Parkinson’s disease patients with heterozygous GBA-mutation: longitudinal phenotyping of motor and non-motor symptoms – more rapid progression compared to Parkinson’s disease patients without GBA-mutation
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Für meine Mutter Lucia Pflederer
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<tr>
<td>$^{123}$I MIBG</td>
<td>$^{123}$I-meta-iodobenzylguanidine ligand</td>
</tr>
<tr>
<td>$^{123}$I-FP-CIT</td>
<td>$^{123}$I-2-β-carbomethoxy-3β-(4-iodophenyl)-N-(3-fluoropropyl)nortropane - a cocaine analogue labeled with $^{123}$I (DaTSCAN)</td>
</tr>
<tr>
<td>A53T</td>
<td>Point mutation in alpha-synuclein gene</td>
</tr>
<tr>
<td>AAO</td>
<td>Age at onset</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>ADL</td>
<td>Activities of daily living</td>
</tr>
<tr>
<td>ALP</td>
<td>Autophagy-lysosomal pathway</td>
</tr>
<tr>
<td>APOE</td>
<td>Apolipoprotein E gene</td>
</tr>
<tr>
<td>ATP13A2</td>
<td>ATPase cation transporting 13A2</td>
</tr>
<tr>
<td>Aβ</td>
<td>Amyloid β protein</td>
</tr>
<tr>
<td>BDI-II</td>
<td>Beck depression inventory-second edition</td>
</tr>
<tr>
<td>CBD</td>
<td>Corticobasal degeneration</td>
</tr>
<tr>
<td>CD/CV</td>
<td>Common disease-common variant hypothesis</td>
</tr>
<tr>
<td>CD/RV</td>
<td>Common disease-rare variant hypothesis</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeters</td>
</tr>
<tr>
<td>CMA</td>
<td>Chaperone-mediated autophagy</td>
</tr>
<tr>
<td>COM</td>
<td>Center of mass</td>
</tr>
<tr>
<td>COMT</td>
<td>Catechol-O-methyltransferase gene</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>CTE</td>
<td>Chronic traumatic encephalopathy</td>
</tr>
<tr>
<td>DGN</td>
<td>German Neurological Society: Deutsche Gesellschaft für Neurologie</td>
</tr>
<tr>
<td>DJ-1</td>
<td>Protein deglycase Daisuke-Junko-1</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>DLB</td>
<td>Dementia with Lewy bodies</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>e.g.</td>
<td>Exempli gratia</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>EOPD</td>
<td>Early onset Parkinson's disease</td>
</tr>
<tr>
<td>ER</td>
<td>Endoplasmic reticulum</td>
</tr>
<tr>
<td>ERAD</td>
<td>Endoplasmic-reticulum-associated protein degradation</td>
</tr>
<tr>
<td>ERT</td>
<td>Enzyme replacement therapy</td>
</tr>
<tr>
<td>ESS</td>
<td>Epworth sleepiness scale</td>
</tr>
<tr>
<td>ETC</td>
<td>Electron transport chain</td>
</tr>
<tr>
<td>FAQ</td>
<td>Functional activities questionnaire</td>
</tr>
<tr>
<td>FBXO7</td>
<td>F-Box Protein 7</td>
</tr>
<tr>
<td>F-Dopa/ FDG-PET</td>
<td>18F-fluorodopa/ fluorodeoxyglucose- positron emission tomography</td>
</tr>
<tr>
<td>FR</td>
<td>Functional reach</td>
</tr>
<tr>
<td>GBA</td>
<td>Glucocerebrosidase gene</td>
</tr>
<tr>
<td>GBAP</td>
<td>Beta-glucosylceramidase pseudogene</td>
</tr>
<tr>
<td>GCase</td>
<td>Beta-glucocerebrosidase enzyme</td>
</tr>
<tr>
<td>GCS inhibitor</td>
<td>Glucosylceramide synthase inhibitor</td>
</tr>
<tr>
<td>GD</td>
<td>Gaucher's disease</td>
</tr>
<tr>
<td>GDS</td>
<td>Geriatric depression scale</td>
</tr>
<tr>
<td>GlcCer</td>
<td>Glucosylceramide</td>
</tr>
<tr>
<td>GlcSph</td>
<td>Glucosylsphingosine</td>
</tr>
<tr>
<td>GP_e</td>
<td>Globus pallidus externus</td>
</tr>
<tr>
<td>GP_i</td>
<td>Globus pallidus internus</td>
</tr>
<tr>
<td>GWAS</td>
<td>Genome-wide association studies</td>
</tr>
<tr>
<td>H&amp;Y</td>
<td>Modified Hoehn &amp; Yahr scale</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
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<tr>
<td>HAAS</td>
<td>Japanese Honolulu-Asia Aging Study</td>
</tr>
<tr>
<td>HRV</td>
<td>Heart rate variability</td>
</tr>
<tr>
<td>IGF-1</td>
<td>Insulin-like growth factor 1</td>
</tr>
<tr>
<td>iPSC</td>
<td>Induced pluripotent stem cell</td>
</tr>
<tr>
<td>L444P</td>
<td>GBA mutation with proline at position 4444 instead of leucine</td>
</tr>
<tr>
<td>LBD</td>
<td>Lewy body disorder</td>
</tr>
<tr>
<td>L-dopa</td>
<td>Levodopa (L-3,4-dihydroxyphenylalanin)</td>
</tr>
<tr>
<td>LED</td>
<td>Levodopa equivalent dose</td>
</tr>
<tr>
<td>LIMP-2</td>
<td>Lysosomal membrane protein 2</td>
</tr>
<tr>
<td>LOPD</td>
<td>Late onset Parkinson’s disease</td>
</tr>
<tr>
<td>LRRK2</td>
<td>Leucine-rich repeat kinase 2</td>
</tr>
<tr>
<td>LSD</td>
<td>Lysosomal storage diseases</td>
</tr>
<tr>
<td>MAPT</td>
<td>Microtubule associated protein tau gene</td>
</tr>
<tr>
<td>MCI</td>
<td>Mild cognitive impairment</td>
</tr>
<tr>
<td>mDA</td>
<td>Midbrain dopaminergic neurons</td>
</tr>
<tr>
<td>MDS</td>
<td>Movement disorder society</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>MiGAP</td>
<td>Markers in glucocerebrosidase -associated Parkinson’s disease</td>
</tr>
<tr>
<td>miRNA</td>
<td>Micro ribonucleic acid</td>
</tr>
<tr>
<td>MoCA</td>
<td>Montreal cognitive assessment</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>MRSI</td>
<td>Magnetic resonance spectroscopic imaging</td>
</tr>
<tr>
<td>MSA</td>
<td>Multiple system atrophy</td>
</tr>
<tr>
<td>mTOR</td>
<td>Mechanistic target of rapamycin</td>
</tr>
<tr>
<td>N370S</td>
<td>GBA mutation with serine at position 370 instead of asparagine</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>NAA</td>
<td>N-acetyl-aspartate</td>
</tr>
<tr>
<td>NGS</td>
<td>Next generation sequencing</td>
</tr>
<tr>
<td>NIBP</td>
<td>Noninvasive blood-pressure</td>
</tr>
<tr>
<td>NMS</td>
<td>Non-motor symptoms</td>
</tr>
<tr>
<td>NMSS</td>
<td>Non-motor symptoms scale</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NPI</td>
<td>Neuropsychiatric Inventory</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>NMSQuest</td>
<td>Parkinson's disease non-motor symptoms questionnaire</td>
</tr>
<tr>
<td>PD</td>
<td>Parkinson's disease</td>
</tr>
<tr>
<td>PDD</td>
<td>Parkinson's disease dementia</td>
</tr>
<tr>
<td>PD&lt;sub&gt;GBA&lt;/sub&gt;</td>
<td>Parkinson's disease associated with heterozygous GBA mutation</td>
</tr>
<tr>
<td>PD&lt;sub&gt;Idiopathic&lt;/sub&gt;</td>
<td>Idiopathic Parkinson’s disease</td>
</tr>
<tr>
<td>PD&lt;sub&gt;LRRK2&lt;/sub&gt;</td>
<td>Parkinson’s disease associated with heterozygous LRRK2 mutation</td>
</tr>
<tr>
<td>PDQ-39</td>
<td>Parkinson’s disease questionnaire</td>
</tr>
<tr>
<td>PDSS</td>
<td>Parkinson’s disease sleep scale</td>
</tr>
<tr>
<td>PIGD</td>
<td>Postural instability and gait difficulty PD-subtype</td>
</tr>
<tr>
<td>PINK1</td>
<td>PTEN-induced putative kinase protein 1</td>
</tr>
<tr>
<td>PLA2G6</td>
<td>Gene encoding for an A2 phospholipase</td>
</tr>
<tr>
<td>PRIPS</td>
<td>German Prospective Evaluation of Risk Factors for Parkinson’s Idiopathic Syndrome</td>
</tr>
<tr>
<td>PSP</td>
<td>Progressive supranuclear palsy</td>
</tr>
<tr>
<td>p-syn</td>
<td>Phosphorylated alpha-synuclein</td>
</tr>
<tr>
<td>p-tau</td>
<td>Phosphorylated- microtubule associated protein tau</td>
</tr>
<tr>
<td>PTEN</td>
<td>Phosphatase and tensin homolog (enzyme)</td>
</tr>
<tr>
<td>q-motor</td>
<td>Quantitative motor system</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
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</tr>
<tr>
<td>RBD</td>
<td>Rapid eye movement sleep behavior disorder</td>
</tr>
<tr>
<td>RBDSQ</td>
<td>Rapid eye movement sleep behavior disorder screening questionnaire</td>
</tr>
<tr>
<td>REM</td>
<td>Rapid eye movement</td>
</tr>
<tr>
<td>SCARB2</td>
<td>Scavenger receptor class B member 2</td>
</tr>
<tr>
<td>SCD</td>
<td>Subjective cognitive deficit</td>
</tr>
<tr>
<td>SCNA</td>
<td>Alpha-synuclein gene</td>
</tr>
<tr>
<td>SN</td>
<td>Substantia nigra</td>
</tr>
<tr>
<td>SNARE-complex</td>
<td>Soluble N-ethylmaleimide-sensitive-factor attachment receptor-complex</td>
</tr>
<tr>
<td>SNc</td>
<td>Substantia nigra pars compacta</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>SNr</td>
<td>Substantia nigra pars reticularis</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single photon emission computed tomography</td>
</tr>
<tr>
<td>SRT</td>
<td>Substrate reduction therapy</td>
</tr>
<tr>
<td>SSR</td>
<td>Sympathetic skin response</td>
</tr>
<tr>
<td>ST</td>
<td>Schellong test</td>
</tr>
<tr>
<td>TCS</td>
<td>Transcranial sonography</td>
</tr>
<tr>
<td>TMT-A/ -B</td>
<td>Trail making test-A/ -B</td>
</tr>
<tr>
<td>t-tau</td>
<td>Total- microtubule associated protein tau</td>
</tr>
<tr>
<td>TUG</td>
<td>Timed-up and go test</td>
</tr>
<tr>
<td>UKBBS</td>
<td>United Kingdom Brain Bank Society</td>
</tr>
<tr>
<td>UMSARS</td>
<td>Unified Multiple System Atrophy Rating Scale</td>
</tr>
<tr>
<td>UPDRS-III</td>
<td>Unified Parkinson’s Disease Rating Scale Part III</td>
</tr>
<tr>
<td>UPR</td>
<td>Unfolded protein response</td>
</tr>
<tr>
<td>wt</td>
<td>Wildtype</td>
</tr>
<tr>
<td>α-syn</td>
<td>Alpha-synuclein</td>
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1. Introduction

1.1 Prolog

The etiology of Parkinson’s disease (PD), the second most frequent neurodegenerative disease, is not sufficiently explained up to the present time [1-4]. Remarkably, even approximately 30 years ago, PD was assumed to represent a classic sporadic disease without providing relevant hereditary components [5, 6]. Nowadays, it corresponds to common knowledge that genetic determinants contribute to the development of PD in a substantial proportion of 27% up to 41% [7-11].

Interestingly, patients with homozygous or compound heterozygous mutations in the glucocerebrosidase gene (GBA) are suffering from the most common lysosomal storage disorder (LSD), Gaucher’s disease (GD), and have been reported to develop Parkinsonian symptoms in the course of their disease [3, 6, 12, 13]. Independently, a striking number of PD patients were objectified in the closer familiar environment of GD subjects. Furthermore, studies revealed PD patients to present with a 5-fold increased frequency of being a GBA-carrier with monoallelic mutational status compared to healthy individuals [14]. Meanwhile, more than 100 years have passed since the first independent reports of both PD and GD cases [15]. Only due to precise clinical characterization of patients – suffering from a rare genetic disorder such as GD – and their familiar environment, it was possible to gain insight into the disease course and pathophysiology of a very common disease such as PD [16].

This intriguing link between GBA mutations and the risk for developing PD was further supported by the finding, that GBA mutations also influence the heterogeneous clinical landscape of PD [12]. Specifically, PD with GBA mutations (PD_{GBA}) present with a younger age at PD-onset (AAO), and more pronounced neuropsychiatric impairments such as PD-associated dementia [17-19]. PD_{GBA} reaches major clinical PD-milestones such as postural instability, dementia and death much earlier in the course of disease than PD cases with GBA wildtype (wt) allele [19, 20]. Therefore, an early, accurate and thorough clinical work-up is of highest importance.
These clinical findings may be due to PD’s pathological hallmark, the Lewy body pathology, which commences at a younger age in PD\(_{\text{GBA}}\) and seems to be more widespread in the brain as Neumann et al reported [19, 21].

Knowledge of the underlying pathophysiology is of utmost importance in order to design disease-modifying, targeted therapies for PD\(_{\text{GBA}}\) – which are currently tested in phase-II-trials.
1.2 Parkinson’s disease

1.2.1 PD – epidemiology

After Alzheimer's disease (AD), PD presents as the second most neurodegenerative disease with a prevalence of about one percent of individuals over the age of 60 years [12, 22]. After all, four percent of people get affected with PD during their lifetime [12]. However, data on PD frequency varies, depending on the applied statistical method and the cohort that was examined. The Global Burden of Disease Study, a systematic analysis, showed that the worldwide burden of PD doubled between 1990 and 2016 and it is hypothesized that numbers will double every 20 years reaching 14 million PD patients in 2024 [23]. Presumably, the reason is our ageing society as age itself represents the major risk factor [23]. Males are more often affected than females in a ratio up to 2:1 [24, 25]. However, there also exist protective factors which are summarized in Table 1 below.

Table 1: Features associated with Parkinson's disease.

<table>
<thead>
<tr>
<th>rather protective factors</th>
<th>risk factors</th>
</tr>
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<tbody>
<tr>
<td>smoking nicotine</td>
<td>positive family history</td>
</tr>
<tr>
<td>caffeine</td>
<td>herbicides &amp; pesticides exposure</td>
</tr>
<tr>
<td>exercising</td>
<td>older age</td>
</tr>
<tr>
<td>treatment with NSAID</td>
<td>(metals like manganese or lead)</td>
</tr>
<tr>
<td>urate</td>
<td>(history of CTE)</td>
</tr>
</tbody>
</table>

Left: risk-decreasing factors whereas nicotine consumption is discussed controversially. Right: risk-increasing factors with some aspects put in brackets due to their insufficient evidence. NSAID: Non-steroidal anti-inflammatory drugs. CTE: chronic traumatic encephalopathy. Data taken from [26-33].

Regarding the underlying mechanisms of PD, various etiologies can be differentiated. Heaped, severe occasions of craniocerebral trauma, e.g. contact sports such as boxing, can lead to the development of chronic traumatic encephalopathy (CTE) and late Parkinsonian symptoms [26]. Furthermore, neurotoxins such as the herbicides paraquat and rotenone as well as the fungicide maneb are reported to favor the development of PD [27, 28]. Metals as manganese and lead are suspected to trigger PD [29, 30, 33]. However, the most graving risk factors for PD are age and a positive family history [31, 32]. Genes associated with PD and PD susceptibility loci are discussed separately because 1) of their relevance to the disease and 2) they play an important role in this dissertation.
On the other side, moderate physical activity was associated reciprocally with a lower PD risk [34]. Smoking, urate and caffeine (relative to men consuming up to four cups of coffee per day) appear to be rather protective as well as the treatment with ibuprofen [35-37].

1.2.2 PD – clinical manifestations
PD presents with heterogeneous clinical features. The course is slowly progressive and characterized by motor and non-motor symptoms (NMS).

Further, PD can be divided into several subtypes, with three of them comprising the majority of PD patients: tremor dominant subtype, akinetic-rigid subtype and postural instability subtype [38].

Motor symptoms
The three classic signs of motor impairment in PD are referred to as cardinal symptoms and include bradykinesia, rigidity and resting tremor [39]. Furthermore, postural instability is another important sign which is more of a late symptom [39]. All motor impairments can occur in varying manifestations [39]. When affected by an overall slowing of movements – or bradykinesia – patients often struggle when tying shoes, buttoning a shirt or getting up from a chair [39]. Over the course, bradykinesia can intensify to freezing phenomena or festination [39]. A physician may further detect reduced amplitude and speed of movement during finger tapping and alternating supination-pronation may be limited as well [39].

An abnormally increased resistance to passive movement of a joint is called rigidity, which – as all motor symptoms - usually begins on one side, which keeps being the dominant (more affected) side during the disease [39, 40]. Rigidity can contribute to pain and reduced swinging of the arms while walking [39]. Associated with rigidity may be a cogwheel phenomenon in which the examined joint moves jerkily and stops intermittently [39, 41]. Alternatively, a lead pipe effect may occur – presenting with rather sustained tonic resistance during the enduring flexing movement [39, 41]. In contrast to spastic dysfunctions, rigidity is independent of the applied speed of passive movement and is best tested in passive slow movements.
The term tremor describes a rhythmic and involuntary movement of a body part, which leads to oscillating motion [42]. It is caused by synchronous or asynchronous muscular actions [42]. Most PD patients present with a resting tremor but some may show an additional action tremor, the latter occurring mainly as a postural tremor [43]. The typical PD-tremor is an asymmetrical rest tremor, which predominantly manifests when the corresponding body part is not performing any action at all and it is also referred to as a pill-rolling tremor (frequency 4-5 Hz) [39, 44].

A patient with postural instability tends to have a misperception of his position in the room – resulting in balance disturbances which may lead to falls which occur more frequently than in a healthy person [39]. As already mentioned, this symptom is more likely to be associated with later stages in PD [39]. In patients who show this aspect very early and report frequent fall events, atypical Parkinsonian disorders, such as progressive supranuclear palsy (PSP), should be considered [39]. Remarkably, an axial deficit such as postural instability is linked to disability and is associated with limited quality of life [39, 45].

In addition, there are other motor symptoms that can occur at varying degrees and times in the course of the disease [39]. Many of these aspects, mentioned in Figure 1 below, are associated with one of the cardinal symptoms stated above.

**Non-motor symptoms (NMS)**

- **craniofacial**: dysphagia, dysarthria, sialorrhea, hypomimia, reduced blink rate
- **visual**: impaired upward gaze, eyelid opening apraxia
- **musculoskeletal**: kyphotic posture, scoliosis, dystonia, micrographia
- **gait**: freezing, small step gait, increased number of turning steps

*Figure 1: Relevant motor impairments in PD*  
Classified into groups according to their localization and symptomatology: craniofacial, visual and musculoskeletal impairment or gait disturbance. According and adapted to [39].
In the past, PD was considered primarily as a movement disorder with pure motor features due to a disturbed dopamine transmitter system, but there is evidence that it rather corresponds to a multisystemic disorder – affecting also other circuits of cholinergic, noradrenergic and serotonergic neurotransmitters [46]. This leads to a huge variety of NMS, which – at least in part - occur very often well before the onset of motor impairment and include autonomic dysfunctions, impaired mood such as depression or apathy, cognitive decline as one of the most important ones encompassing all stages from mild cognitive impairment (MCI) to mild, moderate and severe forms of Parkinson's disease dementia (PDD), further sleep impairments and fatigue, visual impairment such as disturbed contrast sensitivity and also olfactory impairment may occur [17, 46-49]. In the following section, the most important NMS are explained in detail.

Autonomic Dysfunction

Autonomic dysfunctions include constipation, orthostatic hypotension, as well as urinary and sexual dysfunction and also somnolence [50]. In particular, orthostatic hypotension may be worsened by PD drug regimens [39].

Mood disturbance

Among the most serious NMS are mood disorders: such as depression, apathy and anxiety. Depressive dysfunctions in PD affect the quality of life and are often not adequately treated, especially in later disease stages [39, 51, 52]. The fact, that depression can lead to psychomotor slowdown and flattening of emotions on the one hand, and that PD, on the other hand, is characterized by bradykinesia, hypomimia and sleep disorders, can enhance the difficulty in diagnostic attribution.

In addition, anxiety disorders occur in up to 31% of PD patients, with generalized anxiety disorders being one of the most common disturbances [53]. Apathy as a symptom can occur with and without depression and is characterized by decreased motivation with a pooled frequency of about 40% due to a meta-analysis of den Brok et al [39, 54, 55].
Cognitive decline and dementia in PD

If patients themselves or their relatives notice cognitive deficits while professional cognitive testing is inconspicuous, the term subjective cognitive deficit (SCD) is used [56]. Although there is a higher risk for further cognitive decline in SCD subjects in the general population, there are still no established criteria for confirming the diagnosis [46]. About 10-20% of PD patients show symptoms of PD-MCI, a more pronounced form of cognitive impairment compared to SCD, at time of PD diagnosis, while PD-MCI is associated with shortened but variable time to the onset of PDD [57]. After 10 years of illness between 75% and 90% of PD patients present with this subcortical dementia as a complication [58-60]. Due to growing life expectancy in western countries, this proportion can be expected to continue to increase [59].

Clinically, PDD presents with executive dysfunction, impaired working memory and reduced attention and visuo-spatial performance [47, 61].

The separation between the categories SCD, MCI and PDD is not strict [47]. However, only in PDD an impairment of activities of daily life in terms of social and professional interaction is defined – independent of motor and autonomic aspects [47]. Risk factors for a rapid cognitive decline are increasing age and severe, especially non-tremor-associated PD-symptoms [46, 57].

Furthermore, the clear separation between PDD and dementia with Lewy bodies (DLB) is often challenging in everyday clinical practice and poses a phenotypical and biological continuum with Lewy-body pathology in both disease entities [39].

The diagnosis of DLB may be given if the dementia aspect coincides with the onset parkinsonism or occurs even before. In contrast, PDD should be diagnosed if dementia arises in the context of established PD [62]. For research studies, it was formerly recommended to consider the so-called 1-year rule, (motor impairment exists already 12 months or longer before dementia occurs) [62]. However, many authors consider this rule to be rather arbitrary and not the most sensible way to differentiate between the two entities PDD and DLB [63].
Sleep disorders
Another subgroup of NMS in PD comprises various sleep disorders, namely insomnia, daytime sleepiness and rapid eye movement (REM)-sleep behavior disorder (RBD) [39, 64-67]. Most commonly, patients report to wake up at night several times and to wake up very early in the morning as well [39]. Possible reasons for frequent awakening during night sleep may include nocturia, pain, depression and nightmares as well as immobility and thus less turning movements [39, 67-69].

RBD is a specific sleep disorder in PD, in which patients perform movements such as kicking or pounding during their sleep - due to the fact physiological loss of muscle tone is not present in RBD patients, so the dreams can be lived out [39, 70]. A study by Sixel-Döring et al revealed the frequency of RBD in PD to affect up to 46% of patients [70].

Furthermore, up to three quarters of PD patients are affected by excessive daytime somnolence [71]. The reasons for this are manifold and are considered to be both disease-inherent and also induced by dopaminergic therapy or nocturnal sleep dysfunctions [39, 67, 72, 73].

Visual performance
PD patients may show visual acuity and color discrimination limitations as well as contrast sensitivity deficiencies [39, 74]. In addition, disturbances of saccades are possible [39, 74].

Olfactory disturbance
Dysfunctions of the olfactory performance can affect the odor detection, the odor discrimination or the odor sensitivity [75]. However, PD patients often do not realize the reduced olfactory performance [39]. Since olfactory impairments do not only occur in early PD clinical stages but can precede motor symptoms of PD for a long time, patients with idiopathic olfactory dysfunctions are associated with an increased risk for PD [76, 77]. This is why trials often focus on these subjects in prodromal PD studies in order to develop screening tools for evaluating their risk of developing PD later.
1.2.3  PD – from preclinical to prodromal and clinical PD stages
Currently, PD is subdivided into several disease stages that follow one another: the preclinical period is followed by the prodromal phase which in turn is followed by the clinical PD-stages [50]. This delineation expresses that the beginning of PD is not to be equated with the occurrence of motor deficits but that neurodegenerative changes already arise years to decades before [50]. It is assumed that in prodromal PD stage, a disease-modifying neuroprotective therapy – preventing the transfer to clinical stages – could be possible and it is therefore relevant to reliably recognize these early PD stages [50]. According to the classification given by the Movement Disorder Society (MDS) task force,

Figure 2 illustrates the 3-phase model of PD on the next page.
Figure 2: The Parkinson’s disease stages over time. Beginning from potential people at risk to preclinical stage without any clinical signs and further to prodromal phase with varying temporal extent. The clinical PD stage is reached when bradykinesia with rest tremor or rigidity or both are present. The pointed triangle on the right marks the progressive loss of dopaminergic neurons in the substantia nigra. Notably, about 40-60% of nigral neurons might be already lost at the prodromal PD stage – although motor deficits are not obligatory at this time. PD: Parkinson’s disease. SN: substantia nigra. According to the MDS task force [50, 78].

According to this terminology, people at risk for the development of PD show no signs of neurodegeneration [50]. Though, these individuals may present with a genetic composition or may be exposed to a certain environment, associated with increased risk for PD [50]. The challenge of diagnosing preclinical PD requires the positive detection of appropriate bio- or imaging markers, that have still not been validated up to the present time – according to the author’s knowledge [50].
The prodromal PD stage is characterized by progressive neurodegeneration, which may affect the substantia nigra (SN) but does not necessarily result in motor impairment [50].

It is estimated that up to 60% of dopaminergic neurons are destroyed before PD becomes clinically significant [50, 79]. Although, distinct motor deficits are possible, they may be so subtle that they cannot be distinguished from signs of the normal aging processes [50]. Moreover, most of the NMS, which frequently dominate the early PD stages, are fairly unspecific and making it difficult in general to meet PD criteria for diagnosis [50].

1.2.4 PD – prodromal stage and associated markers

Prodromal PD can be described by evidence-based markers, which, however, have to fulfill certain criteria [50]. Therefore, in 2015 Postuma et al defined four criteria for a suitable use of these prodromal markers which are listed in Table 2 below:

| 1. The strength of evidential value of the marker must be known and sufficiently large [50] |
| 2. The specificity or positive predictive value of the marker should be captured as it varies between the individual markers currently used [50] |
| 3. The time between the detectability of a marker and the onset of clinical PD – the marker’s lead time - is of great importance [50] |
| 4. Collecting the marker for a sufficiently large sample must be feasible without going beyond the technical and financial opportunities [50] |

As suggested by Postuma et al in 2015 [50].

In order to investigate prodromal PD-markers, prospective studies such as studies in PD-high-risk groups (due to their statistical power) and population-based studies (according to their unselected, large sample sizes) are assumed to be most appropriate [50]. Examples for population-based examinations include the Japanese Honolulu-Asia Aging Study (HAAS) and the German Prospective Evaluation of Risk Factors for Parkinson’s Idiopathic Syndrome (PRIPS) study [80, 81]. One disadvantage of population-based studies comes about due to the low frequency of PD which in turn requires the screening of relatively large samples over many years to filter out a relatively small number of PD cases – which makes
it time- and cost-intensive [50, 80]. Many conclusions drawn from high-risk studies, which can be generalized only to a limited extent to the overall population, are derived from patients with idiopathic RBD [50, 82].

Multiple times, RBD has been reported to occur in more than 45% of PD-cases and further it seems to be a prodromal marker for synucleinopathy – a neurodegenerative disorder like PD or multiple system atrophy (MSA) – characterized by aberrant accumulation of the phosphoprotein α-synuclein (α-syn) in neuronal or glial cells in general: over 80% of RBD patients developed a synucleinopathy within 10 years since being diagnosed with RBD [83-85].

Findings, such as RBD not representing an exclusive marker for PD, and reports about olfactory disturbance, that can also occur in prodromal AD, require further research in the field of prodromal PD [84, 86]. Today, according to Postuma et al, a combination of several proven clinical markers can be ascertained as Figure 3 illustrates on the next page [50].
Introduction Parkinson’s disease

Figure 3: Overview of the essential markers of prodromal Parkinson’s disease. RBD = Rapid eye movement sleep behavior disorder. PD: Parkinson’s disease. RBD has the highest specificity of current proven markers for prodromal PD. Subtle motor dysfunction presents with high specificity as well and can be measured with the semi-quantitative Unified Parkinson’s disease rating scale. Olfactory impairment affects about 80% of all Parkinson patients, which is why this marker is assumed to be high-sensitive. Autonomy dysfunctions comprise many subfields with rather high prevalence and accordingly rather low specificity. Mood disorders are supposed to correlate with a rather low positive predictive value for PD.

These proven prodromal markers are further elucidated in the next section.

Proven prodromal markers (clinical investigation)

Rapid Eye Movement behavior disorder (RBD)

RBD, which leads to enacted dreaming and can be diagnosed by polysomnography, has the highest specificity of all prodromal PD markers with a rather moderate sensitivity (see also Figure 4 below) [50, 87].

This marker can be clinically evaluated by RBD screening questionnaire, whereas a single-question (“Have you ever been told, or suspected yourself, that you seem to act out your dreams while asleep (for example, punching, flailing your arms in the air, making running movements, etc.)?”) ascertained RBD with a specificity higher that 87% [50, 88].
Subtle motor disturbances

Individuals of the general population with subtle motor disturbances were investigated by using the Unified Parkinson's Disease Rating Scale (UPDRS), and it could be shown that a score higher than 0 points already correlated with a slight PD-risk and a UPDRS score higher than 4 points was associated with an almost tripled relative risk [50, 89, 90].

Olfactory disorders

Several studies have shown the relevance of olfactory dysfunction as prodromal PD marker [76, 91], with a specificity lower than that of both RBD and subtle motor dysfunctions but greater than that of various other clinical indicators and therefore of moderate degree [50]. As already mentioned, olfactory disturbances also occur in advance of other neurodegenerative diseases (such as AD and DLB) [78]. Since hyposmia characterizes a non-motor deficit in 80% of PD patients, it is to be assumed that the sensitivity of olfactory dysfunction as a prodromal marker is greater than that of many other markers [50].

Autonomic dysfunctions

**Constipation** as an autonomic dysfunction was associated with increased PD risk – although the positive value is low due to a prevalence of up to 20% in the general population and the time to PD onset may take more than 20 years [89, 92, 93]. α-syn – a presynaptic phosphoprotein that can abnormally accumulate in e.g. neuronal cells, glia cells and nerve fibers – will be further elucidated due to its outstanding importance for pathogenesis in PD (see 1.2.6) [94]. At this point, it should only be mentioned, that α-syn can also be detected in the enteric system with increased levels (especially in the vermiform appendix) and studies further suggested α-syn’s ability to spread: there is a hypothesis that α-syn-spreading may be favored by a prolonged transit time in the gut – which could eventually contribute to PD-development, therefore [95, 96].

In addition, **orthostatic hypotension** has also been associated with an increased PD-risk, although the sensitivity is probably rather low, as only 10% to one third of patients with early PD stages are suffering from this autonomic dysfunction subtype [97, 98]. Increased PD risk is also associated with urinary
impairment but the specificity is low, as there are other common diseases associated with it – e.g. prostate hypertrophy [50, 97].

**Severe erectile dysfunction** was associated with PD risk in a study without providing information regarding **sexual dysfunction in women** [99]. Due to the low number of conducted studies and the high prevalence in the population, Postuma et al evaluated this marker as low specific as well [50].

Somnolence can also be grouped into autonomic dysfunctions [50]. It is mainly observed in late PD clinical stages and in PDD, with two studies, including the Honolulu-Asia Aging Study, confirming an increased risk of PD associated with daytime sleepiness [50, 100, 101].

**Mood disorders**

Mood disorders, such as depression and anxiety disorders have been reported to correlate with a relative PD risk of 1.5 with an assumed low positive predictive value [102, 103].

