick test assessed olfactory function via kappa-coefficient on 211 PD patients (ParkCHIP II). Within ParkCHIP II, thirteen patients were found to be normosmic. To find evidence for potential misdiagnoses, we screened this group (ParkCHIP III) for special features. Risks of olfactory impairment were estimated with proportional odds models including factors such as age, disease severity and the belonging to a certain PD subtype. Existing literature and national statistics were used to estimate the costs associated with PD.

Results: Impaired olfactory ability was found among the majority of PD patients. Participants of the overall ParkCHIP study group revealed high prevalence rates for smell loss (> 90%). For the ParkCHIP I, we found disease severity as well as age being associated with olfactory disturbance. No statistically significant association could be revealed for PD subtypes and olfactory impairment. Among the 16 Sniffin’ sticks odors coffee, peppermint and anise discriminated best between non-diseased and diseased subjects. With random forest we found a misclassification rate for the full set of Sniffin’ sticks of 22.4% and of 23.8% when applying these top-3 odors. Self-reported olfactory impairment was not found to be a reliable measure with patients significantly underestimating their smell loss. In the ParkCHIP II, prevalence rates of olfactory disturbance obtained by self-assessment substantially were statistically significant lower than the rates obtained by olfactory testing. Besides the preserved olfactory ability among the individuals of the ParkCHIP III, we found further evidence for alternative diagnoses and misdiagnoses respectively. Olfactory testing shows the potential to reduce costs that cause extensive socio-economic burden.

Discussion: Olfactory testing is an important and supportive diagnostic tool for PD. The Sniffin’ sticks test was shown to be a valid and easily applicable tool which can serve as a point-of-care test in the clinical routine. The short version of the test containing only three odors was also shown feasible to discriminate between PD and non-diseased individuals, however with slightly increased misclassification rate. This test can be used in order to improve accuracy of diagnosis and even for differential diagnosis of PD. Thus, the financial socio-economic burden can be decreased. In addition, inappropriate drug therapy can be reduced and early access to potential neuroprotective therapy facilitated.
Dedicated to

Marita Sullivan and all Parkinson patients
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### Abbreviations

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<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
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<td>AWMF</td>
<td>Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften</td>
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<tr>
<td>CGI-S</td>
<td>Clinical Global Impression of Disease Severity</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>CM</td>
<td>Centromedian nucleus</td>
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<td>CMA</td>
<td>Cingulate motor area</td>
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<tr>
<td>COMT</td>
<td>Catechol-O-methyltransferase</td>
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<tr>
<td>CT</td>
<td>Computed tomography</td>
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<tr>
<td>DAT</td>
<td>Dopamine active transporter</td>
</tr>
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<td>DBS</td>
<td>Deep brain stimulation</td>
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<td>DI</td>
<td>Disability Index</td>
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<tr>
<td>EPS</td>
<td>Extrapyramidal symptoms</td>
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<td>FP-CIT</td>
<td>$[^{123}I]$ N-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl) nortropane</td>
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<tr>
<td>GABA</td>
<td>Gamma-amino butyric acid</td>
</tr>
<tr>
<td>GP</td>
<td>Globus pallidum</td>
</tr>
<tr>
<td>GPe</td>
<td>Globus pallidum pars externa</td>
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<tr>
<td>GPi</td>
<td>Globus pallidum pars interna</td>
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<tr>
<td>HAQ</td>
<td>Health Assessment Questionnaire</td>
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<tr>
<td>HAQ-DI</td>
<td>Health Assessment Questionnaire Disability Index</td>
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<td>HC</td>
<td>Healthy controls</td>
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<td>Hz</td>
<td>Hertz</td>
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<tr>
<td>IQR</td>
<td>Inter-quartile range</td>
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<td>L-DOPA</td>
<td>L-3,4-dihydroxyphenylalanine</td>
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<td>MAO-B</td>
<td>Monoamine oxidase B</td>
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<td>MDS-UPDRS</td>
<td>Movement Disorder Society-sponsored revision of the UPDRS</td>
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<td>MPTP</td>
<td>1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridin</td>
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NMDA  N-methyl-D-aspartate
NPH  Normal pressure hydrocephalus
OR  Odds ratio
ORs  Odds ratios
PD  Parkinson’s disease
PET  Positron emission tomography
PIGD  Postural instability and gait difficulty
PMC  Pre-motor cortex
PPN  Pendunculopontine nucleus
REM  Rapid eye movement
SN  Substantia nigra
SNC  Substantia nigra pars compacta
SNR  Substantia nigra pars reticulata
SMA  Supplementary motor area
SPECT  Single photon emission computed tomography
STN  Subthalamic nucleus
UKPDBBC  United Kingdom Parkinson’s Disease Society Brain Bank
Clinical Diagnostic Criteria
UMSARS  Unified Multiple System Atrophy Rating Scale
UPDRS  Unified Parkinson’s Disease Rating Scale
UPSIT  University of Pennsylvania Smell Identification Test
VA  Ventral anterior nucleus
VL  Ventral lateral nucleus
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1. Introduction

1.1 Parkinson’s disease

Parkinson’s disease (PD) was first described by James Parkinson in his publication ‘An Essay on the Shaking Palsy’ in 1817 (Parkinson, 1817). PD is the second most common neurodegenerative disease after Alzheimer’s disease (AD) and is primarily caused by loss of dopaminergic neurons in the substantia nigra pars compacta (SNc). The subsequent dopamine deficiency results in a multitude of symptoms with the hallmark features being tremor at rest, rigidity, akinesia or bradykinesia and postural instability.

The term PD is different from the description of Parkinson’s syndrome, which only describes the symptom complex of tremor, and/or rigidity, bradykinesia, and postural instability. PD is one of the diseases summarized in the broader term Parkinson’s syndrome.

According to the AWMF (Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften e.V./Working Committee of the Professional Association of Medicine) guidelines, (AWMF, 2012) Parkinson’s syndrome is classified into four subgroups: i) idiopathic Parkinson’s syndrome (IPS), ii) genetic forms of Parkinson’s syndrome, iii) Parkinson’s syndrome in the context of other neurodegenerative diseases also known as atypical parkinsonian syndromes, and iv) secondary Parkinson’s syndrome. The first subgroup, IPS, is a synonym for PD with idiopathic classifying that there is no obvious external factor that causes the disease. PD is the most common cause of Parkinson’s syndrome (Christine and Aminoff, 2004). Genetic forms of PD are rare. However, several monogenetic forms of the disease as well as various genetic risk factors have been revealed (Klein and Westenberger, 2012) (see 1.4.2). Subgroup three, the atypical parkinsonian syndromes, include multiple system atrophy (see 1.5.1), progressive supranuclear palsy (see 1.5.2), corticobasal degeneration (see 1.5.3) and
dementia with Lewy bodies (see 1.5.4). The last subgroup, secondary parkinsonism, comprises all pathologies that have symptoms similar to PD but that are caused by various factors such as anti-dopaminergic drugs (Hardie and Lees, 1988) or cerebrovascular diseases (Gupta and Kuruvilla, 2011) (see 1.5.7).

In addition, the AWMF guidelines classify the neurodegenerative Parkinson’s syndromes i) IPS and ii) atypical parkinsonian syndromes according to pathological criteria into tauopathies and synucleinopathies. Both are proteopathies, a group of diseases where protein structures become anomalous resulting in an unphysiological assembly and/or a deposit of specific proteins in the brain (Walker and LeVine, 2000). The characteristic protein for tauopathies such as progressive supranuclear palsy is the tau protein, which accumulates and builds neurofibrillary tangles (Hardy and Gwinn-Hardy, 1998). PD, multiple system atrophy, and dementia with Lewy bodies belong to the so called synucleinopathies (McCann et al., 2014) named after the accumulated protein alpha-synuclein. Accumulated alpha-synuclein is a major component of Lewy bodies and Lewy neurites and it is supposed to be causatively involved in the pathogenesis of PD (Tong et al., 2010).
1.2 Pathological features and etiology of Parkinson’s disease

The pathological hallmarks of PD are the loss of dopaminergic neurons in the SNc and the presence of fibrillar, proteinaceous inclusions known as Lewy bodies. The loss of dopamine is responsible for the occurrence of motor symptoms due to the connection between SNc and basal ganglia circuit (Bernheimer et al., 1973) (see 1.2.1; 1.2.2). In a study on PD patients by Fearnley and Lees (Fearnley and Lees, 1991) the neuronal loss in the SNc was exponential and was more than measured in normal ageing individuals. In these degenerated neurons, intracellular, protein-rich Lewy bodies were detected.

Although Lewy bodies are not exclusively responsible for the cell damage, they are referred to as histological and pathological hallmarks of PD (Schulz-Schaefffer, 2010). Lewy bodies consist of a great variety of proteins with fibrillar alpha-synuclein being the main component (Spillantini et al., 1998). In its native form, alpha-synuclein, which is predominantly expressed at presynaptic terminals, presents with an unfolded structure (Clayton and George, 1999). Conformational changes of alpha-synuclein in the form of oligomerization and fibril accretion are under suspicion to be relevant in the pathogenesis of PD (Conway et al., 1998; Taschenberger et al., 2012). However, the exact pathological changes that occur as a consequence of aggregation, oligomerization, and fibrillization of alpha-synuclein are not entirely elucidated. It is hypothesized that the protofibrils that develop in the course of fibril growth, as an intermediate form of alpha-synuclein, are the toxic species. They are thought to affect regulation of neurotransmitters, synaptic function, and plasticity (Lashuel et al., 2013). Luk and co-workers (Luk et al., 2012) demonstrated that alpha-synuclein fibrils, once implanted in a mice brain, spread over the brain and cause damage in the SNc and are found to be responsible for a neurodegenerative cascade (Masuda-Suzukake et al., 2013). The linkage between alpha-synuclein fibrils and PD is even stronger in familial PD. Studies by Polymeropoulos and co-workers (Polymeropoulos et al., 1997) and Singleton and colleagues (Singleton et al., 2003) revealed mutations in the SNCA gene which encodes for alpha-synuclein in patients with inherited forms of PD. The exact etiology of PD is not completely understood but it is assumed that both genetic and environmental factors play a role in PD.
Apart from Lewy bodies (Spillantini et al., 1998), oxidative stress (Jenner, 2003) for example due to altered iron and neuromelanin homeostasis (Zecca et al., 2001) and neuroinflammation (Whitton, 2007, Hirsch and Hunot, 2009) are other potential factors contributing to the neuronal damage within PD. Additionally, factors like mitochondrial dysfunction, excitotoxicity, neutrophic factors, glia immune modulators and apoptosis are assumed to etiologically contribute to PD (Olanow and Tatton, 1999).

Besides these factors, iron and neuromelanin metabolism are found to be associated with the cell loss in the SNc. Particularly the neuromelanin-containing, or so-called pigmented neurons of the SNc, are vulnerable to degeneration (in contrast to the non-pigmented neurons) (Hirsch et al., 1988; Kastner et al., 1992). In PD, the content of neuromelanin was significantly reduced compared to normal aging individuals (Mann and Yates, 1983). Neuromelanin is the main iron storage in the substantia nigra (SN) (Zecca et al., 2001) and is known to have a strong chelating affinity not only for iron but also for other metals (Zecca et al., 1996). Furthermore, dopamine-melanin, a synthetic model for neuromelanin, was shown in vitro to be able to buffer hydroxyl-radical production which is catalyzed by iron (Zareba et al., 1995). Therefore, neuromelanin can possibly reduce oxidative stress and can be protective against cytotoxic processes that are caused by redox-active metals (Zecca et al., 2001). Due to the reduced amount of neuromelanin in PD, there are less molecules that can act in a neuroprotective manner which can potentially contribute to neuronal loss (Zareba et al., 1995). Further, an altered iron metabolism was found to play a role in the pathological mechanism that takes place in the SNc of PD patients. Sofic and co-workers (Sofic et al., 1988) found increased levels of Fe (III) as well as an overall increase in iron in the SN of PD patients. They hypothesize that iron contributes to the neuronal loss in the SN due to enhanced oxidative processes and the decrease in neuromelanin (Good et al., 1992). Because the SNc is part of the basal ganglia, the function of the basal ganglia circuit is affected.
1.2.1 Functional anatomy of basal ganglia in healthy individuals

There are two subcortical structures that are summarized as the basal ganglia, the striatum and the globus pallidum (GP). The former is comprised of the caudate nucleus and the putamen. The GP is composed of globus pallidum pars interna (GPI) and globus pallidum pars externa (GPe; Figure1).

The main functional assignment of the basal ganglia is the planning and programming of voluntary movement. Based on this functional assignment, there are two more structures being functionally part of the basal ganglia, the SN and the subthalamic nucleus (STN). The SN is made up of SNc and substantia nigra pars reticulata (SNr).

The main projection loop of the basal ganglia includes afferences from the cerebral cortex, especially from the prefrontal, sensory, and motor cortex as well as the thalamus. It projects back into the cortex via the thalamus. There is no immediate connection between the basal ganglia and the spinal cord. The main structure of the basal ganglia receiving afferences is the striatum. Afferences derive from the ipsilateral hemisphere and are arranged in a somatotopic order. Besides the cortical afferences, which form the vast majority, there are also afferences from the SNc and thalamus projecting into the striatum.

In addition to various co-transmitters, the main neurotransmitter of the striatum is gamma-amino butyric acid (GABA). Neurons of the striatum that project to the GPe use encephalin as their co-transmitter and predominantly express D2 receptors, whereas striatal neurons projecting to the GPi use substance P and dynorphin as transmitter and express dopamine receptor D1 (Smith et al., 1998).

L-DOPA is an important modulator emitted by midbrain that influences the incoming corticostriatal glutaminergic activity pre- and postsynaptically (Bamford et al., 2004), thereby supporting the modification of movement.

Outgoing fibers originate from the GPi and the SNr. The GPe sends projections to the STN and the GPi. As in the striatum, the predominant transmitter is GABA whereas the SN projecting to the GPi, SNr, and back to GPe transmits via glutamate and has an excitatory effect. The GPi projects its GABAergic neurons to
the ventrolateral and ventroanterior nuclei of the thalamus as well as to the pendunculopontine nucleus (PPN), which is located in the brain stem.

There are two ways of circuitry between cortex, striatum, GP, STN, and thalamus which serve to modulate movement, namely the direct and the indirect way.

The direct way essentially enables movement. Glutamnergic afferences derived from different parts of the cerebral cortex activate the GABAergic neurons of the striatum which in turn inhibit the SNr and GPi. Thus, the repression of the inhibition of the thalamus by SNr and GPi is reduced and the following excitation of glutamnergic neurons provokes an increase in movement (Chevalier et al., 1985).

The indirect way leads to a decrease in movement (Delong, 1990). It also originates from the cerebral cortex and leads to the striatum via glutamnergic corticostriatal fibers. Activated by glutamine, the striatal neurons release GABA, thereby inhibiting the GPe which acts as a suppressor of the STN. This suppression is consequently abrogated and the STN is activated. As a consequence, SNr and GPi, which receive glutamnergic neurons from the STN, are activated and, in turn, inhibit the thalamus via GABAergic neurons (see Figure 2, left panel).

In addition, basal ganglia are not only integrated into motor control through their reciprocal connection to cortex via the thalamus, but they also project into various other areas such as premotor, occulomotor, prefrontal and inferiortemporal areas of the cortex (Middleton and Strick, 2000). Thus, they are involved in eye movement, cognitive function, emotions and motor learning as well as habit formation (Alexander and Crutcher, 1990; Haber, 2003; Hikosaka et al., 2002; Yin and Knowlton, 2006).

L-DOPA released by the SNc has an activating effect on the direct way via D1 receptors and inhibits the indirect way by means of the D2 receptor. Striatal neurons express either D1 or D2 receptors. D1 and D2 are both G protein coupled receptors. D1 receptors are linked to a stimulatory G protein and D2 receptors to an inhibitory G protein. The D1-expressing neurons influence the direct, movement supporting way in an excitatory manner and project to the GPi and SNr, whereas
D2-expressing neurons inhibit the indirect pathway. L-DOPA inhibits striatal D2 expressing neurons and activates D1 neurons. Thus, dopamine facilitates movement through both ways (Surmeier et al., 2007; West and Grace, 2002).

In order to modify, amplify or inhibit the incoming motor impulses there are many interneurons influencing the striatum. GABAergic interneurons inhibit the striatum with lateral inhibition (Tepper et al., 2004).

**Figure 1. Location of the basal ganglia in the human brain.**

Anatomically, the basal ganglia comprise the striatum and the globus pallidum. The former is made of the caudate nucleus (1) with its substructures caput (2) and cauda (3) as well as the putamen (4). The globus pallidum (GP; 5) is composed of globus pallidum pars interna (GPI) and globus pallidum pars externa (GPe). Based on their functionality, the subthalamic nucleus (STN) and the substantia nigra (SN) are further structures that belong to the basal ganglia (not depicted here).

The Amygdala (6) is part of the basal ganglia from an evolutionary point of view, however, functionally it is part of the limbic system.

From Trepel (Trepel, 2008).
1.2.2 Basal ganglia in Parkinson’s disease

The hallmark feature of PD is the loss of dopaminergic neurons in the SNc and a subsequent decreased amount of dopamine in the striatum. Consequently, it evokes an imbalance in the basal ganglia circuitry and finally an increased activity in the neurons of the major output center of the basal ganglia; the GPi and SNr (see Figure 2; right panel). This in turn leads to the inhibition of thalamus and cerebral cortex (Albin et al., 1989). However, the resulting functional changes are versatile and the influence of dopamine depletion on the direct as well as on the indirect way is discussed controversially.

Obeso and colleagues (Obeso et al., 2008) found that a reduced activity in D1-expressing striatal neurons leads to an inhibition of the direct way. Contrarily, a decreased inhibition of the neurons is revealed in the D2 receptor-carrying neurons which leads to an increased activity of the indirect way. On the other hand, Mallet and colleagues (Mallet et al., 2006) demonstrated in their study on parkinsonian rats an incline of signals in a subpopulation of cortical neurons which reach the striatum via the direct way. They observed an activating impact on the indirect way.

As described above, a hyperactive STN in PD patients leads to increased activity in the output neurons of GPi and SNr. With its well-defined function, it is regarded the clearest defined functional feature of PD (Obeso et al., 2000). In animal models as well as in Parkinson patients it could be shown that a lesion of STN yields a progression in both parkinsonian symptoms and thalamocortical activity (Guridi et al., 1996; Trost et al., 2006).
Figure 2. Schema of the direct and indirect pathway of the basal ganglia motor circuits under normal conditions (left) and in individuals affected by Parkinson’s disease (PD) (right).

The inhibitory pathways are indicated by red arrows, whereas, excitatory pathways are depicted in blue. Thick arrows represent increased and thin arrows decreased activity of the connections. Dashed arrows are used to mark the central issue that occurs in the basal ganglia of PD patients, a diminished amount of dopamine released by the SNc and, as a consequence, decreased amount of dopamine in the striatum. Subsequently, it results a misbalance in the basal ganglia circuitry and finally an increased activity in the neurons of the GPi and SNr. This leads to the inhibition of thalamus and cerebral cortex.

