The remote assessment of parkinsonism supporting the ongoing development of interventions in Gaucher disease

Abigail Louise Higgins¹, Marco Toffoli¹, Stephen Mullin², Chiao-Yin Lee¹,³, Sofia Kolets¹,³, Micol Avenali⁴,⁵, Fabio Blandini⁴,⁶ & Anthony HV Schapira*,¹,³
¹Department of Clinical & Movement Neurosciences, University College London, Queen Square Institute of Neurology, London, UK
²Institute of Translational and Stratified Medicine, University of Plymouth Peninsula School of Medicine, Plymouth, UK
³Aligning Science Across Parkinson’s (ASAP) Collaborative Research Network, Chevy Chase, MD 20815, USA
⁴Department of Brain and Behavioural Sciences, University of Pavia, Pavia, Italy
⁵Neurorehabilitation Unit, IRCCS Mondino Foundation, Pavia, Italy
⁶Cellular and Molecular Neurobiology Unit, IRCCS Mondino Foundation, Pavia, Italy
*Author for correspondence: a.schapira@ucl.ac.uk

Practice points

- The therapeutic focus for Parkinson’s disease (PD) is currently symptomatic, rather than disease modifying or neuroprotective.
- Nonmotor prodromal features of PD may precede the motor symptoms required for clinical diagnosis by several years.
- GBA mutations, which are associated with Gaucher disease in their biallelic form, have emerged as the most common genetic risk factor for PD.
- GBA-associated PD displays a slightly exaggerated phenotype, including earlier age of onset, enhanced cognitive decline, more severe affective disturbances and a greater likelihood of manifesting REM sleep behavior disorder, hyposmia and autonomic dysfunction.
- The RAPSODI GD study is an annual remote assessment which aims to define the clinical prodrome of PD in a large cohort of GBA mutation carriers, to accurately predict clinical diagnosis.
- The secondary objectives of RAPSODI GD are to risk stratify homozygote and heterozygote GBA mutation carriers for PD risk, understand the variable penetrance of the GBA-associated PD phenotype and create a platform for the future evaluation of biomarkers of disease.
- The putative implication of the RAPSODI GD study is to contribute toward earlier diagnosis of GBA-associated PD, to provide a timeframe for delivering neuroprotective interventions.
- Defining prodromal PD in GBA carriers may have wider implications for sporadic PD.

Mutations in GBA which are causative of Gaucher disease in their biallelic form, are the most common genetic risk factor for Parkinson’s disease (PD). The diagnosis of PD relies upon clinically defined motor features which appear after irreversible neurodegeneration. Prodromal symptoms of PD may provide a means to predict latent pathology, years before the onset of motor features. Previous work has reported prodromal features of PD in GBA mutation carriers, however this has been insufficiently sensitive to identify those that will develop PD. The Remote Assessment of Parkinsonism Supporting Ongoing Development of Interventions in Gaucher Disease (RAPSODI GD) study assesses a large cohort of GBA mutation carriers, to aid development of procedures for earlier diagnosis of PD.

Lay abstract: Changes in a gene called GBA cause a rare condition called Gaucher disease and are the most common genetic risk factor for Parkinson’s disease (PD). To diagnose PD, patients must show symptoms of disordered movement which only occur after irreversible brain cell loss. Earlier symptoms may allow for the prediction of PD, years before movement symptoms occur. Previous work has reported earlier symptoms of PD occurring in people with GBA changes, however these studies have not been able to identify those at risk of developing PD. The Remote Assessment of Parkinsonism Supporting Ongoing Development of Interventions in Gaucher Disease (RAPSODI GD) study assesses a large group of people with GBA changes, to help develop a way to diagnose PD earlier.
Background

Challenges facing early diagnosis & intervention in Parkinson’s disease

Parkinson’s disease (PD) is the second most common neurodegenerative disease, with increasing global prevalence which doubled between 1990 and 2016 [1,2]. The clinicopathology of PD is characterized by motor symptoms including bradykinesia, tremor and rigidity, attributed to the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta [3,4]. An important neuropathological hallmark of PD is the presence of filamentous intracellular inclusions of α-synuclein, known as Lewy bodies [5].