Recently, a first update of the research criteria for prodromal PD showed, that in addition to the just mentioned criteria, 4 other prodromal and risk markers are also relevant: (1) type 2 diabetes mellitus, (2) cognitive impairment, (3) low plasma urate levels (<5 milligram/dL) in male individuals and also (4) physical activity (less than one hour weekly of activity causing increased heart and breathing frequency) [104-111]. Especially regarding the last aspect, a meta-analysis of prospective studies showed that physical activity is associated with a lower PD risk and, vice versa, that inactivity can be interpreted as a PD risk [104]. Concerning the prodromal marker cognitive impairment, discrete changes in cognition were already found at time of PD diagnosis – suggesting, that pathological processes may already occur during the prodromal phase [112, 113] and that DLB without Parkinsonian symptoms might be a prodromal PD aspect, according to the altered MDS criteria [1, 50, 78]. **Figure 4** on the next page gives an overview of the markers mentioned:
Potential prodromal markers (clinical investigation)

In addition to the established prodromal markers described above, further potential clinical aspects are being investigated, regarding their predictive value for PD. Promising, but not in use yet, however, is color vision loss [50]. Limited color perception, especially yellow / blue differentiation, has been demonstrated in many PD studies using the Farnsworth-Munsell 100 Hue Test instrument [50, 114].

Although the etiology for this is not fully understood, it is currently considered to be more likely a consequence of cognitive impairment, implying that the 100 Hue test may stronger reproduce cognitive deficits than isolated reduced color discrimination [115]. This may be strengthened by the fact, that the Farnsworth-Munsell 100 Hue test demonstrated a higher predictive value for DLB than for PD patients without an impaired cognition [84, 116].
**Neuroimaging markers**

The methods of imaging of the dopaminergic system using positron-emission tomography (PET) or single-photon emission computed tomography (SPECT), as well as transcranial sonography (TCS) of the SN and specific measurements using magnetic resonance imaging (MRI) - are promising to detect adequate prodromal PD-markers of the brain [50]. Moreover, scintigraphy-based investigations of the heart may also be promising [50].

At time of diagnosis of PD, PET and SPECT imaging techniques show an advanced dopaminergic denervation of more than 50% in the dorsal striatum, suggesting that a reduced innervation might already be detectable before [50, 79]. Subjects with reduced dopaminergic innervation were more frequently reported to develop other prodromal markers such as constipation and olfactory dysfunctions [117] and about 40% of RBD individuals showed conspicuous dopaminergic neuroimaging findings in further investigations [50, 118].

In PD, the SN is in about 90% of the affected patients hyperechogenic on transcranial ultrasound even in early stages and revealed a 20-fold enhanced PD risk in healthy individuals older than 50 years relative to the general population [90, 119]. Nevertheless, no convincing reports are present whether nigral hyperechogenicity is more likely a marker for high-risk patients or for prodromal PD [50]. In any case, it is an inexpensive and fast method but also requires an experienced examiner and appropriate examination conditions, which means a proper bone window [50].

Furthermore, in studies with RBD subjects, the methods (1) diffusion tensor imaging, (2) MRI functional connectivity, (3) $^{123}$I-meta-iodobenzylguanidine (MIBG) cardiac scintigraphy, and also (4) MRI volumetric analysis with measurement of cortical thickness were confirmed to possibly supply prodromal markers for synucleinopathies as PD [120-123]. Statements about the extent of their positive predictive values, the respective lead times and also specificity and sensitivity are not reliable yet [50].
Biomarkers for preclinical and prodromal PD

In addition to the elucidated clinical markers above, biomarkers can be obtained through different sources such as tissue specimens as well as blood and cerebrospinal fluid (CSF) samples [50]. The assignment preclinical is also included, as the following markers cannot always clearly be classified into preclinical or prodromal [50].

Peripheral tissue samples

Although only postmortem studies allow a definitive PD diagnosis, histopathological methods objectify neuronal loss and α-syn deposits already in the premotor phase [89, 124]. A Danish pathology study showed that gastrointestinal biopsies with α-syn deposits were more common in subjects with prodromal and clinical PD than in healthy individuals [125]. Besides that, it was previously described that phosphorylated α-syn (p-syn) within nerve fibers in the skin may be a potential biomarker even in early PD stages [126].

However, α-syn staining of enteric tissues is not yet sufficiently documented to be used routinely [50].

The updated MDS Research Criteria for prodromal PD also criticize the wide range of factors such as the number of tissue samples taken, the location of the sampling and different biopsy techniques – influencing sensitivity as well as specificity [104].

Peripheral blood samples: insulin-like growth factor 1-level

A cross-sectional clinical study compared patients with Idiopathic Parkinson’s disease (PD_{idiopathic}) (n=15) without previous pharmacological treatment with healthy elderly controls including a control-subgroup with PD-at-risk subjects (n = 11) [127]. The latter presented with conspicuous results in Unified Parkinson’s Disease Rating Scale Part III (UPDRS-III) as well as nigral hyperechogenic findings in TCS [127]. Significantly higher levels of insulin-like growth factor 1 (IGF-1) were found in the PD_{idiopathic} group compared to controls in general but no significant differences could be objectified between PD_{idiopathic} subjects and PD-at-risk participants [127]. The authors concluded that serum IGF-1 levels may help to identify potential PD risk patients [127].
Nevertheless, prodromal markers from peripheral blood are not well documented in terms of specificity and sensitivity at the present time, even not for clinical PD [50].

Cerebrospinal fluid samples: Increased fructose, mannose and threonic-acid-levels

Results of a recently published biomarker study, examining CSF from therapy-naive PD subjects in early disease stages, demonstrated PD-specific metabolic alterations after comparing the PD-CSF profile with matched controls [128]. The detected molecular profile (comprising increased levels of fructose, mannose and threonic acid) was associated with (1) inflammation, (2) antioxidant stress response and (3) glycation (non-enzymatic attachment of a sugar molecule to a lipid or protein molecule) [128]. Trezzi et al recommended to add these CSF-markers to increase the accuracy of clinical diagnosis [128].

In conclusion, since there are still no preventive therapies for PD, the knowledge of the extent of prodromal symptoms is of enormous importance. Only via thorough clinical detection of prodromal PD, potentially at-risk individuals can be recognized and included in clinical studies which hopefully lead to a better understanding of the underlying pathology and deliver insight into how PD progression is characterized [82, 129]. The clinical PD-stages with the corresponding diagnostic criteria will be elucidated in the following section.

1.2.5 PD – clinical diagnostic criteria

The PD criteria according to MDS portrayed here are primarily suitable for clinical research but can also be used in clinical routine for the diagnosis [1]. The step-by-step approach to PD diagnosis, according to the MDS Clinical Diagnostic Criteria for PD, starts with examining whether Parkinsonism is present or not [1].

Parkinsonism by definition is bradykinesia with (1) rigor or (2) resting tremor or (3) both [1]. Figure 5 below further elaborates the required criteria for Parkinsonism which can be objectified by the UPDRS-III.
These Parkinsonism-criteria should support the distinction between nonspecific parkinsonism, presented by up to a quarter of all elderly individuals without PD diagnosis, and PD-induced parkinsonism [1]. Bradykinesia in PD-induced parkinsonism, for instance, comprises slowness but also partial decrease in motion amplitude or in velocity when movements are continued – which is objectified less common in other etiologies [1].

Another goal aimed at by these criteria is to clearly differentiate between PD and Parkinsonism due to other reasons (other forms of neurodegeneration or secondary Parkinsonism) [1]. So, the next step is to determine, if PD is present or if other causes for Parkinsonism are much more likely: such as PSP, subcortical arteriosclerotic encephalopathy, MSA or essential tremor [1]. Postuma et al defined these supportive criteria, listed in Table 3 on the next page, in 2015 in addition to absolute exclusion criteria and red flags – which are listed in the manuscript [1]. Conferring to that, a patient meets the MDS-PD criteria for clinically established PD if

- at least 2 out of 4 supportive criteria are given,
- there are no absolute exclusion criteria and
- there are no red flag features [1].

The patient fulfills the diagnostic criteria for merely clinically probable PD if (1) there are no absolute exclusion criteria and if (2) a maximum of 2 red flags persists with a minimum of 2 supportive criteria balancing them [1]. The presence of more than 2 red flags excludes a clinically probable PD [1].

---

**Figure 5: Criteria for Parkinsonism.**

Bradykinesia is obligatory and may be accompanied by either rigidity and/or rest tremor. Below these three major criteria, several clinical aspects are listed in order to support detection of PD-related Parkinsonism on the one hand and rather non-specific Parkinsonism on the other hand. According to Postuma et al [1].

<table>
<thead>
<tr>
<th>Bradykinesia</th>
<th>Rigidity</th>
<th>Rest tremor</th>
<th>Parkinsonism</th>
</tr>
</thead>
<tbody>
<tr>
<td>slow movements</td>
<td>speed independent resistance</td>
<td>4-6 Hertz tremor in fully resting limb</td>
<td>Parkinsonism</td>
</tr>
<tr>
<td>decrement of speed/amplitude</td>
<td>eventually additional cogwheel phenomenon</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Table 3: Supportive Criteria for Parkinson's Disease. |

### Supportive Criteria

<table>
<thead>
<tr>
<th>Supportive criteria</th>
<th>Absolute exclusion criteria</th>
<th>Red flags</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Unambiguous benefit of huge amplitude to dopaminergic therapy</td>
<td>1. Clear cerebellar abnormalities in the investigation</td>
<td>1. Rapid development of gait disorder, use of wheelchair within 5 years since onset</td>
</tr>
<tr>
<td>2. Detection of dyskinesias induced by L-dopa</td>
<td>2. Selective slowing of downward vertical saccades or vertical supranuclear gaze palsy downwards</td>
<td>2. No increase in motor impairment in a minimum 5-year period unless therapy prevents this</td>
</tr>
<tr>
<td>3. Former or current evidence of resting tremor of an extremity in clinical investigation</td>
<td>3. Diagnosis of primary progressive aphasia or frontotemporal type of dementia within first 5 years of disease</td>
<td>3. Early onset severe bulbar syndrome within 5 years since onset</td>
</tr>
<tr>
<td>4. Pathological findings in min. 1 auxiliary diagnostic tests as: a. Impaired smelling ability b. Cardiac sympathetic denervation in metalidobenzylguanidine scintigraphy</td>
<td>4. Parkinsonian symptoms only in lower limbs for ≥ 3 years</td>
<td>4. Inspiratory respiratory disorder (a and/or b) a. Frequent inspiratory sighs b. Diurnal or nocturnal inspiratory stridor</td>
</tr>
<tr>
<td>5. Therapy with dopamine receptor blocker or a dopamine-depleting agent consistent with drug-induced parkinsonism</td>
<td>5. Severe autonomic impairment within 5 years since onset a. Hypotension b. Urinary incontinence/retention</td>
<td></td>
</tr>
<tr>
<td>6. No response to high dose Levodopa ≥ 600mg/day despite moderate disease severity</td>
<td>6. Falls due to balance disturbance within 3 years since onset</td>
<td></td>
</tr>
<tr>
<td>7. Clear cortical sensory loss</td>
<td>7. Anterocollis or contractures of hand/feet within 10 years since onset</td>
<td></td>
</tr>
<tr>
<td>8. Inconspicuous neurometabolic imaging of presynaptic dopaminergic system</td>
<td>8. No typical NMS within 5-year period since onset (i.e. hyposmia, depression, RBD)</td>
<td></td>
</tr>
<tr>
<td>9. Alternative syndrome is more likely than PD (DLB is no alternative syndrome)</td>
<td>9. Unexplained pyramidal tract signs</td>
<td></td>
</tr>
<tr>
<td>10. Bilateral symmetric symptoms</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In order to meet the supportive criterion of profoundly benefiting from dopaminergic therapy (first column), patients should have reached or almost completely regained their baseline status which may be objectified by an UPDRS-III score improvement by more than 30% [1]. Only moderate response to therapy is not sufficient [1]. Rest tremor of a limb is a supportive criterion, because it is less frequently found in other Parkinsonian syndromes and thus more likely to propose PD [1]. At the same time it is often less responsive to dopaminergic therapy, which makes it challenging for especially the tremor-dominant subtype to fulfill the first supportive criterion for PD [1]. Furthermore, for the auxiliary tests mentioned in the 4th supportive criterion, three or more studies from different centers could confirm a specificity of more than 80% for the applied method [1].

Exclusion Criteria
If one of the 9 absolute exclusion criteria is present, the diagnosis PD should be rejected, unless another independent disease clearly causes the corresponding symptom [1]. For exclusion criteria with a time frame, such as tauopathy-associated dementia within 5 years since onset of the disease (third criterion) for example, there is no need to wait and see if the symptom will still occur within that time period – especially if all other PD-criteria are fulfilled [1]. The 7th exclusion criterion, clear cortical sensory loss, implies an existing progressive aphasia or an unambiguous ideomotor apraxia of a limb [1]. The task force, which developed the MDS criteria for PD, also points out that functional neuroimaging is not a compelling diagnostic tool for PD and that diagnosis can be given without it [1]. Finally, regarding 9th exclusion criterion, MSA or PSP do represent alternative Parkinsonian syndromes but DLB does not [1].

Red Flags
A severe bulbar syndrome according to the third red flag criterion may be determined by severe dysarthria or dysphagia [1]. It requires a nasogastric or gastrostomic feeding tube and corresponds to an UPDRS score of 4 for dysarthria and 3 or more for dysphagia [1]. Although autonomic dysfunctions are a typical PD aspect, however, severe autonomic dysfunction with massive hypotension and urinary impairment – not explained by medication, volume depletion or prostate disease – are rather typical for MSA and therefore named as a red flag [1].
Frequent falls more than once a year are considered as a red flag as well, unless they have been caused by loss of consciousness or healthy people would have fallen as well – due to situational circumstances [1]. Lastly, pyramidal signs are red flags unless they present with a slight reflex asymmetry in favor of the affected side – common in PD – or an isolated extensor plantar response which can be found according to a striatal toe in PD as well [1]. Postuma et al validated these MDS clinical diagnostic criteria for PD from 2015 and reported higher levels of specificity and sensitivity than the United Kingdom Brain Bank criteria of 1988 [130]. The diagnostic criteria for clinically probable PD showed a sensitivity of over 94% and specificity of over 88% [130].

In order to be able to provide patients with potentially disease-modifying therapies, the diagnosis must be made as early and as correctly as possible. Therefore, Berg et al developed clinical criteria for this early PD phase, also called “Clinically Established Early PD”, which can be used in clinical trials with high specificity (> 95%) and moderate sensitivity (about 70%) [131]. Concluding, the diagnosis of PD is based on core criteria representing clear motor symptoms [82]. These may be preceded by NMS with an architecture varying in scope and intensity but the following impairments are common: neuropsychiatric aspects such as depression, constipation, hyposmia and sleep disorders such as RBD [82].

1.2.6 PD – pathophysiology: nigral and extra-nigral characteristics

**Motor loop of the basal ganglia**

The basal ganglia (BG) are located in the basal telencephalon and consist of the striatum (putamen and caudate nucleus) and the globus pallidus externus (GP_e) and internus (GP_i) – with some authors adding the SN (mesencephalon) with pars compacta (SN_c) and reticularis (SN_r), the thalamus (diencephalon) and the subthalamic nucleus (diencephalon) (STN) as well [132].

The BG process motor information from cortical regions, such as the premotor and supplementary motor cortex, and are crucially involved in voluntary motor
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function – especially in fine motor skills [132]. Figure 6 attempts to present a simplified model of the BG function:

![Figure 6: Simplified model of basal ganglia circuits. The basal ganglia affect fine motor skills by processing cortical impulses, which mainly arise in the striatum. Summarized, it is shown in here that the direct route via D1 leads to a lesser inhibition of the thalamus, which consequently sends more movement-promoting, exciting impulses to the cerebral cortex. The indirect pathway via D2, on the other hand, leads to a disinhibition of STN via an inhibition of GPe, which ultimately results in an increased inhibition of the thalamus and thus a rather movement-inhibiting information to the cortex. D1: dopamine-1-receptor. D2: dopamine-2-receptor. GPe: globus pallidus internus. SNr: substantia nigra pars reticularis. SNc: substantia nigra pars compacta. GPe: globus pallidus externus. STN: subthalamic nucleus. Orange arrows: excitatory effect. Blue arrows: inhibitory effects.](image)

The striatum is reached via excitatory afferents from cortical areas and is also influenced by dopaminergic excitatory (via D1 receptor) and inhibitory (via D2 receptor) stimuli from SNc [132]. In striatum, the direct pathway begins which is triggered by dopamine: it inhibits Gpi and SNr, which consequently inhibit the thalamus to a lesser degree and enable thalamocortical excitatory stimuli – this promotes enacting of movements [132].

Furthermore, the indirect pathway starts from striatum as well, which is inhibited by dopamine, however: via the indirect pathway, striatal D2-associated neurons inhibit GPe [132].

As a consequence, this diminishes STN-inhibition. Through this disinhibition, the STN stimulates both Gpi and SNr more intensely: these inhibit the thalamus in
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turn, which is why the indirect pathway has a rather movement-inhibiting effect [132]. It is important that dopamine promotes the direct pathway via striatal D1 receptors and inhibits the indirect pathway via striatal D2 receptors, thus acting as a whole as inducing movement [132].

In PD, there is a progressive decline of dopaminergic neurons in SNc and therefore, the direct pathway, promoting movement, is impaired, while at the same time the indirect, movement-inhibiting pathway is intensified [132]. Thus, thalamus increasingly receives inhibitory impulses from GPi and SNr and stimulates cortical motor areas to a smaller degree – causing movement inhibition with symptoms like hypomimia or bradykinesia [132]. This model is supported by the fact that motor symptoms usually respond well to the dopamine precursor L-3,4-dihydroxyphenylalanine (L-dopa)-therapy [48].

At the time when the disease defining PD motor symptoms occur, 60% of nigral neurons are already degenerated [133]. Nevertheless, as the major part of NMS and also some motor symptoms, such as postural instability or tremor, are less responsive to L-dopa, it seems likely that there could be additional extra-nigral neuropathological involvement [48].

And in fact, the locus coeruleus in the pons as well as the dorsal vagal nucleus in the medulla oblongata show signs of neurodegeneration already at early stages in PD subjects, too [134]. Finally, brain atrophy of PD patients witnesses the involvement of the whole brain.

Lewy Body pathology

Cross-sectional postmortem studies performed with brain samples of PD-patients suggested, that PDidiopathic is associated with the formation of inclusion bodies in certain types of neurons that appear to be specifically susceptible to it [124]. These small intraneuronal inclusion bodies mainly consist of the protein α-syn [124] but over 500 other proteins are possible components of these so-called Lewy formations as well [135-137]. α-syn is physiologically located in the cytoplasm of axons and their presynaptic contacts [124].
The protein plays a role in formation of vesicles, it affects the curvature of their membrane and α-syn mediates the presynaptic release of neurotransmitters into the synaptic cleft by binding to the soluble N-ethylmaleimide-sensitive-factor attachment receptor-complex (SNARE-complex) [138, 139]. For reasons that remain still unclear, α-syn can alter its tertiary structure, thereby losing its physiological binding to the cell membrane and consequently lying freely in the cytosol [140, 141]. There, α-syn can form different subtypes of intracellular aggregates which may contribute to formation of elongated structures, Lewy neurites, and the roundish Lewy bodies [124, 141].

In conclusion, the term synucleinopathy describes abnormal accumulation of α-syn in fibrillated configuration, located in the brainstem or in cortical regions [142]. Lewy body pathology is considered to be the pathological hallmark of PD, however, it should be noted that Lewy bodies may also be detected in other neurodegenerative disorders including even tauopathies as AD and PSP – here, a neuropathological co-morbidity is likely [134, 143].

Interestingly, PD subjects exhibit Lewy pathology not only in the SN but, in fact, in the whole nervous system including the nucleus basalis of Meynert in basal frontal lobe, in the pontine locus coeruleus, in cerebral-cortical areas as well as in intestinal and cardiac plexus [134]. This raises the question of where exactly PD pathology begins. Since a loss of nigral dopaminergic nerve cells could be detected early in PD, it was assumed that this might be the origin of PD pathology [133]. However, the German neuroanatomist H. Braak questioned this theory by presenting a 6-step model of PD-spreading, which is explained in the following section.
**Braak staging**

The pathological progress, according to Braak et al, is assumed to spread via synaptic contacts and may start in the anterior olfactory nucleus, or in the dorsal motor nucleus of the cranial nerves IX and X (glossopharyngeal nerve and vagus nerve) as pictured in Figure 7 – or eventually in the enteric nervous system [50, 124, 144]. Based on these apparently very susceptible neuronal structures, the pathological development proceeds, up to subcortical and cortical grey matter until finally the primary sensory and motor fields are involved [124].

*Figure 7: Spreading of pathological Lewy body pattern in PD. With the beginning in the olfactory bulb and in the brainstem. The deposits spread from brainstem to thalamic structures, to mesocortical areas and finally to primary motor and sensory cortical regions. The spreading goes along with specific clinical stages, according to Braak et al. Image reproduced and minimally modified from [124], with kind permission from Elsevier. Copyright © 2002 Published by Elsevier Inc.*
Braak et al. suggested that this process shows only few fluctuations between affected individuals and could therefore be classified in six stages according to the morphological expression of pathological patterns as Table 4 demonstrates below [21, 124, 145].

**Table 4: Braak stages according to affected anatomical structures.**

<table>
<thead>
<tr>
<th>Braak stage</th>
<th>Brainstem</th>
<th>Basal forebrain &amp; limbic system</th>
<th>Cortical regions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Olfactory peduncle</td>
<td>Medulla oblongata</td>
<td>Pons</td>
</tr>
<tr>
<td>1</td>
<td>AON</td>
<td>dmV</td>
<td>LC</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The level of involvement of anatomical regions increases with escalating Braak stages - as indicated by the gradually saturated hues of red. The X marks the involvement of the SN in Braak stage 3. AON: anterior olfactory nucleus, dmV: dorsal motor nucleus of vagal nerve, LC = locus coeruleus, RN = nuclei raphes, SN = substantia nigra, nbM = nucleus basalis of Meynert, CA2 = cornu ammonis. A: amygdala. Cg: cingulum.*

As displayed in Table 4 above, changes in the olfactory bulb and in the medulla are present in stage 1, conferring to a clinical premotor stage [134, 146]. Clinically, NMS, such as early olfactory impairment and autonomic dysfunction, correlate with these neuroanatomically affected areas. In stage 2, clinical manifestations include mood disturbances, such as anxiety and depression, according to neuropathologically affected serotonergic neurons in the brainstem [147]. Additionally, sleep disorders such as RBD may occur [147]. In the third stage, the SN gets involved and motor disturbances may occur, when about 50% of the dopaminergic neurons are lost due to the increasing dopaminergic neuronal decline, while in stage 4 neurodegeneration intensifies [134].
In the final stages 5 and 6, cortical regions of the temporal and frontoparietal lobe of the telencephalon get affected [134]. The impairment of the SN in stage 3 marks a milestone in this model, dividing PD into a premotor or prodromal phase and the motor or manifest phase [147]. However, as mentioned in the chapter on prodromal PD, subtle motor disturbances at this early state are possible and can be objectified by carrying out UPDRS, according to Berg et al [90].

Braak’s staging hypothesis has been controversially discussed, as the common asymmetric presentation of PD cannot be well explained by the model [134]. In addition, older control subjects without PD showed a similar chronological involvement of the mentioned prodromal symptoms [134, 147]. So, it may be challenging to clearly separate PD-associated neurodegeneration from normal aging processes.

1.2.7 PD – etiology: complex environmental and genetic interactions

As already mentioned, traumatic events and neurotoxic substances can contribute to the development of PD [27-29]. Moreover, today genetic factors are known to play an important role in PD. Although PD usually occurs in the sporadic form, it has been shown that up to a quarter of PD_{idiopathic} cases have a first-degree relative, who suffers from PD [148].

Importantly, a risk for PD more than twice as high (odds ratio (OR) = 2.3) could be detected for these first-degree relatives compared to first-degree relatives from healthy controls – especially in case they are male [148].

The discovery of PD-associated mutations in the alpha-synuclein (SCNA) gene represented a milestone in PD-research in 1997 [149] and, over the years, further causative genes were detected to be associated with familial PD, showing autosomal dominant and autosomal recessive or X-linked inheritance [150, 151]. Table 5 on the next page shows the PD forms according to their inheritance, their associated genes and their corresponding clinical hallmarks.
Table 5: Overview of the established and the not yet proven (*) PD forms, their inheritance, their associated gene or gene product and relevant clinical aspects.

<table>
<thead>
<tr>
<th>PD form</th>
<th>Inheritance</th>
<th>Gene/ protein</th>
<th>Clinical aspects</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARK1</td>
<td>AD</td>
<td>SCNA/ α-synuclein</td>
<td>EOPD /DLB</td>
</tr>
<tr>
<td>PARK4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PARK2</td>
<td>AR</td>
<td>Parkin</td>
<td>Dystonia/ Dyskinesia</td>
</tr>
<tr>
<td>PARK3*</td>
<td>AD</td>
<td>?</td>
<td>Onset juvenile to elderly adults</td>
</tr>
<tr>
<td>PARK5*</td>
<td>AD?</td>
<td>UCHL1</td>
<td>Insufficient information</td>
</tr>
<tr>
<td>PARK6</td>
<td>AR</td>
<td>PINK1</td>
<td>EOPD</td>
</tr>
<tr>
<td>PARK7</td>
<td>AR</td>
<td>DJ-1</td>
<td>EOPD</td>
</tr>
<tr>
<td>PARK8</td>
<td>AD</td>
<td>LRRK2/ Dardarin</td>
<td>Similar to PD/idiopathic</td>
</tr>
<tr>
<td>PARK9</td>
<td>AR</td>
<td>ATP13A2</td>
<td>Dementia/ pyramidal signs/ atypical parkinsonism</td>
</tr>
<tr>
<td>PARK10*</td>
<td>AD?</td>
<td>?</td>
<td>LOPD</td>
</tr>
<tr>
<td>PARK11*</td>
<td>?</td>
<td>GIGYF2</td>
<td>Insufficient information</td>
</tr>
<tr>
<td>PARK12*</td>
<td>X-linked</td>
<td>?</td>
<td>LOPD</td>
</tr>
<tr>
<td>PARK13*</td>
<td>AD?</td>
<td>HTRA2</td>
<td>Insufficient information</td>
</tr>
<tr>
<td>PARK14</td>
<td>AR</td>
<td>PLA2G6</td>
<td>Dystonia and parkinsonism in adults</td>
</tr>
<tr>
<td>PARK15*</td>
<td>AR</td>
<td>FBXO7</td>
<td>Parkinsonism/ increasing pyramidal signs</td>
</tr>
<tr>
<td>PARK16*</td>
<td>?</td>
<td>PARK16</td>
<td>LOPD</td>
</tr>
<tr>
<td>PARK17*</td>
<td>AD</td>
<td>VPS35</td>
<td>LOPD</td>
</tr>
<tr>
<td>PARK18*</td>
<td>AD</td>
<td>EIF4G1</td>
<td>Similar to PD/idiopathic</td>
</tr>
<tr>
<td>PARK19 A/B*</td>
<td>AR</td>
<td>DNAJC6</td>
<td>A: EOPD, B: like LOPD</td>
</tr>
<tr>
<td>PARK20*</td>
<td>AR</td>
<td>SYNJ1</td>
<td>EOPD/ seizures/dystonia</td>
</tr>
<tr>
<td>PARK21*</td>
<td>AD</td>
<td>TMEM239</td>
<td>LOPD</td>
</tr>
<tr>
<td>PARK22*</td>
<td>AD</td>
<td>CHCHD2</td>
<td>Similar to PD/idiopathic</td>
</tr>
<tr>
<td>PARK23*</td>
<td>AR</td>
<td>VPS13C</td>
<td>EOPD/ dementia/ autonomy dysfunctions</td>
</tr>
</tbody>
</table>

Studies estimated, that only up to 10% of PD\textsubscript{Idiopathic} show Mendelian inheritance and up to 30% of familial PD are monogenic – which reveals, that the vast majority of PD cases does not arise due to known autosomal dominantly or recessively inherited gene mutations [36, 149, 153-160]. Instead, it is hypothesized that gene-to-gene interactions on the one hand and interactions between genes and environmental components on the other hand must be considered as causative as well [36, 161].

The complexity of PD’s genetic background is further exposed by genetic variants that appear to act more as susceptibility factors for PD.

Validated PD-susceptibility factors include polymorphisms (allele frequency above 1 percent in observed populations) in Leucine-rich repeat kinase 2 (LRRK2) gene, in SCNA gene as well as heterozygous mutations in GBA gene [36, 158, 162-168]. GBA mutations currently are the most important genetic risk factor for developing PD [16, 129, 169]. However, a study, aiming to capture the penetrance of PD among carriers of GBA mutations, objectified a penetrance of about 14% for 60-year-old, about 21% for 70-year-old and about 30% for 80-year-old subjects [170]. Therefore, the authors raised the question whether GBA should be considered as a causative gene with reduced penetrance and autosomal dominant inheritance due to this background [170].

The genetic background of PD and similar complex diseases is tentatively mapped by two different theories. The common disease-rare variant (CD/RV) hypothesis postulates that rare genetic variants, defined by allele frequencies of 1% or less, are common in populations [149]. The CD/RV hypothesis implies, that these pathogenic rare variants exist because they are barely removed by natural selection, however, the identification of these rare variants in sufficiently large samples is quite expensive and therefore challenging [149]. By using the methods of next generation sequencing (NGS) and as NGS has
become better affordable, it became more feasible to test CD/RV hypothesis [149]. Additionally to its expansiveness, the highly homologous Beta-glucosylceramidase pseudogene (GBAP) close to GBA is another pitfall for NGS because of gene-pseudogene rearrangements that can lead to the detection of false positive GBA recombinant variants [171].

The common disease-common variant (CD/CV) hypothesis states, that frequently occurring diseases, such as PD, are caused by frequently occurring (allele frequency greater than 1% in population) interacting genetic variants [172, 173]. According to the CD/CV hypothesis, particularly detrimental alleles are rather sorted out by natural selection due to their abundance [149]. Consequently, many alleles remain which are barely harmful when taken alone – but in their entirety, they can be quite deleterious [149].

The CD/CV hypothesis was the basis for genome-wide association studies (GWAS), aiming to capture alleles associated with a specific characteristic and seeking to identify an association between a haplotype and a phenotype [149]. So, numerous common risk loci and susceptibility genes for PD could be discovered [149]. Technically, genotyping is performed by using common, defined single nucleotide polymorphisms (SNPs), to detect possible susceptibility genes for diseases as PD within very large sample sizes [173]. Interestingly, the detected genetic variants are usually localized within noncoding gene regions, they are linked with little effects and they independently influence the PD-phenotype only to a limited extent [172, 174].

A meta-analysis of a GWAS, carried out for PD until 2014, evaluated nearly 14,000 cases and over 95,000 controls [175]. 28 independent PD risk variants were identified at 24 genetic loci [175].

Another exome-wide study, exploring the association between genes that cause lysosomal storage disorders and the risk of developing PD, could identify a compound for 54 LSD-causing genes [176]. Interestingly, this association was still evident, when GBA as an established lysosomal susceptibility factor was not considered in the analysis [176]. More than 50% of the PD cases studied (n=1156) had at minimum one potentially harmful LSD gene variant and more than a fifth
presented with a variety of LSD-alleles [176]. Therefore, the sum of interactions between genetic variants was assumed to possibly contribute to the reduction of lysosomal capacity and thus increases PD-susceptibility [176].

1.2.8 PD – therapeutic pathways

Background of previous therapeutic approaches

After J. Parkinson published his clinical findings in the early 19th century and after J.M. Charcot renamed Parkinson’s Paralysis Agitans into “Parkinson’s disease” a little later, patients were treated with muscarinic alkaloids for 50 years – without any evidence for underlying pathomechanisms which could be specifically addressed (see Figure 8 on the next page) [177]. 75 years later, anticholinergics such as Artane and Akineton were applied [177] and 140 years later, A. Carlsson recognized the link between nigral cell death and dopamine [177, 178].