CM, centromedian nucleus; CMA, cingulate motor area; GPe, globus pallidus, pars externa; GPi, globus pallidus, pars interna; M1, primary motor cortex; PMC, pre-motor cortex; PPN, pedunculopontine nucleus; SMA, supplementary motor area; SNc, substantia nigra, pars compacta; SNr substantia nigra, pars reticulata; STN, subthalamnic nucleus; VA/VL, ventral anterior/ventral lateral nucleus.

From Galvan and Wichmann and Smith and colleagues (Galvan and Wichmann, 2008; Smith et al., 2012).
1.2.3 Classification of Parkinson’s disease according to Braak

The degenerative process in form of alpha synuclein containing immunoreactive Lewy neurites and Lewy bodies does not only affect the SN, but also extra-nigral structures like the dorsal motor nucleus of glossopharyngeal and vagal nerves. Moreover, there is a specific order in which these structures are affected. Consequently, the brain pathology can be classified into six different stages defined as Braak stages (Braak et al., 2003). At the earliest stage, (stage 1) the medulla oblongata, the dorsal motor nucleus and intermediate reticular zone as well as the anterior olfactory nucleus are prevalently affected (see Figure 3, dark-red shaded portion of the brain). Thus, it can be hypothesized that this relates to the decreased olfactory ability of PD patients. The pathology progresses further to the pontine tegmentum with caudal raphe nuclei and reticular formation being affected (see Figure 3, intermediate red). This characterizes stage 2. Together stage 1 and 2 represent the pre-symptomatic phase of PD with usually no apparent motoric dysfunction.

However, there is a great variety of so-called non-motor symptoms that occur prior to the hallmark motor symptoms of PD. For example patients experience olfactory problems, sleep disorders as well as autonomic and sensory symptoms. Further non-motor symptoms are neuropsychiatric symptoms such as depression, dementia or anhedonia. Affected areas of stage 1 and 2 are thought to be key areas in mediation of non-motor symptoms.

According to Braak, stage 3 and 4 are characterized by the spreading of the pathological symptoms to the SN and other nuclear greys of the midbrain and forebrain (see Figure 3, pathology spreads out from the intermediate to the light red following the white arrows). In these stages typical motor symptoms of PD emerge. During this symptomatic phase the disease progresses and this stage is the predominant point of PD diagnosis (Gibb and Lees, 1988). In stage 5 and 6, Lewy bodies and neurites are present in the mature neocortex as well as in the limbic structures. Here, PD may show all its clinical dimensions (Braak et al., 2003).
Figure 3. Progression of Parkinson’s disease (PD)-related intraneuronal pathology.

This image visualizes how lesions spread as PD progresses. It starts in the dorsal IX/X motor nucleus and in the anterior olfactory nucleus (dark-red) and continues according to the white arrows from the brain stem upward until the lesions finally reach the cortex (light-red, rose). The gradual diminishment in shading intensity depicts the topographical expansion of the lesions.
From Braak and colleagues (Braak et al., 2003).
1.3 Clinical features of Parkinson’s disease

1.3.1 Motor symptoms

The acronym TRAP describes the four cardinal symptoms of PD: tremor at rest, rigidity, akinesia (or bradykinesia) and postural instability (Frank et al., 2006). Besides these primary motor symptoms there are various symptoms in PD, which might occur additionally. Amongst these are secondary motor symptoms such as hypomimia, dysarthria, dysphagia, sialorrhea, micrographia, shuffling gait, festination, freezing, dystonia and glabellar reflexes (Jankovic, 2008).

Zetusky and colleagues (Zetusky et al., 1985) hypothesized the existence of two different subtypes of PD due to the variable clinical expression of PD: a tremor-dominated subtype and a subtype with postural instability and gait difficulty (PIGD). Jankovic and colleagues (Jankovic et al., 1990) supported the classification into these subtypes. Both groups acknowledged that patients presenting with PIGD have a more aggressive course of disease than patients suffering from the tremor-dominated subtype. A different classification approach was taken by Rajput and colleagues (Rajput et al., 2009) who define three subtypes: tremor-dominant, akinetic-rigid and mixed. They found the subtypes to be concordant with the biochemical pathology in the brain. Further, it was reported that the subtypes might differ in their ability to identify odors, suggesting that olfactory function relates to prognosis of PD. The tremor-dominant group outperformed the akinetic-rigid group regarding the detection of odors (Iijima et al., 2011; Stern et al., 1994).

However, the issue of subtype classification remains a controversy in the field (Marras and Lang, 2013). In addition to the motor symptoms, PD goes along with a multitude of further symptoms such as non-motor symptoms.
1.3.2 Non-motor symptoms

The hallmark (motor) symptoms of PD are not present until pathology reaches an advanced stage in which about 50% of the dopaminergic neurons are lost (Fearnley and Lees, 1991). The preclinical, early stage of the disease is often affected by non-motor symptoms and can precede the motor phase by years (up to 10 years) (Gonera et al., 1997; Morrish et al., 1998). Non-motor symptoms are manifold such as autonomic dysfunction, cognitive/neurobehavioral abnormalities, sleep disorder and sensory abnormalities such as anosmia, paresthesia and pain (Jankovic, 2008).

Some of the non-motor symptoms such as olfactory impairment, rapid eye movement (REM) sleep behavior disorder, constipation, fatigue and depression can appear in the preclinical phase of PD (Chaudhuri et al., 2005; Shiba et al., 2000) whereas other symptoms occur during exacerbated pathological progress of disease (Hely et al., 2005). Symptoms are various, however, it was shown that most patients that eventually develop PD presented themselves to their general practitioner with non-motor symptoms years before diagnosis (Schrag et al., 2015). In some cases, non-motor symptoms have been found to have a greater impact of the patient’s quality of life than motor symptoms (Martinez-Martin et al., 2011). Depression as a profound non-motor symptom occurs in up to 30% of PD patients (Slaughter et al., 2001). However, non-motor symptoms are often not recognized by physicians. For example, the presence of sleep disturbance remained unnoticed in more than 40% of patients (Shulman et al., 2002).

1.3.2.1 Olfactory dysfunction

Loss of smell affects up to 90% of PD patients in the course of the disease (Doty et al., 1988; Haehner et al., 2009a). It may even affect all patients taking into account a relevant fraction of patients being misdiagnosed as PD. A decreased olfactory ability in PD was first described by Ansari and Johnson (Ansari and Johnson, 1975).
Further studies showed that olfactory dysfunction was a frequently observed and specific symptom in early PD (Doty et al., 1992). It was valued as a potential means for the early diagnosis of PD, since loss of smell already occurs in the preclinical phase of PD years before motor symptoms become apparent (Hawkes, 2006; Ross et al., 2008). With the high frequency of patients affected, olfactory dysfunction is comparable, if not more common, with the frequency of the occurrence of tremor as a further characteristic feature of PD (Hoehn and Yahr, 1967). As these studies demonstrate, there is a statistically significant relation between olfactory disturbance and subsequent occurrence of PD, thus loss of smell can be seen as a useful screening tool to find individuals with a high risk for developing PD in later life (Ross et al., 2008).

These findings of frequent olfactory impairment among PD patients go along with the pathological staging of PD by Braak and colleagues (Braak et al., 2003) where in Braak stage I Lewy bodies were found inter alia in the olfactory bulb. Further hypotheses concerning loss of olfaction in PD such as dopaminergic as well as nondopaminergic neurotransmitter alterations, microglia initiated inflammation of the olfactory bulb and xenobiotics as welding fume or viruses exist, however, detailed and conclusive pathomechanism of PD remains enigmatic (Doty, 2012). Both, patients with sporadic PD and their asymptomatic relatives showed a decreased olfactory ability (Montgomery et al., 1999). Some hyposmic, however, asymptomatic relatives of PD patients presented themselves with subclinical dopaminergic dysfunction (Berendse et al., 2001). Ponsen and co-workers (Ponsen et al., 2004) detected a 10% risk of olfactorily impaired first-grade relatives of PD patients to develop PD.

In addition, olfactory testing is potentially helpful to differentiate PD from atypical parkinsonian syndromes (see 1.5). Preserved or only mildly impaired olfactory function is more likely to go along with atypical parkinsonian syndromes whereas profound olfactory disturbance is mainly suggestive for PD (Wenning et al., 1995). Further studies support the above-named findings that profound smell loss is associated with PD but not present in progressive supranuclear palsy or corticobasal degeneration. In addition, they found that multiple system atrophy patients can show mild olfactory impairment but patients with dementia with Lewy bodies are often diagnosed with severe smell loss with its extent comparable to
smell loss in PD (Katzenschlager and Lees, 2004). Hence, olfactory testing can be considered as a supportive, cheap, easy-to use and manageable tool in differential diagnosis of PD.

1.4 Diagnosis of Parkinson’s disease

1.4.1 Clinical examination

The diagnosis of PD is based on clinical examination, as there is no test to verify the presence of the disease in living individuals. In order to standardize clinical examination of PD patients, there are common criteria such as the United Kingdom Parkinson’s Disease Society Brain Bank Clinical Diagnostic Criteria (UKPDBBC) (Gibb and Lees, 1988), which aim for more accuracy in diagnosis (Table 1). However, only post mortem neuropathological examination of the brain can verify the diagnosis. With the use of the clinical diagnostic criteria of the UKPDBBC and the majority of diagnoses made by neurologists Hughes and colleagues (Hughes et al., 2001) revealed a diagnostic accuracy for PD of 90%. In a former study by Rajput and co-workers (Rajput et al., 1991) accuracy of PD diagnosis was only 76%.

According to these criteria, the symptoms are evaluated in three steps. The first step of the UKPDBBC is a diagnosis step. To be positively diagnosed in this step, the patients have to display bradykinesia in combination with at least one of the three hallmark features: muscular rigidity, 4-6 Hertz (Hz) rest tremor or postural instability.

Step 2 is an exclusion step. At this level, patients are evaluated on a list of symptoms indicative for a neurological disorder other than PD. For the definite diagnosis of PD at least three of the following criteria have to be fulfilled (Step 3): unilateral onset, rest tremor present, progressive disorder, persistent asymmetry, excellent response (70-100%) to L-3,4-dihydroxyphenylalanine (L-DOPA) (as the principal medication for symptomatic therapy of PD), severe L-DOPA-induced chorea, L-DOPA response for 5 years or more and clinical course of 10 years or
more. The UKPDBBC are evaluated on the basis of an entire neurological examination of the patient. Positive response to L-DOPA, which means amelioration of motor symptoms, is an important feature in PD diagnosis (Calne et al., 1992). With the positive predictive value of L-DOPA response ranging from 67% to 96%, acute challenge tests were estimated to be a useful diagnostic criterion by a committee of experts (Albanese et al., 2001). A negative response should raise suspicion to an alternative diagnosis (Dcosta et al., 1995).
Table 1. United Kingdom Parkinson’s Disease Society Brain Bank Clinical Diagnostic Criteria (UKPDBBC).

From Gibb and Lees (Gibb and Lees, 1988).

<table>
<thead>
<tr>
<th>Step 1 Diagnosis of Parkinsonian syndrome</th>
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<tbody>
<tr>
<td>Bradykinesia (slowness of initiation of voluntary movement with progressive reduction in speed and amplitude of repetitive actions)</td>
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<tr>
<td>And at least one of the following:</td>
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<tr>
<td>• muscular rigidity</td>
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<tr>
<td>• 4-6 Hz rest tremor</td>
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<tr>
<td>• postural instability not caused by primary visual, vestibular, cerebellar, or proprioceptive dysfunction</td>
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<table>
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<tr>
<th>Step 2 Exclusion criteria for Parkinson’s disease</th>
</tr>
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<tbody>
<tr>
<td>• History of repeated strokes with stepwise progression of parkinsonian features</td>
</tr>
<tr>
<td>• History of repeated head injury</td>
</tr>
<tr>
<td>• History of definite encephalitis</td>
</tr>
<tr>
<td>• Oculogyric crises</td>
</tr>
<tr>
<td>• Neuroleptic treatment at onset of symptoms</td>
</tr>
<tr>
<td>• More than one affected relative</td>
</tr>
<tr>
<td>• Sustained remission</td>
</tr>
<tr>
<td>• Strictly unilateral features after 3 years</td>
</tr>
<tr>
<td>• Supranuclear gaze palsy</td>
</tr>
<tr>
<td>• Cerebellar signs</td>
</tr>
<tr>
<td>• Early severe autonomic involvement</td>
</tr>
<tr>
<td>• Early severe dementia with disturbances of memory, language, and praxis</td>
</tr>
<tr>
<td>• Babinski sign</td>
</tr>
<tr>
<td>• Presence of cerebral tumor or communicating hydrocephalus on CT scan</td>
</tr>
<tr>
<td>• Negative response to large doses of L-DOPA (if malabsorption excluded)</td>
</tr>
<tr>
<td>• MPTP exposure</td>
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<table>
<thead>
<tr>
<th>Step 3 Supportive prospective positive criteria for Parkinson’s disease</th>
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<tbody>
<tr>
<td>(Three or more required for diagnosis of definite Parkinson’s disease)</td>
</tr>
<tr>
<td>• Unilateral onset</td>
</tr>
<tr>
<td>• Rest tremor present</td>
</tr>
<tr>
<td>• Progressive disorder</td>
</tr>
<tr>
<td>• Persistent asymmetry affecting the side of onset most</td>
</tr>
<tr>
<td>• Excellent response (70-100%) to L-DOPA</td>
</tr>
<tr>
<td>• Severe L-DOPA-induced chorea</td>
</tr>
<tr>
<td>• L-DOPA response for 5 years or more</td>
</tr>
<tr>
<td>• Clinical course for 10 years or more</td>
</tr>
</tbody>
</table>
For the longitudinal follow-up of diagnosed PD patients there are different rating scales (Ramaker et al., 2002) to evaluate limitations, disease severity and progression of the disease, with the Unified Parkinson’s Disease Rating Scale (UPDRS) being most commonly used. This scale includes four parts which refer to the following categories: Non-motor experiences of daily living, motor experiences of daily living, motor examination and motor complications (see 3.1.4.4) (Fahn et al., 1987). The UPDRS is an essential scale in the diagnostic as well as in the therapeutic process.

### 1.4.2 Genetic tests

PD mostly occurs sporadically; however, there are rare cases of familial parkinsonism. There are mutations in a number of genes such as SNCA, PINK1, LRRK2 that have been shown to be responsible for the occurrence of monogenetic PD. In addition, there are various genetic risk factors which contribute to a higher risk of PD (Klein and Westenberger, 2012; Singleton et al., 2013). Monogenetic forms account for about 5-10% of the PD cases (Lesage and Brice, 2009). Although the different loci contributing to familiar forms of PD are well described, genetic testing plays a minor role in current PD diagnostic. Reasons are the high testing costs and the comparably small group of PD patients affected by familial parkinsonism (Tolosa et al., 2006).

### 1.4.3 Neuroimaging

Although PD diagnosis is mainly based on clinical observations, neuroimaging can help to assure the diagnosis and plays an important role in exploring further information on the pathomechanism of PD. There are numerous techniques available to image brain structures as well as brain function. In case of PD, transcranial sonography (Becker et al., 1995), computed tomography (CT) (Becker et al., 1979), magnetic resonance imaging (MRI) (Laakso et al., 1996), SPECT (Asenbaum et al., 1997) and positron emission tomography (PET) (Morrish et al.,
1998) have been shown applicable. Both SPECT and PET are physiological imaging techniques and provide the opportunity to detect metabolic or neurochemical changes. In general, PET and SPECT comprise the ability to show the in vivo capability of binding and metabolism of compounds that are marked with short-lived positron emitting isotopes. With respect to the diagnosis of PD, PET and SPECT are the most sensitive methods to support the diagnostic process especially in early stages of disease when typical motor symptoms are (not yet) apparent. $^{18}$Fluor dopa is used as a radiotracer in PET and gives information about the praesynaptic dopaminergic uptake in means of the activity of the L-DOPA decarboxylase in the striatum which is decreased in PD. (Piccini and Whone, 2004).

SPECT measures the uptake of tracers, for example the agent $^{[123]}$I-$\omega$-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl) nortropane (FP-CIT). FP-CIT has a high affinity to the dopamine active transporters (DAT). This is relevant as in PD, DAT are decreased in the striatum (Eshuis et al., 2009). According to the German medical fee schedule (German medical fee schedule/Gebührenordnung für Ärzte (online), 2015) a three-dimensional PET can cost more than 800 €. Tang and co-workers (Tang et al., 2010) showed that especially in an early stage of the disease, when symptoms are unspecific and differentiation between PD and atypical parkinsonian syndromes is not possible by clinical examination, the use of a PET CT is helpful. They found a sensitivity of 84% and a specificity of 97% (negative predictive value: 82% positive predictive value: 98%) for image based classification (PET CT) of PD. Similar rates were found for multiple system atrophy and progressive supranuclear palsy but not for corticobasal degeneration.

Due to the in vivo imaging capability, neuroimaging methods can contribute to a better diagnosis as well as an improved understanding of pathology (Thobois et al., 2001). Major limitations of these techniques are the costs and availability which is why they are not routinely used in clinical routine but are utilized in clinical studies. Another option of neuroimaging is transcranial sonography. Employing this technique, hyperechogenicity of the SN can be displayed which is typical for PD. Hyperechogenicity is caused by an increase of glia cells and correlates
positively with the stage of degeneration of the SN (Becker et al., 1995). This symptom is found in about 90% of PD patients but only approximately 10% of the healthy population shows this phenotype (Berg et al., 1999; Berg et al., 2001). Hyperechogenicity was found to be a trait marker for PD featuring the predisposition for PD. It does not change over time as the disease progresses (Berg et al., 2005).

1.4.4 Drug challenging test

L-DOPA or apomorphine, a non-selective dopamine agonist, are dopaminergic drugs which are able to alleviate motor symptoms in PD patients. Both are used to support clinical diagnosis of PD. L-DOPA challenge showed a sensitivity of 70.9% and a specificity of 81.4% to predict clinical diagnosis (Merello et al., 2002). This feature is used in the diagnostic process of PD in order to confirm diagnosis and differentiate it from other diseases. The UKPDBBC describe negative response to large doses of L-DOPA (if malabsorption is excluded) as an exclusion criterion for PD. The positive response to L-DOPA is used as an established supportive criteria for the diagnosis of PD (Gibb and Lees, 1988; Hughes et al., 2001).
1.5 Differential diagnoses of Parkinson’s disease

Due to the wide variety and complexity of symptoms presenting as PD, there are various differential diagnoses. Besides secondary parkinsonism there are sporadic neurodegenerative disorders presenting as parkinson’s syndrome. The characteristics of these diseases that are grouped as atypical parkinsonian syndromes are similar to PD and often also show parkinson-like symptoms such as tremor, bradykinesia, rigidity and postural instability. Thus, differentiating between atypical parkinsonian syndromes and PD is challenging. With tremor being a common feature of PD, essential tremor is a further differential diagnosis of PD. Concerning the gait disturbance that occurs in PD normal pressure hydrocephalus (NPH) must be considered as an additional differential diagnosis.