Current treatments of PD focus primarily on symptom management, rather than disease modification [6]. With an aging population, the putative continuation of the upward trend in cases of PD calls for the development of preventative therapies, of which there currently are none [7,8]. One of the main challenges for developing neuroprotective therapies for PD is that clinical diagnosis relies upon the presentation of defined motor deficits, which manifest after the onset of neurodegeneration [9]. As such, there is increasing interest in characterizing the prodromal features of PD, which may precede motor symptoms by several years [10]. Several latent features of PD’s clinical prodrome have been validated thus far, including REM sleep behaviour disorder (RBD), hyposmia, depression and anxiety, global cognitive impairment, constipation and autonomic dysfunction [11]. In recent years, studies such as PREDICT-PD, have optimized the validation of prodromal features of PD through remote assessment methods, enhancing recruitment and power, and allowing for repeated measures without inter-observer variation [12].

Gaucher disease & GBA

The GBA gene located on chromosome 1q21, encodes for the lysosomal hydrolase glucocerebrosidase (GCase), and has 12 exons (exon 1 is noncoding) [13,14]. Gaucher disease (GD) is a rare autosomal recessive disorder caused by biallelic homoygous or compound heterozygous GBA mutations, of which there have been over 400 pathogenic mutations identified [15–17]. In GD, a loss of function of GBA results in deficient GCase activity, and the subsequent accumulation of its substrate, a sphingolipid called glucosylceramide primarily in macrophages [18]. Literature concerning the epidemiology of GD is highly variable, with birth incidence of GD in the general population falling between 0.39 and 5.80 per 100,000, and prevalence ranging between 0.70 and 1.75 per 100,000 [19]. The prevalence of GD varies with ethnicity and is markedly higher in Ashkenazi Jewish populations, who also have a higher GBA mutation carrier frequency [19]. GD has three clinical subtypes: 1, 2 and 3 [20]. Type 1 GD is the mildest and most common phenotype, accounting for 94% of cases in western populations [19]. Type 1 is characterized by clinicopathological features including bone lesions, hepatosplenomegaly, thrombocytopenia and anemia, in the absence of primary neurological disease [18]. Types 2 and 3 are neuronopathic forms, with mean lethality at <4 years old and <40 years old, respectively [21]. GBA mutations vary in pathogenicity therefore those that cause type 1 GD (such as N370S) are considered mild, whereas those that also cause type 2 and 3 GD (such as L444P) are denoted as severe [22,23].

The link between GBA mutations & PD

Within the last three decades, clinicians have reported the presentation of parkinsonian motor features in a subset of patients with GD [24,25]. An increased incidence of PD within GD kindreds was observed, and PD patients with GBA mutations were more likely to report a first or second degree relative with PD, than those without GBA mutations [26–28]. Parkinsonism has presented in GD patients with both neuronopathic and non-neuronopathic GBA variants [29]. Subsequently, in an international multi-centre analysis a strong association was detected between GBA mutations and PD, with an odds ratio of 5.43 for carrying heterozygous GBA mutations in PD patients versus controls [28]. This work established heterozygous GBA mutations as the most common genetic risk factor for PD. While an increased lifetime risk for developing PD has been established for both biallelic and monoallelic carriers of GBA mutations, it is still only a minority of individuals with these mutations that will go on to develop PD [30,31]. Heterozygous GBA mutation carrier status confers a 3–15% risk of PD by age 80, contingent upon the severity of the variant, however the literature concerning the penetrance of these mutations in PD is variable [23,30,32,33].
Penetration of GBA mutations in PD has been estimated between 10 and 30% and can be stratified by genetic and nongenetic factors, including age-specificity and severity of mutation [33,34].