G. Cotzias optimized motor deficits in PD patients for the first time with oral dopamine administration in 1969 [177, 179]. Additives such the decarboxylase inhibitors carbidopa and benserazide increased dopamine activity 6 years later and already in 1989, sustained-release preparations accomplished persistent L-dopa plasma-levels [177, 178]. The NMDA receptor antagonist amantadine was approved in 1976 in the US as another PD-therapeutic option [180]. Although subcutaneous administration of the dopamine agonist apomorphine has been possible since 1951, pump-based injections for the continuous stimulation of dopaminergic receptors and thus a clear and sufficient reduction of the off-phases were only available in the early 1990s [178, 181-183].

Orally administered L-dopa leads to fluctuating plasma levels peripherally due to its short half-life, which, after crossing the blood-brain barrier and being converted to dopamine, leads to fluctuating dopamine levels at the synapse [184]. Clinically, subsequent motor phenomena such as dyskinesias and wearing-off phases are observed [184].

Prolongation of the short effect duration of L-dopa was achieved with catechol-O-methyltransferase (COMT) inhibitors (tolcapone, entacapone) in the late
1990s, which stabilized L-dopa plasma levels via blocking methylation of the dopamine precursor, thus preventing its degradation and facilitating increased transfer across the blood-brain-barrier [181, 184].

Further, monoamine oxidase (MAO) inhibitors increased the levels of neurotransmitters, including dopamine, by reversible or irreversible inhibition of degrading monoamine oxidase-A or B or both enzyme variants) [185].

In PD, selective MAO-B inhibitors such as selegiline and rasagiline improved motor functions by reducing the off-phase of L-dopa – they were approved in 1974 and in 2005 [186].

Opicapone (Ongentys) followed in 2016, reducing off-phase and prolonging the on-phase without bothersome dyskinesias [187]. Safinamide, a relatively new selective reversible MAO-B inhibitor with glutamatergic effects was launched on the European market in 2015 (Xadago) and was approved in addition to L-dopa and

![Figure 8: Overview of drug therapies for PD. The years below the drug agents correspond to the year or period of first documented use. L-dopa: Levodopa. COMT: catechol-O-methyltransferase inhibitors. MAO-B: monoamine oxidase B inhibitors.](image-url)
other PD drugs in the mid-to-late PD stage with corresponding effect fluctuations [188]. The recognition, that agonists, which are acting on dopamine receptors of the D1-like and D2-like group, cause significantly less dyskinesias, represented another milestone in PD therapy in the 1990s [181]. Ergoline agents such as bromocriptine, cabergoline, pergolide and lisuride as well as non-ergoline agonists such as pramipexole (1997), ropinirole (1996), rotigotine (2006) and piribedil can be differentiated (2006) [181].

However, due to several reports of increased risk of heart failure and fibrotic remodeling of e.g. vessels, pericardium and pleura under the treatment with ergoline derivatives, there are mainly non-ergoline dopamine agonists used nowadays [181, 189, 190].

**Individualized, stage and symptom specific therapy**

Drug therapy today mainly depends on the stage of PD (early or more advanced PD), the prevalence of type of symptoms (motor or non-motor), and on severity of symptoms. Patients with mild motor symptoms at an early PD stage may be mono-treated either with MAO-B inhibitors selegiline and rasagiline (with the latter improving total UPDRS score I-III) or primarily with L-dopa or primarily with dopamine agonists (DA) [181, 191, 192].

For a long time, it was assumed that it would be helpful to start as late as possible with L-dopa in order to minimize motor complications induced by long-term therapy, such as dyskinesias [181]. In 2014, reports of a long-term trial (PD-MED) revealed, however, that initial L-dopa therapy is slightly superior to an L-dopa-sparing regime regarding mobility scores, and that dopamine agonists and rasagiline, used as L-dopa-saving-therapy, are similarly effective [193]. Furthermore, the risk of developing dyskinesia after 7 years of therapy was 33% for levodopa-sparing therapies and 36% for L-dopa regimes, without significant differences between the two study arms in terms of motor fluctuations [193].
Table 6: Overview of drug-based PD-therapy and the advantages (+) and disadvantages (-).

<table>
<thead>
<tr>
<th>MAO-B inhibitors</th>
<th>L-dopa</th>
<th>Dopamine agonists</th>
<th>Anti-cholinergics</th>
<th>COMT inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>• delaying L-dopa requirement for 9 months improving UPDRS score ⚫ reducing off-time</td>
<td>• most effective drug ⚫ better mobility scores</td>
<td>• less motor complications than L-dopa</td>
<td>⚫ optimizing rest tremor in TD-PD</td>
</tr>
<tr>
<td>-</td>
<td>• more dyskinesia (e.g. SG) ⚫ long-term dyskinesias ⚫ fluctuations</td>
<td>• nausea, hallucination ⚫ sleep disturbance ⚫ skin alterations</td>
<td>• More cognitive and neuropsychiatric impairment than placebo</td>
<td>⚫ Nausea ⚫ Diarrhea ⚫ Orthostatic hypotension ⚫ Dyskinesia ⚫ hepatotoxicity (e.g. TC)</td>
</tr>
</tbody>
</table>


As it can be seen in Table 6, the drugs mainly target motor deficiencies in PD. Although there are also both dopaminergic and non-dopaminergic approaches for frequent NMS, reaching sufficient NMS improvement may be cumbersome [181, 195]. If fluctuating NMS are detected, switching to a sustained-release dopaminergic drug or continuous drug supply may be helpful [181]. Sexual dysfunction in men could benefit from sildenafil administration, in hyperhidrosis one should primarily ensure to establish continuous drug administration, constipation could be treated with macrogol and a reduction in the daily amount of dopamine may be reflected [181, 196].

Regarding neuropsychiatric symptoms, depressive symptoms respond well to pramipexole and rivastigmine amended dementia in PD patients [197]. Zeuner et al also reported that psychotic and hallucinatory aspects are less common in L-dopa-therapy than in DA- or amantadine-regimes and should therefore be seen as a side effect of therapy rather than as a stand-alone symptom [181,
In any case, dopamine dose should be reduced due to these symptoms and an atypical neuroleptic, such as clozapine, may be supplemented [181, 197].

Further, there is only limited evidence for the efficacy of modafinil, a psychostimulant, and methylphenidate, a norepinephrine-dopamine reuptake inhibitor, regarding fatigue [197]. Regarding common sleep disorders such as RBD in PD, care should be taken to ensure a safe sleep environment first and, if possible, to reduce or discontinue existing treatment with MAO inhibitors, beta-blockers and antidepressants [198]. In case cognitive deficits, daytime fatigue, fall-down or sleep apnea syndrome are present, melatonin may be given - if not, clonazepam should be given at night [199-204].
1.3 Gaucher’s disease
GD is named after the French dermatologist Philippe Gaucher, who observed splenomegalaly in a young woman in 1882 and attributed it to a form of spleen neoplasia [205]. The correct detection of the underlying pathology and, additionally, the establishment of the world’s first enzyme replacement therapy (ERT) goes back to the American biochemist Roscoe O. Brady in 1965 [206, 207].

Although GD is the most common lysosomal storage disorder, the sphingolipidosis GD is still a rare pathology based on a deficiency of beta-glucocerebrosidase enzyme (GCase) and presents with a huge variety of clinical symptoms [208-211]. Due to autosomal-recessive inherited mutations in GBA gene, the enzyme substrates glucosylceramide and glucosylsphingosine (GlcSph) accumulate and cause mainly visceral, hematological and skeletal disorders [212].

However – drawing conclusions from GBA-genotype to GD-phenotype is only practicable to a limited extent [212]. The patient’s whole genetic makeup, in addition to environmental factors, plays an important role, as modifier genes can have a variety of effects on the clinical phenotype of the formal monogenic Gaucher’s disease [212].

1.3.1 GD – epidemiology
GD occurs in all races, regions and continents with approximately 1 in 40.000 up to 60.000 people worldwide being affected and it is therefore pan-ethnic [211]. However, a subgroup of Ashkenazi-Jewish people demonstrates a much larger disease frequency with 1 out of 855 people affected by GD and in northern Sweden, in the Norrbotten region, the Norrbottian subtype of GD was found especially frequently as well [211, 213].

1.3.2 GD – pathophysiology
Under physiological conditions, the lysosomal wt GCase is synthesized in the endoplasmic reticulum (ER), then it gets folded and undergoes ER quality control [214]. When properly folded, transportation to the Golgi complex follows for further modification and afterwards, it reaches its site of action, the lysosome [214-216].
Due to genetic homozygous or compound heterozygous alterations in the GBA gene, located on chromosome 1, several consequences follow:

- accumulation of glucosylceramides (GlcCer)
- impaired intracellular transport of the mutated GCase from the ER to the lysosome and
- possible early degradation of the misfolded GCase in the proteasome [217-219].

The accumulative aspect occurs due to reduced hydrolysis of GlcCer into the products ceramides and glucose (Figure 9) [208, 220]. Successively, GlcCer accumulates in the lysosomes which leads to a heterogeneous disease with multisystemic clinical aspects [21, 162]. This buildup occurs mainly in macrophages, which are part of the mononuclear phagocyte system, causing them to become Gaucher cells: these cells are huge and show condensed cytoplasm and chromatin if investigated by light microscopy (Figure 10) [211]. Gaucher cells can be objectified in internal organs, mainly in spleen and liver as well as in bone marrow and generate symptoms like hepatosplenomegaly but also inflammatory signals [135, 211].
Another but much rarer etiology is given by PSAP gene mutations, encoding for the activator protein saposin C, which in turn leads to reduced or absent activation of GCase through saposin C [222]. GCase presents itself without functional deficits in case of PSAP mutations [211, 222]. However, this case will not be further deepened in here.

The entity of GD is subdivided into three different clinical phenotypes with special characteristics, which will be further elucidated in the next section [213].

1.3.3 GD – classification

GD-classification is based on a 3-armed system according to clinical history as Table 7 demonstrates on the next page: the postulated non-neuronopathic type 1 with highly variable penetrance, although in recent years it has been associated with neurological aspects such as increased PD risk and mild peripheral neuropathy, the acute neuropathic infantile type 2 with onset within the first 6 months of life and the chronic neuronopathic type 3, involving both juvenile and adult forms [135, 211, 223-227]. However, categorization into these three subtypes seems to be less and less suitable, given the fact that intermediate phenotypes between the three forms are frequently found [21, 210, 228].

Table 7: Characteristics of the three subtypes of Gaucher’s disease.

<table>
<thead>
<tr>
<th>Aspect</th>
<th>Type 1 GD</th>
<th>Type 2 GD</th>
<th>Type 3 GD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuronopathic affection</td>
<td>Non-neuronopathic</td>
<td>Acute neuronopathic</td>
<td>Chronic neuronopathic</td>
</tr>
<tr>
<td>Inheritance</td>
<td>Autosomal recessive</td>
<td>Autosomal recessive</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>Occurrence</td>
<td>Pan-ethnic, increased frequency in Ashkenazi-Jewish</td>
<td>Pan-ethnic</td>
<td>Pan-ethnic, Increased frequency in Norrbotten, Sweden</td>
</tr>
<tr>
<td>Clinical aspects</td>
<td>Hepatomegaly</td>
<td>Hepatomegaly</td>
<td>Hepatomegaly</td>
</tr>
<tr>
<td></td>
<td>Splenomegaly</td>
<td>Splenomegaly</td>
<td>Splenomegaly</td>
</tr>
<tr>
<td></td>
<td>Anemia</td>
<td>Strabismus</td>
<td>Eye movement disorder</td>
</tr>
<tr>
<td></td>
<td>Thrombocytopenia</td>
<td>Opisthotonus</td>
<td>Myoclonic epilepsy</td>
</tr>
<tr>
<td>Symptom</td>
<td>Growth delay-</td>
<td>Brainstem involvement</td>
<td>Cognitive impairment</td>
</tr>
<tr>
<td>---------------------------</td>
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<tr>
<td>Skeletal lesions</td>
<td>Regression of milestones</td>
<td>Psychiatric disorders</td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>Seizures</td>
<td>Aortal calcifications</td>
<td></td>
</tr>
<tr>
<td><strong>PD, polyneuropathy</strong></td>
<td>Aspiration</td>
<td>Hydrocephalus</td>
<td></td>
</tr>
</tbody>
</table>

Proportion: 1. ~90-95% \(^1\) 2. <5% up to 20% \(^2\) 3. ~5-35% \(^3\)

GD: Gaucher’s disease. PD: Parkinson’s disease. Type 1 GD was assumed to be non-neuronopathic, however, there is now evidence for neurological affection regarding e.g. Parkinson’ disease or polyneuropathy. \(^1\) in Europe and North America. \(^2\) <5% of cases in most populations, but up to 20% in some cohorts. \(^3\) commonly 5% of cases, but up to 33% in certain cohorts. Data from [211, 213, 221, 229].

### 1.3.4 GD – distribution of GBA mutations

GBA is located on chromosome 1 on arm q at position 22 (1q22) and consists of 10 introns and 11 exons [211]. Meanwhile, a variety of GBA mutations are known – with N370S (GBA mutation with serine at position 370 instead of asparagine), L444P (GBA mutation with proline at position 4444 instead of leucine) and RecNcil being the most common in general, but there are population-related individual clusters [230]. In Ashkenazi Jewish populations, 90% of all mutations are represented by the following four mutations: N730S, L444P, c.84dup, and IVS2+1G> A, whereas in non-Ashkenazi Jewish subjects, these mutations only represent 60% [211]. Most frequently, Ashkenazi Jewish subjects carry N370S mutations (up to 80%) [211]. N370S causes much milder GD forms in the homozygous mode than the less common but significantly more severe L444P mutations, which frequently set off neurological impairment according to type 2 and 3 [211]. In general, about 300 GBA mutations were detected, which correspond to stop-, frame shift-, missense- and splice-site-mutations or recombinant alleles – with the two common mutational variants, N370S and L444P encompassing for more than 60% of GD cases in European-derived populations [220, 231].
Table 8: Overview of the three subtypes of Gaucher’s disease and further subtypes with their associated most frequent genotypes in relation to different populational clusters and corresponding life expectancy.

<table>
<thead>
<tr>
<th>Aspect</th>
<th>Type 1 GD</th>
<th>Type 2 GD</th>
<th>Type 3 GD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neo-natal</td>
<td>Infant-tile</td>
<td>3a</td>
</tr>
<tr>
<td>common Geno-type</td>
<td>N370S*</td>
<td>2 null Mutations</td>
<td>-</td>
</tr>
<tr>
<td>clusters</td>
<td>Ashkenazi-Jewish</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>prognosis</td>
<td>Normal</td>
<td>Neonatal death</td>
<td>Death within 2 years</td>
</tr>
</tbody>
</table>


Remarkably, the pseudogene GBAP, located close to GBA, is not transcribed but represents a 96% identical exon sequence to the coding GBA gene [233]. The large gene density in this region on the one hand and the pronounced homology of the non-processed pseudogene GBAP on the other hand, contribute to the event of chromosomal rearrangements, such as duplications, inversions or deletions [21, 220]. Due to a heterogeneous clinical portray and a varying life expectancy depending on GD subtype (see Table 8 above) and due to common genotypes in distinct, the question arises as to how far genotype and phenotype correlate in GD.

Genotype-phenotype correlations

Diverse GD genotypes have been detected in similar phenotypes and conversely, patients with identical mutational alleles displayed very heterogeneous phenotypes – which was even reported for twins and siblings [210, 234, 235]. This makes a clear correlation rather unlikely [210, 234, 235]. Nevertheless, rather mild GBA mutations (e.g. N370S, G377S) seemed to be associated with type 1 GD, whereas more severe gene alterations (e.g. L444P, RecNcil, R463C) appear
to be linked with in GD type 2 or 3 [236-238]. Accordingly, genotype impact on GD-phenotype is supported by the fact that homozygous L444P-mutations are linked with an increased frequency of neuronopathic involvement in type 3 GD [21, 239]. Furthermore, patients diagnosed with “non-neuronopathic” type 1 GD intermittently showed symptoms of Parkinsonism in the early course of their disease, such as motor impairments like tremor and gait disturbances but also cognitive deficits [240-242]. These findings suggest, that not every GD patient with his phenotypic character is unambiguously assigned to one of the 3 GD subtypes but there may be intermediate states [243, 244]. So, GD phenotype does not appear to be exclusively influenced by genotype and its Mendelian heredity [235]. Rather, the environment, diverse penetrance of mutations, specific regulated gene expression and other gene loci with modifying functions seem to play a major role [235, 245, 246].

1.3.5 GD – diagnosis

GD-diagnosis is given via testing for reduced GCase activity in leukocytes or fibroblasts, which is usually reduced by 75-80% [247]. In rare cases, if a clinical GD-syndrome (see Table 7 above) comes with a normal GCase function, PSAP gene should be sequenced in order to not miss a deficiency of GCase activator saposin C, encoded by PSAP [211, 222]. Even prenatal diagnosis is possible [248]. Bone marrow biopsy is not routinely recommended but can confirm the diagnosis if Gaucher cells are detected [211]. In addition, biomarkers are available: chitotriosidase levels for intraindividual monitor treatment efficacy, chemokine CCL-18 for estimating prognosis, further the promising marker GlcSph and also ferritin levels – for the prediction of bone involvement [249-252].

1.3.6 GD – therapeutic pathways

In GD, a periodic monitoring is required to initiate any drug therapy prior to the onset of irreversible complications [211]. In principle, there are two different therapeutic options: ERT and substrate reduction therapy (SRT) [253, 254].

*Enzyme replacement therapy (ERT)*

Alglucerase, a modified human GCase, was the first intravenous therapy in type 1 GD in the 1990's, improving symptoms such as anemia, thrombocytopenia and
hepatosplenomegaly with good tolerability [255]. However, alglucerase was derived from human placenta, requiring over 10 tons of placental tissue to treat a single patient for 1 year [256]. Therefore, imiglucerase (Cerezyme), a recombinant GCase from ovarian hamster cells, was introduced as an intravenous, weight-adapted therapy for type 1 GD in 1994 and alglucerase was withdrawn from the market a few years later [256]. Unfortunately, due to viral contamination of the imiglucerase production site in 2009, global supply bottlenecks occurred for one year [257]. In 2010, velaglucerase alfa (Vpriv) was established, based on human sarcoma cell lines [258], and in 2012, taliglucerase alfa (Elelyso) followed – produced from plant cells [256]. Each ERT is given intravenously, administration frequency is variable and may comprise several weeks and further, ERT-dose is adapted to patient’s clinical outcome [211]. Type 1 GD patients should only be treated with ERT in case they are symptomatic regarding clinical and biological aspects, whereas type 2 GD is unfortunately not responsive to ERT [229, 259].

In type 3 GD, ERT should be started in any case [211, 259]. A Russian study analyzed plasma levels of oligomerized α-syn in GD patients and healthy controls and detected significantly higher levels in GD subjects [260]. Interestingly, no plasma level differences were found comparing GD patients with ERT for more than 5 years with healthy controls [260]. The authors therefore concluded that ERT may contribute to the reduction of the plasma α-syn concentration [260]. Further studies revealed, that ERT improves bone impairment, hematological disorders and abdominal involvement of GD patients – without optimizing the neurological symptoms, however [242, 261, 262].

Substrate Reduction Therapy (SRT)

Due the fact, that ERT is expensive, there is only intravenous application and also its immunogenicity, the property of triggering immune response by means of antibody synthesis, is assessed as disadvantageous, SRT also plays a relevant role in GD therapy [256]. The principle of SRT is based on the inhibition of an enzyme, involved in glucosylceramide synthesis: the UDP-glucose ceramide glucosyltransferase [256]. Orally available drugs are the glucose-analogue miglustat (Zavesca) for GD-subtypes 2 and 3 and the ceramide-analogue
eliglustat (Cerdelga) for type 1 GD [211]. Although miglustat crosses the blood-brain barrier, however, it was not superior regarding neurological symptoms in comparison with the "no miglustat therapy" study arm superior in clinical studies with type 3 GD subjects [263].

As far as the author is aware, patients with type 2 and 3 GD are currently receiving miglustat as SRT, who for other reasons are not eligible for ERT [256]. Eliglustat is, like ERT, first choice for type 1 GD, because studies demonstrated comparable efficacy levels as for imiglucerase and adequate safety as well [211].

1.3.7 GD – conclusion
GD is a rare disease that, except of type 2 and 3a GD, is of slow onset and it is often detected rather late [211]. In cases of splenomegaly with or without concomitant thrombocytopenia, this LSD should be considered as a differential diagnosis [211]. In addition, regular monitoring is required – including asymptomatic GD patients as well [211].
1.4 The link between GBA and PD

This section seeks to summarize the key milestones of the discovery of the intriguing link between mutations in GBA gene and increased risk of developing PD, to describe the relationship of GBA mutations with other neurodegenerative diseases, to outline the clinical qualities and neuroimaging findings that characterize PD_{GBA}, to delineate prodromal aspects that are emphasized in PD_{GBA} and to provide insight into how GBA mutations contribute to pathogenesis of PD [264]. Finally, current therapeutic approaches in PD_{GBA} are introduced with focus on targeted therapies.

1.4.1 Overview of key milestones of research

Already in 1996, Neudorfer et al reported on 6 cases of type 1 GD, showing signs of Parkinsonism already in their middle adult age, with a more severe course and poor response to drug treatment (see also Figure 11) [241]. Parkinsonism, as a neuronopathic involvement, thus contradicted the original GD classification according to which only type 2 and 3 exhibit such manifestations [240, 241, 262]. Especially, Parkinsonism in GD linked with the N370S mutation disclosed deficient response or was even refractory to common therapy regimes [240, 241, 262].

Due to close observation of GD patients and their environment, a clustering of PD cases in the familiar background of GD patients was objectified and genetic analyses of these PD cases followed regarding GBA mutations [18, 265]. A pedigree analysis of GD patients revealed their first-degree family members to be concurrently heterozygous Gaucher carriers and also to suffer from PD with increased frequency [18, 158].

Since 2004, this was followed by a variety of studies in different populations, such as North American [18, 166], Chinese / Taiwanese [158], Ashkenazi-Jewish [162, 165, 266] or Caucasian-based samples [21, 167] – using either direct whole-genome-sequencing or screening techniques for the most common GBA mutations to objectify the carrier frequencies in PD patients and controls.
Introduction

The link between GBA and PD

It became remarkably clear due to an extensive multicenter analysis [14], that not only a minor part of GD patients with homozygous mutational status in GBA gene could develop PD over the years but also that heterozygous mutation carriers, thus GD-healthy individuals, had a significant risk of developing PD in their lifetime [14]. Patients with established PD\textsubscript{idiopathic} do also bear GBA mutations with an OR of 5.43 – rendering GBA mutations the most common risk factor for PD [14, 267].

Further studies confirmed these increased GBA carrier-frequencies among patients with familial PD [268] on the one hand and a link between GBA mutations and early-onset PD (EOPD) on the other hand [168, 269]. In 2013, a British study revealed that heterozygous GBA mutations are risk factors for the Lewy body disorders (LBD) PDD and DLB as well [270].
In addition, GBA mutations increase PD-risk in distinctive ethnicities in varying degrees (Figure 12). For N370S, an increased PD risk was shown in Ashkenazi- and non-Ashkenazi-Jewish populations [271].

Further, in Ashkenazi Jewish populations, an elevated risk was observed for the variants 84insGG and R496H, while in non-Ashkenazi Jewish populations the GBA polymorphism E326K as well as the variants L444P, T369M, RecNcil, R120W, D409H, H255Q and IVS2+1G>A increased PD-risk [271].

Figure 12: Different GBA variants/ polymorphisms related to the respective PD-risk. Dark blue: Ashkenazi-Jewish subjects. Light blue: Non-Ashkenazi-Jewish subjects. Presented in logarithmic scale. In Ashkenazi-Jewish samples, N370S mutation was associated with the highest risk for PD, while non-Ashkenazi-Jewish with the GBA polymorphism E326K present with the highest risk for PD, compared to the rest of the portrayed mutations. Data taken from [271].

Besides, L444P increased PD risk in all populations of non-Ashkenazi-Jewish offspring [271]. In Europe and West Asia, E326K, N370S, H255Q, and D409H were the major risk alleles, while in Eastern Asia the GBA variant R120W was significantly associated with a higher PD-risk [271]. A computational analysis of the published literature vis-à-vis GBA-associated PD revealed, that L444P seems to be the most severe of the three most common variants (L444P, N370S, E326K) [272]. A structural analysis of the enzyme’s molecular architecture localized the milder variants N370S and E326K in the area of the alpha helix of GCase, whereas the L444P mutation was located in the start area of the beta sheet [272].
1.4.2 GBA mutations related to further neurodegenerative diseases

Neuropathological studies of cerebral tissue, derived from GD patients of all 3 subtypes, revealed the evidence of Lewy body pathology localized in the cerebral calcarine cortex (layer 4b) of the occipital lobe and in hippocampal subfields (Cornu ammonis area 2-4) of the medial temporal lobe [21, 273]. In type 1GD-cases with Parkinsonian symptoms, synuclein deposits were primary localized in the brainstem and in hippocampal regions — comparable to the synucleinopathic pattern in PD [21, 242, 273]. Further, GBA mutations increase the risk of developing DLB even more — with an OR of 8.28 [270, 274, 275].

No association was identified for GBA variants and tauopathies such as corticobasal degeneration (CBD) and PSP [270, 274]. Especially, a strong association was objectified for E326K with LBD such as PD, PDD and DLB by a Spanish clinicopathologic study [276]. Interestingly, for E236K and the GBA variant T369M, no association was confirmed for GD [277-279].

1.4.3 PD<sub>GBA</sub> — clinical phenotype

PD<sub>GBA</sub> patients share numerous clinical similarities with PD<sub>idiopathic</sub> patients but they also exhibit a partly different phenotypical NMS-syndrome [7]. So, PD<sub>GBA</sub> cases may present with a decreased age at onset, more non-motor impairment — specifically regarding cognitive decline —, a better response to dopaminergic treatment than GD subjects with PD and additionally, they may be affected in different matters by rather mild or rather severe genotypes [13, 14, 17, 165].

**Age at onset:**

Neumann et al stated a significant difference between mean age of PD-onset in PD<sub>idiopathic</sub> and PD<sub>GBA</sub> — with the latter being affected earlier [21]. This finding of a premature disease onset was strengthened by further studies [15, 162, 280]. Conversely, Lesage et al could not confirm a significant difference between PD<sub>GBA</sub> and PD<sub>idiopathic</sub> with respect to early onset in their case-control study with European-descent PD subjects [238].
Response to therapy:
Further, PD\textsubscript{GBA} subjects respond well to L-dopa therapy – in contrast to GD patients, showing poor or no response to L-dopa treatment, as mentioned above [21, 158, 163].

Mild and severe mutations:
A case-control study of Ashkenazi-derived Jewish subjects demonstrated that rather crucial GBA mutations, known to cause more severe GD, were associated with 13-fold increased risk for PD, while milder GBA variants only doubled the risk of developing PD [165]. Though, PD\textsubscript{GBA} carriers of predominantly severe recombinant or null GBA mutations were reported to be affected earlier than carriers of rather moderate GBA gene alterations (mean AAO=39 years vs. mean AAO= 51 years, p = 0.008) [238]. This finding was further confirmed by a large meta-analysis [281]. According to Cilia et al, the risk of dementia in PD\textsubscript{GBA} was increased almost 3-fold by more deleterious mutations (IVS10+1G>T, G377S, L444P) compared with mild variants (e.g. N370S) [3]. Notably, risk of dementia for PD patients was amplified almost 6 times by severe GBA mutations compared to PD\textsubscript{Idiopathic} with wt GBA [3].

PD\textsubscript{GBA} – non-motor symptoms
Since NMS, rather than motor impairments, characterize the early phase of both PD\textsubscript{Idiopathic} and PD\textsubscript{GBA} and they often occur already in prodromal stage, a brief overview of the relevant categories such as autonomic impairments, cognitive deficits, mood and neuropsychiatric disturbances, visual deficits, olfactory performance and the extent of therapy response is given below [12, 17].

Autonomic dysfunctions:
Brockmann et al revealed an increased frequency of autonomic involvement in PD\textsubscript{GBA}, especially regarding orthostatic impairment and bowel dysfunctions (p = 0.001, p= 0.02), compared to PD\textsubscript{Idiopathic} [17]. Also, fatigue and sexual dysfunction were more commonly observed in PD\textsubscript{GBA} than in PD with wt GCase [17, 282, 283]. Nevertheless, conflicting results were obtained by other studies with respect to the frequency of orthostatic dysregulation [3, 14] and cardiac $^{123}$I-MIBG uptake abnormalities in PD\textsubscript{GBA} [12, 284, 285]. The method of $^{123}$I MIBG-uptake revealed
a high sensitivity level in order to assess autonomic dysfunctions in PD_{idiopathic} patients [286].

**Cognitive deficiency:**
Global cognition was impaired more frequently and to a greater extent in PD_{GBA} compared with PD_{idiopathic} [17]. The CORE-PD study confirmed this durable association between GBA mutations and cognitive impairment: PD_{GBA} subjects achieved poorer results in the Mini-Mental State Examination (MMSE), as well as in fields of non-verbal memory and visual-spatial performance than PD_{idiopathic} patients [13]. Neumann et al reported cognitive impairment for 48% of the investigated PD_{GBA} subjects [21], while Zhang et al described PD_{GBA} to be linked with a threefold increased risk regarding dementia [287]. Tsuang et al performed a complete postmortem genetic assessment of GBA in subjects with and without dementia and concluded that GBA mutations present to be a major risk factor for DLB and PD [288]. Although GBA-mutations occurred with an increased frequency in the mixed subgroup of subjects with neuropathological changes related to AD and to LBD, GBA did not appear to act as a susceptibility factor for AD [288].

**Mood and neuropsychiatric disturbances:**
Further, hallucinations occurred significantly more often in PD_{GBA} than in PD_{idiopathic} [289]. A clinical-neuroimaging study revealed accumulated neuropsychiatric disturbances (depression, anxiety, apathy, indifference) as well as increased events of dementia in PD_{GBA} carriers of N370S and L444P – compared to PD_{idiopathic} [17]. Later, an increased incidence of hallucinations and delirium has been confirmed for PD_{GBA} subjects [284]. However, not all studies found significant differences for hallucinations in PD between mutant and wt GBA and some authors even discussed hallucinations to be mainly drug-induced, reflecting side-effects [162, 181, 197]. Besides that, an association between the subject’s gender and the extent of neuropsychiatric morbidity was suggested, as only male PD_{GBA} subjects presented an elevated risk for anxiety and depression compared to PD_{idiopathic} individuals [290]. Based on literature-analysis, the frequency of depressive impairment in PD_{GBA} is inconclusive, as differing results were obtained or in some studies, the effects were not significant [13, 268, 283].
Visual disorders:
According to several studies, visual working memory and, in particular, visual short-term memory is more severely impaired in PD_{GBA} than in PD_{idiopathic} [13, 291]. According to Goker-Alpan et al, patients with both PD and GD presented with reduced blood flow in the cerebral regions “lateral parieto-occipital association cortex” and in the “cortical-parietal precuneus area” [7]. Interestingly, the precuneus area proved to be a central structure for acquiring more accurate visuospatial information [292] and fittingly, visual stimuli are processed in the parieto-occipital-association cortex as well [293].

Olfactory performance:
Group comparisons showed impaired olfactory ability to be more common in GD patients and in GBA-carriers without PD diagnosis than in GBA wt controls [294]. The early onset of impaired olfactory ability is supported by Braak's hypothesis that PD begins in peripheral neuronal structures with retrograde spreading of the synucleinopathy, which includes the olfactory tract, the brainstem and finally cortical structures [12, 124].

PD_{GBA} – motor symptoms
Bradykinesia as an initial symptom was more common in PD_{GBA} than in PD_{idiopathic} [12, 238, 287, 295], whereas controversial results were obtained regarding dyskinesias [238, 287]. Motor impairment in general appears to progress more rapidly if linked with a mutational GBA status and additionally, E326K has been reported to be specifically related with postural instability and gait dysfunction [8, 19, 285]. For a GBA-related emphasis of tremor and rigidity, no sufficient evidence has been objectified so far [12].

A cross-sectional clinical study investigated motor characteristics of early onset PD by using the UPDRS and revealed PD_{GBA} L444P carriers to achieve significantly higher rates in UPDRS-III compared to non-L444P carriers. The authors concluded that the affected GBA allele might influence the motor phenotype in PD patients [296]. Additionally, a Thai case-control study discovered that PD_{GBA} was associated with higher Modified Hoehn & Yahr scale (H&Y) stages during
disease progression compared to PD_{idiopathic} [297]. Angeli et al reported that PD_{GBA} subjects attained the indication for deep brain stimulation (DBS) earlier than PD cases with another genetic background [298].