1.5.1 Multiple system atrophy

Multiple system atrophy presents in two different forms: i) predominant parkinsonian symptoms (multiple system atrophy-p) as a consequence of nigrostriatal degeneration or ii) cerebellar (multiple system atrophy-c) symptoms due to olivoponto cerebellar degeneration combined with early and profound autonomic symptoms (Gilman et al., 1999; Wenning et al., 1994). Multiple system atrophy-p is hard to differentiate from PD. However, there are some diagnostic features that support multiple system atrophy diagnosis: nocturnal stridor, rapid course, early instability and falls, stimulus sensitive myoclonus, pyramidal tract signs, severe dysarthria and insufficient or only transient response to L-DOPA (Colosimo et al., 1995; Gouiderkhouja et al., 1995).

1.5.2 Progressive supranuclear palsy

Progressive supranuclear palsy, formerly known as Steel-Richardson-Olszewski syndrome, is caused by multisystem degeneration. Many symptoms contribute to progressive supranuclear palsy for instance supranuclear gaze palsy in form of
loss of vertical eye movement, parkinsonism, pseudobulbar palsy and frontal lobe syndrome (Litvan et al., 1996; Steele et al., 1964). There are two clinical phenotypes in progressive supranuclear palsy, the Richardson’s syndrome which presents with the typical symptoms mentioned above and the progressive supranuclear palsy-parkinsonism with hallmark symptoms similar to PD (Williams et al., 2005) and their diagnosis can be difficult. When it comes to differential diagnosis between progressive supranuclear palsy and PD the following factors distinguish progressive supranuclear palsy the best: unstable gait, absence of tremor-dominant disease and absence of response to L-DOPA (Litvan et al., 1997b).

1.5.3 Corticobasal degeneration

Corticobasal degeneration is a progressive neurodegenerative disease with multiple clinical symptoms including unilateral parkinsonism (akinese). Best predictors for diagnosis of corticobasal degeneration are the following symptoms: limb dystonia, ideomotor apraxia, myoclonus and asymmetric-rigid syndrome with late onset of gait or balance disturbance (Litvan et al., 1997a). As a tauopathy there are clinical and biological characteristics that are shared between corticobasal degeneration and progressive supranuclear palsy (Houlden et al., 2001). L-DOPA response is rare (Kompoliti et al., 1998).

1.5.4 Dementia with Lewy bodies

Dementia with Lewy bodies presents with early dementia and parkinsonism. Additional diagnostic criteria for dementia with Lewy bodies are cognitive fluctuations, visual hallucinations and sensitivity to neuroleptic drugs (McKeith et al., 1996). In order to differentiate dementia with Lewy bodies from PD accompanied by dementia, the so-called ‘1-year’ rule as a supportive tool for clinical diagnosis according to the consensus guidelines for dementia with Lewy bodies is applied. Parkinsonism followed by dementia within one year after
symptoms occurred qualifies as dementia with Lewy bodies whereas later onset of dementia implies the diagnosis of PD. However, the ‘1-year’ rule has to be applied with caution and individually adapted (McKeith et al., 2005).

1.5.5 Essential tremor

Essential tremor goes along with bilateral postural or kinetic tremor which affects hands and forearms. The tremor is persistent, which is in contrast to the typical unilateral 4-7 Hz tremor at rest as it occurs in PD (Deuschl et al., 1998). Essential tremor might confound PD diagnosis frequently due to the fact that patients suffering from essential tremor can also suffer from tremor at rest (Cohen et al., 2003).

1.5.6 Normal-pressure hydrocephalus

Normal-pressure hydrocephalus (NPH) results from an imbalance of cerebrospinal fluid production and reabsorption (Kiefer and Unterberg, 2012) and is characterized through a classic symptom triad: urine incontinency, gait disturbance and dementia (Adams et al., 1965; Hakim and Adams, 1965). In NPH, the mean basal intracranial pressure is normal or mildly elevated. Because the symptoms are prevalent among the elderly, a large number of cases may remain unrecognized. However, more than 70% of patients with NPH show additional akinetic, tremulous, hypertonic or hyperkinetic movement disorders or even present themselves with parkinsonism (Krauss et al., 1997) or dementia (Kiefer and Unterberg, 2012). The broad based gait pattern of patients with NPH differs from the gait of PD patients (trunk bent forward, reduced arm swing, short steps), thus, analyzing the gait pattern helps to distinguish both diseases (Stolze et al., 2001). Moreover, cerebrospinal fluid diversion is beneficial for NPH patients but shows no alleviation of symptoms in PD patients (Larsson et al., 1991).
1.5.7 Secondary parkinsonism

Secondary parkinsonism can be induced by certain types of medication such as anti-dopaminergic drugs (Hardie and Lees, 1988; Miller and Hiley, 1974) or calciumantagonists (Daniel and Mauro, 1995). Moreover, secondary parkinsonism can appear in patients with cerebral tumors (Polyzoidis et al., 1985), cerebrovascular diseases (Gupta and Kuruvilla, 2011), or cerebral inflammation (Adler et al., 1989). Additionally, it can occur in the course of infectious diseases (Espay and Henderson, 2011; Gonzalez-Duarte et al., 2010; Mirtsattari et al., 1998), post-traumatically (Mendez, 1995), in patients that are exposed to toxins like manganese (Olanow, 2004), 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Davis et al., 1979), or due to metabolic problems in the course of hyperparathyroidism (Uncini et al., 1985).

1.5.8 Misdiagnoses in Parkinson's disease

Due to the large number of possible differential diagnoses and the broad clinical spectrum of symptoms, PD is not well diagnosed. Moreover, elderly people frequently display mild extrapyramidal symptoms (EPS) such as resting tremor, bradykinesia, rigidity or even parkinsonism without having PD (Bennett et al., 1996; Richards et al., 2002). In early stages of PD, misdiagnosis rates were found to be as high as 20-30% (Hughes et al., 1992; Rajput et al., 1991; Brooks, 2012) with an improvement to a positive predictive value of 98.6% for PD when diagnosis was assessed in a special movement disorder service (with a negative predictive value of 90%). In the same clinical and diagnostic setting diseases that can present with symptoms similar to PD such as atypical parkinsonian syndromes, vascular and post encephalitic parkinsonism were also assessed (Table 2). Amongst these diseases, the diagnosis of PD reached the highest positive predictive value. With regards to atypical parkinsonian syndromes, corticobasal degeneration was associated with the lowest positive predictive value (Hughes et al., 2002).
Table 2. Positive predictive value for the diagnosis of Parkinson’s disease (PD) and atypical parkinsonian syndromes assessed in a specialist movement disorder service.

Adapted from Hughes (Hughes et al., 2002).

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Positive predictive value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idiopathic Parkinson´s disease</td>
<td>98.6</td>
</tr>
<tr>
<td>Multiple system atrophy</td>
<td>85.7</td>
</tr>
<tr>
<td>Progressive supranuclear palsy</td>
<td>80.0</td>
</tr>
<tr>
<td>Corticobasal degeneration</td>
<td>33.3</td>
</tr>
</tbody>
</table>

In the aforementioned studies the most common misdiagnoses were: multiple system atrophy, progressive supranuclear palsy and corticobasal degeneration. However, there are many more misdiagnoses such as essential tremor, AD or vascular parkinsonism (Meara et al., 1999). Vascular parkinsonism results from ischemic cerebrovascular disease which can potentially be caused due to high blood pressure (Gupta and Kuruvilla, 2011). Schrag and colleagues (Schrag et al., 2002) showed that at least 15% of PD patients do not match the diagnostic criteria and about 20% of PD patients who have already come to medical attention have not been diagnosed with the disease. These high misdiagnosis rates are primarily caused by the diversity and complexity of the clinical symptoms that are shown in PD. So far, there is no approved diagnostic biomarker for PD and the spectrum of diagnostic means, particularly neuroimaging, are not fully applied due to economic constraints. In any case, misdiagnoses are conjoined with severe burden to the individual caused by non-appropriate treatment and to the economy/health care systems due to the profound amount of expenses that are caused in course of disease. One false-positive case misclassified as PD results in very high costs.

Biomarker tests are essential in order to identify people at risk for developing PD and start neuroprotective therapies early in the course of disease (Miller and O’Callaghan, 2015).
1.6 Therapy

PD treatment includes pharmacological therapy as well as different types of surgery. Although there are many therapeutic options to treat the symptoms, at present, there is no cure for the disease.

1.6.1 Pharmacological treatment

Pharmacological treatments aim to increase the level of dopamine in the patient’s brain. In this respect, the treatment with L-DOPA and decarboxylase inhibitors is the gold standard in the therapy of PD (Mercuri and Bernardi, 2005). Essential contributions to this therapy were the findings of Cotzias and co-workers and Yahr and colleagues (Cotzias et al., 1967; Yahr et al., 1969) showing that L-DOPA is the basis for symptomatic treatment of PD. L-DOPA is a metabolic precursor of dopamine which can - in contrast to dopamine - cross the blood-brain barrier. When L-DOPA is given orally, decarboxylases existing in the periphery (liver and kidney) convert the majority of L-DOPA into dopamine (Khor and Hsu, 2007).

In order to reduce the peripheral effects of dopamine and consequently increase the availability of L-DOPA in the brain, the aromatic amino acid decarboxylase inhibitors carbidopa or benserazide are administered. By the use of those peripheral inhibiting substances the applied dose of L-DOPA could be significantly reduced (Pinder et al., 1976). Simultaneously, the percentage of L-DOPA entering the brain was increased (Bartholi et al., 1967).

The most frequent side effects occurring with the use of L-DOPA are motor complications such as motor fluctuations and dyskinesia. Among PD patients receiving L-DOPA for more than 5-10 years, 50 to 80% are affected by motor complications (Marsden and Parkes, 1977; Rajput et al., 2002). The pathological mechanism of motor complications is not entirely known yet, however, it is suggested that the ability of L-DOPA to trigger pulsatile excitation of dopamine
receptors in addition to its short half-live is a factor that contributes to the motor complications (Albin et al., 1989; Blanchet et al., 1995).

Although the treatment with L-DOPA and decarboxylase inhibitors is the gold standard in the therapy of PD (Mercuri and Bernardi, 2005), there are further substances available such as dopamine receptor agonists (Wolters et al., 1995), catechol-O-methyltransferase (COMT) inhibitors (Kurth and Adler, 1998) and monoamine oxidase B (MAO-B) inhibitors (Riederer and Laux, 2011). COMT as well as MAO-B are involved in the degradation of L-DOPA. COMT can be found in the periphery whereas MAO-B is localized in the brain. The use of those additional drugs either reduces the dose of L-DOPA and decarboxylase inhibitors or helps to retard the beginning of the therapy with L-DOPA and decarboxylase inhibitors. Among non-dopaminergic medication, N-methyl-D-aspartate (NMDA) receptor agonists (Greenamyre and Obrien, 1991) and anticholinergic drugs (Cooper et al., 1992) are administered in different therapeutic strategies. Dopaminergic drugs are capable of alleviating motor symptoms, however, non-motor symptoms stay unaffected by dopaminergic therapy (Goetz et al., 2005).

1.6.2 Surgical treatment

The surgical options available for treatment of PD are numerous but obsolete for the most part as dopaminergic replacement was found to help with parkinsonian symptoms. Only a few cases are indicated for pallidotomy (surgical lesion of a small part of GPi) or thalamotomy (surgical lesion of the thalamic nucleus ventralis intermedius). Most commonly these surgical methods are applied in cases where the side effects of pharmacological therapy or the symptoms of PD are not controllable (Fahn, 2008). In PD, the activity of the GP is increased leading to a slower and decreased body movement. Consequently, pallidotomy takes aim at reducing dyskinesia and rigidity (Laitinen et al., 1992). Due to the inclined activity of the thalamus in PD and the subsequent tremor, thalamotomy is supposed to alleviate tremor (Speelman et al., 1998). In addition, thalamotomy was shown to reduce L-DOPA induced dyskinesia (Narabayashi et al., 1984).
Because of the high complication rate and the availability of deep brain stimulation (DBS), the surgical methods explained above are rare. DBS only includes the implantation of an electrode and consequently only minimal destructive lesions in the brain (Lozano and Mahant, 2004). However, the exact mechanism of action of DBS is not elucidated (Perlmutter and Mink, 2006). There are two main structures at which DBS is applied: the GPi and the STN. However, there are further brain locations, such as pedunculopontine nucleus or zona incerta where DBS has been successfully used (Plaha et al., 2006; Plaha and Gill, 2005).

1.7 Epidemiology of Parkinson’s disease

1.7.1 Prevalence of Parkinson’s disease

PD is the second most common neurodegenerative disease after Alzheimer’s disease with an estimated overall prevalence of 1.8% in the European population of age 65 and older (de Rijk et al., 2000). Additionally, there is a general increase in the prevalence with age. In a review of seven large population-based studies de Rijk and co-workers found prevalence rates (per 100 population) ranging from 0.6% among people 65-96 years to more than four times higher rates for people aged 85-89 years (see Figure 4).

A systematic review of 39 incidence and prevalence studies in Europe by von Campenhausen and colleagues revealed a prevalence rate for PD in the European general population that ranges from 108 to 257/100,000 per year (von Campenhausen et al., 2005). Prevalence for PD in Germany are based on three independent studies with two of the studies carried out on patients that were institutionalized or above the age of 65 years thus, potentially biased towards overestimation of the prevalence rate. Based on these studies, the PD prevalence in Germany was estimated to be 6,607/100,000 (95% CI; range 713-12,500/100,000). With the population ageing there is a predicted rise of 50% of PD in Germany by 2030 (Dorsey et al., 2007).
Figure 4. Prevalence rates of Parkinson’s disease (PD) for both sexes depending on age.

Prevalence rates were obtained from five different European surveys.
From de Rijk and co-workers (de Rijk et al., 1997).

1.7.2 Incidence of Parkinson’s disease

Overall incidence rates of PD in Europe vary. Incidence was calculated in some studies to be 16-19/100,000 persons per year (Twelves et al., 2003). This is similar to findings by von Campenhausen and colleagues (von Campenhausen et al., 2005) who found incidence rates that ranges from 11 to 19/100,000 persons per year. Both of these reviews on epidemiological studies revealed an increase of incidence with age. Peak incidence was 70 to 79 years and mean age regarding occurrence of symptoms was either 60 to 65 years or above the age of 65 depending on the studies (Twelves et al., 2003). Lifetime risk for PD was 4.4% for men and 3.7% for women.
1.7.3 Risk factors of Parkinson’s disease

Some studies found prevalence of PD to be slightly increased in men (Benito-Leon et al., 2003; Shulman, 2007). However, this difference is not consistently found across studies (de Rijk et al., 1997; Tison et al., 1994).

One explanation for the potentially decreased prevalence rate of PD in women, are estrogens and their neuroprotective role (Green and Simpkins, 2000; Saunders-Pullman, 2003). Data on prevalence and incidence regarding ethnicity are various (Morens et al., 1996; Schoenberg et al., 1988) and not conclusive. Some studies revealed PD to be less common in people of African and Asian decent (Van Den Eeden et al., 2003). Other studies, however, found high incidence rates of PD especially for people of African descent (Mayeux et al., 1995). Findings on the linkage between ethnicity and the occurrence of PD are conflicting and results might originate from difference in response rate, survival and case-ascertainment rather than from real differences (de Lau and Breteler, 2006).

Smoking was found to decrease the risk for developing PD (Hernan et al., 2002), naming the potential neuroprotective ability of nicotine as a possible explanation (Park et al., 2007).

1.8 Neuronal ageing

In the undisturbed, non-diseased process of ageing, profound changes take place in the brain of healthy individuals. The symptoms co-inciting with this ageing process can be misclassified as parts of diseases (for example neurodegenerative diseases) instead of non-pathologic features of ageing. Studies on elderly people showed that EPS occur frequently without being associated with PD (Bennett et al., 1996; Richards et al., 2002). EPS result from decline in nigrostriatal dopamine regulation that occurs with ageing (McGeer et al., 1977). There are different
findings on the cellular processes that dopaminergic neurons undergo in ageing and in PD.

Two independent studies showed that the mechanisms of the cellular processes differ and that there is no correlation between normal ageing of the dopaminergic neurons and the degeneration of neurons in the course of PD (Kish et al., 1992; Scherman et al., 1989). However, Collier and colleagues (Collier et al., 2011) hypothesized that the damage of the neurons in the SNc in normal ageing and PD are similar processes with the progression of neuronal loss in PD being an aggravated ageing process that is induced by environmental factors and genetic patterns.

1.9 Olfaction

1.9.1 The olfactory system

The olfactory system consists of cranial nerve I, olfactory bulb and tract, olfactory tubercle, septum and stria diagonalis, lateral entohiral cortex and parts of the amygdala (Shipley and Ennis, 1996; Figure 5). In humans, the superior nasal septum, the cribiforme plate, the superior turbinate and parts of the middle turbinate are lined with the olfactory epithelium which is covered with mucus (Leopold et al., 2000). This specialized olfactory neuroepithelium interacts with odorants (Mombaerts, 2001). Three different cell types can be found within the olfactory epithelium, the basal cells (stem cells), the supporting cells and the bipolar sensory cells (Hatt, 2004). Caudally, the latter send ciliated dendrites towards the nasal cavity: on their cranial side an axon is sent through the lamina cribosa to the olfactory bulb. The olfactory receptors are located on the dendritic ends of the sensory cells. According to the receptor that is expressed by the sensory cells they target into eligible glomeruli in the olfactory bulb. In the glomeruli, information is processed from the sensory axons to the dendrites of mitral cells. The mitral cell axons as the main input-output neuron, however,
project into higher cortical regions. Altogether, the axons build the first cranial nerve (Firestein, 2001).

Detection of an odor is a complex process. Odor molecules bind to a G-protein coupled receptor in the ciliary membrane of the olfactory neurons. Upon binding of the ligand, a stimulatory G-protein is activated (Levy et al., 1991). Through a second messenger cascade the signal is transferred to the olfactory bulb and propagated to cortical regions (Firestein, 2001). From here signals are relayed to the olfactory cortex (Zou et al., 2001) where the signals are continuously processed and result in the perception of smell. There are approximately 1,000 classes of odorant receptors known, encoded by a multigene family (Buck and Axel, 1991; Lancet and Benarie, 1993)
**Figure 5. Olfactory system.**

The panel on the right depicts a sagittal view of the front part of the human head. The enlarged detail on the left shows the olfactory nervous system. On the luminal side which points towards the nasal cavity, olfactory cilia (blue) reach the surface. These cilia are covered with mucus. The olfactory epithelium (left panel) comprises three different cell types: olfactory receptor cells (blue), columnar epithelial cells (also known as supporting cells; light orange) and basal cells (also known as stem cells; brown). The olfactory receptor cells (also known as bipolar sensory cells) send their axons (blue) through the lamina cribosa (grey-brown) to the olfactory bulb (light blue) in which they converge together with dendrites of mitral cells (red). Axons of the mitral cells (red) send projections from the olfactory bulb through the olfactory tract (light blue, panel on the right) to different brain structures.