The prodrome of GBA-associated PD

There is an overrepresentation of literature concerning the natural history of PD associated with the two most frequent GBA pathogenic variants, L444P and N370S, compared with the role of many of the other hundreds of variants [13,22,35–37]. The phenotype of GBA associated PD (GBA PD), differs from sporadic PD (sPD), with a slightly earlier age of onset of approximately 5 years, with a dose effect for severe versus mild mutations [23,38]. It has also emerged that GBA PD is associated with more nonmotor symptoms than its idiopathic counterpart, including a greater likelihood of manifesting cognitive dysfunction concomitant with more aggressive cognitive decline, more severe affective disorder, and an increased risk of experiencing RBD, hyposmia and autonomic dysfunction [30,39–42].

GCase in sporadic PD

While the molecular mechanisms of pathogenesis have not been fully elucidated for GBA PD, much like in GD there is a significant reduction in GCase activity [43]. Histopathological analyses of post-mortem PD brain tissue have identified reduced GCase activity and protein levels in regions with elevated expression of α-synuclein [43], and this was subsequently reproduced [44,45]. The most significant reduction in GCase activity was noted in the substantia nigra, and notably these observations were found in both GBA PD tissue and tissue from individuals with sPD [43]. Work in iPSC models has suggested that the involvement of GCase in both GBA PD and sPD, may result from a bidirectional relationship between reduced lysosomal GCase activity and cytotoxic α-synuclein aggregation [46–48]. Due to the implication of deficient GCase activity in both GBA PD and sPD, GCase has become an attractive therapeutic target for PD [49–51]. Murine and iPSC models of GBA PD and sPD have demonstrated the promise of gene therapies and small-molecule chaperone modulation, to increase GCase activity and subsequently reduce α-synuclein accumulation with neuroprotective outcomes [52,53]. Understanding the mechanisms of GCase-mediated PD pathogenesis is therefore a salient research focus for not only GBA PD but more widely sPD.

Objectives & rationale

The increasing global burden of PD highlights the relevance of utilizing PD’s clinical prodrome for earlier diagnosis, and intervention [2]. As the most common genetic risk factor for PD, with premotor features and putative implications for sPD, defining prodromal PD features associated with GBA is an important target for enhancing detection of disease and earlier intervention timepoints [3,28,39,49].

The RAPSODI GD study aims to define prodromal symptoms of PD in individuals who carry GBA mutations, both homozygote and heterozygote, and to further elucidate the natural history of GBA PD. It will test the hypothesis that the presentation of prodromal features of PD for GBA can predict the onset of clinical diagnosis. In doing so, it may be possible to develop models for the accurate prediction of individuals at-risk of developing GBA PD, affording earlier opportunities for intervention before irreversible neurodegeneration. Secondary objectives of the study include:

- Undertaking PD risk stratification for individuals with biallelic versus monoallelic GBA mutations;
- Serving as a platform for future research to identify at-risk individuals for GBA PD, to explore biomarkers of GBA PD;
- Probing the limited and variable penetrance of the PD phenotype for individuals with GBA mutations.

Design & methodology

RAPSODI GD is a remote, longitudinal, descriptive, natural history, registry-based study. Repeated measures are delivered on an annual or biennial basis following baseline assessment. Assessment is undertaken via the online study portal (www.rapsodistudy.com), and by post. Saliva sample collection and genotyping for GBA and LRRK2 mutations is an independent measure, occurring once at baseline. The study aims to assess participants for the presence and progression of six prodromal features of PD: olfactory dysfunction, motor dysfunction, cognitive decline, anxiety and depression, motor symptoms, REM sleep dysfunction. RAPSODI GD has 25 years of ethical approval from London - Queen Square Research Ethics Committee (REC reference: 15/LO/1155).
**Methodology** Higgins, Toffoli, Mullin et al.

### Sampling
Purposive sampling has been used to recruit participants from the following strata: heterozygous GBA mutation carriers, homozygous GBA mutation carriers, heterozygote GBA mutation carriers with a confirmed clinical diagnosis of PD, idiopathic PD cases with unknown GBA status, controls. These sampling methods were chosen with the aim of optimizing recruitment for each stratum, to develop a large registry of GBA mutation homozygotes and heterozygotes. This method seeks to enhance the statistical power necessary for the detection of subtle prodromal features of PD, allowing for future analysis and comparison of the distribution of premotor symptoms of PD between groups.