Unfortunately, these patients developed more rapid cognitive deficiency after establishment of DBS [298]. However, it is challenging to clearly delineate the impact on cognitive deterioration of DBS on the one hand and GBA mutations on the other hand.

1.4.4 PD_{GBA} – neuroimaging findings

**Transcranial sonography (TCS) findings:**

TCS findings revealed a significant majority of PD_{idiopathic} subjects to present with nigral hyperechogenicity in previous studies – which is why this was considered to be a pathognomonic aspect in PD_{idiopathic} [299]. However, even a small proportion of healthy adults showed increased nigral hyperechogenicity as well [300]. Iron deposits and loss of neuromelanin were considered as histopathological markers for the hyperechogenic degenerative processes in the SN [300]. In a postmortem study, Zecca et al observed a negative correlation between SN echogenicity (increased) and nigral neuromelanin concentration (decreased), while the association between SN echogenicity and iron metabolites (iron, H-/ L-ferritin) correlated positively [300]. A comparison of the sonographic findings of PD_{idiopathic} and PD_{GBA} mutations revealed no differences in nigral hyperechogenicity [301]. However, TCS exposed a reduced echogenicity of the midline raphe structure in the brainstem of PD_{GBA} patients [17], which is known to be associated with depression.

**PET and SPECT findings:**

Neurons, projecting from the rather small SN into the much larger striatum, are perishing in PD_{idiopathic} [302]. For this reason, this phenomenon is also called nigrostriatal degeneration [302]. The ends of these projecting neurons correspond to axon endings and can be detected by SPECT imaging, with the dopamine transporter (DAT) being absolutely relevant: DAT is located in the nigrostriatal axon terminals and responsible for dopaminergic reuptake from the synaptic cleft.
into neurons [302]. In case of nigrostriatal degeneration and dopamine deficiency in the synaptic cleft, intact axon terminations aim to regulate DAT down for compensation of the synaptic dopamine deficiency [302]. In turn, this increases the DAT deficit, representing a marker of nigrostriatal degeneracy in SPECT [303]. McNeill et al reported an asymmetric striatal loss of dopaminergic neurons in PD\textsubscript{GBA}, while PD\textsubscript{Idiopathic} demonstrated relatively symmetric abnormalities [304].

Additionally, positron emission tomography studies, using 18F-fluorodopa (FDG) respectively fluorodeoxyglucose (FDG), revealed an altered metabolic activity [305]. Metabolism was modified either striatal and also in the lentiform nucleus in PD cases of Ashkenazi-Jewish descent with homozygous mutations (N370S) or compound heterozygous mutations (N370S/R496H) [305]. Though, the small sample size of n = 2 and the related possible distortion must be considered here. Further, Goker-Alpan et al investigated possibly altered neurobiological conditions by using 18-F-dopa-PET and they objectified a similar diminished striatal dopamine synthesis in patients with PD and GD as well as for PD\textsubscript{Idiopathic} [7]. The regional cerebral blood flow, expressing synaptic turnover, in the parietal precuneus region was reduced in patients with PD and GD, but not in PD\textsubscript{Idiopathic} subjects [7]. Further, reduced biparietal resting activity as a typical pattern of PD\textsubscript{Idiopathic} was found in all subjects with PD, however, GBA-carrier demonstrated the most significant reduction in this analysis [7].

**Magnetic resonance spectroscopic imaging (MRSI):**

As another modality, magnetic resonance spectroscopic imaging (MRSI) is suitable for the detailed analysis of tissues and their metabolic components [306]. Brockmann et al used the non-invasive method to compare PD-relevant cerebral regions of PD\textsubscript{GBA} cases with healthy controls [307]. The scientists observed significantly lower concentrations of N-acetyl-aspartate (NAA), a marker for neuronal integrity, in the putamen as well as in the midbrain of PD\textsubscript{GBA} individuals [307, 308]. Nevertheless, the levels of energy-rich phosphates were normal, which contradicted any pronounced disruption of mitochondrial integrity at time of examination [135, 307].
1.4.5 PD_{GBA} – prodromal symptoms
Symptoms and features, that precede PD_{Idiopathic}, were also found in PD_{GBA} samples [12]. A prospective clinical study by Beavan et al, analyzing potential prodromal PD features (type 1 GD subjects, healthy GBA carriers and healthy controls) detected that GBA-mutation-positive subjects achieved significantly worse levels of depression, deteriorated RBD findings and also worse outcomes in UPDRS-III [309]. Remarkably, in patients with isolated RBD, GBA variants were more common than in healthy controls [310]. Another study revealed GD subjects, suffering simultaneously from PD, to be associated with a higher risk for poor olfactory performance, with an increased prevalence of RBD and with an enhanced frequency of hallucinations – compared with PD_{Idiopathic} subjects [311].

*Increased frequency of prodromal features in a relatively shorter prodromal stage*
A retrospective study used a validated interview on prodromal PD symptoms, carried out by PD_{GBA} and PD_{Idiopathic} patients as well as healthy elderly subjects [312]. The results comprised three key findings: first, it was shown that PD_{GBA} and particularly L444P-associated PD_{GBA} presents with prodromal symptoms more frequently [312]. Second, PD_{GBA} showed nearly concurrently non-motor and early motor deficits immediately prior to diagnosis and finally, PD_{Idiopathic} demonstrated a relatively longer prodromal phase – which begins with NMS and presents much later with motor impairments [312].

1.4.6 PD_{GBA} – findings from peripheral blood sample and cerebrospinal fluid analysis
Chahine et al objectified elevated levels of inflammatory mediators in plasma samples of PD_{GBA} carriers compared to PD_{Idiopathic} [313]. Further, a whole genome expression analysis with peripheral blood samples from PD_{GBA} subjects of Ashkenazi-Jewish descent and healthy controls showed 26 genes to be significantly altered regarding their expression, mainly corresponding to a downregulation [314]. The extended subanalysis of 5 of these genes, which were all related to B cell function or immune system associated meanings, unveiled downregulation for PD_{GBA} and PD_{Idiopathic} subjects but not for healthy GBA carriers and non-carriers [314].
Further, a case-control study analyzed CSF by gas chromatography and reported different levels of fatty acids in PD<sub>GBA</sub> compared with PD<sub>idiopathic</sub> and healthy controls: significantly lower levels of palmitoleic acid, arachidonic acid and eicosapentaenoic acid were found in PD<sub>GBA</sub> [315].

1.4.7 PD<sub>GBA</sub> – underlying pathology
At present, the underlying mechanisms how GBA mutations contribute to PD-pathology, are still not entirely understood – although there are several causative hypotheses. These considerations are mainly focused on interaction of GCase and α-syn [316], reduced cerebral GCase activity [317], dysfunctional autophagy-lysosomal pathways [6], mitochondrial impairment [318], impaired calcium homeostasis [319], endoplasmic-reticulum-associated protein degradation (ERAD) [142] and dysfunctional lipid metabolism [320]. These hypotheses are deepened and reflected in the discussion section. Therefore, these hypotheses will not be further elaborated here.

1.4.8 PD<sub>GBA</sub> – therapeutic approaches
To date, PD<sub>GBA</sub> subjects receive similar treatment as PD<sub>idiopathic</sub> patients – as ERT, utilized in GD, does unfortunately not optimize patient’s neurological impairment [261]. However, the small molecule venglustat, an inhibitor of the enzyme glucosylceramide synthase (GCS), is currently being studied in a clinical trial (MOVES-PD), that targets PD<sub>GBA</sub> in early stages [321]. MOVES-PD is based on the background, that an increase of glucosylceramides has been demonstrated in both PD<sub>GBA</sub> and PD<sub>idiopathic</sub> and a once-daily administration of venglustat might counteract this increase. The study is expected to continue until 2022 [212, 321].

Another approach investigates the small molecule ambroxol, which pushes the exocytosis of lysosomes out of the cell (possibly optimizing cellular clearance) and further leads to increased GCase concentrations in a murine model [322]. Ambroxol is currently, like venglustat, subject of clinical research (AiM-PD) [323].

Furthermore, Sardi et al reported fascinating effects by using a mouse model, representing type 1 GD case associated with PD: the implementation of normal GBA genes via viral vectors into murine brain tissue and thus cerebral expression of wt GCase, reduced the toxic accumulation of GlcSph, of α-syn aggregates,
protein tau and ubiquitin [324]. Cognitive performance of the type1 GD-PD mice was evaluated by using an object recognition test [324]. After expression of wt hippocampal GCase, the murine cognitive decline had improved [324].

1.5 Aims and objectives of the study
Only a precise knowledge of the clinical phenotype and its trajectories of GBA-associated PD allows an early diagnosis which in turn provides the basis for establishing an early, disease-modifying therapy. Moreover, knowledge on the course of the disease, estimates on the timeframe until reaching milestones such as PDD are needed when planning clinical trials and defining readouts [129].

Centered on these considerations, this study aimed:

- to evaluate the progression of motor and non-motor symptoms in PD patients with heterozygous GBA mutations (N370S, L444P) compared to PD patients with GBA-wt [129] and
- to report on survival rates in PD<sub>GBA</sub> and PD<sub>idiopathic</sub> [129].

Therefore, it was intended to address the following issues:

I. Are there significant differences in disease progression regarding:
   a. motor symptoms in PD<sub>GBA</sub> patients compared with PD patients with GBA-wt mutational status?
   b. non-motor symptoms in PD<sub>GBA</sub> patients compared with PD patients with GBA-wt mutational status?

II. Do the survival rates differ between the investigated subgroups?
2. Material and Methods

2.1 Study design

This clinical prospective cohort study was performed to analyze disease progression and to set up a phenotyping of patients with PD\textsubscript{GBA} compared with PD\textsubscript{Idiopathic} patients without GBA-mutations. The examination was carried out longitudinally over a three-year-period with special regard to non-motor and motor symptoms [129].

This submitted thesis refers to the second follow-up examination in 2013, following a baseline examination in 2010 and a first follow-up in 2011. Clinical assessments were performed from the 24\textsuperscript{th} of June 2013 to the 11\textsuperscript{th} of November 2013 in the Center of Neurology, Department of Neurodegeneration of the University Hospital of Tübingen [129].

2.2 Preparation

Prior to the start of baseline study in 2010, a mutational analysis was performed via deoxyribonucleic acid (DNA) screening of 1000 individuals. Formerly, these subjects had been diagnosed with PD\textsubscript{Idiopathic} according to the criteria of the United Kingdom Brain Bank Society (UKBBS) [129]. Therefore, these two preparatory steps are briefly outlined below. It should be noted that these introductory steps as well as the clinical investigations in 2010 and in 2011 were not performed by the author of this dissertation.

2.2.1 UK Brain Bank Criteria

All patients included in this study were formerly diagnosed as PD\textsubscript{Idiopathic} by a physician before they were incorporated in the baseline study in 2010. Diagnosis was based on the criteria of the UKBBS according to Hughes et al(see appendix I) [325]. However, new clinical diagnostic criteria for PD have been published in the meantime by the MDS – aiming to be operated for both clinical research and clinical routine practice and also stressing the relevance of non-motor symptoms [1, 326].
2.2.2 Mutational screening, subjects and recruitment

It was aimed to include appropriate patients with the required mutational status corresponding to one of the two most frequently found mutations in the GBA gene: N370S and L444P [129, 226]. Therefore, a total of n=1000 PD<sub>Sporadic</sub> patients of Caucasian descent, who agreed to genetic analyses, were used as population – compiled through the Department of Neurodegenerative Diseases and the Hertie Institute for Clinical Brain Research, University of Tübingen, Germany until 2009 [17, 129]. Subjects donated blood samples for a DNA screening test, which analyzed genome with regard to an altered mutational status in several genes associated with monogenic PD: LRRK2, DJ-1, Parkin, PINK1 (PTEN-induced putative kinase protein 1) [129]. If mutations were objectified in these genes, the subjects were not included in the PD<sub>GBA</sub> subgroup [129]. The mutational screening detected n=33 out of 1000 PD-subjects to be heterozygous for the GBA-mutations N370S or L444P [129]. Out of this sample, a number of n=20 (n=14 L444P, n=6 N370S) submitted informed consent and were incorporated in the baseline study 2010 [129].

2.3 Drop out and exclusion of data analysis

As Figure 13 portrays, there has been gradual drop-out (n=5) over the 3-year-period of observation between 2010 and the second follow-up in 2013 due to different reasons [129].

Therefore, only n=15 PD<sub>GBA</sub> subjects were informed by phone, mail or in writing about the objectives and procedure of the planned examinations in 2013 [129]. However, two more subjects did not attend the second follow-up due to different reasons: reduced general condition (n=1), no feedback due to unknown reasons (n=1) [129].
Material and Methods

Drop out and exclusion of data analysis

Figure 13: Overview on stepwise sequence of phases

Study preparations in 2009, start of baseline in 2010, first follow-up investigation in 2011, reasons leading to the exclusion of subjects as well as the second follow-up examination in 2013 with emphasis on survival and progression analysis of patients with PD

PD_{Idiopathic}:

n = sample size.

PD_{GBA}:

- patients with Parkinson’s disease and heterozygous GBA mutation N370S or L444P [129].

2009: mutational screening n = 1000 PD_{Idiopathic}

out n = 967

n = 33 PD_{GBA} heterozygous for either N730S or L444P

out n = 13

- No consent (13)

2010: n = 20 PD_{GBA} gave informed consent

included in baseline examination

out n = 7

- Death in meantime (5)
- Severely reduced general condition (1)
- No feedback due to unknown reasons (1)

2011: first follow-up examination

2013: Second follow-up examination:

“Parkinson’s disease patients with heterozygous GBA-mutation: longitudinal phenotyping of motor and non-motor symptoms – more rapid progression compared to Parkinson’s disease patients without GBA-mutation”

ethical vote 497/2009BO1

survival analysis

n = 20

progression analysis

n = 13

Figure 13: Overview on stepwise sequence of phases. Study preparations in 2009, start of baseline in 2010, first follow-up investigation in 2011, reasons leading to the exclusion of subjects as well as the second follow-up examination in 2013 with emphasis on survival and progression analysis of patients with PD_{GBA}. n = sample size. PD_{Idiopathic}:

patients with idiopathic Parkinson’s disease. PD_{GBA}:

patients with Parkinson’s disease and heterozygous GBA mutation N370S or L444P [129].
In conclusion, n=13 PD_{GBA} patients could be included in the second follow-up study for progression analysis whereas data of n=20 PD_{GBA} patients was integrated for survival analysis [129]. 27 PD_{idiopathic} patients, living in the surroundings of Tübingen, had been matched at baseline for disease duration and sex – after ensuring they had no positive mutational status regarding GBA N370S or L444P – and included as cohort PD_{idiopathic} for comparison [129].

For progression analysis, questionnaires, assessments and quantitative measures were carried out in the three examinations in 2010, 2011 and 2013. However, not every method was performed completely by all subjects in all three examinations [129]. Consequently, not all subjects delivered data over the whole 3-year-period in all subtasks. The underlying reasons for this matter were:

- the subject participated in the examination but was unable to complete the subtask due to severe disease-related general condition,
- the subject missed one or more of the three examinations due to reported reasons in Figure 13 or
- the subtask was not scheduled at the time the subject participated.

2.4 Ethics

Both subgroups submitted written informed consent in accordance with the Helsinki Declaration [129]. The study was approved by the Ethics Committee of the University of Tübingen (Test number 497/2009BO1) [129].

2.5 Performance and clinical examinations

Initially, for each subject a concise medical history was obtained. This was followed by selective examinations of non-motor aspects such as autonomy, cognition, mood, vision and olfactory performance as well as aspects of axial and distal motor performance. Questionnaires, assessments and quantitative analyzes were applied to every participant in the same order.
The investigations were carried out in dopaminergic ON state and are summarized in Table 9 below. Furthermore, some subjects delivered blood samples and agreed to spinal tap for the extraction of CSF and/or dermal punch biopsy.

Table 9: Brief overview of the applied questionnaires, assessments and quantitative measures, content sorted according to thematic focus.

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2.5.1 **History**
A thorough medical history was collected including sex, age at onset, age at inclusion, PD disease duration and antiparkinsonian medication. Furthermore, a pedigree, sketching the subject’s family history with regard to PD, GD and dementia, was created. Nicotine and alcohol consumption were ascertained as well.

2.5.2 **Questionnaires and scales related to autonomic performance**

**Unified Multiple System Atrophy Rating Scale (UMSARS)**
The Unified Multiple System Atrophy Rating Scale (UMSARS), primarily developed to objectify disease progression in multiple system atrophy, consists of parts I-IV whereby in this study only Part III was used. This part evaluates autonomic subfields, such as orthostatic, urinary and bowel functions as well as sexual symptoms [327, 328].

**The Non-motor Symptoms Questionnaire (NMSQuest)**
The Non-motor Symptoms Questionnaire (NMSQuest) was used in the translated German version in order to assess severity and frequency of non-motor affections. Therefore, subjects answered a self-administered 30-item-questionnaire with yes or no, depending on whether the symptoms occurred within the previous month or not [329].

**Non-motor Symptoms Scale (NMSS)**
The Non-motor Symptoms Scale (NMSS) was utilized to ascertain both intensity and frequency of NMS in PD and was applied in the German version [329, 330]. In contrast to the original English form with 9 subscales, the German version comprises 5 NMS-dimensions: 1 – cardiovascular, 2 – sleep/fatigue, 3 – mood/cognition, 4 – perceptual problems/hallucinations and 5 – attention/memory [329].

2.5.3 **Questionnaires and scales related to mood disturbances**

**Beck Depression Inventory-Second Edition (BDI-II)**
In order to measure the intensity of depressive mood disorders, the revised version of BDI-II (*see appendix IV*) was carried out [129]. Patients received the German version of BDI-II, they were instructed to read the 21 items and to choose the most appropriate option out of four given multiple-choice answers. The
subjects were told to select the option that matched most likely with their mood in
the last 14 days – including the day of examination. For evaluation, all single item
scores were summarized to total score from 0 to 63 points [331, 332]. The cutoffs
were implemented as follows: 0-8 points indicate no depression, 9-13 points: min-
imal depression, 14-19 points: mild depression, 20-28 points moderate and 29-
63 severe depressive symptoms [129].

Neuropsychiatric Inventory (NPI)
To discriminate between elderly people and PD patients with dementia, the Neu-
ropsychiatric Inventory (NPI) was performed as an interview with corresponding
caregivers, e.g. spouses [333]. NPI contains different domains, such as delu-
sions, arousal, apathy or disinhibition. Each domain is associated with a key
question and includes additional issues, which were only worked out in case the
Corresponding key question was answered appropriately. Symptom frequency
was assessed as follows: rarely – one point, sometimes – two points, common –
three points, very common – four points, whereas symptom severity was esti-
mated as mild, moderate or severe [333].

Finally, the caregivers feelings, how strong they were bothered about the sub-
ject’s behavior, were scored as caregiver distress from: null (not at all) – five (very
severely or extremely) [333].

Geriatric Depression Scale (GDS)
The Geriatric Depression Scale (GDS) was obtained in German edition to evalu-
ate depressive mood disturbances [334]. The examiner interviewed the patients
due to the 15-item scale, while cut-offs were set as follows: null – five points =
normal, five – ten points = mild to moderate depression, eleven – 15 points: se-
vere depression [334].

2.5.4 Questionnaires related to activities of daily living (ADL)
Functional Activities Questionnaire (FAQ)
The Functional Activities Questionnaire (FAQ) was raised to assess the level of
independence with regard to the activities of daily living (ADL) [335]. FAQ targets
erly people with all forms of dementia, MCI as well as regular cognitive skills
and aims to discriminate between patients with dementia and healthy elderly
subjects. It includes ten categories, rated by the patient, relatives or friends over the past 4 weeks. The items were valued as follows: null – normal or has never done the activity, could do it now, one – has difficulty, but does it self-administered or has never done the activity and would now have difficulty with it, two – needs assistance and three – dependent on others. FAQ was reviewed in studies dealing with AD for validity and reliability [336].

**Parkinson’s Disease Questionnaire (PDQ-39)**

The Parkinson’s Disease Questionnaire (PDQ-39) was raised to assess the impact of PD on 8 different aspects of daily living. Validity and reliability were investigated by previous studies [337, 338]. These eight dimensions are: mobility, activities of daily living, emotional well-being, social support, cognition, communication, stigma and bodily discomfort. The German validated version of PDQ-39 was applied as a self-administered questionnaire [339]. All single scores were added to a total without transforming the subscale raw values.

2.5.5 Questionnaires and scales related to sleep disorders

**Epworth Sleepiness Scale (ESS)**

The Epworth Sleepiness Scale (ESS) offers information about daytime fatigue by assessing the chance to nod or fall asleep in different described situations and was worked out independently by the subjects [340]. Probability to doze was leveled from null (would never doze) to three (high chance of dozing), a total score of 24 points was reachable. The German version of ESS was applied in this study [341]. According to a multicenter study, which intended to work out normative rates for the German ESS, a total score lower than eleven points was taken as cut-off between healthy individuals and subjects with an increased sleepiness [342].

**Rapid eye movement sleep Behavior Disorder Screening Questionnaire (RBDSQ)**

The Rapid eye movement sleep Behavior Disorder Screening Questionnaire (RBDSQ), as a ten-item measurement, was assessed to provide information about the intensity of lively dreams, moving arms or legs while sleeping or knocking over items near the patient’s sleeping site [343]. Patients answered the
questionnaire with yes or no and could reach a total of 13 points. A score of at least six points or more in RBDSQ was reported to be a usable cut-off to detect RBD [344].

**Parkinson’s Disease Sleep Scale (PDSS)**

Sleep disturbances in patients with PD were evaluated with the modified Parkinson’s Disease Sleep Scale (PDSS), a screening tool for nocturnal impairments in PD [345]. Patients answered 15 questions by rating the symptomatic frequency over the last 7-day-period (0 – very often, 1 – often, 2 – sometimes, 3 – occasionally, 4 – never). Score-range was from 0 – 60 points with higher scores corresponding to more intense sleep disturbances [345].

2.5.6 **Assessments related to autonomic performance**

**Schellong Test**

The Schellong Test (ST) was assessed to evaluate orthostatic reactions, caused by a dosed load due to sympatho-adrenergic response. First, systolic and diastolic blood pressure as well as the heart rate were measured after subjects lay down for a 10-minute-period. The measurement of all 3 parameters was repeated immediately after rising (0 min) and after 2, 4 and 6 minutes while standing.

2.5.7 **Assessments related to cognitive functions**

**Montreal Cognitive Assessment (MoCA)**

Cognitive impairment of PD patients was investigated with the Montreal Cognitive Assessment (MoCA, see appendix V), a widely used screening test for diverse mental functions, such as temporally and spatially orientation, attention, memory, executive and visuospatial utilities, concentration, language facilities, calculation as well as conceptual skills [346].

The subjects received the German version of MoCA, which starts with the item “Alternating Trail Making”. One point was given when the subject drew the correct line from one to E in ascending order, null points were given when there was an error. The next task was to duplicate a displayed cube, whereas one point was only allocated when the copied cube had parallel lines of matching length, not more or less lines regarding the original cube and if the three-dimensional aspect was given. The cube-task was followed by the instruction to depict a clock.
Three points were achieved when the clock shape was a closed circuit (one point), when all numbers were displayed in correct order and square (one point) and if both hands were present, showing the correct time (11:10 am/pm) and placed in the middle of the dial (one point).

Afterwards, subjects had to name exactly three displayed animals, up to three points were possible. This naming test was followed by a memory assessment. Five words in five seconds were read out to the patient with the instruction to repeat them. This procedure was replicated immediately, and the patient was told to keep the words in mind. Furthermore, subjects listened to two short sequences of numbers and had to recall them both forward and backward. Two points were given for two correct answers.

The calculation item expected patients to subtract seven from 100 and keep on calculating until they were asked to stop. Three points were allocated for four or five correct subtractions, two points for two or three correct answers, one point for one exact subtraction and null points if there was no right subtraction.

The following subtask required the correct repetition of two sentences. The subjects got one point for each sentence – with special regard to possible substitutions or omissions. Afterwards, a verbal facility investigation followed, whereas as many words as possible had to be listed beginning with the letter “F” within one minute. One point was achieved, if the patient mentioned eleven words or more.

Abstractional thinking was investigated by asking the subjects, which aspect two single words may have in common and why it is reasonable that they form a pair. Two points were given for each correct item pair. At this time, patients were asked to recall the five words they were told before in the memory assessment. The subjects got one point for every word, which was recalled spontaneously. In the end, the patients had to answer questions concerning their temporal and spatial orientation. A total score of 30 points was obtainable, the cut-off of less than 26 points postulated a cognitive impairment [129, 347].
**Trail Making Test (TMT) -A and -B**

Psychomotor functions, such as working memory, visual scanning and inhibition control, were explored by the Trail Making Test (TMT) [348, 349].

The TMT is a paper-based examination with two different subtasks A and B: for the first part (A) patients had to link numbers, distributed over the sheet, from 1 to 25 in rising order. The second part (B) required to draw a line between letters (A to L) and numbers (1 to 13) in an ascending but alternating sequence (from 1 to A to 2 to B to 3 et cetera) [350]. Time was taken for TMT A and B and ΔTMT was calculated as “time B minus time A” under specific conditions (300 seconds were allowed, mistakes were counted separately for both parts, subjects were made aware of possible errors by the examiner) [351].

**2.5.8 Assessments related to visual disturbances**

**The Pelli-Robson contrast sensitivity test**

Visual dysfunction may be impaired in PD regarding visual acuity, color discrimination or contrast sensitivity [352]. The Pelli-Robson contrast sensitivity chart (Figure 14) was used to evaluate patient’s contrast sensitivity vision.

It shows horizontally arranged triplets of capital letters with 2 triplets per row. Contrast decreases from each triplet to the next, both vertically from row to row and horizontally within a row as well.

The subjects were keeping a 1-meter-distance from the board, without removing their glasses or contact lenses. The chart was placed with its center at the subject’s eye level. The eyes were examined monocularly, beginning in the first row with the first triplet. Afterwards, binocular vision was tested. The last triplet was marked if a minimum of 2 of 3 letters were named right [353].
Visual acuity test

The subjects were standing four meters away from a number chart in order to capture their visual acuity. The examiner asked the patients to monocularly read out the first digit of the penultimate line – beginning with the right eye.

Farnsworth Munsell 100 Hue Test

Color discrimination was investigated by utilizing the Farnsworth Munsell 100 Hue test. For this purpose, the subjects were given four different sets, representing the visible spectrum of colors. The subjects had to sort a total of 85 different colored caps in sequence [355]. By mapping the full color spectrum in 4 divided sets, it is assumed to minimize global color confusion and to achieve limited error clusters instead, which further allows a more sensitive detection of the exact subtype of color vision disorder [356].

2.5.9 Assessments related to olfactory disorders

Sniffin Sticks
Once there were no limiting factors, such as infections of the respiratory system or further restrictive aspects at time of investigation, the twelve items test-battery of Sniffin’ Sticks (Burghart Medizintechnik) was used as an olfactory screening test for odor identification [357]. The subjects performed the screening test in a relaxed sitting position and received twelve pens in succession, each filled with a special odor (such as shoe leather, peppermint, liquorice, pineapple, cinnamon, lemon, fish, orange, rose, coffee, banana and cloves). Therefore the cap of each odor-pen was disconnected for 3 seconds and was hold with a distance of two centimeters (cm) to patient’s nose [358]. After each pen, the subject used a multiple-choice sheet with possible answers where he had to choose one out of four given options. The summarized results laid between null and twelve correct answers. The absolute score was transformed to a percentage value, whereas values under 75% indicated hyposmia.

2.5.10 Assessments related to axial and distal motor performance

**Unified Parkinson’s Disease Rating Scale III (UPDRS-III)**

The UPDRS-III, *(see appendix I)* as a semi-quantitative/semi-clinical measure. It was utilized in the German version to evaluate the severity of axial and distal motor symptoms, whereas both the original and the modified version of the MDS were carried out [129]. The modified version consists of 18 single items. The instructions for each exercise were read out clearly to the patient and demonstrated simultaneously. Other factors influencing the motor performance (such as stroke, arthrosis or contractures) and leading to an impaired examination, were taken into account and the task was noted as unable to rate. All subscales were evaluated with integer scores as follows: null = normal, one = slight, two = mild, three = moderate, four = severe [359, 360].

**Perdue Pegboard Test**

Additionally, distal motor performance was assessed with the Perdue Pegboard test. Single- and double-handed dexterity was analyzed by removing and resorting wooden pegs out of or into the board again – according to instructions given by the examiner. Time was taken during each subtask [361].

**Modified Hoehn & Yahr Scale (H&Y)**
The H&Y Scale (see appendix III) represents a widely used instrument to categorize the stage of PD – in contrast to UPDRS, which targets at the severity of motor symptoms [129]. H&Y was used in the modified form with the stages: 1 – 1.5 – 2 – 2.5 – 3 – 4 and 5 [362, 363].

**Levodopa Equivalent Dose (LED)**

PD patients, participating in clinical examinations, are performing under the influence of varying drug regimens – corresponding to different daily doses of levodopa. Therefore, a conversion tool was developed to assess the LED (see appendix VI) for each antiparkinsonian drug by using conversion factors [364]. LED is assumed to bring the identical control of symptoms as caused by 100 milligram (mg) of levodopa. For each subject, a medication-history was assessed and LED was calculated due to the guidelines of the German neurological society (DGN) [129, 365].

2.5.11 Quantitative measures related to autonomic performance

Neurocardiac functions were investigated due to the computerized analysis-system SUEmpathy (SUESS-Medizintechnik, Aue, Germany). An electrocardiogram (ECG) and a non-invasive blood-pressure (NIBP) measurement was carried out in a lying position. For obtaining NIBP, the CBM3000 tool (Nihon Colin Co, Komaki, Japan) was attached. Heart rate, NIBP and respiratory rate were obtained for 30 seconds. Afterwards the investigation of heart rate variability (HRV) was raised, whereas patients were instructed via earphone to breathe in and out for 120 seconds (metronomic breathing). The examination of the sympathetic skin response (SSR) was obtained in laying horizontal position with auditory signals given by earphones as well [366].

2.5.12 Quantitative measures related to axial and distal motor performance

Clinical investigations of motor impairment in PD are subjective and may not be sensitive enough to detect discrete, subtle deficits of motor performance [367]. Since the slightest disturbances of balance and gait can refer to prodromal motor symptoms in PD, instrument-based, objectifiable quantitative measurement methods can help to identify them and enable early PD diagnosis [368, 369]. Therefore, axial motor performance was additionally evaluated by accelerometer-
based measurements – including the Timed-up-and-Go-Test (TUG), the functional reach (FR) test, a balance measurement and a gait analysis without (single task) and with cognitive challenge (dual task).

Distal motor performance was assessed by digitomotography, utilizing a quantitative motor system (q-motor, see below 2.5.12.2) [370].

2.5.12.1 Axial motor performance

Movement analyses were performed by using the computerized system DynaPort Hybrid (McRoberts, The Hague, The Netherlands). Patients were instructed to perform exercises while wearing a portable inertial sensor, an accelerometer – which was integrated in an adjustable belt.

In advance of the examination, a MicroSD card was applied to an adapter, which was connected to the computer. Afterwards, the software MiRA2 (McRoberts, The Hague, The Netherlands), was used to initialize the MicroSD card for each patient. After initialization, the MicroSD card was attached to the DynaPort-Adapter, which itself was placed into the elastic belt. The sensor inside the belt was positioned in the lower back, centered over the L3-4 spine area [371]. This region is assumed to represent the body center of mass (COM), which is supposed to be the region where the desired motions could be detected at best [369].