Adapted from Thuret and colleagues (Thuret et al., 2006).
1.9.2 Olfactory dysfunction

Clinical tests and the application of odor-test sticks in epidemiological studies have shown that the ability to identify odors is influenced by different factors (Doty and Mishra, 2001). On average, women do better in odor identification than men and decreased olfactory ability sets in later. Moreover, a statistically significant impairment of the olfactory function occurs after the age of 65 years. In the age group between 65 and 80 years, more than 50% of the individuals show olfactory impairment whereas in the group of those 80 years and older 75% are affected (Doty et al., 1984a). In a large population-based cross-sectional study on more than 2,000 older adults, Murphy and colleagues (Murphy et al., 2002) found a mean prevalence for olfactory impairment among older adults (mean age 68 years) of approximately 25%. The number increased with age from about 5% in the 53 to 59 year old group to more than 60% in the group aged 85 to 97 years.

The factors that contribute to smell loss in elderly are various and comprise anatomical issues such as restriction/stenosis of the cribiform plate and receptor damage due to multiple infections during life. Genetics, gender, age, diseases, smoking and toxic exposure (Doty and Mishra, 2001) as well as having nasal congestion or upper respiratory infection are factors associated with the prevalence of olfactory impairment. A history of epilepsy and stroke also contributes to a decrease in olfactory ability (Murphy et al., 2002). Moreover, a statistically significant association was not only revealed between olfactory impairment and the five-year incidence of cognitive impairment of older adults (mean age 67 years) (Schubert et al., 2008) but also between olfactory impairment and AD (Serby et al., 1991). In addition, chemotherapeutic agents in cancer therapy were found to cause severe olfactory impairment (Riga et al., 2015).

In contrast to factors having an additional negative impact of the olfactory ability, such as history of nasal polyps and deviated septum as well as heavy alcohol abuse, there are some factors that are connected with a decreased risk of olfactory impairment: use of lipid lowering agents, exercising at least once a week and oral steroid use (Schubert et al., 2011).
The ability to smell is a core factor of enjoyment of food, nature and lives in general, thus, impaired olfaction has a profound impact of the daily life of patients (Miwa et al., 2001).

Although these data point towards olfactory impairment being a general problem among elderly, however, olfactory disturbance in PD patients is still much higher.

1.9.3 Olfactory dysfunction and Parkinson’s disease

Loss of smell affects more than 95% of PD patients during the course of the disease (Haehner et al., 2009a). Decreased olfactory ability in the course of PD was discovered in the 1970s by Ansari and Johnson (Ansari and Johnson, 1975). It was shown that olfactory dysfunction was a robust symptom in early PD (Doty et al., 1992) and beneficial for the early diagnosis of PD since loss of smell already occurs in the preclinical phase of PD (Ponsen et al., 2004). Olfactory disturbance can occur several years before motor symptoms become apparent (Hawkes, 2006).

1.9.4 Testing the olfactory system

Sniffin’ Sticks is a brand name for a test commonly applied in Germany to assess olfactory ability (Hummel et al., 1997). The test kid is produced by Burghart Messtechnik located in Wedel, Germany. The costs of a 16-pen including odor identification test kid amount to 149 € with a minimum durability of 18 month (personal communication via customer service). It is a simple and implementable test used in the clinical routine as well as in clinical and epidemiological studies. There are two alternatives: i) a complete set of Sniffin’ sticks including tests for odor discrimination, odor threshold and odor identification which contains 16 odor pens in order to test odor identification (Hummel et al., 1997) or ii) the reduced version with 12 pens which is only used to test odor identification. In contrast, commonly used as an odor identification test in the United States is the University
of Pennsylvania Smell Identification Test (UPSIT). It contains 40 different odors (Table 3) and is presented as a forced choice test where the proband can choose between four different odors. The odors are encapsulated on a piece of paper and they have to be scratched with a pen in order to release the scent (Doty et al., 1984b).
Table 3. Overlapping and specific odors of different sets of odor identification tests.

<table>
<thead>
<tr>
<th>Sniffin' sticks (12 pens)</th>
<th>Sniffin’ sticks (16 pens)</th>
<th>University of Pennsylvania Smell Identification Test (UPSIT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leather, Banana, Licorice, Rose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coffee, Peppermint, Fish, Clove, Orange, Pineapple, Lemon, Cinnamon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turpentine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anise, Garlic, Apple</td>
<td>Pizza, Gum, Menthol, Cherry, Motor Oil, Mint, Clove, Coconut, Onion, Fruit Punch, Cheddar, Cinnamon, Gasoline, Strawberry, Cedar, Chocolate, Gingerbread, Lilac, Peach, Root Beer, Dill Pickle, Pineapple, Lime, Orange, Paint Thinner, Grass, Smoke, Pine, Grape, Lemon, Soap, Natural Gas, Peanut, Wintergreen, Watermelon</td>
<td></td>
</tr>
</tbody>
</table>
The application time for the 12 odor containing identification test amounts to 2-3 minutes (without explanation time). The 16 item Sniffin’ sticks test takes about 5 minutes to conduct (Eibenstein et al., 2005). The Sniffin’ sticks test is portable and is made for repetitive use. It can be used until expiration date is reached. The Sniffin’ sticks test is applied to test the nasal chemosensory function by pen-like odor dispensing devices.

Odor discrimination defines the ability to distinguish outlying odors out of a series of odors (Kobal et al., 2000). However, it was found that odor identification is more often impaired amongst PD patients than odor discrimination, thus allowing for a more precise discrimination between diseased subjects and healthy controls (HC) (Boesveldt et al., 2008).

The costs outlined in section 4.4 which are caused by PD and the subsequent socio-economic burden emphasize the urgent need for additional research to improve diagnosis and treatment of PD (Spottke et al., 2005), especially with the knowledge of the validity of the smell test. Deeb and co-workers (Deeb et al., 2010) found similar sensitivity rates for smell testing and neuroimaging via SPECT (86% vs. 92%) with the imaging method being superior regarding the anatomical localization, the smell test outperforms the neuroimagig with respect to time, manpower and costs. The costs of a SPECT can be calculated to be around 1,000 € (Van Laere et al., 2008). It has to be taken into consideration that there are numerous PD patients that are not diagnosed yet and not detected as such. As several door-to-door prevalence surveys revealed, the proportion of patients who were first identified through screening was more than 25% (de Rijk et al., 1997; Morgante et al., 1992).
Figure 6. Sniffin’ sticks odor identification test.

This image presents the Sniffin’ sticks odor identification test. It contains 16 pen-like devices containing different odors which have to be identified by the probands. This test was utilized in the ParkCHIP study.

From Burghart Messtechnik (Produktkatalog Sniffin’ sticks, 2015).
2. Objective

The diagnosis of PD is still an exclusive clinical diagnosis and is associated with a high rate of misdiagnoses. In this respect, there are different criteria which are considered such as UKPDBBC or a patient’s positive response to L-DOPA in order to diagnose PD. There are still no distinct markers or tests to assure the diagnosis and the occurrence of the disease can only be proven through a post mortem pathological examination. Misdiagnosis in PD is a serious matter with severe consequences for the individual as well as the society/economy in terms of financial burden. An early and valid diagnosis of PD is fundamentally relevant to the patients in order to apply appropriate therapeutic measures. The quality of life of patients can be improved and psychological stress due to uncertain or delayed diagnosis can be reduced. By an early and accurate diagnosis, costs for the patient and for the society/the healthcare system can be reduced or even avoided. Hence, from both a health care and economic perspective, diagnostic tools for the early detection of PD are crucial. In this respect, the olfactory ability of PD patients plays an important role. It was shown that olfaction decreases within the course of the disease, years before clinical symptoms appear. Thus, the reduced olfactory ability is one of the important symptoms which could serve as a potential target for diagnosis of PD.

The role of olfactory impairment in PD was analyzed. Factors contributing to the loss of olfaction were studied and identified. For the ParkCHIP study, the feasibility of the Sniffin’ sticks as a point-of-care test was investigated and the use of a subset of the Sniffin’ sticks test was assessed and evaluated. The role of self-assessed olfactory impairment was evaluated. Furthermore the problem of misdiagnosis in PD was reviewed by examining a subgroup of the participants of the ParkCHIP study. In a final step, both costs caused by misdiagnoses of PD and costs of diagnostic tools were reviewed.
3. Materials and Methods

3.1 ParkCHIP

3.1.1 The ParkCHIP project

The ParkCHIP project takes aim at the development of a biomarker chip for the early detection of PD. The goal is to determine a disease-specific signature of antibodies in the blood of parkinsonian patients. These miniaturized biomarker chips are implementable in clinical diagnosis as well as in research projects. This minimally invasive test procedure can possibly support accuracy of diagnosis, reveal patients in preclinical stages and can contribute to the evaluation of individual disease progression. Establishing a biomarker chip can be conducive to faster and more reliable diagnosis of PD and can prevent costly, time-consuming and invasive diagnostic. In the course of the study, participants underwent clinical examination, had their blood drawn and answered questionnaires. For this thesis, the results of the blood testing were not analyzed. Particular attention was directed to the questionnaires, clinical investigation and olfactory testing.

3.1.2 Study groups

The ParkCHIP study, a cross sectional study with case-control design was performed at the St. Josef Hospital, Ruhr-University Bochum. Overall, the ParkCHIP I study group consists of 148 PD patients and the same number of HC, the ParkCHIP II study group of 211 PD patients and the ParkCHIP III study group of 13 normosmic individuals of the ParkCHIP II study group.
Recruited probands were in-patients and out-patients of the continuous business of the St. Josef Hospital, Bochum. In case the companions of the patients were not related to them, they were asked to be controls if fulfilling certain criteria. Excluded were all probands suffering from any kind of addiction, infectious disease, severe affected cognition and inadequate language skills. Also, participants that sustained olfactory impairment due to surgery, basilar skull fracture or head trauma were not included in the analysis. Moreover, blood relatives of already participating probands were not allowed to take part in the ParkCHIP study. PD subjects were frequency-matched by sex and age at the time of enrollment. The established inclusion criterion for all of the groups was to obtain a written, informed consent. The local ethic committee of the Ruhr-University Bochum approved the ParkCHIP study.

For the purpose of this thesis, the ParkCHIP study group was divided into three sub-groups, ParkCHIP I, ParkCHIP II and ParkCHIP III. ParkCHIP I and II were divided according to their time of recruitment and to existing controls (Table 4; Figure 7). ParkCHIP III forms a subcategory of ParkCHIP II. All participants underwent the same series of examination and responded to identical questionnaires, which are listed and explained below. Belonging to the PD group implied a more than 90% probability of right diagnosis which was evaluated by a physician according to the UKPDBBC.

ParkCHIP I

ParkCHIP I consists of 148 PD patients and the same amount of HC who were frequency-matched by sex and age. The recruitment took place between January 2010 and September 2011. This study group was object to research on the discriminative value of olfaction in the diagnosis of PD. By comparing and contrasting the general olfactory ability of PD patients and HC within ParkCHIP I, the value of the Sniffin’ sticks test, the possibility to use a short form of Sniffin’ sticks test with only three pens (quick 3-odor test) and the use of olfactory testing as a point-of-care test in PD diagnosis were attained. In addition, we assessed the clinical relevance of olfactory testing and ascertained other risk factors that contributed to olfactory impairment.
ParkCHIP II

This study group was recruited from January 2010 to January 2013 and consists of 211 PD patients including the 148 PD patients of the ParkCHIP I study group. In contrast to ParkCHIP I, this study group contains only PD patients. Before conducting the Sniffin’ sticks odor identification test, participants were asked to evaluate their olfactory function. Answering options were ‘good’, ‘limited’, ‘severely impaired’ and ‘unknown’. Findings with regards to the value of self-assessment of olfaction in comparison to olfactory testing were assessed.

ParkCHIP III

There were 13 PD patients out of the 211 ParkCHIP II subjects (6.2%) that presented with good olfactory function. The questionnaires of the affected participants were particularly reviewed. Suspicion was raised that despite the precise and comprehensive diagnostic process that was applied, missing olfactory dysfunction among these 13 individuals points to misdiagnosis, or to the presence of special cases of PD such as familial parkinsonism.
Figure 7. Graphical overview of the Parkinson patients of the ParkCHIP study groups.

The largest circle of the Venn diagram represents the 211 PD patients of the ParkCHIP II study group. The medium-sized circle depicts the 148 PD patients of the ParkCHIP I study group which is a subgroup of the 211 PD patients of the ParkCHIP II study group. The smallest circle in the middle represents 13 PD patients out of the diseased subjects that present with preserved olfactory ability. The figure was created with Microsoft excel 2007.
Table 4. Objective of the ParkCHIP study groups.

<table>
<thead>
<tr>
<th>Study group</th>
<th>Participating individuals</th>
<th>Objective</th>
</tr>
</thead>
<tbody>
<tr>
<td>ParkCHIP II</td>
<td>211 Parkinson patients</td>
<td>Reliability of self-assessed olfactory ability.</td>
</tr>
<tr>
<td>ParkCHIP III</td>
<td>13 Parkinson patients with preserved olfactory ability</td>
<td>Characteristics of PD patients with preserved olfactory function.</td>
</tr>
</tbody>
</table>
3.1.3 Examinations

3.1.3.1 Blood samples

In order to detect a shared antibody pattern in PD patients as well as in HC being at a higher risk for PD, blood samples were taken. To determine a multiplicity of laboratory parameters seven tubes, each containing 3 to 5 ml blood, were drawn. Blood samples were taken by the study nurse or the physician.

3.1.3.2 Examination by the physician

First, the physician assured that the proband was contractually capable and had not participated in another study within the past 60 days. Subsequently, a detailed past medical history concerning medication, history of L-DOPA response, former diseases, allergies and age of onset of PD was inquired. Elaborate examination was performed to assess the severity of disease and look for symptoms that potentially indicate atypical parkinsonian syndromes. Detailed examining procedures are described in 3.1.4 and 3.1.5.

3.1.4 Measurement instruments

All participants of the ParkCHIP study underwent the same interviews and cognitive tests. The interview contains three different questionnaires. Unless stated otherwise, a study nurse filled in the questionnaires according to the patients’ answers and test results and examined the patients for the necessary physical tests. The first questionnaire collects information about the study subjects’ socio-demographic situation. It also inquires information about the coffee, tea and nicotine consumption, family history concerning neurodegenerative diseases, received blood transfusions, number of births, sobriety, sleep and dream behavior and existence of restless-legs syndrome. Within this questionnaire, a physical examination concerning pulse and blood pressure measured after 2 minutes in a lying position and 2 minutes after the change over into a standing position is
conducted. Measures deviating from average expectations based on patients’ health in this test indicate autonomic dysfunction.

Further tests in the framework of this assessment were the Mini Mental State Examination (Folstein et al., 1975) and Clock Drawing Test (Goodglass and Kaplan, 1983) to analyze cognitive impairment as well as the Sniffin’ sticks odor identification test in order to evaluate olfactory function. The second questionnaire assesses the patients’ health status according to the Health Assessment Questionnaire (HAQ) (Fries et al., 1980) disability index (HAQ-DI) (Bruce and Fries, 2005). A last questionnaire contains the UPDRS (Goetz et al., 2003) and the Movement Disorder Society-sponsored revision of the UPDRS (MDS-UPDRS) (Goetz et al., 2008). The latter was conducted by the physician.

3.1.4.1 Health Assessment Questionnaire Disability Index

In order to determine the patients’ health status and the extent of their functional limitations, the HAQ-DI was utilized. In its elaborate version, the HAQ distinguishes five different categories: mortality, disability, pain, medication effects and cost of care (Fries et al., 1980). However, in the course of the ParkCHIP study only the Disability Index (DI) was required. The DI is widely used for research purposes in both experimental and observational studies, as well as in clinical settings. The participants were asked questions which had to be answered regarding to their health status of the previous week. The questions can be classified into eight categories: dressing, arising, eating, walking, hygiene, reach, grip and common activities. In order to allow for a quantitative analysis of the answers, the probands could only choose from the following four answering options: ‘without any difficulty’, ‘with some difficulty’, ‘with much difficulty’ and ‘unable to do’. The scoring convention of the DI contains a score from zero to three. The answers were assigned to increasing scores from: without any difficulty being equal to zero and to be unable to do being equal to three (Bruce and Fries, 2003).
3.1.4.2 Mini Mental State Examination

The participants had to undergo the Mini Mental State Examination to assess their cognitive status. The Mini Mental State Examination was first introduced Folstein and colleagues in 1975 (Folstein et al., 1975). The Mini Mental State Examination consists of 30 questions which can be answered in 5 to 10 minutes. A key factor of the test is a comfortable environment in which the patients do not feel put under pressure. In case a question was found difficult to answer the question could be left out. The test is divided into two parts, the first consisting of 21 and the second of nine questions. In the former, the participants were asked questions concerning orientation, memory and attention. The answers were given vocally. The second part examines the ability to name and follow both verbal and written comments as well as writing a sentence andportraying a geometrical figure. For the two parts, the answering options were either ‘right’ or ‘false’/ ‘I don’t know’/ ‘no answer’. The answers were evaluated as right or wrong with ‘no answer’ and ‘I don’t know’ being allocated to wrong. Each question answered correctly equaled to one point. Consequently, the maximum total score is 30. A score of 24 points or lower assumes a declined cognitive function (Adunsky et al., 2002). There was no time limit for the duration of the Mini Mental State Examination. In the ParkCHIP study, we excluded the questions that were associated with manual tasks since some probands were handicapped due to hand and arm injuries or presented themselves with restricted mobility and were consequently not able to participate in the total Mini Mental State Examination. Therefore we reduced the Mini Mental State Examination by all exercises with manual tasks resulting in a maximal score of 24 points. A score of 19 points or less indicated for cognitive impairment.

3.1.4.3 Clock Drawing Test

In addition to the Mini Mental State Examination, the Clock Drawing Test was performed as a further cognitive test including auditory comprehension, planning, visual memory and reconstruction within a graphic image. In addition, visuo-spatial abilities, motor programming and execution, numerical knowledge, abstract thinking, concentration and frustration tolerance are tested (Shulman, 2000). The
Clock Drawing Test is subdivided into three parts. At the beginning, participants were asked to draw a clock face into a pre-drawn circle. Subsequently, the time 11.10 had to be put onto the clock. Last 11.10 had to be written as it was usually found in a timetable (Libon et al., 1993). The test was scored according to Thalmann and colleagues (Thalmann et al., 2002) which is based on a maximum score of 7 points. Probands received one point if 12 numbers were drawn on the clock and two points for each of the following criteria: the number 12 is drawn on top, they drew two distinguishable hands of the clock and the time is written down correctly. Reaching 5 points or less points towards cognitive impairment.

