Abbreviations

BDI Beck's Depression Inventory

CBI Copenhagen Burnout Inventory

CISS Coping Inventory for Stressful Situations

CHAPO Challenges and Opportunities model

CHAPO Challenges and Opportunities for Parkinson's Disease Patients

CRF case report form

EPI Echo planar imaging

EC Ethics Committee

HRQOL health-related-quality-of-life

ICF Informed consent form

iPS idiopathic Parkinson's syndrome

MFI-20 Multidimensional Fatigue Inventory

MDS-UPDRS Movement Disorder Society Unified Parkinson's Disease Rating Scale

MoCa Montréal Cognitive Assessment

MRI Magnetic resonance imaging

NMSQ Non-Motor Symptom Questionnaire

PD Parkinson's disease

PHQ Patient Health Questionnaire

PDCB Parkinson's disease caregiver burden Questionnaire PDCB

PPMI Parkinson's Progressive Marker Initiative

PSS Perceived Stress Scale

QoL Quality-of-Life

TE *echo time*

TR *repetition time*

WHOQoL World Health Organization Quality of Life

ZBI-22 Zarit Burden Interview

1 Preamble

1.1. Contact information

Role	**Contact**
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Data protection officer	XXX XXX XXX XXX

1.2. Version

Revision Version	**Protocol Date**	**Template number and version**	**Protocol Section modified**	**Summary of Changes**	**Justification for Modification**
A	Mai 19th, 2022		-	Initial version	Initial version

2 Protocol

2.1. Introduction

2.1.1. Background

Parkinson's disease (PD) represents a chronic neurodegenerative condition manifesting with both motor and non-motor symptoms. The disabilities in PD have a major psychosocial impact and lead to considerable losses in patients' quality of life and a high burden on (informal) caregivers. To date, various instruments have been developed to assess health-related-quality-of-life (HRQOL), some of which are specific to PD (Quelle Stuerenberg 2022). Nevertheless, none of the models takes into account the positive aspects of well-being, as well as the personal attitiude (e.g., optimism) and social resilience factors like social support, degree of integration, to name a few. In the context of this project, an investigation of the hollistic quality of life of PD-patients will be carried out. For this purpose, life quality will be assessed primarily longitudinally over a longer period of time with established and validated HRQOL questionnaires. In a further step, holistic observations of life quality will aim at providing meaningful statements in terms . The envisaged instrument for recording this, the so-called Challenges and Opportunities model (CHAPO) model, an approach initially developed for evaluating the quality of life of the very old (NRW80+ (Quelle?)). By adapting it to aspects of PD-patients, the so called CHAPO model (Quelle Thieken et al. 2021) will find application in this cohort study. Thus, the aim of this project is establishing a digital Quality-of-Life (QoL) monitoring system with the goal of assessing PD-patients in a standardised way throughout the course of their disease and to evaluate their quality of life. We would like to relate the data obtained in this way to the annual follow-up cranial MRI and the biomedical markers obtained from stool, urine, saliva, hair and blood samples. This would conceivably identify imaging or biomedical markers with predictive value for QoL-change. In addition, this longitudinal study will include a follow-up assessment of necessary support services to improve understanding the needs of family members of PD-patients. This should make it possible to develop a demand-oriented support offer according to the different phases of the disease. The stress experience, changes in sleep behavior and the losses in quality of life over the observation period are also included in the analysis in order to detect a surrogate for adequate support.

2.1.2. Geographic context

Approximately 400 000 people are diagnosed with PD in Gernany with an incidence of 84.10 per 100 000 patients annually [12]. The University Hospital of Gießen and Marburgreates up to 1500 patients. In order to understand the special recruitment peculiarities of the University Hospital of Marburg, the knowledge about the location of Marburg in Germany is essential, because Marburg is situated in the countryside in the western center of Germany with 77,129 inhabitants [18], although it is a university city and district town in Hesse. Due to its location about 77 kilometers of direct distance between the metropolitan areas of Frankfurt am Main and Kassel, the importance of the University Hospital of Marburg in the medical care of the district 70 km around Marburg is essential. To ensure patient's access to care in the district of Marburg, the Parkinson Network Alliance Marburg (PANAMA) was created in 2016 by the Department of Neurology at the Marburg University Hospital. Within this care network different stakeholders work together to facilitate the integration of care services and improve outcomes for patients.