[369]The sequence of the following examinations was standardized, and it started with timed single tasks (1 and 2) for calibration:

(1) get up from sitting, remain standing for 10 seconds and calculate (172 minus 7 continuously)

(2) get up from sitting as fast as possible, remain standing for 10 seconds and marking crosses on a sheet of paper while time was taken for calibration

Timed-up-and-Go (TUG)-test : Time was taken for the TUG-Test, a fast and uncomplicated method for measuring mobility, performed in 4 subtasks [372]. The subjects had to:

(3) get up from sitting, start walking a 3-meter-distance with right foot and regular speed, circling right, go back to the chair and sit down
(4) get up from sitting, start walking a 3-meter-distance with left foot and regular speed, circling left, go back to the chair and sit down
(5) get up from sitting, start walking a 3-meter-distance with right foot and rapid speed, circling right, go back to the chair and sit down
(6) get up from sitting, start walking a 3-meter-distance with left foot and rapid speed, circling left, go back to the chair and sit down

Functional reach (FR) test: FR-test, discriminating between healthy subjects and patients with PD [373], required the subjects to stand straight next to a wall and to keep both feet in a parallel position. Then subjects were asked to raise their right upper extremity and keep it parallel to the ground. The position of the fingertip was marked as the starting position and the patients were told to reach out with their right arm as far as possible, without losing their balance or moving their feet. This position was marked as the maximum position. Subjects had to keep their individual position for 15 seconds. Afterwards patients remained standing still for additional 15 seconds, then this trial was stopped.

Balance measurement: Balance analysis was performed by using a foam mat, AIREX Balance-pad (Airex AG, Sins, Schweiz). Subjects had to stand with both feet on the mat and take a slightly diagonal staggered position. This position was investigated with either open as well as closed eyes for a predetermined duration between 10 or 30 seconds. The last task started with eyes opened and the subjects had to close and open their eyes every 10 seconds due to the examiner’s command. This final task was finished after a period of 80 seconds.

In-Circuit exercise: Subjects were told to circumnavigate a circular blanket of 120cm-diameter, which was placed on the floor. Patients had to move around that blanket in different directions while keeping a clipboard and a pen in their hands. They should avoid touching the blanket and had to absolve special secondary tasks (calculating, marking with crosses) while circling. Time was taken during both single and dual tasks.

Gait analysis: subjects had to walk down a corridor with a defined distance of 20 meters (m). The subjects performed these 20m several times under varying
conditions (starting with different feet, varying walking speed, additional tasks such as calculating or marking crosses to effect cognitive challenges).

2.5.12.2 Distal motor performance
Fine motor skills were additionally examined by digitomotography, utilizing a quantitative motor system (q-motor) [370]. A pre-calibrated sensor (Sensor Mini40 / ATI Industrial Automation / Apex, NC 27539 USA) registered the force applied over the time, the frequency of tapping and the time intervals. Subjects were guided by the examiner to perform six subtasks, which are further explained below.

Lift light left and right (1/6)
At the beginning, the subjects were asked to sit as straight as possible in front of the sensor. The investigation began with the subtask "lift light left and right". Starting with the left hand, the right hand was placed in the lap because supporting the hand or the forearm was not allowed. The patient was asked to grasp the sensor-device with his left thumb and forefinger, only as light as a glass of water. After the first auditive signal from the computer the sensor should be raised and dropped off again after the second computer signal was given. Then, the pressure sensor was replaced and positioned on the patient's right side in a way, that the lead-out cable did not disturb the subject’s movements and the performance was repeated with the right hand.

Tapping right and left (TPD) (2/6)
The sensor was positioned horizontally for the next subtask and the patient was asked to tap the sensor as quickly and as regularly as possible with his forefinger after an auditive computer signal. After 10 seconds, another sound marked the end of the exercise and the subject had to remove his finger from the sensor.

Tapping met right and left (3/6)
The patient was instructed to knock rhythmically with his forefinger, together with a computer signal. Although the signal stopped after 5 seconds, the subject should continue with tapping until a second signal told him to stop.

Pronate and supinate left and right (4/6)
Subjects were asked to rotate the inside and outside surfaces of their hands on the sensor. In doing so, no great force should be applied. This pronation and supination movement should be performed as fast as regular, with the emphasis being on turning-movement – instead of speed. Any wristwatches were removed before starting the exercise.

**Foot tapping right and left (5/6)**
For tapping right and left, a foot block was placed on the floor and positioned in front of the seated patients, with the pressure-sensor plugged in. The patient was asked to put his ball of the foot on the sensor. The knee joint should be bent slightly more than 90 degrees. Further, the patient was instructed to tap the sensor as quickly and as steady as possible, after the start signal was given by the examiner, and to keep tapping until a second signal terminated the exercise. Again, no excessive force was allowed. The patient was also instructed to properly lift the foot off the sensor during tapping.

To prevent the foot block from tipping over, the patient should hold it with the other foot if possible. Thereafter, the exercise was performed with the other foot.

**Tremor measurement (6/6)**
For this last part, patients sat up a little, so that the legs could dangle freely. Afterwards, 4 tremor-sensors were applied. One sensor each was attached on the left and right forefoot and on the left and right index finger as well. The patient was asked to shore up the forearms on the thighs to hang his hands freely. The instruction was to sit calm and relaxed for 20 seconds after the first auditory signal. After the second auditory signal, the patient should perform calculations for distraction.

With the third auditory signal, the arms and legs should be stretched out and the fingers should be spread. This position ought to be kept for 20 seconds. After the fourth auditory signal was given, patients had to calculate again over an episode of 20 seconds. Finally, the cables were removed, the software program Tremor TPD was selected and the patient was asked to relax again for 20 seconds. After that, the exercise was completed.
2.5.13 Biomarkers: blood, cerebrospinal fluid and fibroblast samples

The implementation of this clinical prospective study described in here also coincided with the baseline investigation of a further study called “Markers in GBA-associated PD (MiGAP)”. However, the multicenter study MiGAP is independent from this presented study and was not part of this submitted dissertation. The subjects, included in this longitudinal study presented here, were informed about the background, the goals and course of MiGAP and included in MiGAP – as they all gave their informed consent for participation.

Accordingly, from all subjects (n=13), additionally included in MiGAP, several biomarkers were obtained: a peripheral blood sample, cerebrospinal fluid and a skin punch biopsy. Notably, the analysis of these samples was not part of this submitted thesis.
2.6 Devices

Table 10 below summarizes all devices, utilized in this presented study.

Table 10: Overview of the applied devices (left) and the corresponding manufacturer (right) [129]

<table>
<thead>
<tr>
<th>Device</th>
<th>Producer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelli Robson Contrast Sensitivity Test Chart</td>
<td>Clement Clarke International Cartel Business Estate Edinburgh Way Harlow CM20 2TT</td>
</tr>
<tr>
<td>Farnsworth Munsell 100 Hue Test</td>
<td>Gretag Macbeth LLC 617 Little Britain Road New Windsor, NY 12553-6148 United States</td>
</tr>
<tr>
<td>Sniffin' Sticks Screening 12 Test</td>
<td>Burghart Messtechnik GmbH Tinsdaler Weg 175 22880 Wedel Deutschland</td>
</tr>
<tr>
<td>Q-Motor F/T Sensor Mini40</td>
<td>ATI Industrial Automation 1031 Goodworth Dr., Apex, NC 27539 USA</td>
</tr>
<tr>
<td>WinSCP, Open Source Client Software for q-motor</td>
<td>M. Prikryl, Prague</td>
</tr>
<tr>
<td>DynaPort Hybrid</td>
<td>McRoberts, The Hague, The Netherlands</td>
</tr>
<tr>
<td>SUEmpathy™</td>
<td>SUESS-Medizintechnik, Aue, Germany</td>
</tr>
<tr>
<td>AIREX Balance-Pad</td>
<td>Airex AG, Sins, Schweiz</td>
</tr>
</tbody>
</table>

2.7 Data analysis and statistics

Statistical analysis was performed with the software SPSS 21.0 for Microsoft Windows (IBM, Armonk, New York, US), whereby different statistical methods for the analysis of disease progression and survival were applied [129].

2.7.1 Disease progression analysis

To assess PD-specific disease progression over the 3-year period, motor properties were measured by using UPDRS-III, H&Y, and LED, whereas non-motor characteristics were evaluated by using MoCA for cognition and BDI-II for mood disorders [129].
Progression analysis was applied only to those subjects who could be observed over the entire 3-year period.

This was applicable for \( n=13 \) out of 20 PD\(_{GBA}\) subjects, initially included at baseline [129]. Therefore, the examined sample included the cohort PD\(_{GBA}\) \((n = 13)\) and the control group PD\(_{Idiopathic}\) \((n = 26)\), the latter controlled to present with neither GBA L444P nor N370S. Both groups were matched for disease duration and sex [129].

For the entire sample of \( n = 39 \) subjects \((n=13 + n=26 = n=39)\), a regression model was applied to describe possible independent effects of age at examination, disease duration, age at disease onset and GBA-mutational status on the applied clinical measures [129]. Statistical analysis of disease progression within each subgroup was performed with the paired T-test, whereas comparisons between both subgroups were analyzed separately for each time point (0, 1- or 3-year examination) parametrically by using the T-test or non-parametrically by using the Mann-Whitney U test.

Whether or not data showed normal distribution, was verified by the Kolmogorov-Smirnov test with p-value > 0.05. Normally distributed data is given as mean and standard deviation, while non-normal distributed variables are given as median with range [129].

A mutation-specific analysis of the PD\(_{GBA}\) group \((n=13)\) comparing the N370S carrier \((n=3)\) with L444P carrier \((n=10)\) was not carried out due to the small sample size [129].

2.7.2 Survival analysis
Survival analysis, equivalent to the influence of GBA mutational status on natural history of PD, was performed by using chi-square test for group comparison PD\(_{GBA}\) \((n = 20)\) versus PD\(_{Idiopathic}\) \((n=27)\) [129].

2.7.3 Variables
Since significantly more investigations were carried out during this prospective study than were finally statistically analyzed, only the following variables listed
below (Table 11) were taken into account for statistical evaluation in this dissertation for reasons of clarity:

Table 11: Applied variables for statistical analysis in this submitted thesis

<table>
<thead>
<tr>
<th>mood/ cognition</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) MoCA</td>
<td></td>
</tr>
<tr>
<td>(2) BDI-II</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>motor performance</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) UPDRS-III</td>
<td></td>
</tr>
<tr>
<td>(2) H&amp;Y</td>
<td></td>
</tr>
<tr>
<td>(3) LED</td>
<td></td>
</tr>
</tbody>
</table>

Instead, the variables below (Table 13) are available for further analyses:

Table 12: Variables not taken into account for statistical analysis.

<table>
<thead>
<tr>
<th>autonomy</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) UMSARS</td>
<td></td>
</tr>
<tr>
<td>(2) NMS-Quest and NMSS</td>
<td></td>
</tr>
<tr>
<td>(3) Schellong Test</td>
<td></td>
</tr>
<tr>
<td>(4) HRV and SSR</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>mood/ cognition</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) NPI</td>
<td></td>
</tr>
<tr>
<td>(2) GDS</td>
<td></td>
</tr>
<tr>
<td>(3) NMS-Quest and NMSS</td>
<td></td>
</tr>
<tr>
<td>(4) Trail Making Test A and B</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>daily living</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) FAQ</td>
<td></td>
</tr>
<tr>
<td>(2) PDQ39</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>sleep behavior</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) ESS</td>
<td></td>
</tr>
<tr>
<td>(2) RBDSQ</td>
<td></td>
</tr>
<tr>
<td>(3) PDSS</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>visual Performance</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Pelli-Robson Test</td>
<td></td>
</tr>
<tr>
<td>(2) Visual acuity Test</td>
<td></td>
</tr>
<tr>
<td>(3) Farnsworth Munsell 100 Hue test</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>olfactory performance</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Sniffin stick Test</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>motor performance</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Perdue Pegboard Test</td>
<td></td>
</tr>
<tr>
<td>(2) Accelerometer-based TUG, FR and Balance</td>
<td></td>
</tr>
<tr>
<td>(3) Gait Analysis</td>
<td></td>
</tr>
<tr>
<td>(4) Q-Motor</td>
<td></td>
</tr>
</tbody>
</table>
3. Results

In this section, the outcomes of this prospective study are presented - structured into the sections according to the aims of the study. Further, the formation of the analyzed samples is described, and also which strategies were used to avoid data distortion. This is followed by the results of progression and survival analysis. Finally, a short summary of all results will be given.

3.1 Definition of samples

A representative random sample was drawn from the population of patients living in Germany with delimited characteristics (diagnosed with PD, altered mutational status in GBA-Gene corresponding to L444P or N370S) defined as target population PD\textsubscript{GBA} [129]. This PD\textsubscript{GBA} sample included \( n = 20 \) subjects for statistical analysis for survival and \( n = 13 \) for statistical analysis of disease progression. This subgroup was compared with a PD\textsubscript{Idiopathic} control sample – including \( n=27 \) for survival analysis and \( n=26 \) for disease progression analysis [129]. Both groups were matched for sex and disease duration [129].

3.2 Strategies for avoiding distortion

Individuals of the PD\textsubscript{GBA} sample were contacted in a manner appropriate to their health condition to avoid systematical favoring or excluding of certain patients with visual or auditory impairments. Since observational studies, such as this presented examination over a 3-year period, allow the occurrence of confounding variables that can influence the results obtained, the aim was to work with subject pairs that match in eminent variables (here, sex and disease duration). Further, measures were only applied in case their reliability, objectivity and validity were evaluated to a sufficient extent in previous studies.
3.3 Disease progression analysis

According to the study design both groups, PD_{GBA} with n = 13 and PD_{Idiopathic} with n = 26, showed no significant differences in sex (PD_{GBA} 69% male, PD_{Idiopathic} 62% male, \( p = 0.73 \)) and disease duration (PD_{GBA} 7.5 years, PD_{Idiopathic} 6.7 years, \( p = 0.65 \)) as illustrated in Table 13 below [129]. However, PD_{GBA} group showed a lower AAO compared with PD_{Idiopathic} (PD_{GBA} = 50.0 years, PD_{Idiopathic} = 60.0 years; \( p = 0.006 \)) [129]. Since disease duration was one of the two matching criteria, on average, PD_{GBA} subjects were younger at time of baseline 2010 and in the first follow-up examination in 2011 and in the second follow-up in 2013 [129]. A general demographic overview of the two subgroups investigated is shown in Table 13 [129].

Table 13: Overview of demographic characteristics and disease duration

<table>
<thead>
<tr>
<th>Variable</th>
<th>( \text{PD}_{\text{GBA}} ) ( n=13 )</th>
<th>( \text{PD}_{\text{Idiopathic}} ) ( n=26 )</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at onset (years)</td>
<td>50.0 (28-65)</td>
<td>60.0 (35-67)</td>
<td>0.006</td>
</tr>
<tr>
<td>Age_0 (years)</td>
<td>57.0 (40-71)</td>
<td>66.0 (38-75)</td>
<td>0.004</td>
</tr>
<tr>
<td>Age_1 (years)</td>
<td>58.0 (41-73)</td>
<td>67.0 (39-76)</td>
<td>0.004</td>
</tr>
<tr>
<td>Age_3 (years)</td>
<td>60.0 (43-75)</td>
<td>69.0 (41-78)</td>
<td>0.003</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>69</td>
<td>62</td>
<td>0.73</td>
</tr>
<tr>
<td><strong>Disease duration</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease Duration_0 (years)</td>
<td>7.5 (6.1)</td>
<td>6.7 (3.8)</td>
<td>0.65</td>
</tr>
<tr>
<td>Disease Duration_1 (years)</td>
<td>8.5 (6.4)</td>
<td>7.7 (3.8)</td>
<td>0.66</td>
</tr>
<tr>
<td>Disease Duration_3 (years)</td>
<td>10.2 (6.5)</td>
<td>9.7 (3.8)</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Normal-distributed data are given as mean with standard deviation in brackets. Non-normally distributed data are given as median with range in brackets [129]. \( n= \) sample size. PD_{GBA}: patient with Parkinson’s disease and heterozygous GBA mutation. PD_{Idiopathic}: patient with Parkinson’s disease with wildtype GBA gene. Age at onset: age in years when PD diagnosis was given to the patient. Age_0: age at time of baseline investigation 2010. Age_1: age at first follow-up investigation 2011. Age_3: age at second follow-up investigation 2013. The same applies to disease duration_0 to _3 [129].

At baseline, there were no significant differences in motor symptoms and disease stage in both groups (\( \text{UPDRS} - III: p = 0.92, \text{H&Y}: p = 0.39 \)) [129]. Non-motor impairment also did not differ significantly with respect to cognition (\( \text{MoCA}: p = 0.56 \)) and mood (\( \text{BDI} - II: p = 0.48 \)) and LED was similar in both subgroups (\( \text{LED} p = 0.62 \)) [129].
The distribution of PD subtypes was similar in both groups, as outlined in Figure 15 below:

![PD subtype distribution]

Figure 15: Overview of the distribution of the three PD subtypes: Equivalent, akinetic-rigid and tremor-dominant – compared for both groups PD_{GBA} and PD_{Idiopathic} assessed in 2010 (baseline examination), \( p = 0.75 \) [129]. PD: Parkinson’s disease. GBA: glucocerebrosidase gene. The intra-group analysis of PD_{GBA} showed a significantly faster progression over the whole 3-year observation period in terms of severity of motor symptoms (UPDRS – III \( p = 0.03 \)), disease staging (H&Y \( p \leq 0.001 \)), medication (LED \( p = 0.01 \)), and cognition (MoCA: \( p = 0.04 \)) as outlined in Figure 16 up to Figure 19 below [129].

![Progression of severity of motor symptoms: UPDRS-III]

Figure 16: Longitudinal intragroup analysis of disease progression UPDRS-III from 2010 up to 2013 of UPDRS-III, according to paired t-test. PD (GBA) in blue and PD (Idiopathic) in light grey. The P-value specifies the intragroup difference for PD_{GBA} with * \( p \leq 0.05 \) and for PD_{Idiopathic} with \( p = 0.94 \). UPDRS-III: Unified Parkinson’s disease rating scale part 3. Permission granted by John Wiley and Sons [129].
Figure 17: Longitudinal intragroup analysis of disease progression H&Y from 2010 up to 2013 of Hoehn and Yahr Staging (H&Y), according to paired t-test. 
PD (GBA) in blue and PD (Idiopathic) in light grey. The P-value specifies the intragroup difference for PD_{GBA} with *** $p = \leq 0.001$ and for PD_{Idiopathic} with $p = 0.08$ [129]. Permission granted by John Wiley and Sons.

Figure 18: Longitudinal intragroup analysis for disease progression LED from 2010 up to 2013 of Levodopa equivalent dosage (LED) according to paired t-test. 
PD (GBA) in blue and PD (Idiopathic) in light grey. The P-value specifies the intragroup difference for PD_{GBA} with ** $p = \leq 0.01$ and for PD_{Idiopathic} with *** $p = 0.001$ [129]. Permission granted by John Wiley and Sons.
The PD\textsubscript{Idiopathic} control group showed a comparable development with regard to medication only ($LED: p = 0.001$) but not in terms of severity of motor symptoms ($UPDRS - III p = 0.94$), disease staging ($H\&Y p \leq 0.08$ and cognition ($MoCA p = 0.95$). The non-motor characteristic mood was stable within both groups in the longitudinal evaluation ($BDI - II: PD\textsubscript{GBA} p = 0.22$, $PD\textsubscript{Idiopathic} p = 0.94$), being illustrated in Figure 20 below:
Finally, **Table 14** below gives a demographic overview of the intra- and inter-group results of progression analysis below:

Table 14: Overview on intergroup (white) and intragroup (blue) differences between PD\textsubscript{GBA} and PD\textsubscript{Idiopathic} regarding motor and non-motor symptoms over the 3-years-observation period.

<table>
<thead>
<tr>
<th>Variable</th>
<th>PD\textsubscript{GBA} n=13</th>
<th>PD\textsubscript{Idiopathic} n=26</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Severity of motor symptoms</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UPDRS-III 0</td>
<td>27.9 (11.4)</td>
<td>27.5 (11.1)</td>
<td>0.92</td>
</tr>
<tr>
<td>UPDRS-III 1</td>
<td>29.3 (6.9)</td>
<td>29.7 (11.5)</td>
<td>0.91</td>
</tr>
<tr>
<td>UPDRS-III 3</td>
<td>36.4 (8.0)</td>
<td>27.4 (9.2)</td>
<td>0.005</td>
</tr>
<tr>
<td>UPDRS-III 0-3 Progression Paired T-test / p-Value</td>
<td>0.03</td>
<td>0.94</td>
<td>-</td>
</tr>
<tr>
<td><strong>Disease staging</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H&amp;Y 0 (years)</td>
<td>2.1 (0.5)</td>
<td>2.3 (0.6)</td>
<td>0.39</td>
</tr>
<tr>
<td>H&amp;Y 1 (years)</td>
<td>2.3 (0.5)</td>
<td>2.5 (0.6)</td>
<td>0.43</td>
</tr>
<tr>
<td>H&amp;Y 3 (years)</td>
<td>2.7 (0.7)</td>
<td>2.5 (0.7)</td>
<td>0.26</td>
</tr>
<tr>
<td>H&amp;Y 0-3 Progression Paired T-test / p-Value</td>
<td>≤0.001</td>
<td>0.08</td>
<td>-</td>
</tr>
<tr>
<td><strong>Medication</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-dopa-equivalent-dosage_0</td>
<td>484.6 (358.5)</td>
<td>537.5 (291.0)</td>
<td>0.62</td>
</tr>
<tr>
<td>L-dopa-equivalent-dosage_0</td>
<td>620.8 (407.6)</td>
<td>639.5 (296.7)</td>
<td>0.88</td>
</tr>
<tr>
<td>L-dopa-equivalent-dosage_0</td>
<td>950.0 (714.1)</td>
<td>687.3 (338.2)</td>
<td>0.13</td>
</tr>
<tr>
<td>L-dopa-equivalent-dosage 0-3 Progression Paired T-test/ p-Value</td>
<td>0.01</td>
<td>0.001</td>
<td>-</td>
</tr>
<tr>
<td><strong>NMS – cognition</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MoCA_0</td>
<td>26.6 (2.8)</td>
<td>27.1 (2.1)</td>
<td>0.56</td>
</tr>
<tr>
<td>MoCA_1</td>
<td>25.0 (3.2)</td>
<td>26.5 (2.8)</td>
<td>0.18</td>
</tr>
<tr>
<td>MoCA_3</td>
<td>24.9 (2.8)</td>
<td>26.9 (3.2)</td>
<td>0.07</td>
</tr>
<tr>
<td>MoCA 0-3 Progression Paired T-test/ p-Value</td>
<td>0.04</td>
<td>0.95</td>
<td>-</td>
</tr>
<tr>
<td><strong>NMS – mood</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDI-II 0</td>
<td>10.5 (6.2)</td>
<td>8.7 (7.8)</td>
<td>0.48</td>
</tr>
<tr>
<td>BDI-II 1</td>
<td>10.4 (7.2)</td>
<td>8.3 (7.3)</td>
<td>0.44</td>
</tr>
<tr>
<td>BDI-II 3</td>
<td>12.5 (7.8)</td>
<td>8.8 (6.3)</td>
<td>0.13</td>
</tr>
<tr>
<td>BDI-II 0-3 Progression Paired T-test/ p-Value</td>
<td>0.22</td>
<td>0.94</td>
<td>-</td>
</tr>
</tbody>
</table>

*Normal-distributed data are given as mean with standard deviation in brackets, non-normally distributed data are given as median with range in brackets. UPDRS-III: Unified Parkinson’s disease rating scale part 3. H\&Y: Hoehn and Yahr scale. MoCA: Montreal Cognitive Assessment. BDI-II: Becks Depression Inventory II. PD\textsubscript{GBA}: patients with Parkinson’s disease with heterozygous GBA mutation. PD\textsubscript{Idiopathic}: patients with Parkinson’s disease with wildtype GBA gene [129].*
Regression analysis showed that L444P and N370S mutations in GBA gene independently affect motor performance ($UPDRS - III: p = 0.005$) and cognition ($MoCA: p = 0.01$) [129]. Notably, there was no significant association between the severity of clinical signs and the variables AAO, age at examination, and disease duration [129].

3.4 Survival analysis

At time of baseline, both groups, PD$_{GBA}$ with $n = 20$ and PD$_{Idiopathic}$ with $n = 27$, showed no significant differences in sex (PD$_{GBA}$ 60% male, PD$_{Idiopathic}$ 63% male, $p = 0.3$), age (PD$_{GBA}$ 62.7 years, PD$_{Idiopathic}$ 65.3 years, p-value = 0.30) and disease duration (PD$_{GBA}$ 9.8 years, PD$_{Idiopathic}$ 7.3 years, $p = 0.15$) [129].

A total of 5 subjects of subgroup PD$_{GBA}$ (25%) died within the 3-years-investigation period between 2010 and 2013, whereas nobody died in subgroup PD$_{Idiopathic}$ (0%) with $p = 0.01$ (see also Table 15 below) [129].

Table 15: Mutational status by the endpoints death or no death within 3-year-period of observation in both subgroups with $n=47$ [129].

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Death (n)</th>
<th>No death (n)</th>
<th>Total (n)</th>
<th>Percentage Death (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD$_{GBA}$</td>
<td>5</td>
<td>15</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>PD$_{Idiopathic}$</td>
<td>0</td>
<td>27</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>42</td>
<td>47</td>
<td>10.64</td>
</tr>
</tbody>
</table>

The cause of death events of the deceased PD$_{GBA}$ subjects was due to PD-associated secondary complications (3x pneumonia, 2x pulmonary embolism), since the subjects were confined to bed by severe and late stages of PD [129]. On average, the 5 individuals who died of PD$_{GBA}$ subgroup presented with age at onset = 56.4 years and age at death = 72.6 years, corresponding to an average disease duration of $dd = 16.2$ years [129]. The last objectified results of these patients, in terms of non-motor characteristics cognition and mood as well as of motor performance and disease stage, corresponded to $MoCA = 15.0$, $BDI - II = 11.0$, $UPDRS III = 49.4$ and $H&Y staging 4$ [129].

In order to consider a possible relation between the two categorical variables "mutational status" and the event "death", a contingency table was created to
demonstrate the proportion of the examined variables. In the following, Pearson's chi-square test was used with the intention of whether the association between the variables only exists in the random sample examined here or whether it is also present in the general population and, if so, up to which extent of probability. The hypotheses $H_0$ and $H_1$ regarding the variables “death” and “mutational GBA status” were defined as follows:

- $H_0$: [death] is not associated with [mutational GBA status]
- $H_1$: [death] is associated with [mutational GBA status]

Chi-square statistic was applied, with $p = 0.01$. Therefore, it was concluded that it exists a significant correlation between the mutational GBA status and the endpoint death, so that $H_0$ had to be rejected.
3.5 Summary of results

In summary, progression analysis for \( n=20 \) PD\textsubscript{GBA} was based on motor and non-motor symptoms, with 7 dropouts over the 3-years-observation period due to the endpoints "death"(\( n=5 \)), "disease-related reduced general condition"(\( n=1 \)) or "no feedback for reasons unknown"(\( n=1 \)) \[129\]. Therefore, longitudinal analysis was carried out with \( n = 13 \) PD\textsubscript{GBA} subjects, compared to 26 PD\textsubscript{Idiopathic} – both groups were matched for the criteria sex and disease duration \[129\].

Both groups were similarly distributed at the time of baseline examination for the variables sex, disease duration, UPDRS-III, H & Y, LED, and MoCA \[129\]. The distribution of PD subtypes was similar in both groups \[129\]. During the 3-year follow-up period, the paired t-test showed a faster progression of motor and non-motor impairment compared to baseline and second follow-up 3 years later in intragroup analysis for PD\textsubscript{GBA} \[129\]. For PD\textsubscript{Idiopathic}, significant alterations were only found considering LED – whereas severity of motor impairment (UPDRS-III, \( p =0.94 \)), disease stage (H & Y, \( p =0.08 \)), and cognitive impairment (MoCA, \( p =0.95 \)) did not significantly worsen in this control group \[129\]. Mood disorders, documented by the BDI-II, appeared to be stable in both groups with PD\textsubscript{GBA} \( p =0.22 \) and PD\textsubscript{Idiopathic} \( p =0.94 \) \[129\]. Regression analysis showed that L444P and N370S mutations in GBA gene independently affect motor performance (\( \text{UPDRS} - \text{III} : p = 0.005 \)) and cognition (\( \text{MoCA} : p = 0.01 \)) \[129\].

There was no significant association between the severity of clinical signs and the variables AAO, age at examination, and disease duration \[129\].

Survival analysis revealed that 25\% (\( n=5 \)) of PD\textsubscript{GBA} subjects reached the endpoint “death” within the 3-year observation period and 0\% of the PD\textsubscript{Idiopathic} Control group, \( p = 0.01 \) \[129\]. The reasons for deceasing in PD\textsubscript{GBA} subgroup were due to complications resulting from the severity of the underlying disease PD\textsubscript{GBA} \[129\]. On average, the following clinical rates were found for the 5 PD\textsubscript{GBA} subjects at their last examination: UPDRS-III 49.4, H & Y staging 4.0, MoCA 15.0 and BDI-II 11.0 \[129\]. Chi-square statistic showed that survival of patients with PD is significantly influenced by the examined GBA mutations N370S and L444P \[129\].
In conclusion, this study led to the following findings:

I Are there significant differences in disease progression regarding:

a. motor symptoms in PD\textsubscript{GBA} patients compared with PD patients with GBA-wt mutational status?
   – Statistical analysis revealed a more rapid disease progression in terms of motor impairment (severity and stage of disease). Significant changes regarding Levodopa equivalent dosage were evident for both PD\textsubscript{GBA} and PD\textsubscript{Idiopathic}.

b. non-motor symptoms in PD\textsubscript{GBA} patients compared with PD patients with GBA-wt mutational status?
   – Statistical analysis revealed a more rapid disease progression in terms of cognitive impairment (global cognition). No significant changes regarding mood disorders were evident for both PD\textsubscript{GBA} and PD\textsubscript{Idiopathic}.

II Do the survival rates differ between the investigated subgroups?
   – Statistical analysis showed that survival of patients with PD is significantly influenced by the GBA mutations N370S and L444P.
4. Discussion

4.1 Key findings of this study

This study phenotyped PD\textsubscript{GBA} subjects in a longitudinal setting in direct comparison with PD\textsubscript{Idiopathic} subjects and yielded the following 2 key findings: First, PD\textsubscript{GBA} subjects show a faster progression of disease in terms of motor impairment and cognitive decline [129]. Second, PD\textsubscript{GBA} subjects have relatively decreased survival rates compared to PD\textsubscript{Idiopathic} [129].

4.2 Accelerated disease progression in PD\textsubscript{GBA}

4.2.1 PD\textsubscript{GBA} – associated with younger age at disease onset

The PD\textsubscript{GBA} patients included in this study showed a younger AAO, compared to the PD\textsubscript{Idiopathic} group [129]. Since disease duration (in addition to gender) was used as a matching criteria at baseline, PD\textsubscript{GBA} subjects were consequently younger at time of follow-up examinations in 2011 and 2013 than PD\textsubscript{Idiopathic} individuals [129]. In order to not obscure the remarkable fact that younger age of PD\textsubscript{GBA} was also accompanied by a faster progression of disease and reduced survival, the two groups were not corrected for age [129]. These findings are in line with the results of a large GWA study, that revealed a difference of AAO between PD\textsubscript{GBA} and PD\textsubscript{Idiopathic} of 6 years – to the disadvantage of PD\textsubscript{GBA} [374] – suggesting that GBA mutations might be associated with a more aggressive pathological pathway – leading to an earlier disease manifestation.

Given the fact that PD\textsubscript{GBA} and PD\textsubscript{Idiopathic} are barely distinguishable at the time of diagnosis, the important question arises as to when the process of rapid deterioration in PD\textsubscript{GBA} begins [19, 129]. This 3-year-period study showed a greater extent of severity of motor symptoms, disease staging and cognitive impairment for PD\textsubscript{GBA} after 8-9 years of disease duration, proposing that the process of aggravation in PD\textsubscript{GBA} may be associated with later disease stages [129]. This hypothesis was recently confirmed by a Thai case-control study [297]. The underlying mechanisms for a rather late acceleration of progression may comprise compensatory processes that can be sustained at the young onset of PD but become overloaded and ultimately fail as PD progresses [129]. This theory is supported
by the finding of an age-dependent decreased GCase activity in SN and putamen in healthy individuals, suggesting that this may additionally lower PD threshold in GBA carriers [375, 376] and might be the reason for an accelerated disease progression in later PD stages on the one hand and for a clinical manifestation at a relatively younger age on the other hand. Controversial results of a retrospective study, indicating an acceleration much earlier in the course of the disease, will be further discussed below (see 4.5 Prodromal characteristics of PD and the role of PDGBA) [312].