3.1.4.4 The Unified Parkinson’s Disease Rating Scale

The UPDRS is commonly used both in clinical routine and in studies to evaluate the degree of disability and impairment in parkinsonian patients. Ramaker and colleagues (Ramaker et al., 2002) assessed different rating scales for impairment and disability in PD and found the UPDRS to be among the most frequently used and the most accurately rated scale. The UPDRS consists of four parts, each assessing another feature of cognition and motor symptoms (Goetz et al., 2003). In 2008, the Movement Disorder Society (MDS)-sponsored a re-adaptation of the UPDRS and developed a revised form of the UPDRS, the MDS-UPDRS (Goetz et al., 2008). The intended purpose was to eliminate existing weaknesses such as lack of clarity in written text and unclear instructions for raters as well as to add missing items which can evaluate non-motor symptoms of PD such as ahedonia, bradyphrenia, anxiety, hypersexuality, sleep disorders, fatigue, dysautonomia, and dysregulation (Goetz et al., 2003). Although, the former UPDRS-established, four parts constitution remained, several items have changed. Part I assesses ‘non-motor aspects of experiences of daily living’ whereas Part II addresses ‘motor aspects of experiences of daily living’. Part III regards ‘motor examination’ and part IV ‘motor complications’. Part I consists of 13 items regarding non-motor features such as cognition, depression, sleep problems, pain, fatigue and autonomic dysfunction (Goetz et al., 2008). It was shown that Part I of the MDS-UPDRS, appropriately reflects the strain of non-motor symptoms that occurs in PD patients (Gallagher et al., 2012). Overall, the MDS-UPDRS incorporates 65 items. For 48 items the answering options are: 0=normal, 1=slight, 2=mild, 3=moderate and
4=severe whereas seven items can be answered with yes/no (Goetz et al., 2008). In order to identify the severity of PD of the individuals participating in the ParkCHIP study, the physician used a conglomerate of the UPDRS, the MDS-UPDRS and the Unified Multiple System Atrophy Rating Scale (UMSARS) (Wenning et al., 2004).

3.1.4.5 Hoehn and Yahr scale

As a further device to describe mainly motor impairment and clinical disability arising as a consequence of PD, physicians used the Hoehn and Yahr scale in an adapted version in which stages range from 0-V instead of originally from I-V (Hoehn and Yahr, 1967). Patients classified as stage zero have no signs of disease. Stage I implies a unilateral involvement with almost no functional impairment whereas in stage II both sides are involved. Balance, however, remains unaffected. Stage III can be distinguished from I and II by a mild to moderate bilateral disease manifestation including some postural instability but patients stay physically independent. In stage IV, patients are severely disabled but are still able to walk and stand on their own. Stage V comprises all patients that rely on a wheelchair or are bed-ridden.

3.1.4.6 Clinical Global Impression of Disease Severity Scale

The Clinical Global Impression of Disease Severity (CGI-S) scale is a widely used scale to assess disease severity. With a score ranging from zero to six the examiner assesses the current disease severity of the diseased patient. Zero equals normal/no disease and six extremely ill (Guy, 1976).
3.1.4.7 REM Sleep Behavior Disorder Screening Questionnaire

The occurrence of REM sleep behavior disorder, which is possibly associated with early stages of different neurodegenerative diseases, is tested by the REM Sleep Behavior Disorder Screening Questionnaire (Stiasny-Kolster et al., 2007). In order to assess the individual’s sleep behavior the questionnaire is comprised of 10 items with the possible answering options: ‘no’, ‘sometimes’, ‘often’ and ‘I do not know’. Items one to four refer to the quantity and quality of dreams and their correlation to movement and behavior during the night. Item five focuses on occurred self or foreign injuries. Item six is subdivided into four sub-items that inquire precise information about nocturnal movements. Items seven and eight deal with nocturnal awakenings whereas item nine relates to generally disturbed sleep. Ultimately, probands are asked if they suffer from any other neurological disease. Answering options ‘sometime’ and ‘often’ are rated one point, so the maximum total score amounts to 13 points. The cut off value for an existing REM sleep behavior disorder was a value greater (or equal) than five.

3.1.4.8 Assessment of tremor and rigor dominance

UPDRS question 20 approaches the issue of tremor at rest. The patient’s actual extent of tremor at rest was assessed by the physician on a scale from zero which equals none to four which equals distinct amplitude and present most of the time. Question 22 of the UPDRS concerning rigidity was evaluated in analogy. In case the physician could not find one of these two features to be dominant, the patients were grouped as ‘other PD patients’.

3.1.5 Olfactory testing

The Sniffin’ Sticks identification test was performed as a multiple forced choice test with 16 common odors which were successively presented to the patients. The different odors were as follows: coffee, peppermint, anise, banana, licorice, fish, leather, clove, rose, orange, pineapple, lemon, turpentine, apple, garlic and
cinnamon. In order to eliminate distracting odors, the room was well ventilated. The opened pen was held approximately 2 cm under both nostrils for approximately 2-3 seconds. Subsequently, the probands had to identify the right odor from a list of four options (Hummel et al., 1997). The total time to accomplish the full test amounts to approximately 5 minutes (Eibenstein et al., 2005). In order to ensure that occurring difficulties of odor identification are not due to an existing sickness or an already established decreased ability to smell, prior to performing the test patients were asked to evaluate their overall ability to smell with the answering options: ‘normal/good’, ‘restricted’, ‘very bad’ and ‘I don’t know’. The interpretation of the results was conducted according to Wolfensberger and Schnieper (Wolfensberger and Schnieper, 1999). With 0-7 correctly identified odors an anosmia is assumed, 8-12 indicate hyposmia and 13-16 correctly identified odors are evaluated as normosmia.
3.2 Statistical analysis

Continuous variables were characterized by median and inter-quartile range (IQR). To assess statistical significant differences between the study groups, we used non-parametric Kruskal-Wallis test for comparison of continuous variables and chi-square tests or Fisher’s exact test for categorical variables (Trampisch and Ehle, 2000). P-values <0.05 were classified as statistically significant.

In order to examine the relative risks of olfactory impairment (hyposmia and anosmia, as reference served normosmia), we modeled proportional odds ratios (ORs) (McCullagh, 1980). The proportional odds ratio (OR) associated with each predictor can be interpreted as a summary of the risk estimates obtained from separate binary logistic regression using all cut-points of the ordinal outcome. Four distinct age groups were defined (<45, 45-65, 66-79, ≥80 years). In this set-up, the risk of the impairment of olfaction was determined for diseased and non-diseased subjects compared to a common reference group (non-diseased subjects aged 45-65 years). In combination with PD subtype, age was implemented as a binary variable with 65 years as cut-off. HC below the age of 65 were used as a reference group. In both models, the association between movement disorders and age was adjusted for cognitive impairment based on the results of the Clock Drawing Test (Goodglass and Kaplan, 1983).

Proportional ORs were always depicted with its 95% confidence interval (95% CI). In order to determine the three odors of the Sniffin’ stick test which in combination would allow for the best discrimination between the study groups (PD and HC), we trained random forest classifiers using the package randomForest (Breiman, 2001; Liaw and Wiener, 2002) in R 2.13 (Breiman, 2001; R Development Core Team, 2001). We evaluated their accuracy by 10-fold cross validation according to the following scheme: We recorded the misclassification rate with and without each permutation of each odor for the random forest. We then defined the accuracy as the difference between the two misclassification rates and normalized by the standard deviation of the differences. The higher the values of the accuracy, the more important the variables.
In order to estimate the agreement between self-assessed olfactory function and Sniffin' sticks test results, we computed the kappa coefficient across all individuals from the ParkCHIP II study group. Individuals answering ‘I don’t know’ when asked about olfactory function were excluded from the analysis. Initially designed to estimate agreement between different raters, the kappa coefficient takes the agreement between the raters and subtracts the probability of agreement by chance. This difference is then normalized to one minus the agreement by chance. Hence, the kappa coefficient is equal to one when there is complete agreement between raters. Kappa values between zero and one suggest an agreement that exceeds agreement by chance, with increasing values indicating higher agreement. Kappa is negative when the observed agreement is less than agreement by chance (Cohen, 1960).

All other analyses were performed with SAS/STAT and SAS/IML software, version 9.3 (SAS institute Inc., Cary, NC).
4. Results

The objective of this thesis was to analyze the role of olfactory impairment and olfactory testing in PD. Olfactory impairment was assessed from a variety of angles, both self-assessed and measured by the ability of patients to identify odors. The latter was conducted in the framework of the Sniffin’ sticks test, whose feasibility as a point-of-care test for clinical diagnosis was subject of this thesis.

4.1. Results of ParkCHIP I

The results presented in this part of the thesis (4.1.1 to 4.1.8) were published by Casjens and colleagues (Casjens et al., 2013) in the journal PLoS One in 2013 under the title ‘Diagnostic value of the Impairment of Olfaction in Parkinson's disease’. My contributions to this publication are as follows:

- Participation in the recruitment and examination of probands including drawing blood samples, performing schellong testing and completing questionnaires
- Conduction of the olfactory test (Sniffin' sticks)
- Contribution to data evaluation and interpretation
- Detailed review of patient questionnaires
- Discussion of the results in the paper including literature research

4.1.1 Characteristics of the ParkCHIP I study group

Table 5 depicts the characteristics of PD patients and HC. The median age of PD patients and HC was 67 years and 62 years, respectively. The number of people with a background of higher education and those who have been life-long non-smokers were more common among PD patients than in HC. PD patients and HC performed equally well in cognitive tests but the number of PD patients with severe disability was higher than the one in the HC.
Table 5. Characteristics of Parkinson patients and healthy controls (HC).

Their cognitive ability was assessed by the Mini Mental State Examination and the Clock Drawing Test (described in section 3.1.4.2 and 3.1.4.3). The Disability Index (DI) of the Health Assessment Questionnaire (HAQ) estimates the extent of functional disability (see 3.1.4.1). IQR: Inter-quartile range.

<table>
<thead>
<tr>
<th></th>
<th>PD (N=148)</th>
<th>% / Median (IQR)</th>
<th>HC (N=148)</th>
<th>% / Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>148</td>
<td>67 (59;73)</td>
<td>148</td>
<td>62 (56;72)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>78</td>
<td>52.7</td>
<td>81</td>
<td>54.7</td>
</tr>
<tr>
<td>Female</td>
<td>70</td>
<td>47.3</td>
<td>67</td>
<td>45.3</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>80</td>
<td>54.1</td>
<td>60</td>
<td>40.5</td>
</tr>
<tr>
<td>Former</td>
<td>58</td>
<td>39.2</td>
<td>62</td>
<td>41.9</td>
</tr>
<tr>
<td>Current</td>
<td>10</td>
<td>6.8</td>
<td>26</td>
<td>17.6</td>
</tr>
<tr>
<td>Education (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 10</td>
<td>75</td>
<td>50.7</td>
<td>82</td>
<td>55.4</td>
</tr>
<tr>
<td>10</td>
<td>25</td>
<td>16.9</td>
<td>33</td>
<td>22.3</td>
</tr>
<tr>
<td>&gt; 10</td>
<td>48</td>
<td>32.4</td>
<td>33</td>
<td>22.3</td>
</tr>
<tr>
<td>Mini Mental State Examination excluding manual tasks (max=24)</td>
<td>147</td>
<td>23 (21;23)</td>
<td>144</td>
<td>23 (22;24)</td>
</tr>
<tr>
<td>Clock Drawing Test (max=7)</td>
<td>147</td>
<td>7 (5;7)</td>
<td>141</td>
<td>7 (5;7)</td>
</tr>
<tr>
<td>HAQ-DI (max=3)</td>
<td>148</td>
<td>0.5 (0;1.4)</td>
<td>143</td>
<td>0 (0;0)</td>
</tr>
</tbody>
</table>
4.1.2 Olfactory impairment in Parkinson patients and healthy controls

Table 6 shows that PD patients identified on average 7 odors out of the 16 odors of the Sniffin’ sticks test battery in contrast to a median of 12 correctly identified odors in HC. More than 90% of PD patients were either hyposmic or anosmic in contrast to HC where less individuals showed these impairments. The majority of PD patients were anosmic (56.8 %), whereas less than a tenth of HC displayed anosmia (6.8%). There was no difference with respect to increased susceptibility to anosmia observed when comparing patients with rigor dominance to patients with tremor dominance.
Table 6. Impairment of olfaction (odor identification) in the ParkCHIP I study group assessed with 16 Sniffin' sticks.

Classification of olfaction according to Wolfensberger and Schnieper (Wolfensberger and Schnieper, 1999).

\(^a\) P value of Kruskal-Wallis test; \(^b\) P value of \(\chi^2\) test; IQR: Inter-quartile range.

<table>
<thead>
<tr>
<th>Identified odors with Sniffin' sticks (max=16)</th>
<th>Normosmia (13-16 odors)</th>
<th>Hyposmia (8-12 odors)</th>
<th>Anosmia (0-7 odors)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study groups</strong></td>
<td><strong>N</strong></td>
<td><strong>Median (IQR)</strong></td>
<td><strong>P value(^a)</strong></td>
</tr>
<tr>
<td>Parkinson patients (PD)</td>
<td>148</td>
<td>7 (5;9)</td>
<td></td>
</tr>
<tr>
<td>Healthy controls (HC)</td>
<td>148</td>
<td>12 (10;13)</td>
<td></td>
</tr>
<tr>
<td>PD vs. HC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD with tremor dominance</td>
<td>38</td>
<td>7.5 (5;9)</td>
<td></td>
</tr>
<tr>
<td>PD with rigor dominance</td>
<td>90</td>
<td>6 (4;9)</td>
<td></td>
</tr>
<tr>
<td>Tremor vs. rigor dominance (PD)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.1.3 Age-dependent risk of olfactory impairment

In Table 7, proportional odds models were applied to investigate confounder variables that contribute to olfactory impairment. In all groups, age was revealed to be a strong confounder with a non-linear shape of the impairment of olfaction (hyposmia and anosmia). HC aged 45-65 years were used as reference group. The OR for PD patients aged 45-65 years was 12.74 (95% CI 5.81-27.94). The OR for aged PD patients (≥80 years) was 74.01 (95% CI 8.17-670.68) and for the same age group among the HC OR was 11.24 (95% CI 2.23-56.76).

Table 7. Estimates of proportional odds ratios (ORs) with 95% confidence intervals (CI) of Parkinson’s disease (PD) and age with joint effects on impairment of olfaction (anosmia and hyposmia) adjusted by the Clock Drawing Test result.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;45 years</td>
<td>7</td>
<td>0.25</td>
<td>0.05 1.38</td>
</tr>
<tr>
<td>45-65 years</td>
<td>71</td>
<td>1</td>
<td>Reference group</td>
</tr>
<tr>
<td>66-79 years</td>
<td>56</td>
<td>1.19</td>
<td>0.59 2.40</td>
</tr>
<tr>
<td>≥80 years</td>
<td>7</td>
<td>11.24</td>
<td>2.23 56.76</td>
</tr>
<tr>
<td>Parkinson patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;45 years</td>
<td>5</td>
<td>1.30</td>
<td>0.21 7.95</td>
</tr>
<tr>
<td>45-65 years</td>
<td>60</td>
<td>12.74</td>
<td>5.81 27.94</td>
</tr>
<tr>
<td>66-79 years</td>
<td>73</td>
<td>18.00</td>
<td>8.31 38.98</td>
</tr>
<tr>
<td>≥80 years</td>
<td>9</td>
<td>74.01</td>
<td>8.17 670.68</td>
</tr>
</tbody>
</table>
4.1.4 Risk of olfactory impairment among the Parkinson’s disease subgroups

Defining HC aged <65 years as the reference group, table 8 depicts that PD patients with rigidity dominance showed higher ORs for olfactory impairment than patients with tremor dominance. The ORs differed in all age groups.

### Table 8. Estimates of proportional odds ratios (ORs) with 95% confidence intervals (CI) of Parkinson’s disease (PD) subtypes and age with joint effects on impairment of olfaction (anosmia and hyposmia) adjusted by the Clock Drawing Test result.

<table>
<thead>
<tr>
<th>Effect</th>
<th>N</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;65 years</td>
<td>78</td>
<td>1.00</td>
<td>Reference group</td>
</tr>
<tr>
<td>≥65 years</td>
<td>63</td>
<td>1.60</td>
<td>0.82</td>
</tr>
<tr>
<td>PD with rigor dominance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;65 years</td>
<td>45</td>
<td>12.72</td>
<td>5.67</td>
</tr>
<tr>
<td>≥65 years</td>
<td>44</td>
<td>30.28</td>
<td>12.45</td>
</tr>
<tr>
<td>PD with tremor dominance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;65 years</td>
<td>15</td>
<td>6.56</td>
<td>2.12</td>
</tr>
<tr>
<td>≥65 years</td>
<td>23</td>
<td>18.63</td>
<td>6.73</td>
</tr>
<tr>
<td>Other PD patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;65 years</td>
<td>5</td>
<td>19.14</td>
<td>2.85</td>
</tr>
<tr>
<td>≥65 years</td>
<td>15</td>
<td>7.36</td>
<td>2.73</td>
</tr>
</tbody>
</table>

4.1.5 Risk of olfactory impairment and disease severity

Table 9 presents the assessment of the disease severity according to different scales: UPDRS, UPDRS-III motor scale, MDS-UPDRS, Hoehn and Yahr rating scale and CGI-S. The risk of olfactory impairment increased with disease severity in PD patients. For the latter two scales, these changes were statistically
significant. For the UPDRS and the MDS-UPDRS the results were marginally significant. A weaker association was observed for the UPDRS-III motor scale.

Table 9. Estimates of proportional odds ratios (ORs) with 95% confidence intervals (CI) of disease severity on impairment of olfaction (anosmia and hyposmia) in Parkinson patients with age adjustment.

<table>
<thead>
<tr>
<th>Change per</th>
<th>N</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unified Parkinson’s Disease Rating Scale (max. score=199)</td>
<td>33 points</td>
<td>145</td>
<td>1.75 0.99 3.09</td>
</tr>
<tr>
<td>UPDRS-III motor scale (max. score=108)</td>
<td>18 points</td>
<td>148</td>
<td>1.38 0.88 2.16</td>
</tr>
<tr>
<td>Movement Disorder Society-sponsored revision of the Unified Parkinson’s Disease Rating Scale (max. score=260)</td>
<td>43 points</td>
<td>145</td>
<td>1.78 0.99 3.21</td>
</tr>
<tr>
<td>Hoehn and Yahr rating (max. score=5)</td>
<td>1 point</td>
<td>148</td>
<td>1.87 1.26 2.77</td>
</tr>
<tr>
<td>Clinician Global Impression of Disease Severity (max. score=6)</td>
<td>1 point</td>
<td>115</td>
<td>1.65 1.06 2.57</td>
</tr>
</tbody>
</table>

4.1.6 Risk of olfactory impairment and cognitive function

Besides age and disease severity, we observed an association between olfactory impairment and cognitive function. For the Clock Drawing Test, the OR was 0.83 with 95% CI ranging from 0.71 to 0.98 and for the Mini Mental State Examination we found OR 0.96 (95% CI 0.85-1.09). Thus, low cognitive performance estimated with the Clock Drawing Test was significantly associated with decreased ability to identify odors. The latter findings could not be shown for cognitive impairment.
assessed with the Mini Mental State Examination and the linkage to olfactory impairment.

4.1.7 Group differences (diseased and healthy subjects) in identifying individual odors of the Sniffin’ sticks test

Table 10 shows the results of the Sniffin’ sticks odor identification test. PD patients were able to identify 7 out of 16 possible odors whereas HC identified 12 odors (Table 6). There was a statistically significant difference in identification for 15 out of the 16 individual odors between the PD patients and the HC (Table 10). PD subjects had an impaired ability to identify all odors except for cinnamon when compared to HC. For this odor no statistically significant difference between the two groups was observed. The odor apple was rarely detected in both groups. HC showed best detection for peppermint and fish whereas PD patients were best at detecting garlic and orange. Among the PD subtypes, statistically significant differences were shown in detecting the odors orange and apple.
Table 10. Correctly identified odors in Parkinson patients and healthy controls (HC).