To represent diversity of the real-life population of PD patients in the district of the Marburg University Hospital, and to guarantee a balanced study cohort, it is intended to include all patients with parkinsonism in the district. Therefore, successfully approved recruitment strategies used in past clinical trials needed to be adopted and refined. First, the patients are directly asked to participate within their appointments in the outpatient clinic or the hospital stay in the Department of Neurology in the University Hospital Marburg. Second, the PANAMA healthcare network will bring the study to the attention of its members, so that the associated healthcare providers will further promote the study. Third, media appearance of the University Hospital will give detailed information (https://www.uni-marburg.de). Additionally, the associated caregivers are requested to participate.

2.2. Protocol synopsis

Longitudinal digital observation of the holistic quality of the life of patients with PD and their caregivers: a prospective observational cohort study	
Study objectives	This study aims at observing quality of life of 1000 patients suffering from PD and their relatives over the course of 20 years and relating this to a objetifiable changes in the metabolism but also to structural imaging changes in this period
Study design	Prospective single-center cohort study
Planned Number of Subjects	1000
Primary Endpoint	
Secondary Endpoints	
Enrollment of participants	Patients suffering from PD may be enrolled together with their relatives at any point in time
Study visits schedule	• Screening • Baseline Visit • Yearly follow-up • Visit at year 2042 will be the End of Study Visit
Study Duration	The study will be considered complete after all subjects complete their visit in the year 2042. The total study duration is estimated to be at most 20 years.
Inclusion criteria PD-patients	• Patients with a clinical diagnosis of idiopathic Parkinson's syndrome according to the recent clinical diagnostic criteria (Quelle Postuma) • iPS-stages of I - IV according to the Hoehn & Yahr scale (without medication, i.e. in the OFF stage) (Quelle Hoehn und Yahr 1967). • Patients aged between between 30 and 100 years

	• Patients with the ability to provide informed consent. In cases where participants lose their capacity to consent at follow-up visits (e.g., due to dementia, etc.), this participant will only be allowed to continue if a legal representative (proxy, guardian) provides informed consent to further participation on behalf of the participant. In this case, the legal representatives will be provided with a separate consent form. • Patients with a good knowledge of German
Exclusion criteria PD-patients	• Patients suffering from a clinical diagnosis of atypic Parkinson's syndrome in a first instance. Patient's enrolled who were later characterized as atypical Parkinson syndroms will not be excluded. • iPS-stages of V according to the Hoehn & Yahr scale (without medication, i.e. in the OFF stage) (Quelle Hoehn und Yahr 1967) • The use of magnetic fields in the MRI examination excludes the participation of persons who have electrical devices (e.g. cardiac pacemakers, medication pumps, etc.) or metal parts (e.g. screws after bone fracture) in or on their bodies. • Women who are pregnant will not receive MR imaging. • Subjects who do not want to be informed about possible incidental findings are also not allowed to participate in the imaging part of the study.
Inclusion criteria PD-patients' relatives	• Relatives of patients included in the study according to the abovementioned criteria • Relatives with the ability to give informed consent • Relatives with a good knowledge of German
Exclusion criteria PD-patients' relatives	Relatives who are unable to give informed consent can't be included in the study
Statistical methods	• lorem ipsum

Primary Statistical Hypothesis	• PD patients quality of life correlates with the well being of their relatives
Statistical test method	• Linear regression
Sample Size Parameters	

2.3. Study objectives and Endpoints

2.4. Study design

The HessenKohorte Study is a prospective 20-year cohort study. The HessenKohorte study aims to recruit patients of all genders suffering from clinically probable PD (n = 1000) along with their relatives in the German region of Hessen. All patients will be recruited from the treated patients in an in- and outpatient setting between 2022 and 2042.