Fascinating findings of Thomas et al strengthen the idea of a gradually overloaded concept: they studied the abundance and the metabolism of proteins, involved in the formation of extracellular vesicles (EV) in an animal model with mutant GCase [377]. According to the authors, EVs are assumed to represent a possible way for aggregated proteins to spread [377]. Spreading of protein-based molecules between cells is a physiological process but it is also discussed intensively regarding neurodegenerative diseases [377]. Thomas et al reported a higher frequency of EV-proteins in mutant-GCase-flies, leading to an increased formation and finally to an amplified release of EVs [377].

What do these EVs contain and where do they go? According to Thomas et al, they include molecules such as α-syn, which rather support protein aggregation, as well as chaperones, which rather inhibit aggregation [377-380]. Neurons both sent and receive these loaded EVs [377]. If proteins increasingly tend to aggregate, the corresponding cell becomes a kind of aggregate donor towards other recipient cells [377]. These cells receive this aggregatory package and are in need for adequate quality controls now, to keep the aggregation-promoting factors at bay [377]. If this fails, e.g. due to reduced GCase activity in the donor cell, accumulation of glucosylceramides and altered cell membrane configuration occurs, thereby increasing EV transfer from donor cells to recipient cells [377]. These, in turn, are increasingly overwhelmed under the burden of intensified protein aggregation [377]. In conclusion, the authors suggested that mutant GCase contributes to an altered cellular lipid membrane, which might amplify the transmission rate of EVs towards other neuronal cells [377]. These recipient cells in
Discussion
Accelerated disease progression in PDGBA

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turn might get along with this enlarged amount of aggregating factors only up to a certain point until the collapse due to neurotoxic effects [377].

Are these developments natural consequences due to normal aging processes? This hypothesis is supported by the fact that older flies with wt GCase also tend to present protein aggregation [381, 382]. However, a comparison of protein turnover and protein abundances of elderly and younger flies with wt GCase revealed that only 4% of the EV-associated proteins reached accelerated or elevated levels in older flies [377]. In the mutant GCase model, however, almost 60% of the EV-associated proteins presented with a more rapid turnover or increased frequency [377]. Therefore, Thomas et al reported these effects to be related with GBA mutations but not to be an expression of regular aging process. Therefore, GCase-related increased EV-release with consecutive overload of recipient neurons might explain the more rapid disease progression in PD\textsubscript{GBA} compared to PD\textsubscript{idiopathic} – especially in later PD stages as reported in this submitted study [129, 377].

4.2.2 PD\textsubscript{GBA} – associated with impaired olfactory performance

Due to the fact that olfactory loss is an important marker in prodromal PD and often occurs in combination with impaired cognition in PD\textsubscript{GBA} (see below 4.2.3), this symptom is briefly discussed here [104, 383].

An impaired olfactory ability is more common in GD and in GBA-carriers compared to GBA wt individuals [294, 311]. Further, olfaction is reduced in the early stages of PD and GD subjects, suffering simultaneously from PD as well, are associated with a higher risk for poor olfactory performance, compared with PD\textsubscript{idiopathic} subjects [294, 311]. The early onset of impaired olfactory ability is supported by Braak’s hypothesis that PD begins in peripheral neuronal structures with retrograde spreading – including the olfactory tract, the brainstem and finally cortical structures [12, 124]. The finding that olfaction may be generally limited in early PD stages but is more common in cases of altered GBA status, may be due to a mutation-specific neurodegenerative pattern [12]. This pattern may cause an early and more intense neuronal loss in the olfactory pathway in PD\textsubscript{GBA} subjects who consequently reach the symptomatic threshold faster than PD\textsubscript{idiopathic} patients
[12]. Of course, it cannot be ruled out that the above-mentioned additionally reduced GCase function in GBA mutations may have extra accelerating effects regarding the decline of olfactory performance.

4.2.3 PD\textsubscript{GBA} – associated with impaired cognition

This present study revealed a significant and independent association between the altered GBA mutational status and global cognition [129]. A multicenter study with a cross-sectional design confirmed this result and found especially the subfields visuospatial abilities, working memory as well as executive functions to be impaired in PD\textsubscript{GBA} [384]. These are represented in parieto-occipital as well as frontal cerebral regions [385, 386]. The functions of working memory and executive performance were assessed in particular by a large clinical study, investigating elderly but neurodegenerative healthy individuals between 50 and 80 years: subjects with poorer skills in working memory and executive performance prioritized in favor of cognitive challenge and to the disadvantage of motor power if they were exposed to cognitive and motor stress simultaneously [351]. The authors therefore assumed a possible correlation between the prioritization behavior on the one hand and cognitive flexibility and working memory on the other hand in elderly people [351]. Therefore, regarding cognitive impairment based on frontal and parieto-occipital dysfunction, PD\textsubscript{GBA} may present with an extra affection, in addition to normal aging processes, with the described cognitive impairments.

Regarding that PD\textsubscript{GBA} is also linked with a greater risk of dementia [13, 19, 285, 313], it is thrilling to discuss this dual syndrome hypothesis for cognitive decline also against this background. According to this hypothesis, the posterior cortical syndrome is mainly linked with dementia whereas the frontostriatal dopamine-mediated syndrome leads to executive dysfunctions [387]. Studies examined the influence of various PD-associated genes on both the frontostriatal dopamine-mediated dysfunction and the posterior cortical syndrome: PD\textsubscript{GBA} has been shown to be associated with the development of dementia as well as with an altered frontostriatal metabolism (ascertained by using $^{123}$I-2-β-carbomethoxy-
3β-(4-iodophenyl)-N-(3-fluoropropyl)-nortropane (123I-FP-CIT SPECT) [388]. No other investigated gene, namely apolipoprotein E gene (APOE), microtubule-associated protein tau (MAPT) gene, COMT gene and SCNA) presented this double role [388]. Is this particular role of GBA regarding cognitive decline possibly based on a GBA-specific neuropathological pattern?

Indeed, it has been reported several times that clinical symptoms in PD seem to be related to the extent of the disease’s hallmark – Lewy body pathology [21]. Neuropathologically, Lewy body disorder is seen in both PD_{GBA} and PD_{Idiopathic} but PD_{GBA} demonstrated a more diffuse neocortical distribution in comparison [21, 129]: all brain samples out of 17 investigated PD_{GBA} patients showed PD-typical changes in terms of α-syn-immunopositive Lewy bodies and Lewy neurites [21]. Each specimen examined corresponded to Braak Stage 5 or 6, indicating that there was neocortical involvement in all PD_{GBA} cases – although the frequency of the single Braak stages did not differ significantly between PD_{Idiopathic} and PD_{GBA} [21, 145]. Alcalay et al confirmed a more pronounced cortical Lewy Body pathology for PD_{GBA} as well [13].

However, the results of a recent longitudinal clinical-pathological study rather weaken this assumption of a specific underlying neuropathological pattern [389]. It was reported that, although the enrolled PD_{GBA} subjects died 5 years earlier on average than PD_{Idiopathic} patients while presenting with the same disease duration, no neuropathological differences were obtained with respect to whole or region-specific Lewy body pathology [389]. Furthermore, no differences were seen regarding senile plaques, leukoaraiosis and fibrillary tangles, even though it should be noted that only the limited sample size of n = 12 PD_{GBA} could be included in the analysis [389]. Another study, raising the actual cortical densities of Lewy bodies, also revealed that PD_{GBA} did not present with a more severe Lewy body pathology in sense of increased density of proteins [390]. It is therefore uncertain whether it is the existence of pathological Lewy substrates that verifiably leads to an impaired cognition in PD and if these substrates are actually of diverse distribution in GBA carriers and non-carriers – which is why further studies, investigating this important issue, are urgently needed [384].
4.2.4 PD\textsubscript{GBA} – heterogenous phenotype due to severe and mild GBA mutations

Although it is now clear, that the presence of homozygous and heterozygous GBA mutations is associated with a significantly increased risk of PD, the gene dose effect is discussed controversially. Some studies reported that gene dose does not seem to have an overly effect on the extent of PD-risk [375], while others observed the severity of PD-phenotype to be related to the strain of GBA mutations [311]. Further, some GBA mutations were stated to be associated with more severe types of GD (including L444P) and others with rather mild forms (such as N370S) [165]. Intriguingly, case-control studies with Ashkenazi-derived Jewish subjects demonstrated that severe GBA mutations increased the risk of developing PD nearly 14-fold whereas milder GBA mutations only doubled the risk of developing PD and GBA polymorphism E326K presented with the lowest PD-risk (OR = 1.7) [165, 391, 392]. Furthermore, PD\textsubscript{GBA} subjects with predominantly recombinant or null GBA mutations were reported to exhibit a decreased AAO compared with PD\textsubscript{GBA} individuals with milder GBA variants: mean AAO=39 years vs. mean AAO= 51 years, p = 0.008 [36, 165, 238].

Also, PD\textsubscript{GBA} cases associated with more severe mutations showed a stronger association with dementia, motor and olfactory symptoms as well as an accelerated cognitive decline than patients with rather mild mutations [3, 393, 394]. Fittingly, a Norwegian clinical study with a 7-year-observation period revealed the carriers of GBA variants T369M, L444P and E326K to progress more quickly to PDD than subjects with wt GBA status [395]. According to more detailed findings from Cilia et al, the risk of dementia was increased almost 3-fold, comparing more deleterious PD\textsubscript{GBA} mutations (e.g.\text{IVS10+1G>T, G377S, L444P}) with rather mild PD\textsubscript{GBA} variants (e.g.\text{N370S}), whereas PD-risk was amplified almost 6 times through severe GBA mutations compared to PD\textsubscript{Idiopathic} [3].

Hence, there is increasing evidence for parallels between GBA genotype and PD phenotype so that PD\textsubscript{GBA} intragroup analyzes would have been also desirable in this presented study. However, even though the majority (77%) of PD\textsubscript{GBA} subjects presented with the more severe GBA mutation L444P, a mutation-specific analysis comparing the N370S-carrier (n=3) with L444P-carrier (n=10) was not performed due to the small sample size and expected bias [129].
Nevertheless, a review of previous GWAS revealed that single nucleotide polymorphisms in the GBA gene are very likely associated with the risk of developing PD [396]. Sequence analyzes of several susceptibility genes as well as of genes associated with autosomal dominant or recessive PD (SNCA, LRRK2, PARK2, PINK1, PARK7, MAPT) and GBA, discovered a total of 47 rare variants [374]. The authors of this GWAS concluded that:

- genetic variants of GBA significantly increase PD-risk,
- the presence of multiple GBA variants may be a greater risk compared to other PD-associated variants and
- the influence of unexamined GBA variants on PD-risk is probably higher than previously estimated [374].

Regarding the fact, that the greater part of GD patients does not present symptoms of PD in the course of their disease [135] and there are asymptomatic carriers of GBA-mutations as well, one could assume that asymptomatic subjects do either provide useful instruments against aggregation of glucocerebrosides or to use another suitable pathway for metabolizing the accumulating glycoproteins [162]. A further explanation might be related to the phenomenon of genetic variance referring to interactions of genes with each other and with environmental factors as well [162].

The special role of the polymorphism E326K
While there is growing evidence for an association between genetic GBA-variants and synucleinopathies, no association was identified for tauopathy entities such as CBD or PSP [274]. A strong association for the GBA polymorphism E326K with both Lewy body disorders PDD and DLB was objectified by a Spanish clinicopathological study [276]. Interestingly, for E326K and another GBA mutation T369M, no association with GD could be shown – even in homozygous individuals – but for PD subjects, although to varying degrees [277-279]. This might be due to either two independent pathological mechanisms of PD and GD or due to a common pathway of GD and PD, which alone is too weak to trigger GD, but
may lead to PD along with other genetic or environmental aggravating factors [277]. This presumption is supported the CD/RV hypothesis as Mitsui et al suggested:

“[...] We should emphasize a paradigm shift from the common disease–common variants hypothesis to the common disease–multiple rare variants hypothesis in our search for disease susceptibility genes in sporadic PD, which may be applicable to studies of other diseases [...]” [397].

4.2.5 PD$_{GBA}$ – associated with impaired motor performance

Although both groups, PD$_{GBA}$ and PD$_{Idiopathic}$, were similarly affected at the time of baseline investigation (regarding UPDRS, H&Y, LED), subjects with mutated GBA status progressed more rapidly to a higher severity of motor impairment after 3 years and reached also higher H&Y stages – which was also reflected in increased dopaminergic medication levels [129]. Similar to the results described here, further studies confirmed GBA mutations to be associated with an increased risk of motor impairment (UPDRS-III) [8, 384]. In addition, some GBA variants together with the polymorphism E326K were associated with postural instability and gait difficulty (PIGD) and presented with more rapid progression in PIGD scores in the longitudinal evaluation [8]. A pilot study by Srulijes et al, focused on gait analyzes during the simultaneous execution of single and dual task assessments showed a worse motor performance under dual motor task conditions in PD$_{GBA}$ than in PD$_{Idiopathic}$ [398]. As mentioned above, cognitive impairment and also a higher chronological age may comprise possible reasons for poorer dual task motor performance. Therefore, it should be discussed if poorer gait performance in PD$_{GBA}$ is rather due to more severe cognitive impairment instead of presenting an independent motor deficit due to the mutational GBA status. Ensuring posture and gait requires a complex interplay of cortical extrapyramidal impulses, visual stimuli and information from brain stem, cerebellum and spinal afferents. Considering, that PD$_{GBA}$ is associated in particular with frontostriatal and parieto-occipital dysfunction as mentioned above, corresponding lesions in these areas may explain an emphasized axial motor involvement. This reflection might be strengthened by results of a Chinese neuroimaging study showing a correlation between axial motor deficits on the one hand and particularly
pronounced deep white matter hyperintensities in frontal and occipital lobes of PD patients – possibly impairing the nerval pathways due to cerebral microangiopathic lesions [399]. However, it should be noted that this MRI study was designed only cross-sectional and that PD_{GBA} was not examined as a separate subgroup – as PD patients were enrolled in general without carrying out a mutational screening in advance.

Although tremor analysis was carried out for both groups in this study, it was not statistically analyzed in order to not go beyond the scope. However, other reports showed that PD_{GBA} did not achieve worse levels regarding progression in tremor scores compared with PD_{idiopathic} [8]. Rest tremor was even found less frequently in PD_{GBA} compared to PD_{idiopathic} [129, 400]. According to a report by Helmich et al, the pacemaker for rest tremor in PD may be formed by thalamic neurons that get hyperpolarized due to reasons, that are not sufficiently understood until now and are oscillating at the typical PD-frequency of 5-6 Hz [43, 401]. Another hypothesis suggests, that the PD-tremor pacemaker might rather be in the basal ganglia and perhaps related with the dopaminergic deficiency [43]. Contradictory to this thesis, however, are the findings that rest tremor usually responds less well to dopaminergic therapy and that the deep brain stimulation of the posterior region of the ventrolateral thalamus achieved beneficial results in terms of tremor reduction [43].

4.3 Reduced survival in PD_{GBA}

A prospective study, aiming to identify independent risk factors for mortality in PD_{idiopathic}, could identify psychotic symptoms, dementia, motor deficits, chronological age and AAO as possible predictors [402]. Further, PD_{idiopathic} patients were revealed to reach the PD-milestones:

- postural instability corresponding to H&Y stage 3,
- dementia and
- death
within 10 years from diagnosis [403]. The finding of PD_{GBA}, progressing more rapidly to the key milestones dementia and H&Y stage 3 by Winder-Rhodes et al, is augmented by this presented study [19, 129]. Therefore, one might hypothesize that GBA mutations are an important predictor for the rate of disease progression in PD [129]. Perchance, this genetic component may be even more relevant than higher AAO or overall advanced age [129]. Nevertheless, the sample size of n=20 PD_{GBA} matched with 27 controls might be too small to allow undistorted conclusions about the influence of GBA mutations on survival in PD [129]. However, in a PD-genotype-phenotype study, Cilia et al recently showed on the one hand that the GBA carriers (n=123) had a greater mortality risk than PD patients with wt status and on the other hand, that carriers of mild (n=67) and severe mutations (n=56) had a comparable mortality risk [3]. This strengthens the assumption of a significant influence of GBA status on survival, based on this study with a relatively small sample size – although even larger samples for the comparison of mild and severe GBA mutations in terms of associated mortality risk may be required [3, 129]. Furthermore, Cilia et al explored the exciting issue whether the increased risk of dementia-development in GBA carriers also influences PD-mortality risk [3]. Cox regression analysis for the endpoint death showed a significantly increased risk of mortality for GBA carriers, no matter if the time-dependent variable "dementia" was brought in or not [3]. Cilia et al concluded that besides dementia, other components may contribute to the increased mortality rates in PD_{GBA}.

4.4 GBA and its contribution to the pathogenesis of PD
At present, the underlying mechanisms, how GBA mutations contribute in particular to PD-pathology, are still not fully understood – although there are several causative hypotheses. These considerations are mainly focused on interaction of GCase and α-syn [316, 317], dysfunctional autophagy-lysosomal pathways[6], mitochondrial impairment [318], impaired calcium homeostasis [319], ERAD [142] and dysfunctional lipid metabolism [320]. In some cases, these hypotheses
may appear contradictory or overlap each other as they all describe different ways of accessing the same complex pathological architecture of PD.

4.4.1 The spectrum between reduced cerebral GCase activity and alpha-synuclein

The reasons for neurometabolic changes of α-syn turnover in PD_{GBA} are not sufficiently understood yet. A possible explanation is provided by experimental studies of Mazzuli et al, using both murine and human GD stem cell model and describing a bidirectional loop via α-syn [225]. Due to wt GBA allele, normal physiological GCase binds α-syn and mediates its degradation. A reduced neocortical GCase activity due to genetic variants, however, had several consequences (see also Figure 21):

- glucosylceramide deposits,
- lower lysosomal degradation efficiency,
- deposition of α-syn and
- oligomerization and fibrillization of α-syn, which is considered to be neurotoxic [225, 404].
Glucocerebrosidase gene (GBA) mutations impact enzymatic function of Glucocerebrosidase enzyme (GCase), which decreases the lysosomal capacity. Glucosylceramides (GlcCer) increase due to this dysfunction and support the accumulation of alpha-synuclein (α-syn). This decreases GCase activity even more and further, α-syn deposits oligomerize and fibrillize - which has neurotoxic effects to the cell. Also, reactive oxygen species, mutation in the alpha-synuclein gene (SCNA), mitochondrial dysfunction and nitric oxides (NO) may contribute to α-syn accumulation as well. Further, this accumulation affects GCase trafficking from the endoplasmic reticulum (ER) to the lysosome, which decreases the lysosomal GCase capacity even more and leads to GCase retention in the ER.

Westbroek et al suggested the latter effect to be a gain-of function – by proposing that the mutated GCase increases α-syn’s tendency to aggregate [135]. This hypothesis was strengthened by a neuropathological study that indicated a varying proportion of α-syn in Lewy bodies, stratified by genotype: In GBA-homozygous subjects, 90% of the Lewy bodies were GCase-positive, in heterozygous PD_{GBA} 75% of the Lewy bodies were positive for the mutant enzyme, whereas only 4% of Lewy bodies were positive in PD_{idiopathic} with wt GCase [316].

Further, α-syn accumulation in turn impaired the trafficking of GCase between the ER and the lysosome – even in PD-models with wt GBA as in-vitro studies revealed: The interaction of GCase with membrane-fixed α-syn in acidic
environment had inhibitory effects on GCase [225, 375, 405]. The authors hypothesized that α-syn might alter or hinder conformational changes of GCase which are essential for unrestricted enzymatic capacity [405]. Consequently, the intralysosomal GCase activity decreases even more due to this bidirectional loop [225, 404, 406]. Further, other factors can lead to accumulation, oligomerization or fibrils of α-syn: mitochondrial diseases with formation of free radicals and nitric oxides or mutations in the SCNA gene, which encodes α-syn (Figure 21) [406].

Dehay et al therefore concluded that a potential therapeutic approach in PD could exist in increasing or restoring GCase capacity and thus to prevent α-syn accumulation [404]. Notably, a study based on neuroblastoma cell lines showed that a direct inhibition of GCase led to accumulation of α-syn, but decreased GBA gene expression did not result in a relevant α-syn gathering [407, 408]. Direct inhibition of GCase at the lysosomal level therefore appears to have a different effect than epigenetic influence, highlighting the complexity of the epigenetic architecture of PDGBA and interaction of regulatory mechanisms between GBA, GCase and other proteins such as α-syn.

Brain sample analyses revealed a clear reduction of cerebral GCase activity in PDGBA with a regional focus on putamen and SN, which emphasizes the relevance of lysosomal metabolism in this dopaminergic brain region for PD [317]. Nevertheless, one could argue that this is just an expression of a nonspecific aging process. Still, two features contradict the assumption that these findings were simple an expression of general neurodegeneration: there was also, due to unknown reasons, a significant lower cerebellar enzyme activity, which is an atypical localization of neurodegenerative changes in PD [317]. Further, there was no decrease of GCase activity in those areas, that are affected by neurodegenerative remodeling by other diseases such as AD [317].

Repeatedly, a negative correlation was pronounced between GCase activity and α-syn levels [12]. GBA homozygous and compound heterozygous individuals showed lower enzymatic activity than GBA heterozygous subjects and these, in turn, had lower GCase activity than non-GBA carriers [409]. In an experimental study, a group of primary cortical murine neurons were analyzed for their rate of...
α-syn-degradation [408]. Neuronal heterozygosity for L444P resulted in a reduced GCase activity of only 40% and led to enrichment of α-syn up to 77%, due to a half-life increase [408]. It was hypothesized, that even a moderate degree of reduced GBA activity and subsequently only a partial loss of lysosomal function may also be conducive to the onset of PD [408]. Interestingly, a heterozygous L444P status also exacerbated the motor and enteric impairments in a murine model, representing the point mutation A53T in SCNA [408]. The authors concluded that GBA mutations may additionally worsen PD phenotypes, that are already associated with an impaired α-syn turnover [408].

Intriguingly, the implementation of normal GBA genes via viral vectors into murine brain tissue and thus the cerebral expression of wt GCase reduced the toxic accumulation of GlcSph, the levels of α-syn aggregates and protein tau and ubiquitin in type 1 GD-PD-mouse-model [324]. This demonstrates the biochemical impact of reduced and normal GCase performance on α-syn metabolism and it further suggests, that normalizing GCase function at an early stage of PD may prevent further neuronal loss. In addition, even a partial reversal of neuronal damage, that already occurred, seems to be possible – according to experimental findings of Sardi et al [324]. They evaluated the cognitive performance of type1 GD-PD mice by using an object recognition test [324]. After expression of wt hippocampal GBA the murine cognitive decline was found improved [324].

Nevertheless, in a postmortem study, Murphy et al studied the anterior cingulate and occipital cortex of PDidiopathic subjects, assigned to earlier and to later Braak stages [396]. They found, especially in regions with elevated α-syn levels, lower GCase protein levels and lower also GBA activity [396]. As GCase activity was also diminished in brain samples from DBL subjects and there are GBA-carriers never developing PD, one could suggest that both synucleinopathies PD and DBL may be due to a common pathway – influenced by GBA mutations which are more likely to be understood as aggravating and accelerating factors in PD and less causally initiate the pathology of PD [317, 410].
4.4.2 Interaction with the autophagy-lysosomal pathway (ALP)

It was hypothesized, that neurodegenerative pathology in type 2 GD may be due to decreased autophagy performance and dysfunctional proteasome [318]. This might lead to more defective mitochondria, more ubiquitinated metabolites and to α-syn accumulation [318].

In addition, increased rates of cortical, cerebellar, and hippocampal cell death were detected which lead to the assumption of a causal link between impaired GCase function, α-syn accumulation and neuronal survival [318, 411]. Autophagy, as a cellular pathway for degradation, comprises the transport of metabolites into the lysosome as the site of degradation, whereas three distinct mechanisms are differentiated: (1) chaperone-mediated autophagy (CMA), (2) macroautophagy, and (3) microautophagy [6, 412, 413].

- Via CMA, metabolites such as abundant or dysfunctional α-syn, get bound and transported to the lysosomal membrane, where they are entered receptor-associated into the intralysosomal domain for degradation [413].
- Macroautophagy is a multistep intracellular signaling cascade to degrade ubiquitin-labelled metabolites such as proteins and cell organelles [12, 135]. The protein complex mechanistic target of rapamycin (mTOR) controls the formation of autophagosomes, which are fused afterwards with lysosomes becoming autophagolysosome [135]. They contain acid hydrolases for decomposing their content [135].
  - Further, macroautophagy includes the mitophagy pathway, selectively discarding dysfunctional mitochondria [6, 413].
- Microautophagy defines the direct internalization of cytoplasm-localized metabolites through the lysosomal membrane [6].

Neurons as post-mitotic cells are particularly susceptible to defective, impaired autophagy pathways, as they are not able to redistribute defective proteins or cell organelles to daughter cells [414]. Fascinatingly, lysosomal degradation pathways are increasingly associated with the development of neurodegenerative diseases such as PD [413]: several PD-associated genes (such as GBA-, LRRK2, SCNA- and Scavenger receptor class B member 2 (SCARB2) gene) are involved
in the autophagy-lysosomal pathway (ALP) [6, 217, 404, 415, 416]. Their products play a role in different functional areas of the ALP such as in mitophagy, as lysosomal enzymes or as trafficking-components [6].

 Particularly, the SCARB2 gene encodes for the lysosomal membrane protein 2 (LIMP-2), that targets GBA to lysosomes [217]. A deficiency of this trafficking receptor LIMP-2 can lead to reduced GBA activity in mouse models and thus to increased α-syn levels as well [417].

Interestingly, mitophagy pathway is regulated by the ligase Parkin and the kinase PINK1, whereas mutations in these genes cause familial recessive PD [135]. In intact mitochondria, PINK1 passes from the outer to the inner membrane [135]. In defective mitochondria, PINK1 remains on the outer membrane – accumulating and recruiting cytosolic parkin to the outer mitochondrial membrane [135, 418]. In turn, this causes ubiquitination as well as unwanted aggregation of mitochondrial proteins [135, 419]. Thus, several studies suggested that mitochondrial dysfunction plays a crucial role in PD [420] and emphasized distinct similarities in mitochondrial dysfunction in GD and familial PD models [318].

### 4.4.3 Dysfunctional lipid metabolism

If one refers to glucosylceramide, it is about a glycolipid that is also considered to be a structure or membrane lipid. Not surprisingly, limited GBA function leads to an altered lipid profile in the cell [142, 267, 421]. In a GD model characterized by limited degradation of glucosylceramide and its subsequent accumulation, an altered cellular lipid metabolism as well as a transformed membranous lipid composition was objectified [422]. Further, it was suggested that an altered lipid composition of the lysosomal membrane may possibly interfere with the formation of an CMA receptor, which internalizes α-syn into the lysosome for further degradation [6]. The altered lipid composition may subsequently favor intracellular α-syn enrichment [6]. The increased glucosylceramide accumulation due to a mutated GCase also affects the sphingolipid composition of the cellular membrane, which might impair α-syn’s binding to the membrane and additionally promotes its intracellular accumulation [16, 231, 423]. Therefore, sphingolipid metabolism is assumed to play a relevant role in neuronal integrity: it influences synaptic stability.
and, in case of dysregulation due to GBA mutations, it may lead to the development of fibrillar α-syn with neurotoxic effects, contributing to PD pathology [424].

4.4.4 ER-associated degradation aspects
GBA mutations provide the blueprint for misfolded GCase, whereas misfolded proteins in general undergo protein quality control by the ER in order to prevent protein toxicity [425]. The ER, if necessary, restrains dysfunctional proteins and routes them to a specific degradation pathway, the ERAD [425].

In case of an increased burden of misfolded GCase, due to GBA mutations, the dysfunctional enzyme can accumulate within the ER, resulting in a lack of lysosomal GCase as well as in triggering the unfolded protein response (UPR), which is part of ERAD [12, 218, 426]. Based on the findings with induced pluripotent stem cell lines of PD\textsubscript{GBA} subjects, Fernandes et al reported a dysfunctional processing of mutant GCase in ER, ER-stress and general increase of lysosomal capacities in dopaminergic neurons compared with cell lines from healthy individuals [427].

The interaction of parkin and GCase
In addition to an increased ER stress in PD\textsubscript{GBA}, another fascinating feature is represented by the connection of the ERAD-associated ligase parkin with mutated GCase. The E3 ubiquitin ligase parkin is involved in misfolded protein degradation as it is part of ERAD [428].

Wt parkin supports degradation of mutant forms of GCase dose-dependently in dopaminergic neurons – by labelling these misfolded enzymes with an ubiquitin appendage [428]. These ubiquitinated molecules are now able to get into lysosomes or proteasomes for degradation. GBA mutations may therefore challenge wt parkin function by reducing its capacity to mark and they disturb indirect the degradation of other misfolded proteins, which consequently accumulate and cause cytotoxic effects to dopaminergic cells [428].

Mutant parkin (due to PARK2 mutations, leading to autosomal recessive PD), however, interacts with mutated GCase by even stabilizing the defective GCase [428]. Normal GCase is not altered in its stability by either wt parkin or mutant parkin [428]. One could suggest that mutated parkin and GBA mutations may
additionally reinforce each other in a vicious circle – similar to the positive feedback mechanism between α-syn and GCase described above. This also supports the thesis of underlying genetic interactions, according to which PD as a sporadic disease is caused by the interference of several and sometimes rare mutations, respective their gene products and associated cellular pathways.

4.4.5 PD$_{GBA}$ – associated with impaired calcium homeostasis and mitochondrial impairment

Experimental studies with induced pluripotent stem cell (iPSC) models, representative for mesencephalic dopaminergic neurons in GD and PD$_{GBA}$, revealed a disturbed calcium metabolism and increased neuronal stress vulnerability in mutant neurons [319]. Further, mitochondrial dysfunction has been reported for PD as well as for GD [208, 407]. Possible causes, why deficient GCase may lead to mitochondrial deficits, could be due to the described impaired calcium metabolism, neuroinflammatory processes, changes in lipid metabolism or due to impaired ALP [208].

Of course, combinations of the individual factors with a cumulative effect are conceivable [208]. According to Larsen et al, mitochondrial quality control can be affected at 3 different levels: first at molecular level via chaperone-related UPR [429]. If this response is not sufficient, the next higher organellar level is involved, where dysfunctional mitochondria or defect mitochondrial components are degraded by mitophagy [429]. Thus, amongst others, the familial PD-associated kinase PINK1 and the E3 ubiquitin ligase parkin recognize defective mitochondria, that must be degraded [154, 157]. However, if mitophagy also fails, more reactive oxygen species arise due to impaired electron transport chain (ETC) and the last, the cellular level with induction of apoptosis corresponding to oxidative stress is reached [208, 416, 429].

Contrary to one’s expectation, an impaired mitophagy did not affect all 4 complexes of the ETC as a significant decrease was observed only for the first 3 complexes but not for the fourth – why one could suggest that a solitaire mitochondrial approach to PD might not be conclusive [208]. Experimental findings derived from murine models, characterized by the loss of neuronal GCase,
exhibited an accumulation of ceramides [208]. This accumulation seemed to activate microglia and astrocytes [208]. Afterwards, these cells produced reactive species such as nitric oxide [208]. In turn, this might impair mitochondrial ETC and lead to cellular damage [208].

In conclusion, there is more and more evidence that mitochondrial dysfunction plays a crucial role in neuronal survival and may therefore contribute to PD development.

4.4.6 GBA and iPSC-technology based findings in twin-studies

Since monozygotic twins often present with phenotypical similarities in many respects due to their shared genotype, they are regularly examined in the context of studies with regard to environmental-genetic interactions [430]. Thus, PD twin studies reported a PD concordance-rate of 15.5% for monozygotic twins in the US [431] and 11% in Sweden [432]. In addition, there are twin pairs with one sibling suffering from PD while the other is not affected – with the underlying reasons for this dysconcordance being likewise unclear as the mechanisms, why some GBA carriers develop PD and others do not [430].

Based on iPSC technology, Woodard et al gained skin fibroblasts from a monozygotic twin pair that was PD-disconcordant and heterozygous for the GBA mutation N370S [430]. iPSC-derived midbrain dopaminergic (mDA) neurons were generated from these fibroblast samples, corresponding to a model of human neurons with a heterozygous N370S-mutation [430].