<table>
<thead>
<tr>
<th>Odor</th>
<th>Total % (HC) minus % (PD)</th>
<th>HC</th>
<th>PD</th>
<th>PD vs. HC</th>
<th>PD with tremor dominance</th>
<th>PD with rigor dominance</th>
<th>Tremor vs. rigor dominance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>% P value</td>
<td>%</td>
<td>%</td>
<td>P value</td>
</tr>
<tr>
<td>Coffee</td>
<td>59.0</td>
<td>44.6</td>
<td>77.7</td>
<td>53.1</td>
<td>31.1</td>
<td>0.360</td>
<td>39.5</td>
</tr>
<tr>
<td>Peppermint</td>
<td>77.9</td>
<td>40.6</td>
<td>93.9</td>
<td>52.7</td>
<td>50.0</td>
<td>0.818</td>
<td>52.2</td>
</tr>
<tr>
<td>Anise</td>
<td>61.6</td>
<td>39.2</td>
<td>73.7</td>
<td>34.5</td>
<td>26.3</td>
<td>0.212</td>
<td>37.8</td>
</tr>
<tr>
<td>Banana</td>
<td>68.3</td>
<td>36.5</td>
<td>84.5</td>
<td>48.0</td>
<td>52.6</td>
<td>0.464</td>
<td>45.6</td>
</tr>
<tr>
<td>Licorice</td>
<td>68.9</td>
<td>35.8</td>
<td>85.1</td>
<td>49.3</td>
<td>50.0</td>
<td>0.645</td>
<td>45.6</td>
</tr>
<tr>
<td>Fish</td>
<td>80.7</td>
<td>31.1</td>
<td>95.3</td>
<td>64.2</td>
<td>71.1</td>
<td>0.194</td>
<td>58.9</td>
</tr>
<tr>
<td>Leather</td>
<td>63.4</td>
<td>30.4</td>
<td>78.4</td>
<td>48.0</td>
<td>55.3</td>
<td>0.315</td>
<td>45.6</td>
</tr>
<tr>
<td>Clove</td>
<td>68.7</td>
<td>29.0</td>
<td>82.4</td>
<td>53.4</td>
<td>47.4</td>
<td>0.616</td>
<td>52.2</td>
</tr>
<tr>
<td>Rose</td>
<td>78.1</td>
<td>26.3</td>
<td>91.2</td>
<td>64.9</td>
<td>68.4</td>
<td>0.433</td>
<td>61.1</td>
</tr>
<tr>
<td>Orange</td>
<td>78.5</td>
<td>23.0</td>
<td>88.5</td>
<td>65.5</td>
<td>79.0</td>
<td>0.023</td>
<td>57.8</td>
</tr>
<tr>
<td>Pineapple</td>
<td>38.7</td>
<td>20.3</td>
<td>46.0</td>
<td>25.7</td>
<td>29.0</td>
<td>0.503</td>
<td>23.3</td>
</tr>
<tr>
<td>Lemon</td>
<td>34.1</td>
<td>18.9</td>
<td>37.8</td>
<td>18.9</td>
<td>21.1</td>
<td>0.139</td>
<td>11.1</td>
</tr>
<tr>
<td>Turpentine</td>
<td>42.2</td>
<td>15.5</td>
<td>47.3</td>
<td>31.8</td>
<td>21.1</td>
<td>0.165</td>
<td>33.3</td>
</tr>
<tr>
<td>Apple</td>
<td>19.1</td>
<td>14.9</td>
<td>25.7</td>
<td>10.8</td>
<td>0.006</td>
<td>0.013</td>
<td>14.4</td>
</tr>
<tr>
<td>Garlic</td>
<td>74.9</td>
<td>14.2</td>
<td>81.1</td>
<td>66.9</td>
<td>63.2</td>
<td>0.703</td>
<td>66.7</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>45.2</td>
<td>-4.1</td>
<td>40.5</td>
<td>44.6</td>
<td>0.481</td>
<td>1.42</td>
<td>55.3</td>
</tr>
</tbody>
</table>
4.1.8 Implementation of a virtual and functional subset of the Sniffin’ sticks test (peppermint, coffee and anise) which allows for a quicker testing

Table 11 shows the three odors that showed the biggest difference with respect to identification of PD patients and HC. These three odors were peppermint, coffee and anise. With these three odors we analyzed the performance for subsets of odors. The ten-fold cross-validated misclassification rate (misclassification PD vs. HC) for the 3-odor Sniffin’ sticks test of peppermint, anise and coffee rose by about 1% compared to the full set of 16 odors. Sensitivity and specificity of the 3-odor test was estimated depending on the decision rule of odor impairment. We achieved a specificity of 99% and a sensitivity of 28% when the decision rule for impairment was to identify none of the three odors correctly.

We compared the performance of our 3-odor Sniffin’ sticks test to previously published results on different short odor tests. Hummel and co-workers (Hummel et al., 2010) performed a short odor identification test called ‘q-Sticks’. It was based on the odors coffee, clove and rose and reached a misclassification rate of 27.0%. With the use of five odors as a short screening for decreased olfactory ability Mueller and Renner achieved a misclassification rate of 24.1% (Mueller and Renner, 2006). Their test consisted of the odors orange, leather, peppermint, rose and fish. Boesveldt and co-workers (Boesveldt et al., 2008) analyzed the use of licorice, cinnamon and anise and found the misclassification rate to be 26.4%. Non-food related odors were taken into consideration as they were proven to be less helpful to classify healthy subjects from PD patients which is concordant with findings of Boesveldt and co-workers (Boesveldt et al., 2011).
Table 11. Discrimination of patients with Parkinson's disease (PD) and healthy controls (HC) based on Sniffin' sticks using random forests on all and selected odors with ten-fold cross-validation.

<table>
<thead>
<tr>
<th>Sniffin' sticks</th>
<th>Misclassification (%)</th>
<th>Top-3 odors</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 odors</td>
<td>22.4</td>
<td>Peppermint, coffee, anise</td>
</tr>
<tr>
<td>Data-driven top-3 odors&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.8</td>
<td>Peppermint, coffee, anise</td>
</tr>
<tr>
<td>Peppermint</td>
<td>29.6</td>
<td></td>
</tr>
<tr>
<td>Coffee</td>
<td>27.6</td>
<td></td>
</tr>
<tr>
<td>Anise</td>
<td>30.3</td>
<td></td>
</tr>
<tr>
<td>Non-food odors (leather, turpentine, rose)</td>
<td>31.9</td>
<td>Leather, rose, turpentine</td>
</tr>
<tr>
<td>q-Sticks (coffee, clove, rose)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.0</td>
<td>Coffee, rose, clove</td>
</tr>
<tr>
<td>5-odor set (orange, leather, peppermint, rose, fish)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.1</td>
<td>Peppermint, fish, rose</td>
</tr>
<tr>
<td>3-odor set (cinnamon, licorice, anise)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>26.4</td>
<td>Anise, licorice, cinnamon</td>
</tr>
</tbody>
</table>

<sup>a</sup>Odors with the three highest variable importance measures according to random forest; <sup>b</sup>Hummel et al. 2010, <sup>c</sup>Mueller and Renner (Mueller and Renner, 2006); <sup>d</sup>Boesveldt et al. 2008.
4.2 Self-assessed olfactory impairment in the ParkCHIP II group

For the ParkCHIP II individuals, olfactory function was not only assessed by the Sniffin’ sticks test but also self-assessed by the use of a question in the ParkCHIP questionnaire. Patients were asked to judge their olfactory ability as ‘good’, ‘limited’, ‘severely impaired’ and ‘I don’t know’/ ‘unknown’. Table 12 depicts the results of the self-assessed olfactory ability compared to the results of the Sniffin’ sticks test. In the ParkCHIP II group we observed different results for self-assessed and measured olfactory ability with the Sniffin’ sticks test (Fisher's exact test $p<0.0001$). Fifty-five PD patients declared severe olfactory dysfunction. Eight of these 55 PD patient were diagnosed hyposmic (14.5%) and 47 anosmic (85.5%). However, individuals with self-assessed good olfaction were more likely wrong with their assessment. Out of 80 PD patients with declared good olfaction only ten (12.5%) were diagnosed normosmic.

To estimate the agreement between the Sniffin’ sticks test and self-assessed olfactory function, we calculated a kappa coefficient for all individuals in this study group who did not answer ‘I don't know’ in the self-assessment (N=197). The analysis yielded a kappa of 0.1315 which also indicates poor agreement between the two different assessments of olfactory function.
Table 12. Self-assessed olfactory ability in comparison to Sniffin' sticks test results of the ParkCHIP II study group.

Columns represent the answers of the ParkCHIP II individuals given to the question about their olfactory ability. Rows show the results of the odor identification test (Sniffin' sticks test).

<table>
<thead>
<tr>
<th>Self-assessed olfactory ability</th>
<th>Good</th>
<th>Limited</th>
<th>Severe</th>
<th>Unknown</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sniffin’ sticks assessed</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>Normosmia</td>
<td>10 (12.5)</td>
<td>2 (3.2)</td>
<td>0 (0)</td>
<td>1 (7.1)</td>
<td>13 (6.2)</td>
</tr>
<tr>
<td>Hyposmia</td>
<td>37 (46.3)</td>
<td>20 (32.3)</td>
<td>8 (14.5)</td>
<td>4 (28.6)</td>
<td>69 (32.7)</td>
</tr>
<tr>
<td>Anosmia</td>
<td>33 (41.2)</td>
<td>40 (64.5)</td>
<td>47 (85.5)</td>
<td>9 (64.3)</td>
<td>129 (61.1)</td>
</tr>
<tr>
<td>Total</td>
<td>80 (100)</td>
<td>62 (100)</td>
<td>55 (100)</td>
<td>14 (100)</td>
<td>211 (100)</td>
</tr>
</tbody>
</table>

4.3 Results of ParkCHIP III study group

Thirteen PD patients out of the ParkCHIP II study group (6.2%) showed no olfactory dysfunction. We compared the ParkCHIP III study group to olfactory impaired participants of the ParkCHIP II study group. We analyzed this study groups with respect to age, sex, L-DOPA response and the presence of other non-motor symptoms. In the ParkCHIP III study group we found that a great proportion showed a missing or reduced response to L-DOPA.
In addition, the questionnaires of the affected participants were reviewed in particular to see if they contained a reference to characteristics that point towards abnormalities in the cases or potential misdiagnoses.

### 4.3.1 Characteristics of the normosmic and the olfactory impaired subjects of the ParkCHIP II study group

Table 13 presents the characteristics of the subjects of the ParkCHIP II and ParkCHIP III study group. Statistically significant differences were revealed in the response to L-DOPA therapy, with the normosmic patients having a limited ‘best ever response to L-DOPA’ as well as a shorter duration of L-DOPA response. Twelve out of the 13 patients of ParkCHIP III showed a reduced response to L-DOPA (<50%). Only one individual had a best ever response to L-DOPA of >75%. In addition, ParkCHIP III subjects differed from the olfactory disturbed PD patients in displaying less non-motor symptoms. They scored significantly lower in the subdivision ‘Non-motor aspects of experiences of daily living’ of the MDS-UPDRS. A statistically significant difference in age was revealed. The 13 normosmic patients of the ParkCHIP III study group were on average 59 years old in contrast to 67 years of age amongst the olfactory impaired subjects of ParkCHIP II. Patients of the ParkCHIP III group presented with statistically significant shorter disease duration, with an average of two years in contrast to six years among the olfactory impaired subjects since diagnosis of PD. Disease severity was more distinct in the olfactory impaired group presenting with statistically significant higher scores in the Hoehn and Yahr rating scale, in the UPDRS-score and the MDS-UPDRS. There were no statistically significant differences with respect to gender distribution. With regards to the smoking status and the PD subtypes, there was no difference between the two groups.
Table 13. Characteristics of the normosmic and olfactory impaired subjects of the ParkCHIP II study group.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>Normosmia</th>
<th>Hyp.-Anosmia</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>% / Median (IQR)</td>
<td>N</td>
<td>% / Median (IQR)</td>
</tr>
<tr>
<td>Age at the beginning of the study (years)</td>
<td>211</td>
<td>67 (58;73)</td>
<td>13</td>
<td>59 (45;68)</td>
</tr>
<tr>
<td>Age at PD diagnosis (years)</td>
<td>209</td>
<td>59 (50;66)</td>
<td>13</td>
<td>57 (41;67)</td>
</tr>
<tr>
<td>Disease duration since diagnosis (years)</td>
<td>209</td>
<td>6 (2;10)</td>
<td>13</td>
<td>2 (1;3)</td>
</tr>
<tr>
<td>Hoehn and Yahr – Rating</td>
<td>211</td>
<td>3 (2;3)</td>
<td>13</td>
<td>2 (2;3)</td>
</tr>
<tr>
<td>UPDRS-Score</td>
<td>200</td>
<td>53 (38;62)</td>
<td>13</td>
<td>36 (29;50)</td>
</tr>
<tr>
<td>MDS-UPDRS</td>
<td>211</td>
<td>66 (48;80)</td>
<td>13</td>
<td>49 (34;61)</td>
</tr>
<tr>
<td>MDS-UPDRS - Non-motor aspects of daily living</td>
<td>211</td>
<td>9 (5;13)</td>
<td>13</td>
<td>7 (1;9)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>118</td>
<td>55.9</td>
<td>8</td>
<td>61.5</td>
</tr>
<tr>
<td>Female</td>
<td>93</td>
<td>44.1</td>
<td>5</td>
<td>38.5</td>
</tr>
<tr>
<td>REM sleep behavior disorder</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>108</td>
<td>51.4</td>
<td>4</td>
<td>30.8</td>
</tr>
<tr>
<td>No</td>
<td>102</td>
<td>48.6</td>
<td>9</td>
<td>69.2</td>
</tr>
<tr>
<td>Missing</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Last response to L-DOPA therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25%</td>
<td>41</td>
<td>19.7</td>
<td>5</td>
<td>38.5</td>
</tr>
<tr>
<td>25-50%</td>
<td>132</td>
<td>63.5</td>
<td>7</td>
<td>53.9</td>
</tr>
<tr>
<td>Unknown</td>
<td>35</td>
<td>16.8</td>
<td>1</td>
<td>7.7</td>
</tr>
<tr>
<td>Best ever response to L-DOPA therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25%</td>
<td>35</td>
<td>16.8</td>
<td>5</td>
<td>38.5</td>
</tr>
<tr>
<td>25-50%</td>
<td>42</td>
<td>20.2</td>
<td>6</td>
<td>46.2</td>
</tr>
<tr>
<td>51-75%</td>
<td>51</td>
<td>24.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&gt;75%</td>
<td>45</td>
<td>21.6</td>
<td>1</td>
<td>7.7</td>
</tr>
<tr>
<td>Unknown</td>
<td>35</td>
<td>16.8</td>
<td>1</td>
<td>7.7</td>
</tr>
<tr>
<td>Duration of L-DOPA response</td>
<td>Total</td>
<td>Normosmia</td>
<td>Hypo-/Anosmia</td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-------</td>
<td>-----------</td>
<td>---------------</td>
<td></td>
</tr>
<tr>
<td>%  / Median (IQR)</td>
<td>%  / Median (IQR)</td>
<td>%  / Median (IQR)</td>
<td>P-value</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>27</td>
<td>14.6</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td>&lt;6 month</td>
<td>9</td>
<td>4.9</td>
<td>2</td>
<td>16.7</td>
</tr>
<tr>
<td>6-11 month</td>
<td>17</td>
<td>9.2</td>
<td>5</td>
<td>41.7</td>
</tr>
<tr>
<td>1-2 years</td>
<td>20</td>
<td>10.8</td>
<td>4</td>
<td>33.3</td>
</tr>
<tr>
<td>3-5 years</td>
<td>38</td>
<td>20.5</td>
<td>1</td>
<td>8.3</td>
</tr>
<tr>
<td>&gt;5 Jahre</td>
<td>74</td>
<td>40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Missing</td>
<td>26</td>
<td>1</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td>Never</td>
<td>112</td>
<td>53.1</td>
<td>8</td>
</tr>
<tr>
<td>Former</td>
<td>83</td>
<td>39.3</td>
<td>4</td>
<td>30.8</td>
</tr>
<tr>
<td>Current</td>
<td>16</td>
<td>7.6</td>
<td>1</td>
<td>7.7</td>
</tr>
<tr>
<td>Tremor dominance</td>
<td>56</td>
<td>26.5</td>
<td>3</td>
<td>23.1</td>
</tr>
<tr>
<td>Rigor dominance</td>
<td>123</td>
<td>58.3</td>
<td>6</td>
<td>46.2</td>
</tr>
<tr>
<td>No dominance</td>
<td>32</td>
<td>15.2</td>
<td>4</td>
<td>30.8</td>
</tr>
</tbody>
</table>
4.3.2 Results of the detailed review of the questionnaires of the ParkCHIP III subjects

Reviewing the questionnaires of the 13 ParkCHIP III individuals in detail, we found six patients who were diagnosed before the age of 50 years (two patients before the age of 40). Four individuals (31%) had a positive family history for PD. None of the 13 individuals took calcium-channel blocker and anti-dopaminergic drugs like antipsychotic drugs. Four subjects took antidepressant drugs. In addition, there were two individuals with a former cranial trauma with proven damage.

Five subjects among the ParkCHIP III study group were found to suffer from hypertension which in turn, can lead to cerebrovascular disease.
Figure 8. Graphical overview of characteristics of the ParkCHIP III study group.

The circles represent possible reasons for parkinsonian symptoms as well as characteristics of the 13 individuals of the ParkCHIP III study group. Edges between the circles indicate overlapping features.