2.4.1. Scale and duration

The study will accompany up to 1000 patients over as much as 20 years in order to enable a good insight into the course of the individual patients and their relatives.

2.4.2. Justification for study design

This study is a single-center prospective and longitudinal cohort study to monitor PD-patients development over the course of the disease and particularly to assess their quality of life in a holistic approach. The comparatively large number of subjects will enable a better insight into PD with its multifacetted phenotypes

2.5. Subject selection

2.5.1. Study population and Eligibility

Study candidates will be drawn from the patients treated in the Neurology Department of the University Hospital of Gießen and MarburgMarburg site) as either in- or outpatients. Moreover, patients suffering from PD may submit a request for participation in the study. The inclusion and exclusion criteria (cf. Section 2.5.1) are checked by one of the study physicians, who are responsible for the final decision. Advertising for the study can be found in the form of a flyer, which is available in the Department of Neurology, but also in the form of an Internet, where the project is presented.

2.5.2. Inclusion criteria PD-patients

Subjects who meet all of the following criteria (cf. Table **??**) may be given consideration for inclusion in this cohort study, provided no exclusion criteria (cf. Section 2.5.3) are met.

- Patients suffering from a clinical diagnosis of idiopathic Parkinson's syndrome according to the recent clinical diagnostic criteria (Quelle Postuma).
- iPS-stages of I - IV according to the Hoehn & Yahr scale (without medication, i.e. in the OFF stage) (Quelle Hoehn und Yahr 1967).
- Patients with the ability to provide informed consent. In cases where participants lose their capacity to consent at follow-up visits (e.g., due to dementia, etc.), this participant will only be allowed to continue if a legal representative (proxy, guardian) provides informed consent to further participation on behalf of the participant. In this case, the legal representatives will be provided with a separate consent form.
- Patients with a good knowledge of German

2.5.3. Exclusion criteria PD-patients

Subjects who meet any one of the following criteria (cf. Table **??**) cannot be included or will be excluded from this cohort study.

- Patients suffering from a clinical diagnosis of atypic Parkinson's syndrome in a first instance. Patient's enrolled who were later characterized as atypical Parkinson syndroms will not be excluded.
- iPS-stages of V according to the Hoehn & Yahr scale (without medication, i.e. in the OFF stage) (Quelle Hoehn und Yahr 1967).
- The use of magnetic fields in the MRI examination excludes the participation of persons who have electrical devices (e.g. cardiac pacemakers, medication pumps, etc.) or metal parts (e.g. screws after bone fracture) in or on their bodies.
- Women who are pregnant will not receive MRI scans.
- Subjects who do not want to be informed about possible incidental findings are also not allowed to participate in the imaging part of the study.

2.5.4. Inclusion criteria PD-patients' relatives

Subjects who meet all of the following criteria (cf. Table **??**) may be given consideration for inclusion in this cohort study, provided no exclusion criteria (cf. Section 2.5.5) are met.

- Relatives of patients included in the study according to the abovementioned criteria
- Relatives with the ability to give informed consent
- Relatives with a good knowledge of German

2.5.5. Exclusion criteria PD-patients' relatives

Subjects who meet any one of the following criteria (cf. Table **??**) cannot be included or will be excluded from this cohort study.

- Relatives who are unable to give informed consent can't be included in the study

2.6. Subject accountability

2.6.1. Point of enrollment

A subject will be considered enrolled at the time of the study-specific informed consent form (ICF) execution. No study-related procedures or assessments can take place until the ICF is signed.

2.6.2. Withdrawal

All subjects enrolled in the HessenKohorte (including those withdrawn from the clinical study) shall be accounted for and documented. If a subject withdraws from the clinical investigation, the reasons shall be reported.

Reasons for withdrawal include but are not limited to:

- subject or relative choice to withdraw consent
- lost to follow-up
- pregnancy*
- implantation of electrical devices or metal parts in or on the body *

* Only the MR-imaging will be discontinued during pregnancy or from the moment of an implantation onwards.