GCase activity of both twins was reduced to approximately 50% while α-syn levels of both subjects were approximately tripled [430]. Additionally, the mDA neurons of both twins produced lower levels of dopamine [430]. It is hypothesized, that the reduced dopamine release was a secondary effect due to the increased α-syn levels and that α-syn-overexpression inhibited dopamine release up to 80% due to impaired synaptic vesicle regulation – as previous findings from mouse models had revealed [430, 433]. However, both twins with the same GBA mutational status showed different levels of dopamine, which supports the thesis of further, possibly epigenetic factors that affect dopamine metabolism [430].
4.5 Prodromal characteristics of PD and the role of PD\(_{\text{GBA}}\)

Considering, that studies exposed a reduced GCase activity in cases of PD\(_{\text{idiopathic}}\) as well [317], it is relevant to explore the existence of a specific prodromal, clinical and biological fingerprint in PD\(_{\text{GBA}}\). An in-depth investigation of prodromal aspects in PD\(_{\text{GBA}}\) might allow conclusions to be drawn on the further course of PD\(_{\text{GBA}}\), as well as conclusions about the development of PD in general [12]. A retrospective study, using a validated interview on prodromal PD symptoms carried out with PD\(_{\text{GBA}}\) and PD\(_{\text{idiopathic}}\) patients as well as healthy elderly subjects, showed that PD\(_{\text{GBA}}\) and particularly L444P-associated PD\(_{\text{GBA}}\) presents with prodromal symptoms more frequently [312]. Further, it was exposed that PD\(_{\text{GBA}}\) shows nearly concurrently non-motor and early motor deficits immediately prior to diagnosis [312]. PD\(_{\text{idiopathic}}\), however, demonstrated a relatively longer prodromal phase which began with NMS and presented much later with early motor impairments [312]. Therefore, Zimmermann et al concluded that PD\(_{\text{GBA}}\) may exhibit its own histopathological characteristics due to the faster and more severe course of the disease and the shorter prodromal phase as well [312]. This finding also questions the previously discussed thesis of a rather late acceleration of PD-progression (see 4.2 above).

So, which conclusions can be drawn from the findings of potential biomarkers related to PD\(_{\text{GBA}}\)?

4.5.1 PD Biomarkers

Although previous biomarker studies disclosed altered lysosomal enzymatic activities in the CSF of PD subjects compared to healthy controls, these parameters as a singular feature were not able not distinguish between PD and controls [434]. An extended combination of several biomarkers, such as mitochondrial dysfunction or α-syn species, with CSF-markers may be helpful in the diagnosis of PD in future [434]. Furthermore, studies investigating blood plasma α-syn levels in PD\(_{\text{idiopathic}}\) patients, described that plasma α-syn levels are rather not suitable as biomarkers for PD and that they do not differ significantly between the both analyzed subtypes (1) tremor-dominant and (2) postural instability and gait disorder [435].
**PD\textsubscript{GBA} and the role of fatty acids as biomarkers in cerebrospinal fluid samples**

According to the fact that GBA mutations are a susceptibility factor for PD and both GBA and α-syn do interrelate with fatty acids [315], levels of 13 different fatty acids were analyzed by gas chromatography. A significant lower level of fatty acids in the PD\textsubscript{GBA} group was assessed, both in comparison with PD\textsubscript{Idiopathic} patients and healthy controls. Furthermore, in the PD\textsubscript{GBA} group, significantly lower levels of palmitoleic acid, arachidonic acid and eicosapentaenoic acid were found, suggesting an altered cerebral lipid metabolism [261, 315].

**PD\textsubscript{GBA} and the role of neurodegenerative markers in cerebrospinal fluid samples**

Furthermore, a longitudinal study investigated the potential and intriguing link between the genetic composition of various subgroups (PD\textsubscript{Idiopathic}, PD\textsubscript{GBA}, PD\textsubscript{LRRK2}, healthy controls) and neurodegenerative markers in CSF [436]. While all 3 PD subgroups presented a decreased Amyloid β protein (Aβ) 1-42 level, only PD\textsubscript{Idiopathic} and PD\textsubscript{GBA} showed lower total-microtubule associated protein tau (t-tau) and phosphorylated-microtubule associated protein tau (p-tau) [436]. An association between an accelerated cognitive decline and higher p-tau levels in the baseline investigation was found for PD\textsubscript{GBA} but not for PD\textsubscript{Idiopathic} [436]. In contrast, another study recently objectified similar levels of Aβ1-42, p-tau, and t-tau for both PD\textsubscript{GBA} and healthy controls in CSF samples [437]. The authors concluded that the highlighted cognitive impairment in PD\textsubscript{GBA} may rather not be associated with specific CSF profile of neurodegeneration parameters [437].

**PD\textsubscript{GBA} and the role of skin punch biopsies in prodromal PD\textsubscript{GBA}**

A recent study with RBD-subjects, considered to be at high-risk for developing PD, was carried out to investigate the appropriateness of skin biopsies for prodromal PD\textsubscript{GBA} and to detect potential differences in dermal p-syn deposition between PD\textsubscript{GBA} and PD\textsubscript{Idiopathic}[85, 126]. Doppler et al found no relevant differences between PD\textsubscript{GBA} and PD\textsubscript{Idiopathic} in p-syn pathology, consistent with other skin biopsy studies [126]. In addition, no significant association could be demonstrated for any of the GBA variants studied (N370S, E326K and L444P) [126].
Remarkably, p-syn deposits correlated with PD\textsubscript{GBA} disease duration and are possibly related to the faster disease progression of PD\textsubscript{GBA}, which is reported in this present study [126, 129]. The authors concluded that the involvement of the peripheral nervous system is similarly heterogeneous as are the clinical and neuro-pathological features in PD\textsubscript{GBA} and PD\textsubscript{Idiopathic} and further, that skin punch biopsies are suitable as a simple tool for future analysis of prodromal PD\textsubscript{GBA} [126].

4.6 PD\textsubscript{GBA} – targeted therapy

To mitigate the accumulation of α-syn and thus to protect midbrain dopaminergic neurons from damage, the effects of GBA gene delivery were investigated in mouse models [438]. In a wt SNCA model, cerebral GBA gene injection increased GCase activity and further, it reduced striatal and nigral α-syn accumulation [438]. The authors concluded, that reinforcing the clearance of α-syn may be associated with neuroprotective effects and might verify lysosomal genes like GBA as components of a targeted therapy [438]. However, as it is still not fully understood how exactly GBA mutations increase the risk for PD and DLB and what precisely originates the accelerated disease course of PD\textsubscript{GBA}, GBA-related therapies continue to be challenging [439]. Therefore, various approaches are being pursued. At present, the increase of GCase activity and the alteration of GBA-associated substrates – the glycosphingolipids – are the most promising attempts [439].

4.6.1 PD\textsubscript{GBA} – lysosomal therapeutic strategies

One possible approach to optimize GCase function is to influence the signal pathways, leading to degradation of the mutated or physiological GCase [440]. Consequently, more GCase can be transported to the lysosome [440]. One suggested strategy comprises the regulation of chaperones. These molecules support both the correct folding and unfolding of proteins or direct them to a pathway of degradation, e.g. the proteasome, in case the proteins are misfolded [441]. Therefore, a possible approach aims to prevent specified chaperones from recognizing mutated GCase and initiating its degradation [440].
4.6.2 PD\textsubscript{GBA} – the role of pharmaceutical chaperons

As ER mediates the degradation of mutated GCase, this leads to ER stress and further, the increasing appearance of unfolded or misfolded proteins inside of ER initiates the UPR [442]. UPR is a complex interaction of multiple signal cascades and aims to maintain or restore the cell integrity [442]. More specifically, UPR aims to:

- reduce or stop protein translation
- degrade the misfolded proteins and
- increase the chaperon production in order to sustain a correct protein folding [442]

If these steps fail to restore the physiological cell function within a distinct period of time, programmed cell death (apoptosis) is the goal [214]. Both accumulation of misfolded proteins and increased UPR have been described for PD\textsubscript{idiopathic} cases [443, 444]. An experimental study, using a transgenic Drosophila model, revealed that flies with the human GBA variants N370S and L444P presented signs of UPR as well and developed Parkinsonian symptoms [214]. These effects were partly reversible due to the addition of the chaperone ambroxol hydrochloride [214]. How did the administration of ambroxol – a substance previously used as a mucolytic agent (Mucosolvan) to increase mucus clearance in bronchopulmonary disorders – come on board [445]? Over 1000 approved drugs were screened for potential enzyme-enhancing therapy in GD [445]. Ambroxol was shown to inhibit the denaturation of wt GCase in a pH-dependent manner: it was found that the enzyme-stabilizing effect of ambroxol is greatest in neutral ER milieu but did not exist in an acid lysosomal environment [445]. As a result, an ambroxol-induced increase of enzymatic function and a reduced GlcCer deposition could be demonstrated for wt and for mutant N370S GCase [445]. Further studies proved that ambroxol supports the correct folding of mutated GCase in the ER, shows an association with increased lysosomal levels of GCase, presumably through facilitated transport from ER to the lysosome and presents with an increased activity of the mutated GCase in type 1 and 2 GD skin fibroblast models [446]. Further, improvement of anemia and thrombocytopenia as well as a
reduction of hepatosplenomegaly were observed in patients with type 1 GD, who underwent 6 months of therapy with ambroxol 150 mg per day [446-449].

In 2014, McNeill et al described that ambroxol can increase GCase activity and reduce markers of oxidative stress in mutant GCase cells [447]. The mechanism for this is seen in an activation of the so-called CLEAR (coordinated lysosomal expression and regulation) system, which, in addition to GBA, comprises more than 400 other genes, encoding for lysosomal enzymes, transport proteins or membrane proteins [447]. As a CLEAR activator, ambroxol is thus seen as an activator of the lysosomal autophagy system in PD

The pioneering fact, that small chaperones can cross the blood-brain barrier, in opposite to ERT used in GD therapy, suggests that they might be helpful in ERT-unaffected neurodegenerative pathology [214]. This finding is strengthened by the effects of a clear reduction in ER stress and prevention of loss of motor functions in PD

Isofagomine interacts with the active GCase center, thus stabilizing GCase and theoretically increasing its activity. However, it did not provide any relevant clinical improvement in GD in corresponding studies, so this approach was discontinued [439, 451]. Nevertheless, ambroxol is currently being investigated in two clinical studies with regard to its tolerability, efficacy and safety (trial identifier: NCT02941822, NCT02914366) [439]. In addition, a non-inhibitory chaperone, NCGC607, was developed, leading to decreased substrate accumulation and increased transport of GCase to the lysosome in mesencephalic dopaminergic neurons in GD- and PD-models [439, 452]. Furthermore, the treatment with NCGC607 increased lysosomal activity, it effectively reestablished GCase activity and reduced both GlcSph and GlcCer levels in macrophages and dopaminergic neuron models [452].

4.6.3 PD

Studies, based on fly models, suggested that rapamycin (Sirolimus, a mTOR-inhibitor) might be a potential option as the substance increased the clearance of
α-syn in GBA-models of primary cortical neurons [453]. However, long-term rapamycin therapy worsened muscle weakness in mouse models and induced apoptosis of iPSC-derived neurons with GBA mutation [454, 455]. Kinghorn et al called for further studies to characterize rapamycin-induced effects and to learn more about the optimal PD stage as to when this therapy regime can be applied to PD patients [311].

4.6.4 PD\textsubscript{GBA} – the role of glucosylceramide synthase (GCS) inhibitors

Another approach to influence the bi-directional loop between mutant GCase and α-syn accumulation aims to alter the sphingolipid turnover [439]. A cell biological study, using mouse models, showed that the administration of a novel GCS inhibitor (GZ667161) was able to cross the blood-brain barrier and that it lowered glucosylceramide levels in a GD-related cell model [456].

The GCS inhibitor further decreased the hippocampal enrichment of neurodegenerative metabolites such as α-syn and tau and improved murine memory performance [456]. The authors therefore suggested that sustained GCS administration might affect cerebral α-syn metabolism and proposed GCS inhibition to be a disease modifying therapy [456]. Notably, it is essential to check the extent to which the insights gained from rodent models can actually be transferred to complex human organisms [457]. NMS, which are of great relevance in PD and even more in PD\textsubscript{GBA}, are barely characterized in murine models, as noted by Vingill et al [457]. An interventional multicenter phase 2 study by Sanofi (MOVES-PD) is investigating the safety and tolerability of the small molecule GZ/SAR402671 from 2016 to presumably 2022 in a placebo-controlled setting (ClinicalTrials.gov Identifier: NCT02906020) [185].

4.6.5 PD\textsubscript{GBA} – the role micro ribonucleic acid’s (miRNA)

Transcription is the transfer of DNA information into ribonucleic acid (RNA), while translation is the transfer of RNA information into amino acid sequences as part of protein synthesis. One type of RNA is the miRNA, which consists of only few nucleotides and does not encode for protein synthesis [458]. Instead, it regulates post-transcriptional translation [458]. Studies have shown that over- and under-expressing of miRNA can lead to an altered expression of NO synthases – whose
NO-species can cause DNA damage, synaptic lesions and also apoptosis [458]. Oxidative stress makes dopaminergic neurons vulnerable, may contribute to their cell death and is therefore a link to PD pathology, as mentioned above [458].

Thus, miRNAs were also discussed as potential target-directed therapy options in PD [458]. As recently reported by Martinez et al, numerous down-regulated miRNAs were detected in brain biopsies from PD patients, which targeted genes such as GBA as well as SCNA, LRRK2, LAMP-2A or PARK 2 [458].

In fact, in PD-animal-models it was recently demonstrated that change in certain miRNA levels can have favorable effects on PD-outcome [458]. Synthetically-engineered molecules that either mimic human miRNAs (agomir) or inhibit miRNA (antagomir) may be therefore therapeutic targets to positively affect early neuropathological processes in PD [458].

4.6.6 PDGBA – the role of adeno-associated Virus (AAV)-based gene therapies

Transferring genes in order to treat complex diseases is an approach that has already been applied for neurodegenerative disorders such as AD or FTD [459]. According to Hudry et al, AAV seems to be best suited for being applied regarding disorders of the central nervous system, due to its properties as a sufficiently safe vector and its profound neurotropism [459]. Therefore, PDGBA patients are currently being recruited in a phase 1/2 trial, which investigates AAV9 serotype as a gene-based therapeutic method transferring the whole wt-GBA gene into the cells of PDGBA patients [459-461].
4.7 Limitations of this study

Like many studies carried out so far, also this presented study has limitations that must be considered for evaluation of the results reported. These limitations are identified below:

- The survival rates of the included PD\textsubscript{idiopathic} subjects are higher than general PD survival rates assessed after literature review. In order to avoid a potential distortion, clarity should be gained on the number of subjects who could not be included and why this was not possible [129].

- The small number of cases: a larger sample would be desirable, which is not easy to accomplish due to the rarity of GBA mutations and the disease-related severe general condition of PD patients [129]. Nevertheless, this would possibly allow mutation-related intragroup analyzes and increase the external validity in general.

- Only the two most common mutations were investigated: Further, only the two most common GBA mutations were investigated in this study due to organizational and financial feasibility. However, a whole gene screening would be useful for detection of mutations-specific effects.

- Short follow-up period: a longer follow-up period would be desirable.

4.8 Outlook

Further studies in a longitudinal prospective design with shorter examination intervals are needed to adequately explore the more rapid progression in PD\textsubscript{GBA} – especially with regard to its shorter and more severe prodromal phase [129]. In particular, it is necessary to further investigate prodromal phase of PD\textsubscript{GBA} for possible risk factors as well as bio- and progression markers. Early diagnosis and the inclusion in clinical trials enable to investigate disease-modifying therapies, to establish and to reduce or even prevent irreversible neurodegenerative degradation. After PD diagnosis, including PD\textsubscript{GBA}, is still based on clinical aspects, although supported by laboratory, neuroimaging and histopathological methods, the use of quantitative diagnostic methods should be reinforced – to compensate for the subjectivity of the examiner and to increase the accuracy of research.
5. Summary

The following comprises a short summary of this clinical observation study including the objective, the applied methods and results as well as the discussion.

A common disease such as Parkinson's disease, which is now understood as a systemic disease and goes far beyond pure motor disturbance, is clearly associated with the rare lysosomal disorder Gaucher's disease. At first glance, GD has little in common with the second most frequent neurodegenerative disease worldwide. Nevertheless, the genetic origin of this compound is based on mutations in the GBA gene that lead to an increased risk of PD. Profound acknowledgement of prodromal and clinical symptoms of PD\textsubscript{GBA} as well as of the progression characteristics of this PD subgroup is of essential importance.

Otherwise, one will not be able at all to detect subjects with the most relevant risk factor for PD and – as the next step – these subjects at risk for PD might not be included in clinical and experimental trials. This, however, is the only way to hopefully expand and deepen the current understanding of the underlying mechanisms on how GBA mutations exactly contribute to PD pathology. Based on these required investigations, the development of promising therapeutic options, that go far beyond the present symptomatic level, are conceivable and are expected to slow down or even stop PD progression in the future.

Therefore, a clinical phenotyping of GBA patients was performed in this study. It revealed that the PD\textsubscript{GBA} group presented not significantly different from the PD\textsubscript{id-iopathic} group at the beginning of the 3-year period regarding motor and non-motor performance. However, at time of the examination in 2013, the PD\textsubscript{GBA} group was affected more severely than the comparison group: motor and cognitive impairment had worsened more rapidly. Moreover, higher doses of dopaminergic drugs were required, and H\&Y disease stages reflected a faster progression of PD\textsubscript{GBA} to one PD-milestone that can be life-changing for PD patients: the endpoint of postural instability. Further, higher mortality rates for PD\textsubscript{GBA} patients were demonstrated in this study.
Epigenetic and environmental factors may seem to play a relevant role in this subgroup of PD, as well as complex gene-gene interactions. Theories, attempting to explain the underlying pathology, range from the causal linkage of common diseases with common genetic variants (CDCV hypothesis) to the currently more probable assumption that common diseases, such as Parkinson's disease, are caused by a variety of singular and separately rare variants (CDRV). At the cellular level, moreover, several approaches are pursued, including the pathological interaction of GCase and α-syn, the impairment of lysosomal clearance, dysfunctional lipid metabolism, disturbances in the area of the proteasome as well as deficits in mitochondrial function.

The primary background of this prospective study was to contribute to a better understanding of this neurodegenerative disease by phenotypically characterizing the subtype PD$_{\text{GBA}}$. This is of crucial importance for following steps as to be able to make a diagnosis at a preferably early disease stage and thus, to prevent disease-associated and irreversibly neuronal cell loss by means of future disease-modifying, targeted therapies. Currently, promising therapeutic studies are in progress with the aim of increasing GCase activity or alternatively, minimizing its pathogenic substrate glucosylceramide.
6. Deutsche Zusammenfassung


Eine solche klinische Phänotypisierung von GBA-Patienten wurde durch diese Studie vorgenommen. Es zeigte sich, dass sich die PD_{GBA} Gruppe zu Beginn einer 3-jährigen Untersuchungsperiode sowohl motorisch, als auch nicht-motorisch von der PD_{idiopathic} Gruppe nicht signifikant unterschied. Zum Zeitpunkt der Untersuchung in 2013 waren PD-Patienten mit einer der beiden Mutationen N370S oder L444P deutlich schwerer betroffen, als die Vergleichsgruppe: die motorische und kognitive Einschränkung hatte sich rascher verschlechtert.

Es waren höhere Dosen dopaminerge Medikamente erforderlich, auch die Krankheitsstadien nach H&Y spiegelten den rascheren Verlauf von PD_{GBA} hin zu einem der drei großen Meilensteine wieder, die für PD-Patienten lebensverändernd sein können: das Erreichen des Endpunktes posturale Instabilität. Höhere Mortalitätsraten für PD_{GBA} Patienten wurden in dieser Studie ebenso belegt. Möglicherweise spielen epigenetische und umwelt-assoziierte Faktoren eine relevante Rolle sowie nicht zuletzt komplexe Gen-Gen-Interaktionen.
Theorien, die die zugrunde liegende Pathologie zu erklären versuchen, reichen hierbei von der ursächlichen Verknüpfung häufiger Erkrankungen mit häufig auftretenden genetischen Varianten (CDCV-Hypothese) bis zur derzeit wahrscheinlicheren Annahme, dass häufige Erkrankungen wie Morbus Parkinson durch eine Vielzahl einzelner und für sich genommen seltener Varianten bedingt sein könnten (CDRV). Auf zellulärer Ebene werden des Weiteren mehrere Ansätze verfolgt, die die pathologische Interaktion von GCase und alpha-Synuklein, die Beeinträchtigung lysosomaler Clearance, einen dysfunktionalen Lipidstoffwechsel und Störungen im Bereich des Proteasoms sowie Defizite in der mitochondrialen Funktion umfassen.

Der führende Hintergrund dieser prospektiven Studie bestand darin, durch phänotypische Charakterisierung des Subtyps PD\textsubscript{GBA} zu einem besseren Verständnis dieser neurodegenerativen Erkrankung beizutragen. Dies ist von entscheidender Bedeutung, um möglichst frühzeitig eine Diagnose stellen zu können und hoffentlich den krankheitsassozierten, irreversiblen neuronalen Zellverlust durch zukünftige, vielversprechende krankheitsmodifizierende, zielgerichtete Therapien verhindern zu können. Es erfolgen derzeit vielversprechende Studien mit dem Ziel, die GCase Aktivität zu steigern oder alternativ deren pathologisch anfallendes Substrat, nämlich Glucosylceramid, zu minimieren.
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SOD1(G93A) mouse model: A (1)H-magnetic resonance spectroscopic study.

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Early increase in marker of neuronal integrity with antidepressant treatment of
major depression: 1H-magnetic resonance spectroscopy of N-acetyl-aspartate.

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R, Ariza A, Tolosa E, and Beyer K (2018) Glucocerebrosidase gene variants are
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8. Appendix

I. UK Parkinson’s disease Society Brain Bank Clinical Diagnostic Criteria (UKBBC)

**UK PARKINSON’S DISEASE SOCIETY BRAIN BANK CLINICAL DIAGNOSTIC CRITERIA*\(^a\)**

**Step 1. Diagnosis of Parkinsonian Syndrome**
- Bradykinesia
- At least one of the following
  - Muscular rigidity
  - 4-6 Hz rest tremor
  - Postural instability not caused by primary visual, vestibular, cerebellar, or proprioceptive dysfunction

**Step 2 Exclusion criteria for Parkinson’s disease**
- History of repeated strokes with stepwise progression of parkinsonian features
- History of repeated head injury
- History of definite encephalitis
- Oculogyric crises
- Neuroleptic treatment at onset of symptoms
- More than one affected relative
- Sustained remission
- Strictly unilateral features after 3 years
- Supranuclear gaze palsy
- Cerebellar signs
- Early severe autonomic involvement
- Early severe dementia with disturbances of memory, language, and praxis
- Babinski sign
- Presence of cerebral tumor or communication hydrocephalus on imaging study
- Negative response to large doses of levodopa in absence of malabsorption
- MPTP exposure

**Step 3 supportive prospective positive criteria for Parkinson’s disease**
Three or more required for diagnosis of definite Parkinson’s disease in combination with step one
- Unilateral onset
- Rest tremor present
- Progressive disorder
- Persistent asymmetry affecting side of onset most
- Excellent response (70-100%) to levodopa
- Severe levodopa-induced chorea
- Levodopa response for 5 years or more
- Clinical course of ten years or more

II. MDS-Unified Parkinson’s Disease Rating Scale III (UPDRS-III)

<table>
<thead>
<tr>
<th>Teil III: Motorische Untersuchung</th>
</tr>
</thead>
<tbody>
<tr>
<td>Übersicht: Dieser Skalenabschnitt evaluiert die motorischen Symptome der Parkinson-Krankheit. Bei der Anwendung des Teils II der MDS-UPDRS soll der Untersucher folgende Richtlinien einhalten:</td>
</tr>
<tr>
<td>Auf der oberen Seite des Formulars notieren Sie bitte, ob der Patient Medikamente zur Behandlung der Symptome der Parkinson-Krankheit erhält. Falls Levodopa eingenommen wird, geben Sie bitte die Zeit seit der letzten Dosage an.</td>
</tr>
<tr>
<td>Falls der Patient Medikamente zur Behandlung der Symptome der Parkinson-Krankheit erhält, notieren Sie bitte den klinischen Status des Patienten unter Verwendung folgender Begriffe:</td>
</tr>
<tr>
<td><strong>ON</strong> ist der typische funktionelle Status, wenn die Patienten Medikamente bekommen und gut auf sie ansprechen.</td>
</tr>
<tr>
<td><strong>OFF</strong> ist der typische funktionelle Status, wenn die Patienten trotz Medikamenteneinnahme schlecht auf sie ansprechen.</td>
</tr>
<tr>
<td>Der Untersucher soll genau das „bewerten, was er sieht“ („rate what you see“). Allerdings können einzelne Bereiche der motorischen Untersuchung durch gleichzeitig vorhandene medizinische Probleme wie Schlaganfall, Lähmung, Arthritis, Kontrakturen und orthopädische Probleme wie Hüftgelenks- oder Knie-Erersatz und Skoliose beeinflusst werden. In Situationen, in denen eine Bewertung absolut unmöglich ist (z.B. Amputationen, vollständige Lähmung, Extremität im Gipsverband), verwenden Sie bitte den Vermerk „UR“ für „Nicht zu Bewerten (unable to rate)“. Ansonsten bewerten Sie die Ausführung jeder Aufgabe so, wie sie der Patient im Kontext der Begleiterkrankungen verrichtet.</td>
</tr>
<tr>
<td>Alle Fragen müssen eine ganzzählige Bewertung aufweisen (keine halben Punkte, keine fehlenden Werte).</td>
</tr>
<tr>
<td>Am Ende der Bewertung geben Sie bitte an, ob Dyskinesien (Chorea oder Dystonie) während der Untersuchung aufgetreten sind, und falls dem so ist, ob diese Bewegungen einen Einfluss auf die motorische Untersuchung hatten.</td>
</tr>
<tr>
<td>3a Erhält der Patient Medikamente zur Behandlung der Symptome der Parkinsonerkrankung?</td>
</tr>
<tr>
<td>□ Nein □ Ja</td>
</tr>
<tr>
<td>3b Falls der Patient Medikamente zur Behandlung der Symptome der Parkinson-Krankheit bekommt, geben Sie bitte den klinischen Status des Patienten unter Verwendung folgender Begriffe an:</td>
</tr>
<tr>
<td>□ ON: ON ist der typische funktionelle Status, wenn die Patienten Medikamente bekommen und gut auf sie ansprechen.</td>
</tr>
<tr>
<td>□ OFF: OFF ist der typische funktionelle Status, wenn die Patienten trotz Medikamenteneinnahme schlecht auf sie ansprechen.</td>
</tr>
<tr>
<td>3c Nimmt der Patient Levodopa ein?</td>
</tr>
<tr>
<td>□ Nein □ Ja</td>
</tr>
</tbody>
</table>
| 3.C 1 Falls ja, geben Sie bitte die Minuten seit der letzten Levodopa-Dosis an: Minutens
### 3.1. SPRACHE

Instruktionen für den Untersucher: Beurteilen Sie die spontane Sprachproduktion des Patienten und beginnen Sie, falls erforderlich, ein Gespräch. Mögliche Themenvorschläge: Fragen Sie nach der Arbeit des Patienten, Hobbys, Sport oder danach, wie er in die Arztpraxis gekommen ist. Beurteilen Sie Umfang, Modulation (Prosodie) und Deutlichkeit, einschließlich undeutlicher Artikulation, Palilalie (Silbenwiederholung), Tachypnoe (Sprachbeschleunigung), Zusammenfassung von Silben.