This figure was created using Microsoft power point 2007.
Review of the 13 individual questionnaires:

i) This male subject was born in 1966 and was diagnosed with PD at the age of 41 years. Belonging to this age group raised suspicion of the existence of familial PD since early-onset PD is more often associated with genetics than late-onset PD (Tanner et al., 1999). However, in this case familial report on PD was negative. This proband did not smoke. He suffered from hypertension. There were no anti-dopaminergic drugs or further, potentially parkinsonism-causing medications present in his medical history. Response to L-DOPA was reduced to 25-50% regarding the best-ever response to L-DOPA and duration of response was diminished to 1-3 years. According to the UKPDBBC a L-DOPA response for 5 years or more is a supportive criterion for diagnosis of PD (Gibb and Lees, 1988).

ii) This female proband was born in 1941. She was 67 years old at the time PD was diagnosed. No further comorbidities exist. She was a former smoker with 7 pack years. The patient took mirtazapine on a regular basis, an antidepressant drug which has the potential to rarely cause drug-induced parkinsonism (Gony et al., 2003). There was no family history of PD. L-DOPA response (specifically marked by her physician) was reduced with her best-ever response being less than 25%. The duration of response lasted only 1-2 years.

iii) This male subject, born in 1951, was diagnosed at the age of 57 years. He never smoked and had no further diseases. His familial report on PD was without findings. His best-ever L-DOPA response was 25-50% and PD symptoms improved for 1-2 years.

iv) Born in 1963, this male patient was diagnosed at the age of 45 years. Familial report on PD was negative. He never smoked and has no further diseases. He only took PD medication. His best L-DOPA response was 25-50% with a duration of effectivity of L-DOPA of 6-11 month.

v) This woman was born 1970 and was diagnosed with PD at the age of 38 years, with no family history of PD. She had never smoked, had no other comorbidity and only took PD medication. Her L-DOPA response was reduced to less than 25% for the best-ever response and to 6-11 month for the duration.
vi) This female proband was born in 1937 and diagnosed with PD at the age of 70. She never smoked. The following comorbidities existed: depression, diabetes and hypertension. She had a positive family history of PD with two of her brothers having been diagnosed with PD. She took trimipramine, a tricyclic antidepressant which has the potential to cause drug-induced Parkinsonism (Gony et al., 2003). Her history of L-DOPA response was not assessed.

vii) This woman, born in 1966 was first diagnosed with PD at the age of 38 years. With no family history of PD. She was a former smoker with five pack years. Information on her L-DOPA response was missing.

viii) This male subject was born in 1940 and was diagnosed with PD at the age of 70 years. He was a former smoker with two pack years. He suffered from hypertension and took multiple drugs. Amongst others, he took antidepressants (venlafaxine and mirtazapine) which are potential causes for drug-induced parkinsonism (Gony et al., 2003). He had no family history of PD. His best L-DOPA response was decreased to 25-50% and the duration of response was 6-11 month.

ix) This woman was first diagnosed at the age of 45 years with PD. She never smoked and no further comorbidities. Her family history with respect to PD was without pathological findings. Her best L-DOPA response was counted to less than 25% and the duration of response was 6 to 11 months.

x) This male subject was born in 1939 and diagnosed at the age of 71. He suffered from diabetes and hypertension and never smoked. Family anamnesis was PD-positive for the father of the patient. His best L-DOPA response was 25-50% for a duration of response of 3-5 years.

xi) Born in 1949 this patient was diagnosed with PD at the age of 63 years. Additionally, he suffered from diabetes. He was a present smoker with about 50 pack years. There was no family history of PD. His best-ever L-DOPA response was less than 25% for a duration of response of less than 6 month.

xii) This male patient, born 1968 and diagnosed with PD at the age of 41, had a head trauma with proved damage in his past medical history. He never smoked. His father and two uncles suffered from PD. Best L-DOPA response was more than 75% and the duration of response was 1-2 years.
This male subject was born in 1953. He was 58 years old when he was diagnosed with PD. He had a head trauma with proved damage in his medical history. In addition, he suffered from hypertension. He took mirtazapine which can potentially account for drug-induced parkinsonism (Gony et al., 2003). He is a former smoker with 23 pack years. His mother suffered from PD (first diagnosed at the age of 79 years). His best-ever response to L-DOPA was 25-50% and the duration of response was less than 6 month.

4.4 Costs of Parkinson’s disease

PD is a progressive and chronic disease with frequent emergence of severe disability (Schrag et al., 2000). Thus, most patients rely on expensive and numerous anti-parkinsonian drugs as well as care either by institutional caretakers or family members (Jennum et al., 2011). There are two types of costs associated with PD which describe the financial burden for both patients and the society/economy. First, the direct costs which describe the expenses that are caused by preventing, detecting and treating health impairment or its effects. Second, the indirect costs which represent different components such as productivity loss due to illness, premature retirement or death (Goossens et al., 2000). Direct costs originate from various sources such as drug costs, inpatient care, outpatient care and costs for non-medical treatment, transportation and special equipment. Further cost-generating factors are formal and informal care. Financial support in form of formal care is provided by health insurance and covers the costs for ambulatory professional care according to the degree of disability of the patient. Informal care is home care and is provided by family and friends of the patient (Winter et al., 2010).

In the following, a short review on the study of Winter and colleagues (Winter et al., 2010) is presented. Results of their study are used in conjunction with data of the Federal Statistical Office in Germany (Statistisches Bundesamt, 2015).
In their longitudinal study on 145 PD patients, Winter and colleagues (Winter et al., 2010) determined the costs of PD in Germany (Table 14). The average annual costs per patient added up to 20,095 € (95% CI 17.294-23.967) including both, direct (13,158 €) and indirect (6,973 €) costs. Considerable costs were antiparkinsonian drugs with mean annual costs that totaled to 3,526 €, out-of-pocket costs of 4,053 € (which were defined as informal care plus co-payments for drugs and treatment), hospitalization with an average amount of 2,315 € per year and rehabilitation with 1,132 € per year. However, the indirect costs for the national economy due to PD-related unemployment and retirement add up to estimated 2.1 billion €. Regarding the costs for medical treatment, further studies by Mueller and co-workers (Mueller et al., 2004) and Keller and co-workers (Keller et al., 2003) supported the latter findings.

**Table 14. Annual costs of Parkinson's disease (PD) in €.**

Costs are a subset of the described annual costs per Parkinson patient of the study by Winter and colleagues (Winter et al., 2010).

<table>
<thead>
<tr>
<th>Direct costs</th>
<th>13,158 (€)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Health insurance</td>
<td>9,105</td>
</tr>
<tr>
<td>Hospitalization</td>
<td>2,315</td>
</tr>
<tr>
<td>Antiparkinsonian Drugs</td>
<td>3,526</td>
</tr>
<tr>
<td>Out of pocket costs in total</td>
<td>4,053</td>
</tr>
<tr>
<td>Informal care</td>
<td>2,479</td>
</tr>
<tr>
<td>Patient’s co-payment</td>
<td>1,574</td>
</tr>
<tr>
<td>Indirect costs</td>
<td>6,937</td>
</tr>
<tr>
<td>Total costs</td>
<td>20,095</td>
</tr>
</tbody>
</table>
In different studies, life expectancy of Parkinson patients was shown to be reduced. In a Swedish community study, Fall and co-workers (Fall et al., 2003) found the mean disease duration between diagnosis and death to be 13 years (with a mean age at onset of 66 years). This is similar to findings of Elbaz and colleagues (Elbaz et al., 2003) who found the median survival of PD patients with an age at onset between 41-60 years to be 17 years. With the average annual costs of 20,000 € per Parkinson patient and an approximated disease duration of up to 17 years one misdiagnosed case can cause costs of up to 340,000 €.

Considering only people above the age of 65 years, which account for approximately 20% of the German population (Statistisches Bundesamt, 2015), results in about 16 million people in this age group. De Rijk and colleagues (de Rijk et al., 2000) showed in a review of seven population-based European studies an overall prevalence of PD of 1.8% in persons ≥ 65 years. With an overall prevalence of PD of 1.8%, there would be approximately 300,000 PD patients in Germany. Assuming that misdiagnosis in PD (here as false-positive cases) is as high as 30% (Hughes et al., 1992; Rajput et al., 1991; Brooks, 2012) there are potentially 90,000 PD patients that live with a wrong diagnosis. Even at a lower false-positive rate of only 10% there would be about 30,000 misdiagnosed cases. This would eventually sum up to costs for the economy, society and the patients of 600,000,000 € per year.
5. Discussion

By analyzing the ParkCHIP I study, a cross-sectional study with case-control design, we were able to highlight the importance of olfactory impairment in PD. Among 148 PD patients and the same number of HC we found disturbed olfaction being present in more than 90% of the PD patients reflecting findings of other studies and highlighting the importance of this symptom as a supportive criterion for diagnostic in PD.

We used the Sniffin’ sticks odor identification test with its 16 pen-like odor devices to test olfaction. We assessed the opportunity of using a subset of odors that could potentially allow for quicker testing. Using random forest classifiers, we found three odors to discriminate best between PD and HC. These were peppermint, coffee and anise with a misclassification rate of 23.8%. Moreover, we investigated factors that potentially contributed to olfactory disturbance and were able to find age, disease severity and cognitive function to be associated with decreased olfactory ability.

In addition, we evaluate the role of self-assessment of olfactory function among the individuals of the ParkCHIP II study group. Self-assessment was shown to hold the risk for underestimation of olfactory impairment. These results highlight the importance and necessity of olfactory testing of every individual where PD is a potential diagnosis.

Since olfactory impairment is a strong symptom in PD patients, we analyzed the questionnaires of 13 patients of the ParkCHIP III study group (6.2%) who showed a normal olfactory ability more profoundly. We found potential indicators for other diagnoses than PD in most of the 13 patients. Importantly, among most of these 13 individuals L-DOPA response was decreased or even missing. Since amelioration of symptoms after L-DOPA treatment is a marker for PD, this is indicative for a potential marker for misdiagnosis.
These findings underline the importance of assessing olfactory impairment in PD in any clinical and diagnostic setting. In order to make the use of olfactory testing easier in the clinical routine we recommended a short form of the Sniffin’ sticks test utilizable as a point-of-care test containing the odors peppermint, coffee, and anise. This simple and cheap point-of-care test allows for improved diagnostic work due to its high specificity of 99%.

Annual costs of PD treatment amount to approximately 20,000 € per year and patient. Consequently, a crucial amount of money can be saved by the use of olfactory testing.
5.1 Discussion

5.1.1 Characteristics of the Parkinson patients

The patients of ParkCHIP I study group were on average 67 years old with an age distribution between 59 to 73 years. This distribution falls into the range of the mean age at onset of PD from early to mid 60s (Inzelberg et al., 2002). De Rijk and colleagues (de Rijk et al., 2000; de Rijk et al., 1997) showed that there is no gender difference for the occurrence of PD, which is in contrast to other studies (Baldereschi et al., 2000; Claveria et al., 2002) who found men to suffer from PD more often than women. A possible explanation for this gender distribution is the neuroprotective effect of estrogens which is discussed in the work of Haaxma and colleagues and Saunders-Pullman and colleagues (Haaxma et al., 2007; Saunders-Pullman, 2003). In the ParkCHIP I study group distribution of gender was balanced with slightly more men participating. About 50% of the ParkCHIP I study group went to school for 10 years or more which is above the German average where only 12.3% in 1970 and 20.7% in 1982 of the German population went to school for more than 10 years (Frietsch, 2004). Rocca and co-workers (Rocca et al., 1996) revealed no association between low educational level and PD, however, Frigerio and colleagues (Frigerio et al., 2005) detected that people with higher education and physicians have an increased risk for PD. The authors hypothesized that educated people are more attentive to mild symptoms of the disease and therefore more often diagnosed. However, further studies have to be conducted since these findings regarding the correlation between education and PD are not yet conclusive. In this study, a participation bias is a possible factor that causes the higher educational level of the ParkCHIP I study group, as well-educated people are more likely to participate in research studies (Ganguli et al., 1998).

In the ParkCHIP I study group we found more ‘never-smokers’ than in the HC, with the proportion of ‘never-smokers’ in the ParkCHIP I study group being above the average of ‘never-smokers’ in the German population (Lampert et al., 2013). Smoking is discussed as a potential neuroprotective factor with smokers having a
lower risk of PD. One explanation for this correlation is the potential neuroprotective nature of nicotine (Park et al., 2007). In a large meta-analysis of studies from 1966 to 2002, a reduction of the risk for developing PD of 60% was found when comparing current smokers to 'never-smokers' (Hernan et al., 2002). Our findings that there are less smokers among the ParkCHIP I study group than in the average German population, support the results mentioned above. The neuroprotective effects of smoking are possibly due to the dopamine releasing effect of nicotine (Wonnacott, 1997), this effect was shown on mice models (Zhou et al., 2001). Further effects are its activity as an antioxidant and potential ability to alter the activity of monoamine oxidase (Quik, 2004). Beneficial effects of integrating nicotine agonists in PD therapy were shown in several studies (Itti et al., 2009; Villafane et al., 2007). Nicotinic therapy can potentially alleviate motor symptoms and dyskinesias and could potentially be neuroprotective but must be tested more for future therapeutic support.

5.1.2 Olfactory impairment in the ParkCHIP I study group

Olfactory impairment was a strong symptom in PD patients of the ParkCHIP I study group with more than 90% of patients suffering from either hyposmia or anosmia. This is concordant with the results of other studies where the majority of PD patients suffered from olfactory disturbance (Doty et al., 1988; Haehner et al., 2009a). In the average elderly population, olfactory impairment is less frequent compared to PD patients (Murphy et al., 2002). In their population-based study, Murphy and colleagues also found olfactory impairment to be prevalent in less than 20% of people aged 60-69 years. Similar results were revealed in further large population-based studies (Brämerson et al., 2004; Landis et al., 2004). These results are not concordant with our findings of olfactory function in HC. 62% of the individuals were found to be hyposmic and 6.8% to be anosmic. A possible explanation could be that the HC are not entirely free from further diseases such as AD or further disorders which are potential contributors to loss of olfaction (Doty, 1995). Further, the HC of the ParkCHIP study are older (median 62 years) than the participants of the study by Landis and colleagues (Landis et al., 2004). Their study population was on average 42 years old which could be conductive to
the different findings as prevalence of olfactory impairment increases with age (Murphy et al., 2002). In addition, the OR for olfactory impairment of the PD patients was considerably higher than the one for the HC.

The early and robust occurrence of olfactory impairment in PD may be explained through the pathological process that was described by Braak. The Braak staging (Braak et al., 2003) is oriented on the Lewy body distribution in the brain. In stage I the olfactory bulb and the anterior olfactory nucleus are affected which can subsequently lead to the clinical symptom of smell loss. Smell loss was shown to be a strong indicator for PD.

Diseases that belong to the complex of atypical parkinsonian syndromes are less probable to present with olfactory dysfunction (Wenning et al., 1995) with the exception of dementia with Lewy bodies and multiple system atrophy (Mueller et al., 2002). Dementia with Lewy bodies is often associated with statistically significant olfactory impairment (Williams et al., 2009) and a differentiation between dementia with Lewy bodies and PD based on olfactory testing is not possible (Liberini et al., 2000). Together, these findings in dementia with Lewy bodies and PD emphasize the role of Lewy bodies in the pathomechanism that leads to smell loss (Hawkes, 2003). In addition, it was shown that patients suffering from drug-induced parkinsonism can present with olfactory disturbance as well (Kruger et al., 2008). In terms of familial parkinsonism there is evidence for milder olfactory impairment (Katzenschlager and Lees, 2004); however, the ability to smell differs depending on the type and location of mutation. In contrast to the findings regarding PD and olfactory function, vascular parkinsonism which develops linked to cerebrovascular disease, does not go along with loss of olfaction (Katzenschlager and Lees, 2004). The relation between olfactory impairment and essential tremor has yet to be clearly defined. A study by Busenbark and colleagues (Busenbark et al., 1992) revealed normal olfactory test scores for patients with essential tremor. However, a more recent study showed mild olfactory impairment among essential tremor patients (Louis and Jurewicz, 2003).

Thus, when using olfactory testing as a supporting tool in differential diagnosis, it has to be taken into consideration that some Parkinson-like diseases potentially
display mild olfactory impairment. However, in this disease complex severe smell loss is most likely associated with PD (or dementia with Lewy bodies) (Wenning et al., 1995). Hence, examining olfactory function within the diagnosis process of PD could serve as a further means for precise diagnosis as well as differential diagnosis.

5.1.3 Olfactory impairment and confounding risks

We ascertained other risk factors that contributed to olfactory impairment. As shown in several population studies (Murphy et al., 2002; Schubert et al., 2009) increasing age is strongly associated with olfactory impairment. The trend of age-related impairment was non-linear with a steeper gradient for PD patients than for healthy controls. The estimate of the olfactory impairment related to age escalated to a high OR in diseased subjects in comparison to the HC aged 45-65 years.

Our findings that the PD subtype that is dominated with rigor has a higher (but not statistically significant) OR for olfactory impairment than the tremor-dominant subtype are in agreement with others (Iijima et al., 2011; Stern et al., 1994) but remain in contrast to Verbaan and colleagues (Verbaan et al., 2008). Studies concerning PD subtypes are rare and are mostly comprised of only a small number of patients. As a consequence, the small differences in olfaction we found could be an artifact finding due to limited statistical power.

Furthermore, we observed a statistically significant relation between olfactory impairment and disease severity. Especially for disease severity assessed with the Hoehn and Yahr rating scale and the CGI-S, a statistically significant correlation with loss of smell was shown. Similar results were presented by Tissingh and co-workers (Tissingh et al., 2001) who revealed a negative correlation between odor discrimination and disease severity according to the Hoehn and Yahr stage. This impact could not be revealed for the UPDRS motor scale. However, the latter correlations could not be replicated in further studies (Haehner et al., 2009a; Haehner et al., 2009b). Boesveldt and colleagues (Boesveldt et al., 2008) found different results for odor identification and odor discrimination with respect to
disease severity and disease progression. Odor identification did not correlate with disease progression, but odor discrimination increased as disease progressed.

There are several factors that contribute to whether an association can be detected or not. In general, the number of participants, the characteristics of the study population, the scales that were used and the adjustment for observer or study center can influence the results. The ParkCHIP study was robust with respect to potential bias introduced by the observer or study center with only two physicians at a single study center being responsible for rating issues. Additionally, all patients fulfilled the UKPDBBC (Table 1). The findings that the correlation between Hoehn and Yahr as well as the CGI-S and olfactory impairment were stronger than the one between the UPDRS and olfactory impairment could be related to the structure and content of the scales. The UPDRS is a more extensive scale which includes more unspecific, less PD-related questions that can lead to altered scores.

In this study, we also found that impaired cognitive function assessed with the Clock Drawing Test but not with the Mini Mental State Examination showed a statistically significant correlation with a decrease in odor identification. This correlation was shown in further studies (Bohnen et al., 2010; Damholdt et al., 2011). A potential explanation for the difference between the two tests is the observation that the Clock Drawing Test was shown to be more sensitive to reveal cognitive impairment than the Mini Mental State Examination. In a large cross-sectional study on PD patients, the Mini Mental State Examination was found to have a lower sensitivity than the Clock Drawing Test to reveal cognitive impairment in PD patients (Riedel et al., 2008).

### 5.1.4 Utility of the Sniffin’ sticks test to discriminate diseased from healthy individuals

The Sniffin’ sticks test as a robust test of odor identification was performed on the 148 PD patients of ParkCHIP I as well as on the same amount of HC. When comparing the olfactory function of PD patients to the one of HC there was a
statistically significant difference in the ability to detect odors for 15 of the 16 individual odors. No difference was found for the odor cinnamon. In conclusion, the Sniffin’ sticks test allows for a good discrimination between PD and HC. Most of the single odors were suitable to distinguish HC from PD patients. Based on the decreased ability to identify odors discrimination between diseased and healthy subjects was possible.

5.1.5 Use of a subset of the Sniffin’ sticks test

We found that a brief version of the Sniffin’ stick odor identification test is suitable to detect severe olfactory dysfunction. In a study conducted by Boesveldt and colleagues (Boesveldt et al., 2011), it was shown that testing the olfactory ability by a sole odor identification test was highly effective. The application of an additional odor discrimination test was not able to improve sensitivity or specificity of olfactory testing. The complete Sniffin’ sticks odor identification test contains 16 odor devices with an application time per device estimated to be 20 seconds, thus a total of approximately 5 minutes to conduct the full test is required (Eibenstein et al., 2005). In order to implement a test in the clinical diagnostic routine it has to be easy to apply, not time intensive, cheap and its utility has to be proven.