Subjects may of course withdraw at any time, with or without reason, and without prejudice to further treatment. All applicable case report forms case report form (CRF) up to the point of subject withdrawal and an "End of Study" form must be completed. Any subject deemed

"lost to follow-up" should have a minimum of three documented attempts to contact him/her prior to completion of the "End of Study" form. Additional study data may no longer be collected after the point at which a subject has been withdrawn from the study or withdraws consent, for whatever reason. Data collected up to the point of subject withdrawal may be used. Subjects withdrawn after completing the implant procedure will not be replaced

2.6.3. Lost to follow-up

2.6.4. Subject status and classification

A subject will be considered enrolled in this study at the time of the study-specific Informed consent form (ICF) execution.

2.6.5. Enrolment control

The overall enrollment in the study will be capped at 1000 participants.

2.6.6. End-of-study definition

The study is considered complete when 20 years from the first enrolment are over.

2.7. Study methods

2.7.1. Data collection

The data collection schedule is shown in Table **??**

Tabelle 2.2.: *Data Collection Schedule for PD-patients enrolled in the* HessenKohorte

Visit	**Screening**	**Baseline visit**	**Half-year visit**	**Year 1,2,3,4, ..., 20 Visit**	**Unscheduled Visit**
Informed Consent Process	X				
Eligibility Criteria		X			
Subject Demographics		X	X	X	
MDS-UPDRS		X		X	X*
NMSQ		X		X	
CHAPO		X		X	
Hair sample		X		X	
Saliva sample		X		X	
Blood sample		X		X	
Stool sample		X		X	

*may be ascertained and entered into database

Tabelle 2.4.: *Data Collection Schedule for patients' relatives enrolled in the* HessenKohorte

Visit	**Screening**	**Baseline visit**	**Half-year visit**	**Year 1,2,3,4,5, ..., 20 Visit**	**Unscheduled Visit**
Informed Consent Process	X				
Eligibility Criteria		X			
Subject Demographics		X	X	X	
Hair sample		X		X	
Saliva sample		X		X	
Blood sample		X		X	
Stool sample		X		X	

*may be ascertained and entered into database

2.7.2. Candidate Screening

Subjects will be screened for participation in the study based on study Inclusion and exclusion criteria as listed in Section 2.5. Subjects who have provided informed consent and who have been determined to not meet all eligibility requirements will be withdrawn.

2.7.3. Informed consent

Written informed consent must be obtained from potential study candidates and enrollment is only valid, after subjects sign and date the ICF.

- Subjects will be asked to sign the ICF before study-specific tests or procedures are performed;
- The idea of the study must be explained, and subjects must be given the time and

opportunity to ask questions and have those questions answered to their satisfaction.

- The ICF is study specific and has been approved by the Ethics Committee (EC).
- Written informed consent must be recorded appropriately by means of the subject's dated signature.

2.7.4. Questionnaires

a. Challenges and Opportunities for Parkinson's Disease Patients

b. Movement Disorder Society Unified Parkinson's Disease Rating Scale

The MDS-UPDRS evaluates various aspects of PD-patients, including non-motor and motor symptoms. It consists of four parts:

- Part I: Experiences of daily living (non-motor symptoms), including 13 items.
  - A: Behavioral problems of the patient, as evaluated by the examiner.
  - B: Part on non-motor symptoms completed by the patient, with the assistance of a caregiver if necessary, but independent of the investigator.
- Part II: Experiences of daily living (motor aspects) with 13 items. This part is also a self-report questionnaire to be completed by the patient, with the assistance of a caregiver if necessary, but independent of the investigator.
- Part III: Motor examination with 18 items. All instructions are read to the patient by the examiner or demonstrated directly, so that this part is completed by the examiner.
- Part IV: Motor Complications with 6 items. This part contains instructions for the examiner and also instructions to be read to the patient. It combines patient-related information with clinical observations and assessments by the examiner.

c. Montréal Cognitive Assessment (MoCa)

Montréal Cognitive Assessment is a screening tool that can quickly identify hints on cognitive decline according to mild cognitive impairment or dementia. The entire test consists of approximately 10-minutes of questions around different domains of cognitiion. The 30 questions test cognitive abilities such as memory, language production , contextual thinking, attention and concentration, behavior, arithmetic, temporal and spatial orientation, and the ability to

recognize complex shapes and patterns. The test is validated is extensively applied in clinical routine.