<table>
<thead>
<tr>
<th>Wert</th>
<th>Erklärung</th>
</tr>
</thead>
<tbody>
<tr>
<td>0: normal</td>
<td>Keine Sprachprobleme.</td>
</tr>
<tr>
<td>1: angedeutet vorhanden</td>
<td>Verlust von Modulation, Diktion oder Lautstärke, alle Wörter sind aber noch leicht zu verstehen.</td>
</tr>
<tr>
<td>2: leicht ausgeprägt</td>
<td>Verlust von Modulation, Diktion oder Lautstärke mit einigen unklaren Wörtern, aber insgesamt leicht verständlichen Sätzen.</td>
</tr>
<tr>
<td>3: mäßig ausgeprägt</td>
<td>Sprache ist schwer zu verstehen, da einige, jedoch nicht die meisten Sätze schlecht zu verstehen sind.</td>
</tr>
<tr>
<td>4: schwer ausgeprägt</td>
<td>Der Großteil des Gesprochenen ist schwer zu verstehen oder unverständlich.</td>
</tr>
</tbody>
</table>

### 3.2. GESICHTSAUSDRUCK


<table>
<thead>
<tr>
<th>Wert</th>
<th>Erklärung</th>
</tr>
</thead>
<tbody>
<tr>
<td>0: Normal</td>
<td>Normaler Gesichtsausdruck.</td>
</tr>
<tr>
<td>1: angedeutet vorhanden</td>
<td>Minimaler maskenhafter Gesichtsausdruck, der sich nur durch die reduzierte Frequenz des Augenbinzels manifestiert.</td>
</tr>
<tr>
<td>3: mäßig ausgeprägt</td>
<td>Maskenhafter Gesichtsausdruck mit zeitweise geöffnetem Mund, wenn nicht gesprochen wird.</td>
</tr>
<tr>
<td>4: schwer ausgeprägt</td>
<td>Maskenhafter Gesichtsausdruck mit überwiegend geöffnetem Mund, wenn nicht gesprochen wird.</td>
</tr>
</tbody>
</table>
### 3.3. RIGOR


<table>
<thead>
<tr>
<th>Wert</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nacken</td>
</tr>
<tr>
<td>RGE</td>
</tr>
<tr>
<td>LOE</td>
</tr>
<tr>
<td>RUE</td>
</tr>
<tr>
<td>LUE</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>0: Normal: Kein Rigor.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: angedeutet vorhanden: Rigor lässt sich nur durch ein Bahnungsmanöver feststellen.</td>
</tr>
<tr>
<td>2: leicht ausgeprägt: Rigor ist ohne Bahnungsmanöver feststellbar, der volle Bewegungsumfang ist jedoch erhalten.</td>
</tr>
<tr>
<td>3: mäßig ausgeprägt: Rigor ist ohne Bahnungsmanöver feststellbar, voller Bewegungsumfang wird nur durch Anstrengung erreicht.</td>
</tr>
<tr>
<td>4: schwer ausgeprägt: Rigor ist ohne Bahnungsmanöver feststellbar und ein voller Bewegungsumfang wird nicht erreicht.</td>
</tr>
</tbody>
</table>

### 3.4. FINGERTIPPEN


<table>
<thead>
<tr>
<th>Wert</th>
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<tbody>
<tr>
<td>R</td>
</tr>
<tr>
<td>L</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>0: Normal: Keine Probleme.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: angedeutet vorhanden: Mindestens eine der folgenden Schwierigkeiten: a) Regulärer Rhythmus ist gestört durch eine oder zwei Unterbrechungen oder Verzögerungen während des Fingertippens; b) angedeutete Verlangsamung; c) Amplitudendekrement kurz vor dem 10ten Tippen.</td>
</tr>
<tr>
<td>2: leicht ausgeprägt: Mindestens eine der folgenden Schwierigkeiten: a) 3 bis 5 Unterbrechungen beim Fingertippieren; b) leichte Verlangsamung; c) Amplitudendekrement mitten in der 10er Tippssequenz.</td>
</tr>
<tr>
<td>3: mäßig ausgeprägt: Mindestens eine der folgenden Schwierigkeiten: a) mehr als 5 Unterbrechungen beim Fingertippieren oder mindestens eine längere Pause (&quot;Einfrieren&quot;) in der Ausführung; b) mäßige Verlangsamung; c) Amplitudendekrement bereits nach dem ersten Tippen.</td>
</tr>
<tr>
<td>4: schwer ausgeprägt: Patient kann die Aufgabe nicht oder nur schwerlich durchführen aufgrund von Verlangsamung, Unterbrechungen oder Dekrement.</td>
</tr>
</tbody>
</table>
### 3.5 Handbewegungen


<table>
<thead>
<tr>
<th>WERT</th>
<th>R</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0: Normal</td>
<td>Keine Probleme.</td>
<td></td>
</tr>
<tr>
<td>1: angezeiget vorhanden</td>
<td>Mindestens eine der folgenden Schwierigkeiten: a) Regulärer Rhythmus ist gestört durch eine oder zwei Unterbrechungen oder Bewegungsverzögerungen; b) angedeutete Verlangsamsam; c) Amplitudenabfall zum Ende der Aufgabe.</td>
<td></td>
</tr>
<tr>
<td>2: leicht ausgeprägt</td>
<td>Mindestens eine der folgenden Schwierigkeiten: a) 3 bis 5 Bewegungsunterbrechungen; b) leichte Verlangsamsam; c) Amplitudenabfall bei wechselnden Bewegungen.</td>
<td></td>
</tr>
<tr>
<td>3: mäßig ausgeprägt</td>
<td>Mindestens eine der folgenden Schwierigkeiten: a) mehr als 5 Bewegungsunterbrechungen oder mindestens eine längere Pause („Einfrieren“) in der fortlaufenden Bewegung; b) mäßige Verlangsamsam; c) Amplitudenabfall nach erster „Offen und Schließen“-Sequenz.</td>
<td></td>
</tr>
<tr>
<td>4: schwer ausgeprägt</td>
<td>Patient kann die Aufgabe nicht oder nur schwerlich durchführen aufgrund von Verlangsamsam, Unterbrechungen oder Dekrement.</td>
<td></td>
</tr>
</tbody>
</table>

### 3.6 Pronations-Supinationsbewegungen der Hände


<table>
<thead>
<tr>
<th>WERT</th>
<th>R</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0: Normal</td>
<td>Keine Probleme.</td>
<td></td>
</tr>
<tr>
<td>1: angezeiget vorhanden</td>
<td>Mindestens eine der folgenden Schwierigkeiten: a) Regulärer Rhythmus ist gestört durch eine oder zwei Unterbrechungen oder Bewegungsverzögerungen; b) angedeutete Verlangsamsam; c) Amplitudenabfall zum Ende der Aufgabe.</td>
<td></td>
</tr>
<tr>
<td>2: leicht ausgeprägt</td>
<td>Mindestens eine der folgenden Schwierigkeiten: a) 3 bis 5 Bewegungsunterbrechungen; b) leichte Verlangsamsam; c) Amplitudenabfall in der Übung.</td>
<td></td>
</tr>
<tr>
<td>3: mäßig ausgeprägt</td>
<td>Mindestens eine der folgenden Schwierigkeiten: a) mehr als 5 Bewegungsunterbrechungen oder mindestens eine längere Pause („Einfrieren“) in der fortlaufenden Bewegung; b) mäßige Verlangsamsam; c) Amplitudenabfall nach erster „Supination-Pronation“-Sequenz.</td>
<td></td>
</tr>
<tr>
<td>4: schwer ausgeprägt</td>
<td>Patient kann die Aufgabe nicht oder nur schwerlich durchführen aufgrund von Verlangsamsam, Unterbrechungen oder Dekrement.</td>
<td></td>
</tr>
</tbody>
</table>
### 3.7 Vorfußstippen


<table>
<thead>
<tr>
<th>Wert</th>
<th>Inhalt</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>Keine Probleme.</td>
</tr>
<tr>
<td>L</td>
<td>Mindestens eine der folgenden Schwierigkeiten: a) Regulärer Rhythmus ist gestört durch eine oder zwei Unterbrechungen oder Verzögerungen der Tipppbewegungen; b) angedeutete Verlangsamung; c) Amplitudenrekriment kurz vor dem 10. Tippen.</td>
</tr>
<tr>
<td></td>
<td>leicht Verlangsamung; c) Amplitudenrekriment mitten in der Übung.</td>
</tr>
<tr>
<td>L</td>
<td>Mindestens eine der folgenden Schwierigkeiten: a) mehr als 5 Bewegungsunterbrechungen oder mindestens eine längere Pause („Einfrieren“) in der fortlaufenden Bewegung; b) mäßige Verlangsamung; c) Amplitudenrekriment nach dem ersten Tippen.</td>
</tr>
<tr>
<td>L</td>
<td>Patient kann die Aufgabe nicht oder nur schwerlich durchführen aufgrund von Verlangsamung, Unterbrechungen oder Dekrement.</td>
</tr>
</tbody>
</table>

### 3.8 Beweglichkeit der Beine


<table>
<thead>
<tr>
<th>Wert</th>
<th>Inhalt</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>Keine Probleme.</td>
</tr>
<tr>
<td>L</td>
<td>Mindestens eine der folgenden Schwierigkeiten: a) Regulärer Rhythmus ist gestört durch eine oder zwei Unterbrechungen oder Bewegungsverzögerungen; b) angedeutete Verlangsamung; c) Amplitudenrekriment zum Ende der Aufgabe.</td>
</tr>
<tr>
<td></td>
<td>Mindestens eine der folgenden Schwierigkeiten: a) 3 bis 5 Bewegungsunterbrechungen; b) leichte Verlangsamung; c) Amplitudenrekriment mitten in der Übung.</td>
</tr>
<tr>
<td>L</td>
<td>Mindestens eine der folgenden Schwierigkeiten: a) mehr als 5 Bewegungsunterbrechungen oder mindestens eine längere Pause („Einfrieren“) in der fortlaufenden Bewegung; b) moderate Verlangsamung; c) Amplitudenrekriment nach dem ersten Aufstampfen.</td>
</tr>
<tr>
<td>L</td>
<td>Patient kann die Aufgabe nicht oder nur schwerlich durchführen aufgrund von Verlangsamung, Unterbrechungen oder Dekrement.</td>
</tr>
</tbody>
</table>
### 3.9 Aufstehen vom Stuhl


<table>
<thead>
<tr>
<th>Wert</th>
<th>Beschreibung</th>
</tr>
</thead>
<tbody>
<tr>
<td>0: Normal</td>
<td>Keine Schwierigkeiten. Patient kann schnell und ohne Verzögerung aufstehen.</td>
</tr>
<tr>
<td>1: angedeutet vorhanden</td>
<td>Das Aufstehen erfolgt langsamer als normal oder es wird mehr als ein Versuch dazu benötigt, oder eine Bewegung zum Stuhlsitz ist erforderlich, um aufstehen zu können. Benutzung der Armlöcher ist jedoch nicht nötig.</td>
</tr>
<tr>
<td>2: leicht ausgeprägt</td>
<td>Patient drückt sich mit Hilfe der Armlöcher ohne Schwierigkeiten hoch.</td>
</tr>
<tr>
<td>3: mäßig ausgeprägt</td>
<td>Patient drückt sich hoch, aber neigt zum Rückfallen; oder er muss es mehrmals unter Benutzung der Armlöcher versuchen, Aufstehen ist jedoch ohne fremde Hilfe möglich.</td>
</tr>
<tr>
<td>4: schwer ausgeprägt</td>
<td>Kann nicht ohne Hilfe aufstehen.</td>
</tr>
</tbody>
</table>

### 3.10 Gehen/Gangbild


<table>
<thead>
<tr>
<th>Wert</th>
<th>Beschreibung</th>
</tr>
</thead>
<tbody>
<tr>
<td>0: Normal</td>
<td>Keine Probleme.</td>
</tr>
<tr>
<td>1: angedeutet vorhanden</td>
<td>Patient geht ohne Hilfe mit leichter Gangstörung.</td>
</tr>
<tr>
<td>2: leicht ausgeprägt</td>
<td>Patient geht ohne Hilfe, jedoch mit erheblicher Gangstörung.</td>
</tr>
<tr>
<td>3: mäßig ausgeprägt</td>
<td>Patient benötigt eine Gehhilfe für sicheres Gehen (Gehstock, Gehwagen). Ist aber in der Lage, ohne fremde Hilfe zu gehen.</td>
</tr>
<tr>
<td>4: schwer ausgeprägt</td>
<td>Patient kann gar nicht gehen oder nur mit fremder Hilfe.</td>
</tr>
</tbody>
</table>
### 3.11 BLOCKADEN BEIM GEHEN


<table>
<thead>
<tr>
<th>Wert</th>
<th>Beschreibung</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐</td>
<td>Keine Blockade beim Gehen.</td>
</tr>
<tr>
<td>0: Normal</td>
<td>Ein Blockade beim Gehen tritt entweder beim Starten, Umdrehen oder Gehen durch den Türeingang auf und zeigt sich als nur eine Bewegungsunterbrechung bei einer dieser Bewegungsabläufe; danach werden fortlaufende fließende Bewegungen ohne Blockade beim Geradeausgehen ausgeführt.</td>
</tr>
<tr>
<td>4: schwer ausgeprägt</td>
<td>Eine Blockade tritt einmal beim Geradeausgehen auf.</td>
</tr>
</tbody>
</table>

### 3.12 POSTURALE STABILITÄT


<table>
<thead>
<tr>
<th>Wert</th>
<th>Beschreibung</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐</td>
<td>Keine Probleme: Patient fängt sich nach einem oder zwei Schritten auf.</td>
</tr>
<tr>
<td>0: Normal</td>
<td>3-5 Schritte, Patient fängt sich jedoch ohne Hilfe auf.</td>
</tr>
<tr>
<td>2: leicht ausgeprägt</td>
<td>Mehr als 5 Schritte, Patient fängt sich jedoch ohne Hilfe auf.</td>
</tr>
<tr>
<td>4: schwer ausgeprägt</td>
<td>Sicherer Stand, posturale Antwort ist jedoch nicht vorhanden, fällt, wenn er nicht vom Untersucher aufgefangen wird.</td>
</tr>
<tr>
<td>4: schwer ausgeprägt</td>
<td>Sehr instabil; neigt dazu, das Gleichgewicht sportartig bzw. auf ein leichthes Ziehen an den Schultern hin zu verlieren.</td>
</tr>
</tbody>
</table>
### 3.13 Körperhaltung

In Anlehnung an den Untersucher: Die Haltung wird an dem aufrecht stehenden Patienten beurteilt, nachdem er von einem Stuhl aufgestanden ist sowie beim Gehen und ebenso während der Untersuchung der posturalen Reflexe. Wenn Sie eine schlechte Körperhaltung bemerken, fordern Sie den Patienten auf, gerade zu stehen und beobachten Sie, ob sich die Körperhaltung bessert (siehe Punkt 2 unten). Bewerten Sie die schlechteste Körperhaltung, die Sie während dieser drei Beobachtungspunkte sehen. Beobachten Sie die Flexion und die Seitenneigung.

<table>
<thead>
<tr>
<th>Wert</th>
<th>Beschreibung</th>
</tr>
</thead>
<tbody>
<tr>
<td>0: Normal</td>
<td>Keine Probleme.</td>
</tr>
<tr>
<td>1: Leicht</td>
<td>Nicht ganz aufrechte Haltung; die Körperhaltung könnte jedoch für eine ältere Person normal sein.</td>
</tr>
<tr>
<td>2: Leicht ausgeprägt</td>
<td>Eindeutige Flexion, Skoliose oder Seitenneigung, aber der Patient kann die Haltung nach Aufforderung korrigieren.</td>
</tr>
<tr>
<td>3: Mäßig ausgeprägt</td>
<td>Gebückte Haltung, Skoliose oder Seitenneigung, die vom Patienten willentlich zu einer aufrechten Haltung nicht korrigiert werden kann.</td>
</tr>
<tr>
<td>4: Schwer ausgeprägt</td>
<td>Flexion, Skoliose oder Seitenneigung mit ausgeprägter Haltungsstörung.</td>
</tr>
</tbody>
</table>

### 3.14 GLOBALE SPONTANITÄT DER BEWEGUNG (BRADYKINESIE DES KÖRPERS)


<table>
<thead>
<tr>
<th>Wert</th>
<th>Beschreibung</th>
</tr>
</thead>
<tbody>
<tr>
<td>0: Normal</td>
<td>Keine Probleme.</td>
</tr>
<tr>
<td>1: Angedeutet vorhanden</td>
<td>Angedeutete globale Verlangsamung und Verarmung der Spontanbewegungen.</td>
</tr>
<tr>
<td>2: Leicht ausgeprägt</td>
<td>Leichte globale Verlangsamung und Verarmung der Spontanbewegungen.</td>
</tr>
<tr>
<td>3: Mäßig ausgeprägt</td>
<td>Mäßige globale Verlangsamung und Verarmung der Spontanbewegungen.</td>
</tr>
<tr>
<td>4: Schwer ausgeprägt</td>
<td>Schwere globale Verlangsamung und Verarmung der Spontanbewegungen.</td>
</tr>
</tbody>
</table>
### 3.15 HALTETREMOR DER HÄNDE


<table>
<thead>
<tr>
<th>Wert</th>
<th>Beschreibung</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td></td>
</tr>
<tr>
<td>L</td>
<td></td>
</tr>
</tbody>
</table>

0: Normal: Kein Tremor.
1: Angedeutet vorhanden: Tremor ist vorhanden, die Amplitude ist jedoch geringer als 1 cm.
2: Leicht ausgeprägt: Tremor mit einer Amplitude von mehr als 1 cm, aber geringer als 3 cm.
3: Mäßig ausgeprägt: Tremor mit einer Amplitude von mindestens 3 cm, jedoch geringer als 10 cm.
4: Schwer ausgeprägt: Tremor mit einer Amplitude von mindestens 10 cm.

### 3.16 BEWEGUNGSTREMOR DER HÄNDE


<table>
<thead>
<tr>
<th>Wert</th>
<th>Beschreibung</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td></td>
</tr>
<tr>
<td>L</td>
<td></td>
</tr>
</tbody>
</table>

0: Normal: Kein Tremor.
1: Angedeutet vorhanden: Tremor ist vorhanden, die Amplitude ist jedoch kleiner als 1 cm.
2: Leicht ausgeprägt: Tremor mit einer Amplitude von mehr als 1 cm, aber geringer als 3 cm.
3: Mäßig ausgeprägt: Tremor mit einer Amplitude von mindestens 3 cm, jedoch kleiner als 10 cm.
4: Schwer ausgeprägt: Tremor mit einer Amplitude von mindestens 10 cm.
3.17 AMPLITUDE DES RUHETREMORS

Intructionen für den Untersucher: Dieses und das folgende Item wurden absichtlich an das Ende der Untersuchung gestellt, um dem Untersucher die Möglichkeit zu geben, die Beobachtungen zum Ruhetremor zu sammeln, die jederzeit während der Untersuchung auftreten können, wie etwa beim ruhigen Sitzen, beim Gehen und bei Aktivitäten, bei denen sich nur bestimmte Körperente bewegen, während andere hingegen in Ruhe bleiben. Bewerten Sie die maximale Amplitude, die während der Untersuchung aufgetreten ist, als Endwert. Bewerten Sie nur die Amplitude und nicht die Persistenz bzw. die Periodizität des Tremors.


<table>
<thead>
<tr>
<th>Bewertung der Extremitäten</th>
<th>Wert</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kein Tremor</td>
<td>ROE</td>
</tr>
<tr>
<td>≤ 1 cm maximale Amplitude</td>
<td>LOE</td>
</tr>
<tr>
<td>&gt; 1 cm, aber &lt; 3 cm maximale Amplitude</td>
<td>RUE</td>
</tr>
<tr>
<td>3-10 cm maximale Amplitude</td>
<td>LUE</td>
</tr>
<tr>
<td>&gt; 10 cm maximale Amplitude</td>
<td>Lippe/Kiefer</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bewertung der Lippen/ des Kiefers</th>
<th>Wert</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kein Tremor</td>
<td></td>
</tr>
<tr>
<td>≤ 1 cm maximale Amplitude</td>
<td></td>
</tr>
<tr>
<td>&gt; 1 cm, aber ≤ 2 cm maximale Amplitude</td>
<td></td>
</tr>
<tr>
<td>&gt; 2 cm, aber ≤ 3 cm maximale Amplitude</td>
<td></td>
</tr>
<tr>
<td>&gt; 3 cm maximale Amplitude</td>
<td></td>
</tr>
</tbody>
</table>

3.18 KONSTANZ DES RUHETREMORS

Intructionen für den Untersucher: In diesem Item wird der gesamte Ruhetremor mit nur einem Wert versehen. Der Fokus liegt hierbei auf der Konstanz des Runetremors während der Untersuchungszeit, in der sich unterschiedliche Körperente abwechselnd in Ruhe lage befinden. Diese Bewertung erfolgt absichtlich am Ende der Untersuchung, so dass verschiedene Informationen in die Bewertung einfließen können.

<table>
<thead>
<tr>
<th>Bewertung</th>
<th>Wert</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kein Tremor</td>
<td></td>
</tr>
<tr>
<td>≤ 25% der gesamten Untersuchungszeit vorhanden</td>
<td></td>
</tr>
<tr>
<td>≥ 26-50% der gesamten Untersuchungszeit vorhanden</td>
<td></td>
</tr>
<tr>
<td>≥ 51-75% der gesamten Untersuchungszeit vorhanden</td>
<td></td>
</tr>
<tr>
<td>≥ 75% der gesamten Untersuchungszeit vorhanden</td>
<td></td>
</tr>
</tbody>
</table>
III. Modified Hoehn & Yahr scale (H&Y)

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>Unilateral involvement only</td>
</tr>
<tr>
<td>1.5</td>
<td>Unilateral and axial involvement</td>
</tr>
<tr>
<td>2.0</td>
<td>Bilateral involvement without impairment of balance</td>
</tr>
<tr>
<td>2.5</td>
<td>Mild bilateral disease with recovery on pull test</td>
</tr>
<tr>
<td>3.0</td>
<td>Mild to moderate bilateral disease; some postural instability; physically independent</td>
</tr>
<tr>
<td>4.0</td>
<td>Severe disability; still able to walk or stand unassisted</td>
</tr>
<tr>
<td>5.0</td>
<td>Wheelchair bound or bedridden unless aided</td>
</tr>
</tbody>
</table>

According to [462]

IV. Becks Depression Inventory - II (BDI-II)


1. Traurigkeit
   0 Ich bin nicht traurig.
   1 Ich bin oft traurig.
   2 Ich bin ständig traurig.
   3 Ich bin so traurig oder unglücklich, dass ich es nicht aushalten kann.

2. Pessimismus
   0 Ich bin nicht mutlos, was meine Zukunft angeht.
   1 Ich bin mutloser als früher, was meine Zukunft angeht.
   2 Ich glaube nicht, dass sich meine Lage verbessert.
   3 Ich habe das Gefühl, dass es keine Hoffnung gibt für meine Zukunft und es nur noch schlimmer wird.

3. Frühere Misserfolge
   0 Ich fühle mich nicht als Versager.
   1 Ich habe öfter versagt als ich sollte.
   2 Wenn ich zurückblicke, sehe ich eine Menge Misserfolge.
   3 Ich fühle mich persönlich als totaler Versager.
4. Verlust von Freude

0 Ich habe so viel Freude wie immer an den Dingen, die mir Spaß machen.
1 Ich habe nicht mehr so viel Freude an den Dingen wie früher.
2 Ich habe sehr wenig Freude an den Dingen, die mir früher Spaß gemacht haben.
3 Ich habe keine Freude an den Dingen, die mir früher Spaß gemacht haben.

5. Schuldgefühle

0 Ich habe keine besonderen Schuldgefühle.
1 Ich habe bei vielen Dingen, die ich getan habe oder hätte tun sollen, Schuldgefühle.
2 Ich habe die meiste Zeit Schuldgefühle.
3 Ich habe ständig Schuldgefühle.

6. Gefühle, bestraft zu werden

0 Ich habe nicht das Gefühl, für etwas bestraft zu werden.
1 Ich habe das Gefühl, dass ich vielleicht für etwas bestraft werde.
2 Ich glaube, dass ich für etwas bestraft werde.
3 Ich habe das Gefühl, für etwas bestraft zu werden.

7. Abneigung gegen sich selbst

0 Meine Gefühle mir gegenüber sind die gleichen geblieben.
1 Ich habe das Vertrauen in mich verloren.
2 Ich bin von mir selbst enttäuscht.
3 Ich mag mich nicht.

8. Selbstvorwürfe

0 Ich bin mir selbst gegenüber nicht kritischer als sonst und mache mir nicht mehr Vorwürfe als sonst.
1 Ich bin mir selbst gegenüber kritischer als früher.
2 Ich mache mir Vorwürfe für alle meine Fehler.
3 Ich gebe mir die Schuld für alles Schlimme, was passiert.

9. Selbstmordgedanken oder -wünsche

0 Ich denke nie daran, mich umzubringen.
1 Ich habe Selbstmordgedanken, aber ich würde sie nicht ausführen.
2 Ich möchte mich umbringen.
3 Ich würde mich umbringen, wenn ich die Möglichkeit hätte.

10. Weinen

0 Ich weine nicht mehr als früher.
1 Ich weine mehr als früher.
2 Ich weine wegen jeder Kleinigkeit.
3 Mir ist nach Weinen zumute, aber ich kann nicht.
11. Unruhe
0 Ich bin nicht unruhiger oder erregter als sonst.
1 Ich bin unruhiger oder erregter als sonst.
2 Ich bin so unruhig oder erregt, dass es schwer ist, mich nicht zu bewegen.
3 Ich bin so unruhig oder erregt, dass ichständig in Bewegung bleiben oder etwas tun muss.

12. Interesselosigkeit
0 Ich habe das Interesse an anderen Menschen oder anderen Tätigkeiten nicht verloren.
1 Ich bin weniger an anderen Menschen oder Dingen interessier als vorher.
2 Ich habe mein Interesse an anderen Menschen oder Dingen zum größten Teil verloren.
3 Es ist schwer, für Irgendetwas Interesse aufzubringen.

13. Entschlussunfähigkeit
0 Ich treffe Entscheidungen etwa so leicht wie immer.
1 Ich fällt mir schwerer als sonst, Entscheidungen zu treffen.
2Ich habe viel größere Schwierigkeiten, Entscheidungen zu treffen, als früher.
3 Ich habe Mühe, überhaupt Entscheidungen zu treffen.

14. Wertlosigkeit
0 Ich fühle mich nicht wertlos.
1 Ich halte mich nicht für so wertvoll und nützlich wie früher.
2 Ich habe das Gefühl, weniger wert zu sein als andere Menschen.
3 Ich habe das Gefühl, völlig wertlos zu sein.

15. Verlust an Energie
0 Ich habe so viel Energie wie immer.
1 Ich habe weniger Energie als früher.
2 Ich habe nicht genügend Energie, sehr viel zu tun.
3 Ich habe nicht genügendEnergie, irgendetwas zu tun.

16. Veränderungen der Schlafgewohnheiten
0 Meine Schlafgewohnheitenhaben sich nicht geändert.
1a Ich schlafe etwas mehr als sonst., 1b Ich schlafe etwas weniger als sonst.
2a Ich schlafe viel mehr als sonst., 2b Ich schlafe viel weniger als sonst.
3a Ich schlafe die meiste Zeit des Tages., 3b Ich wache 1-2 Stunden zu früh auf und kann dann nicht mehr einschlafen.

17. Reizbarkeit
0 Ich bin nicht reizbarer als sonst.
1 Ich bin reizbarer als sonst.
2 Ich bin viel reizbarer als sonst.
3 Ich bin ständig reizbar.
18. Veränderungen des Appetits
   0 Mein Appetit hat sich nicht verändert.
   1a Mein Appetit ist etwas kleiner als sonst., 1b Mein Appetit ist etwas größer als sonst.
   2a Mein Appetit ist viel kleiner als vorher., 2b Mein Appetit ist viel größer als vorher.
   3a Ich habe überhaupt keinen Appetit., 3b Ich habe ständig großen Hunger.

19. Konzentrationsschwierigkeiten
   0 Ich kann mich so gut konzentrieren wie immer.
   1 Ich kann mich nicht so gut konzentrieren wie sonst.
   2 Es fällt mir schwer, mich sehr lange auf etwas zu konzentrieren.
   3 Ich kann mich auf gar nichts mehr konzentrieren.

20. Müdigkeit
   0 Ich bin nicht müder als sonst.
   1 Ich werde schneller müde als sonst.
   2 Ich bin für viele Dinge, die ich früher getan habe, zu müde.
   3 Ich bin für die meisten Dinge, die ich früher getan habe, zu müde.

21. Verlust des Interesses an Sex
   0 Ich habe in der letzten Zeit keine Veränderungen meines Interesses an Sex bemerkt.
   1 Ich habe weniger Interesse am Sex als früher.
   2 Ich habe jetzt viel weniger Interesse am Sex als früher.
   3 Ich habe das Interesse am Sex völlig verloren
V. Montreal Cognitive Assessment (MoCA)

### VI. Levodopa equivalent dose (LED)

*Levodopa equivalent dose according to DGN [365]*

<table>
<thead>
<tr>
<th>Medikamentenklasse</th>
<th>Medikament</th>
<th>Einzeldosen (mg/100 mg L-Dopa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Dopa</td>
<td>L-Dopa (LD)</td>
<td>100</td>
</tr>
<tr>
<td>Retardierte L-Dopa</td>
<td></td>
<td>133</td>
</tr>
<tr>
<td>Duodopa</td>
<td></td>
<td>90</td>
</tr>
<tr>
<td>COMT-Inhibitoren*</td>
<td>Entacapone</td>
<td>LD x 0.33</td>
</tr>
<tr>
<td></td>
<td>Tolcapone</td>
<td>LD x 0.5</td>
</tr>
<tr>
<td>Dopaminagonisten</td>
<td>Pramipexol</td>
<td>1 mg Salz</td>
</tr>
<tr>
<td>(non-Ergot)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ropinirol</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Rotigotin</td>
<td>3,3</td>
</tr>
<tr>
<td></td>
<td>Piribedil</td>
<td>100</td>
</tr>
<tr>
<td>Dopaminagonisten</td>
<td>Lisurid</td>
<td>1</td>
</tr>
<tr>
<td>(Ergot)</td>
<td>Bromocriptin</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Pergolid</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Cabergolin</td>
<td>1,5</td>
</tr>
<tr>
<td></td>
<td>DHEC</td>
<td>20</td>
</tr>
<tr>
<td>MAO-B Inhibitoren</td>
<td>Selegilin 10 mg (oral)</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Selegilin 1,25 mg (sublingual)</td>
<td>1,25</td>
</tr>
<tr>
<td>andere</td>
<td>Rasaglin</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Amantadine</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Apomorphin (Infusion oder Injektion)</td>
<td>10</td>
</tr>
</tbody>
</table>
9. Declaration of authorship

The study was performed in the Department of Neurodegeneration of the Neurological University Clinic of Tübingen under the supervision of Professor Daniela Berg and Dr. Kathrin Brockmann. The conception of the study was designed by Dr. Kathrin Brockmann, Dr. Karin Srulijes and Professor Daniela Berg. The initial training of essential examination procedures was carried out by Dr. Kathrin Brockmann and Dr. Karin Srulijes as well as the experienced study nurses I. Wolfstädter, T. Heger, C. Haaga, N. Runge, S. Nussbaum and K. Gauss.

S. Kober contacted the subjects in advance and arranged examination appointments. K. Brockmann and S. Kober obtained the case history related to antiparkinsonian medication and created the pedigree, describing the subject’s family history concerning PD, GD and dementia. K. Brockmann assessed motor severity via Unified Parkinson’s Disease Rating Scale Part III, Hoehn & Yahr staging and accomplished the medical examination.

S. Kober carried out vis-a-vis Parkinson-specific Questionnaires and scales

- related to autonomic performance: Part three of the Unified Multiple System Atrophy Rating Scale (UMSARS-III), the Parkinson’s Disease Non-Motor Symptoms Questionnaire (NMS-Quest) and the Non-Motor Symptoms Scale (NMSS),
- related to mood disturbances: Beck’s Depression Inventory II (BDI II), the Neuropsychiatric Inventory-Questionnaire (NPI-Q) and the Geriatric Depression Scale (GDS),
- related to activities of daily living (ADL): Functional Activities Questionnaire (FAQ) and the 39-Item Parkinson’s Disease Questionnaire (PDQ-39)
- related to sleep disorders: the Epwort Sleepiness Scale (ESS), the REM Sleep Behavior Disorder Screening Questionnaire (RBDSQ) as well as the Parkinson’s Disease Sleep Scale (PDSS)

S. Kober performed assessments related to

- autonomic performance: the Schellong Test (ST)
- cognitive functions: Montreal Cognitive Assessment (MoCA) and the Trail Making Test A/ B (TMT-A/ TMT-B),
- visual disturbances: the Pelli-Robson Contrast Sensitivity Test, the visual acuity test and the Farnsworth-Munsell 100 hue test,
- olfactory disorders: the Sniffin’ stick test,
- axial and distal motor performance: Perdue pegboard test, levopdopa equivalent dose (LED)

S. Kober performed quantitative measures related to

- autonomic performance: heart rate variability (HRV) as well as sympathetic skin response (SSR),
- axial and distal motor performance: accelerometer-based measurements including the Timed-up-and-Go-Test (TUG), functional reach (FR) test, balance measurement and a gait analysis with and without cognitive challenge, digitomotography utilizing a quantitative motor system (q-motor)
Data from the former studies in 2010 and 2011, obtained by the medical students Caroline Denise Merten and Senait Ogbamicael, was included in the longitudinal statistical analysis of this submitted thesis.

S. Kober took blood samples, both K. Brockmann and S. Kober obtained skin biopsies and K. Brockmann collected cerebrospinal fluid by lumbar puncture from subjects who were additionally included in MiGAP – “Markers in GBA-associated PD” after they gave their informed consent. It is clearly stated that the analysis of these samples obtained was not part of this presented study and dissertation. S. Kober and K. Brockmann accomplished matching of the subjects with appropriate controls. K. Brockmann and S. Kober performed the statistical analyses. K. Brockmann did the revision of the hereby submitted manuscript.

S. Kober hereby declares to have written the manuscript independently and to have used no further than the sources specified by her.

Tübingen, den 27.02.2020

[Unterschrift]
10. Publications and lectures

Parts of this dissertation have already been published in the following publication:

03/2015

Brockmann K¹, Srulijes K, Pflederer S, Hauser AK, Schulte C, Maetzler W, Gasser T, Berg D.

*GBA-associated Parkinson's disease: reduced survival and more rapid progression in a prospective longitudinal study.*


Abstract:

Parkinson's disease (PD) patients with GBA mutations show an earlier age at onset and more severe non-motor symptoms compared with PD patients without GBA mutations.

This study was undertaken to evaluate progression of motor and non-motor symptoms in sporadic PD patients depending on the mutational GBA status.

We used regression analysis to evaluate independent effects of the mutational GBA status, age at onset, age at examination, and disease duration on motor (Unified Parkinson's Disease Rating Scale [UPDRS]-III, Hoehn and Yahr [H&Y] stage, Levodopa [L-dopa]-equivalent-dosage) and non-motor characteristics (cognition and mood). Disease progression was assessed prospectively over 3 years.

The GBA-associated PD patients compared with non-mutation PD patients, although younger and with an earlier age at onset, show (1) a more rapid disease progression of motor impairment and cognitive decline and (2) reduced survival rates.

The mutational GBA status, rather than older age and age at onset, presents an important predictor for disease progression in this specific subgroup of PD patients.

09/2013

Joint lecture with Ms. Gauss: "GBA: Gaucher and Parkinson" at the 13th Gaucher patient meeting of the Austrian Gaucher Society
Danksagung

Die Phase des Verfassens dieser Dissertation liegt nun hinter mir - geprägt vom Herantasten an wissenschaftliches Arbeiten und unzählige damit verbundenen Fragen. Es führte mich heran an aktuelle Forschungsarbeiten zum Thema Morbus Parkinson, einem komplexen, neurodegenerativen Krankheitsbild, was ich gleichermaßen als faszinierende und spannende, jedoch auch als herausfordernde Aufgabe empfand.


Des Weiteren danke ich den zum Teil schwerst betroffenen Patienten, die diese Studie mit ihrer Teilnahme trotz damit verbundener Mühen überhaupt erst realisierten.


Mein besonderer Dank gilt schließlich meiner Mutter Lucia Pflederer, die mich über viele Jahre auf vielfältigste Weise unterstützte, meinen Weg bis zum Medizinstudium erfolgreich zu gehen. Ohne sie wäre diese Promotion nicht möglich gewesen.

Zuletzt danke ich meinem Mann Stefan für seine Wärme, Hilfe und Unterstützung, wann immer ich sie nötig habe.