Thus, we implemented a short test composed of three (peppermint, coffee, anise) out of the original 16 sticks including Sniffin’ sticks odor identification test. Three odors were sufficient to discriminate between diseased and non-diseased subjects as they reached similar misclassification rates as the full set of Sniffin’ sticks (22.4%). In order to find the best odors out of the 16 existing ones, we applied a random forest classification algorithm with ten-fold cross-validation to evaluate the best discriminating odors. We found the three odors, peppermint, coffee and anise to discriminate best between PD patients and HC with a misclassification rate of 23.8%. These three odors are categorized as food-related odors which were previously shown to be more useful to classify PD patients. Non-food odors performed worse in classifying PD (Boesveldt et al., 2011).
When implementing a test, it has to be evaluated how well the test performs. Sensitivity and specificity are both statistical measures of test performance. Sensitivity or true positive rate describes the proportion of diseased people among the ones having a positive test result. The specificity or true negative rate is the proportion of healthy people that were correctly identified as such. There is usually a trade-off between improving specificity and sensitivity. False positive test results can potentially lead to high costs for additional and more invasive diagnostic to falsify or support the diagnosis of the disease. In our study we applied a strong decision rule, for example a test result was only considered positive provided none of the three best classifying odors was detected. Based on this criterion, we achieved a specificity of 99% and a sensitivity of 28% for the odor identification test containing only a subset of three odors. In summary, testing the ability to identify odors is a reliable and conclusive tool to discriminate between non-diseased subjects and subjects who suffer from PD.

This reduction of the full set of the Sniffin’ sticks test leads to quicker examination and more acceptance in the clinical routine. The short form of the Sniffin’ sticks test allows for a wider application (for example in epidemiological studies), more compliance and agreement and is suitable as a point-of-care test where short testing time, acceptability to participants and manageability of application are key features.

Odors from our top-3 set have also been proposed in quick-test applications in other studies. Hummel and co-workers (Hummel et al., 2010) implemented an identification test composed of three odors, also called q-Sticks, containing coffee, clove and rose. When applied to PD patients and HC of the ParkCHIP I study group misclassification rate rose up to 27% due to lower group differences in identification of rose and clove than of anise and peppermint. A different study found three odors anise, cinnamon and licorice to be the best discriminating odors between HC and PD patients (Boesveldt et al., 2008). With this quick test we revealed a misclassification rate of 26.4%. A different subset of odors was chosen by Mueller and Renner (Mueller and Renner, 2006), who used orange, leather, peppermint, rose and fish as classification odors. With this set, misclassification rate in the ParkCHIP I study group was 24.1%. Most important, when odor identification is used as a discriminator between HC and PD, the odors must be
well detected by HC but only a small proportion of PD patients should be able to identify them. The reduced set of the Sniffin’ sticks odor identification test is able to detect severely decreased olfactory ability.

Olfactory testing should be used in any clinical setting where movement disorders or neurological disorders are diagnosed. There should be definite guidelines for the clinical routine that explain the diagnostic benefit of olfactory testing. Physicians have to become more aware of and informed about this robust and frequent symptom. Moreover, olfactory testing has to be considered as a supportive tool in differential diagnosis of PD with some limitations (including dementia with Lewy bodies which also presents with severe olfactory loss or multiple system atrophy, drug-induced parkinsonism and essential tremor where mild olfactory impairment is possible). It can be used as an in-expensive and easy to apply adjunct tool in diagnosis. It was shown that olfactory testing is as important in the diagnostic process of PD as the clinical examination due to the high frequency in which olfactory disturbance is present in subjects with PD.

5.1.6 Value of self-assessment of olfactory ability

In order to emphasize the importance of olfactory testing we evaluated the value of self-assessment of olfactory disturbance on the ParkCHIP II study group. Many of the individuals of the ParkCHIP II study group who categorized their olfactory function as good were shown to suffer from hyposmia or anosmia. The analysis of rater agreement (kappa coefficient) to assess the agreement between self-assessed olfactory function and Sniffin’ sticks test, showed poor agreement. This analysis further strengthens the point of poor reliability on self-assessed olfactory function.

Our findings are concordant with findings of Murphy and colleagues (Murphy et al., 2002) who found that self-assessment holds the risk for underestimation of olfactory impairment. In a large population based study on older adults, they revealed that prevalence rates of olfactory impairment assessed through self-
report significantly underestimate the prevalence rates obtained by olfactory testing. Further, Murphy and colleagues reported low sensitivity rates for self-assessment of olfactory impairment with the sensitivity of self-report decreasing with age. The aforementioned findings of the limitation of self-assessment are in line with findings from Nordin and co-workers (Nordin et al., 1995) who investigated olfactory dysfunction in elderly subjects and patients with Alzheimer’s disease and with results on detecting taste dysfunction by Soter and colleagues (Soter et al., 2008). Self-assessment in general is fraught with the risk of underestimation, as findings on musculoskeletal diseases (Hughes et al., 1993) and on older population regarding the impact on disability that is caused by neurodegenerative diseases (Waite et al., 2001) have shown.

We conclude that self-assessment is not a reliable measure for individual disability including decreased olfactory ability. Thus, self-assessment of olfactory ability is not an appropriate replacement (for example by the simple anamnestic question about the probands’/patients’ ability to smell) for olfactory testing. This highlights the importance of the Sniffin’ sticks test as an essential tool in the diagnostic process of PD which should be implemented in the clinical routine.

5.1.7 Characteristics of the ParkCHIP III study group

Olfactory impairment is a strong symptom in PD, thus the lack of olfactory malfunction points towards potential misdiagnosis (Hawkes, 2003). Among the ParkCHIP II study group we found 13 PD patients with preserved olfactory ability and grouped them together as ParkCHIP III. Although the diagnoses were assessed according to the UKPDBBC, they still comprise a potential risk of misdiagnoses due to the specific constellation of symptoms that can occur and the amount of diseases with similar clinical appearance. The missing olfactory impairment can be explained by either a misdiagnosis or a correct diagnosis of PD with the patient belonging to the 10% of PD patients that have normosmia (based on findings indicating that more than 90% of PD patients suffer from olfactory loss; Haehner et al., 2009a). Possible misdiagnoses could be multiple system atrophy, progressive supranuclear palsy, corticobasal degeneration or secondary
parkinsonism (for detailed information, see 1.5). In case of PD, clinical diagnoses cannot provide 100% accuracy due to missing special diagnostic markers. Diagnostic accuracy varies according to the diagnostic criteria applied. However, it does not reach 100% certainty even in a setting where specialists for movement disorders are involved (Hughes et al., 2002). Only post mortem pathological examination can provide a definite diagnosis.

First, we compared these 13 individuals (ParkCHIP III) to the ParkCHIP II study group to reveal differences between both groups. Secondly, we reviewed their questionnaires in detail in order to detect potential irregularities or indication for alternative diagnoses or abnormalities in recruitment or examination process.

Patients of the ParkCHIP III study group were on average eight years younger than the individuals of the ParkCHIP II study group. With their mean age of 59 years they were younger than the average PD population (Inzelberg et al., 2002). Age at diagnosis was on average 57 years for the ParkCHIP III subjects and 59 years for the ParkCHIP II group; however, the distribution of age was different. The age distribution of normosmic subjects ranged from 45 to 68 years (versus 58 to 73 years among the olfactory impaired individuals). Early-onset of PD is defined differently in literature, with the age threshold being set at either under the age of 40 years or 50 years (Samii et al., 2004). Independent of the threshold, the early occurrence of PD raises suspicion of familial parkinsonism. A twin study has shown that genetics are more likely to be involved in PD with onset below the age of 50 years (Tanner et al., 1999). More recent studies revealed that early-onset autosomal recessive familial PD is often associated with a mutation in the Parkin gene (PARK2) and it was found that within these patients, disease progressed more slowly than in patients without mutation (Lucking et al., 2000). In addition, there is evidence for reduced occurrence of smell loss in some forms of familial parkinsonism. Khan and colleagues (Khan et al., 2004) demonstrated that patients which are Parkin-positive (PARK2) had preserved olfactory function. Missing olfactory impairment could be due to the absence of Lewy bodies in Parkin-associated parkinsonism (Mori et al., 1998; Takahashi et al., 1994) with the Lewy bodies playing an important role in the pathomechanism that lead to smell loss (Hawkes, 2003). Some PARK8 subjects –the most frequent mutation in autosomal dominant parkinsonism presented with olfactory impairment but they also had
Lewy bodies in the rhinencephalon (Silveira-Moriyama et al., 2008). Studies on familial or genetic parkinsonism respectively are rare and mostly conducted in a small study group. Moreover, genetic causes are manifold and cannot be generalized. But with the PARK2 mutation being the most common mutation among recessively inherited early-onset parkinsonism (Hedrich et al., 2004) there is slight evidence that our patients could suffer from this kind of PD. However, in course of the ParkCHIP study we did not conduct genetic testing.

In addition, duration of disease differed significantly between both groups. The differences in actual age and in disease duration could contribute to milder symptoms in the ParkCHIP III individuals since PD is a chronic and progressive disease with the symptoms’ severity increasing with age and disease duration. One could assume that some subjects of ParkCHIP III are in a beginning stage of the disease, especially because the disease severity, evaluated with the Hoehn and Yahr rating scale and the UPDRS is significantly less severe than in the ParkCHIP II study group. The olfactory impairment could be delayed or the disease could progress in another sequence (Burke et al., 2008) than published by Braak and colleagues (Braak et al., 2003). It was found that diagnosis of PD is frequently delayed (Breen et al., 2013); therefore, the age at onset as well as the disease duration might be liable to incorrect information through the patients.

We found statistically significant differences between the groups with regard to best ever response to L-DOPA and the duration of L-DOPA response. In a study by Rajput and co-workers (Rajput et al., 1990) it was shown that 94% of PD patients responded well to L-DOPA. In addition, the excellent, positive reaction to L-DOPA is one of the supportive prospective positive criteria for PD in the UKPDBBC of which three or more criteria are required for diagnosis of definite PD (Gibb and Lees, 1988). Both missing or reduced L-DOPA response as well as decreased duration of response in PD patients can be an indicator for misdiagnosis since, on average, motor symptoms improve after treatment with dopaminergic drugs (Albanese et al., 2001; Calne et al., 1992). In some diseases of the atypical parkinsonian syndromes, reduced response to L-DOPA is common. However, about 20% of patients with multiple system atrophy initially show a good response to L-DOPA and about 13% a sustained response. Positive response to L-DOPA in progressive supranuclear palsy, corticobasal degeneration and
vascular parkinsonism is usually negligible; however, it can occur. For patients with dementia with Lewy bodies, it is typical that they show psychiatric side effects after the intake of L-DOPA; however, motor symptoms can potentially be alleviated (Lang and Lozano, 1998). It has to be taken into consideration that it is not possible to discriminate between multiple system atrophy and PD based on L-DOPA response; however, it is a helpful tool for further differential diagnosis.

Furthermore, we found statistically significant differences between the two groups with respect to non-motor symptoms. Non-motor symptoms increase with age and disease severity; however, some symptoms such as olfactory disturbance, constipation, depression and REM sleep behavior disorder occur early in the course of the disease (Chaudhuri et al., 2005). Since patients of ParkCHIP III have shorter disease duration we should find some non-motor symptoms to be present while others are not. We found statistically significant differences between the two groups for the general assessment of non-motor symptoms with the MDS-UPDRS scale. We evaluated REM sleep behavior disorder and olfactory impairment as early non-motor symptoms and found a statistically significant difference for olfactory ability but not for REM sleep behavior. REM sleep behavior disorder was assessed by the study nurse and was possibly exposed to uncertain and reluctant information through the patient due to this personal topic. We were able to apply olfactory testing to all patients of the ParkCHIP study by Sniffin’ sticks odor identification test and were able to evaluate this non-motor feature thoroughly. Therefore, we cannot draw any conclusion from the assessment of non-motor symptoms, except for olfactory impairment.

The analysis of the questionnaires of the individuals of the ParkCHIP III study group revealed some abnormalities. We found indication that six participants could suffer from early-onset PD (<50 years). Another group of four (one overlap with the previous group) had a positive family history for PD.

Secondary parkinsonism, can be drug-induced with the most common drugs associated with this effect being calcium-channel blocker and anti-dopaminergic drugs like antipsychotic drugs or anti-emetics (Jimenez-Jimenez et al., 1996). Applied to our study, none of the 13 participants took any of the aforementioned medication. However, there are rare cases in which drugs such as anti-epileptic
drugs, choline esterase inhibitors and antidepressants were found to be able to induce parkinsonism (Mena and de Yebenest, 2006). In our study, three subjects took antidepressants. We could assume that parkinsonism in these three individuals could be a consequence of the intake of antidepressant medication (Gony et al., 2003).

We found two individuals with a former cranial trauma with proven damage. Cranial trauma could account for the appearance of parkinsonism in both individuals since head trauma is known to potentially cause secondary parkinsonism (Mendez, 1995).

In one study, vascular parkinsonism was found to usually have preserved olfaction (Lang and Lozano, 1998) so we could assume that this is a further potential diagnosis for some of the individuals of ParkCHIP III. Among the ParkCHIP III individuals, there were five patients that suffer from hypertension. Hypertension is a frequent disease (Wolf-Maier et al., 2003) and a common vascular risk factor. Thus, hypertension can cause vascular parkinsonism (Gupta and Kuruvilla, 2011).

In summary, although some features such as positive family history with respect to PD, hypertension, head trauma, early-onset of PD, or taking special medication overlap among the individuals, for most of the participants of the ParkCHIP III study group we found abnormalities that point towards potential misdiagnosis or alternative diagnosis which in terms could explain for the missing olfactory dysfunction.

However, further diagnostic measures such as neuroimaging would be needed to support our suspected diagnoses.

5.1.8 Crucial benefits of early and correct diagnosis of Parkinson’s disease

Misdiagnosis in PD is a serious matter with severe consequences for the individual as well as the society in terms of socio-economic burden that is caused by the
high direct and indirect costs for PD. In the study by Winter and colleagues (Winter et al., 2010), the economic impact of PD in the German health care system was evaluated.

Based on data from the Federal Statistical Office in Germany (Statistisches Bundesamt, 2015) and the study of Winter and co-workers annual costs of 600,000,000 € are estimated assuming a false positive rate of 10%. This seems to be a valid assumption given the fact that in the ParkCHIP II study group the false positive rate was more than 6%. The underestimation of the false positive rate would be expected since the ParkCHIP study was a study with the elaborate examination of the probands and patients being performed by a specialist of movement disorders. In addition, one focus of the study was the precise diagnostic process of PD.

Schrag and colleagues (Schrag et al., 2002) found false negative cases to sum up to 22%. In 15 out of 69 cases they found that the previous diagnosis not-PD could not be confirmed and the diagnosis had to be corrected to PD as the final diagnosis.

By an early and accurate diagnosis, both the direct and the indirect costs for the patient and for the society/the economy/the healthcare system can be reduced or even avoided. Hence, from both a health care and economic perspective, robust and inexpensive diagnostic tools for the early detection of PD are crucial. In this respect, the olfactory ability of PD patients plays an important role. It was shown that decreased olfactory ability is present years before clinical symptoms such as tremor at rest, postural instability, rigor and akinesia appear (Chaudhuri et al., 2005). Thus, the reduced olfactory ability is one of the important symptoms which could serve as a potential target for diagnosis of PD. Integrating a test for olfaction into clinical routine such as the 16-odor Sniffin’ sticks identification test, or the time-saving, inexpensive, reliable and easy-to apply short version, the 3-odor Sniffin’ sticks test could thus alleviate clinical diagnosis. The costs of a 16-pen including, 18 month durable Sniffin’ sticks test kid are 149 € (personal communication via customer service) compared to costs of neuroimaging of more than 800 € for a PET-CT (German medical fee schedule/Gebührenordnung für Ärzte (online), 2015). Misdiagnosis of PD causes extreme socio-economic burden.
Consequently, a crucial amount of money can be saved by the use of olfactory testing.

In addition, an early and valid diagnosis of PD is fundamentally relevant to the patients in order to apply appropriate therapeutic measures. The disease modifying and potentially neuroprotective therapies are supposed to be applied as early as possible (Lang, 2011; Ravina et al., 2003) with early treatment having the potential of slowing down disease progression (Riederer et al., 2000). As such, the quality of life of patients can be improved and psychological stress due to uncertain or delayed diagnosis can be reduced.
6. Conclusion

The development of PD is accompanied by loss of olfaction. We analyzed the diagnostic role of olfactory testing with the 16 Sniffin’ sticks odor identification test in PD patients compared to HC within the framework of the ParkCHIP study. For this analysis we used a subgroup of all patients subjected to stringent diagnostic criteria (ParkCHIP I). The Sniffin’ sticks test allowed for a quick testing with high specificity (90%) and moderate sensitivity (28%). This simple and cheap test can contribute to the diagnostic workup as a point-of-care test. Even a subset of only three odors consisting of coffee, peppermint and anise out of the full set of the Sniffin’ sticks test yielded a good performance with regard to discrimination between PD patients and HC. We could show that olfactory dysfunction is a robust and frequent symptom in PD and discriminates well between HC and PD.

Furthermore, we analyzed whether self-assessment of olfaction is a reliable question in the anamnesis of patients in the diagnostic workup for PD. In the analysis of the full set of PD patients recruited for ParkCHIP (ParkCHIP II) we found self-assessment to hold the risk for underestimation. Thus, self-assessment of olfactory ability is not a reliable measure for olfactory function and is not an appropriate replacement for olfactory testing.

We found that the olfactory impairment was not associated with PD subtype.

The diagnostic accuracy of PD is still subject of concern. We carefully explored the characteristics of 13 patients with normal olfaction (ParkCHIP III) to find evidence of diagnostic problems. We found a lower response to L-DOPA and characteristics (such as younger age at onset, comorbidities and medical treatment) in the majority of these patients indicating a probable misdiagnosis of PD or special PD cases respectively.

People diagnosed with impaired olfaction but no further symptoms can be easily followed over the years or one can even proceed with more detailed and cost-intensive diagnosis such as neuroimaging. In addition, a more specific assessment
of the potential occurrence of mild motor symptoms can be conducted. Misdiagnosis of PD contributes to high costs for the society/economy and causes profound burden for the patient in terms of missing special treatment and final diagnosis.

With PD being the second most common neurodegenerative disease and its chronic progression of symptoms there are about 300,000 PD patients in Germany who need qualified care and correct medical treatment. Using rough estimates and calculate with a false positive rate of only 10% (30% in literature) we found the annual indirect and direct cost to sum up to more than 600,000,000 € per year. A cheap, non-invasive and easily applicable test as the Sniffin’ sticks test, with high specificity and consequently a little amount of wrong positive results is substantial. In addition, the Sniffin’ sticks odor identification test could even be applied by medical assistants or nurses and does not require a doctor’s presence. With costs for the 16 pen containing Sniffin’ sticks test of 149 € and a minimum durability of 18 month the calculated costs per testing are rather low. It is fundamental for both, the patients and the economy to incorporate olfactory testing in the clinical routine.
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9. Curriculum vitae

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