d. Non-Motor Symptom Questionnaire, (NMSQ)

The NMSQ is a 30-item rater-based scale designed to assess a broad spectrum of non-motor symptoms in patients with Parkinson's disease (PD). (PD). The NMSQ measures the severity and frequency of non-motor symptoms across nine dimensions.

e. Beck's Depression Inventory (BDI)

The Beck's Depression Inventory (BDI) is a questionnaire which aims at assessing the severity of depressive symptoms in case depression exists. It is not intended to assess depression per se, but only its severity. Hence, it cannot be used as a screening method in the normal population so taht other alternatives should be contemplated. The applied second version of the BDI consists of 21 questions which are supposed to be evaluated for the previous two weeks.

Scores:

- 0–12: no depressive symptoms or clinically inapparent
- 13–19: mild depressive syndrome
- 20–28: moderate depressive syndrome
- $>$ 29 severe depressive syndrome

f. Copenhagen Burnout Inventory (CBI)

The Copenhagen Burnout Inventory (CBI) is a questionnaire for measring personal burnout, work-related burnout, and client-related burnout with a very high internal reliability. It has shown to correlate with future sickness absence, sleep problems, the use of pain-killers, and intention to quit the job.

g. Coping Inventory for Stressful Situations (CISS)

The Coping Inventory for Stressful Situations (CISS) is an instrument for the assessment of coping style. It assesses three different styles: task-oriented coping, emotion-rienting coping and avoidance-oriented coping which in turn can be subdivided into social distraction-oriented coping and more general distraction-oriented coping.

h. Multidimensional Fatigue Inventory

Fatigue is a state where a person experiences a reduced level of energy in daily life activities. The Multidimensional Fatigue Inventory (MFI-20) is an instrument which measures fatigue via 20 different items on five subscales: general fatigue, physical fatigue, mental fatigue, reduced motivation and reduced activity.

i. Parkinson's disease caregiver burden Questionnaire PDCB (PDCB)

The Parkinson's disease caregiver burden Questionnaire PDCB (PDCB) is a questionnaire specifically aiming at caregiver burden for caregivers of patients with Parkinson's disease (PD). It assesses the caregivers burden on the seven subscales of physical burden, sleep disruption, patient symptoms, responsibilities, patient medications, social burden as well as patient and self-relationship. Furthermore a global measure of caregiver burden is given.

j. Patient Health Questionnaire

k. Perceived Stress Scale (PSS)

The perceived stress scale is an instrument for the assessment of the subjective stressfulness of different life events across the last month. A global score is obtained which can be used to subdivide subjects into groups of low stress (0-13 points), moderate stress (14-26points) and high stress (27-40 points).

l. World Health Organization Quality of Life (WHOQoL)

The World Health Organization Quality of Life (WHOQoL) is a detailed, standardized measure of ones Quality of Life (QoL) which is available in many languages and puts an emphasis on cross-cultural comparability. It features 100 questions which can be assessed with six domain scores, 24 *facet* scores as well as a global QoL *facet* score. The domain scores describe QoL on the following dimension: physical, psychological, level of independence, social relationships, environment, and spirituality.

m. Zarit Burden Interview (ZBI-22)

The Zarit Burden Interview (ZBI-22) is a questionnaire assessing the burden of a caregiver caring for another person on a 22 item scale. It is one of the moust commonly used tools to assess this type of burden.

2.7.5. Baseline visit PD-patients

All potential candidates will undergo screening procedures as listed in Section 2.7.2 to determine their eligibility in the study. Subjects may neither have to be on stable anti-parkinsonian medications prior to informed consent nor have to be regularly treated at the University Hospital of Gießen and Marburg Those subjects who meet all inclusion criteria and none of the exclusion criteria (cf. 2.5) may be enrolled. The baseline visit may occur anytime within the screening period and will serve as the final determination of eligibility in the study.

The following data from questionnaires should be collected from patients:

- General Assessments
  - Demographic data and personal information
  - Medication schedule
- CHAPO
- NMSQ

2.7.6. Half year visit PD-patients (± 100 days)

2.7.7. Annual visit PD-patients (± 100 days)

2.7.8. Baseline visit relatives

This study is intended as inclusion of diades of patients and relativesAll potential candidates will undergo screening procedures as listed in Section 2.7.2 to determine their eligibility in the study. Subjects may neither have to be on stable anti-parkinsonian medications prior to informed consent nor have to be regularly treated at the University Hospital of Gießen and Marburg Those subjects who meet all inclusion criteria and none of the exclusion criteria (cf. 2.5) may be enrolled. The baseline visit may occur anytime within the screening period and will serve as the final determination of eligibility in the study.

For the relatives, the following data from questionnaires should be collected:

- General Assessments
  - Demographic data and personal information
  - Relationship to patients
  - Experiencing respect in the patient-family relationship

- Beck's Depression Inventory, (part II)
- CBI
- CISS
- MFI-20
- Montréal Cognitive Assessment (MoCa)
- PDCB
- Patient Health Questionnaire (PHQ)
- Perceived Stress Scale (PSS)
- WHOQoL
- ZBI-22

2.7.9. Half year visit relatives (± 100 days)

2.7.10. Annual visit relatives (± 100 days)

2.7.11. MRI

Every PD-patient will receive MR-imaging if no contraindication exists and at the request of the respective patient. With the aim of producing the greatest possible synergistic effects with other large studies at the centre and to ensure a high quality of the sequences, the programme to be run was based on the PPMI study (`https://www.ppmi-info.org/`). Further details are disclosed below.

a. Overview of MR-imaging

b. Procedure of the imaging

Participants should be positioned comfortably and correctly to minimize motion during the scan. Furthermore, technicians will be instructed to comply with the following:

- Participant should be informed about the total acquisition time and positioned for maximum comfort.
- Subjects must be positioned comfortably and supine in the head coil to minimize any motion during the scan.

Tabelle 2.6.: *Overview on the MRI-sequences in use during the* HessenKohorte*

Sequence Name	**Series Description**
T1-weighted, 3D volumetric sequence	3D T1-weighted
2D Gradient-echo T2*-weighted EPI (BOLD)	rsfMRI_RL
Repeat 2D Gradient-echo T2*-weighted EPI (BOLD)	rsfMRI_LR
NM-MT	2D GRE-MT
DTI	DTI_RL
Repeat DTI	DTI_LR
3D T2 FLAIR	3D T2 FLAIR

*protocol is identical to the one used by the PPMI-study

- Proper back support, and support under the knees will ensure greater comfort, and lead to less motion in the scan.

- There should be no left-right or ear-to-shoulder head tilt, and the participant's neck should not be hyper- extended or retracted.

- Subject's head should be centered in the head coil using the nasion (see example to the right) as an anatomical landmark. Ensure the participant is high enough in the coil to avoid loss of signal at the inferior aspects of the brain.

- Immobilization devices, such as velcro straps, or foam padding should be used to reduce motion.

- The positioning lasers should be used to send the nasion to the magnets isocenter.

If a participant's neck length is such that it does not permit proper positioning in the head coil, please document this on the MRI Acquisition Document along with any other pertinent information regarding the participants scanning session.

c. T1-weighted, 3D volumetric sequence

Tabelle 2.8.: *Details on T1-weighted MRI-sequence*

T1-weighted, 3D volumetric MRI-sequence during the HessenKohorte, e.g. MP-RAGE!, IR-FSPGR!	
Series description	3D T1-weighted
Plane	Sagittal
Slice thickness (mm)	1.0 (slice thickness must remain consistent across time-points)
Number of slices	192 (slice thicksness may be adjusted to 1.2 mm to cover brain iff absolutely necessary. No adjustments of number of slices)
Voxel size (mm)	1.0*1.0 mm in plane resolution
Phase encode direction	Anterior Posterior (AP)
Matrix	256 × 256 (the use of interpolation, zero-filling or a ZIP factor is not permitted)
TR/TE/FA/ other parameters	Will be defined by Invicro according to the scanner
FoV	256 mm (full FoV required, no rectangular FoV)
Scan time	∼ 7 min
Further explanations	The FOV must include the entire brain anatomy including the vertex, cerebellum and pons. Slices should be oblique sagittal, angled along the longitudinal fissure on both the axial and coronal localizers. To avoid artifacts, position the participant such that there is sufficient empty space around the head: approximately 1.5 cm of air or more above the top of the head, and leave 3 - 4 blank slices on either side of the head. Avoid nose ghosting.

*protocol is identical to the one used by the PPMI-study

d. 2D Gradient-echo T2*-weighted EPI

Tabelle 2.10.: *Details on T2-weighted MRI-sequence*

2D-Gradient-echo T2*-weighted EPI (e.g., ep2d_BOLD)	
Series Description	rsfMRI_RL
Plane	Axial Oblique, plane parallel to AC-PC line
Slice thickness (mm)	3.5 with no gap
Number of Slices	∼40
Phase encode dir.	R >>L
Matrix	64 × 64
FOV	224 × 224 mm
Repetition Time (ms)	2500
Echo Time (ms)	30
Flip angle	80
Slice order	Interleaved
Number of measurements	240 (10 min total scan time)
In-plane acceleration	GRAPPA or SENSE (factor of 2)
Instructions	Keep the eyes open and remain still
Scan Time	∼10 min
Further explanations	Please instruct the participant to keep their eyes open during the entire scan. You can instruct them to focus on a point on the mirror or scanner. Check with the participant immediately after the scan to verify they kept their eyes open and did not fall asleep. No audio or video presentation should be made during the scan.Position the axial resting state fMRI slices along the AC-PC plane with care that there is one slice above the vertex, and then cover the rest of the brain and as much of the cerebellum as possible with the remaining slices. The slices should be centered in the axial plane to prevent aliasing in the Anterior/Posterior direction (see Figure 4 ??). TR/TE should not be changed.

e. REPEAT 2D Gradient-echo T2*-weighted EPI

Tabelle 2.12.: *Details on REPEAT T2-weighted MRI-sequence*

REPEAT 2D Gradient-echo T2*-weighted EPI	
Series Description	rsfMRI_LR
Plane	Axial Oblique, plane parallel to AC-PC line
Slice thickness (mm)	3.5 with no gap
Number of Slices	∼40
Phase encode dir.	L >>R
Matrix	64x64
FOV	224 x 224 mm
Repetition Time (ms)	2500
Echo Time (ms)	30
Flip angle	80
Slice order	Interleaved
Number of measurements	10 (25 sec total scan time)
In-plane acceleration	GRAPPA or SENSE (factor of 2)
Instructions	Keep the eyes open and remain still
Further explanations	Repeat the above scan with the phase encoding direction updated to L » R, and the number of measurements updated to “10”. All other parameters should be held constant. Recommended imaging parameters for the repeat resting state fMRI sequence can be referenced in Table 6.

f. 2D Gradient recalled echo with MT preparation

Tabelle 2.14.: *Details on REPEAT T2-weighted MRI-sequence*

2D-Gradient-echo T2*-weighted EPI (e.g., ep2d_BOLD)	
Series Description	rsfMRI_RL
Plane	Axial Oblique, plane parallel to AC-PC line
Slice thickness (mm)	3.5 with no gap
Number of Slices	∼40
Phase encode dir.	R >>L
Matrix	64x64
FOV	224 x 224 mm
Repetition Time (ms)	2500
Echo Time (ms)	30
Flip angle	80
Slice order	Interleaved
Number of measurements	240 (10 min total scan time)
In-plane acceleration	GRAPPA or SENSE (factor of 2)
Instructions	Keep the eyes open and remain still
Scan Time	∼10 minutes
Further explanations	Please instruct the participant to keep their eyes open during the entire scan. You can instruct them to focus on a point on the mirror or scanner. Check with the participant immediately after the scan to verify they kept their eyes open and did not fall asleep. No audio or video presentation should be made during the scan.

g. 2D Diffusion-weighted EPI

Tabelle 2.16.: *Details on 2D Diffusion-weighted EPI*

2D Gradient-echo T2*-weighted EPI (eg ep2d_BOLD)	
Series Description	**DTI!**_RL (and **DTI!**_LR for the repeated scan with reverse PE)
Plane	Straight Axial
Slice thickness (mm)	2.0 with no gap
Number of Slices	∼80
Phase encode dir.	R >>L
Matrix	128x128*
FOV	256x256 mm
Repetition Time (ms)	∼10000
Echo Time (ms)	∼80
Flip angle	90
Slice order	Interleaved
Number of directions	32
b-VALUE	0 and 1000 s/mm2 (B=0 images interleaved throughout if possible in product sequence)
Instructions	Keep still
Scan Time	∼8 minutes
Further explanations	Please instruct the participant to keep still during the entire scan. **DTI!** should be acquired with 32 directions. Slices should cover top of the brain down to base of cerebellum. Two sequences with reversed phase encoding direction should be acquired in full to correct for susceptibility induced distortions. If acquiring a phantom scan, only one sequence with reverse phase encoding direction should be acquired

h. 3D T2 **FLAIR!** (**FLAIR!**) Sequence

Tabelle 2.18.: *Details on T2-weighted **FLAIR!** Sequence*

3D T2 FLAIR! Sequence	
Series Description	3D T2 FLAIR
Plane	Sagittal
Slice thickness (mm)	1.0 – 1.2 (slice thickness must remain consistent)
Number of slices	192 (please adjust slice thickness up to 1.2 mm to cover brain, not the number of slices)
Voxel size (mm)	1.0*1.0 mm in plane resolution
Phase encode dir.	Anterior-Posterior (AP)
Matrix	256 x 256 (the use of interpolation, zero-filling or a ZIP factor is not permitted)
TR/TE/FA/other parameters	Will be defined by Invicro according to the scanner
FOV	256 mm (full FOV required, no rectangular FOV)
Scan Time	∼7 minutes
Further explanations	The FOV must include the entire brain anatomy including the vertex, cerebellum and pons. To avoid artifacts, position the participant such that there is sufficient empty space around the head: approximately 1.5 cm of air or more above the top of the head, and leave 1 - 2 blank slices on top of the head. Avoid nose ghosting.

2.7.12. Biosamples

a. Hair

b. Saliva

c. Urine

d. Blood

e. Stool

2.8. Statistical considerations

2.9. Data management

2.10. Amendments

In case of protocol changes possibly affecting the rights, safety or welfare of any subjects or scientific integrity of the data, a protocol amendment will be completed. Appropriate approvals (especially from the EC) of the revised protocol must be obtained prior to its implementation.

2.11. Compliance

2.11.1. Statement of Compliance

This study will be conducted in accordance with ICH-GCP and with the ethical principles originating in the Declaration of Helsinki.

2.11.2. Investigator responsibilities

a. Delegation of responsibilities

When specific tasks are delegated, the Principal Investigator is responsible for providing appropriate training if necessary and adequate supervision of those to whom tasks are delegated. The investigator is accountable for regulatory violations resulting from failure to adequately supervise the conduct of the clinical study.

2.11.3. Ethics committee

The investigational site has obtained the approval of the local ethics commitee for the clinical investigation. A copy of the written approval of the protocol can be found in the Appendix (cf. chapter **??**). Any amendment to the protocol will require review and approval by the ethics commitee before any changes are implemented to the study. Besides, all changes to the ICF will have to be approved, as well. In case of an extension of the study to further centers, an ethics approval must be obtained by the respective ethics commitee.

2.12. Monitoring

2.13. Potential Risks and Benefits

2.13.1. Anticipated Adverse Events

2.13.2. Risks associated with the study participation

2.13.3. Risks associated with the MRI

2.14. Safety Reporting

2.15. Informed consent

2.16. Suspension or termination of the study

2.17. Study registration and Results

2.18. Bibliography

3. Appendix