RAPSODI GD has eight Patient Identification Centres (PICs), where we are partnered with lysosomal storage disorder units, (Royal Free Hospital, Addenbrooke’s Hospital, Queen Elizabeth Hospital Birmingham, Salford Royal Hospital, Cardiff and Vale University Health Board, University College Hospital, Great Ormond Street Hospital and Manchester Children’s Hospital). From these PICs GD patients with homozygous GBA mutations are recruited. These participants act as index cases, to recruit first-degree relatives from. These relatives once consented are subsequently screened and determined to be homozygous or heterozygous for GBA mutations, or controls. Our sister study, PD FRONTLINE, is a newly established offshoot which screens idiopathic PD cases for GBA mutations. All idiopathic PD cases will initially be recruited and screened via the PD FRONTLINE platform to ensure GBA mutation status. These GBA mutation positive individuals will then be asked to reconsent and reassign from PD FRONTLINE to RAPSODI GD and will act as index cases to contact first degree relatives through. Relatives will be determined to be homozygous or heterozygous for GBA mutations, or controls. Family tracing from index cases is only pursued with permission from the participant. Due to the descriptive nature of RAPSODI GD, we envision that recruitment will remain active throughout the full course of the study period, up to the limit of 10,000 participants.

The inclusion criteria for RAPSODI GD are as follows: homozygous GBA mutation carriers, heterozygous GBA mutation carriers, first degree relatives of heterozygous or homozygous GBA mutation carriers, spouses of individuals matching one of inclusion criteria 1–3, heterozygous or homozygous GBA mutation carriers with a confirmed clinical diagnosis of PD, idiopathic PD cases with unknown GBA status, age 18–90, informed consent.

The exclusion criteria for RAPSODI GD are as follows: GBA mutation negative individuals with a confirmed clinical diagnosis of PD, a history of parkinsonism, individuals with other neurological disorders, individuals on drugs known to be associated with parkinsonism.

### Remote annual & biennial assessments
Annual remote assessments within RAPSODI GD take place online, via the RAPSODI GD portal (www.rapsodistudy.com). The battery of assessments takes approximately 1 h to complete.

### Informed consent
Participant consent is gained upon enrolment within the online RAPSODI GD portal (www.rapsodistudy.com), or the PD FRONTLINE portal for participants with idiopathic PD (www.pd frontline.com).

### Self-report rating scales
The RAPSODI GD study uses three self-administered scales to assess: anxiety and depression, motor symptoms of PD, REM sleep disturbance. Anxiety and depression are examined through the Hospital Anxiety and Depression Scale (HADS) [54]. This 14-item scale determines separate scores for anxiety and depression, between 0 and 21, distinguishing between mild, moderate, severe and noncases. Motor symptoms of PD are assessed via the Movement Disorder Society-Unified Parkinson’s Disease Rating Scale (MDS-UPDRS) part II [55]. This 13-item section of the MDS-UPDRS, rates motor experiences in daily living such as eating and walking, on a scale of 0–4 from normal to severely dysfunctional. REM sleep disturbance is measured via the REM Sleep Behaviour Disorder Screening Questionnaire (RBDSQ) [56]. This 10-item scale assesses clinical features of REM sleep behaviour disorder, such as dream content and sleep movement.

### Bradykinesia akinesia incoordination test
Motor dysfunction is assessed via the Bradykinesia Akinsia Incoordination (BRAIN) Test [57]. On a standard physical computer/laptop keyboard, participants must alternate between tapping the ‘s’ key and ‘;’ key for 30 s per hand. Incoordination, akinesia, dysmetria and kinesia are assigned scores based on the variance in time travelling...
between the keys, the time spent on keys, the accuracy of pressing the correct keys and the total number of key presses, respectively.

**Cognitive testing**

Several aspects of cognitive function are assessed via a battery of precision cognitive tests. This was historically delivered via the online platform ‘CogTrack™’ [58], until the platform expired, and cognitive testing moved to a new platform ‘NeurOn’ in November 2020 (www.neuropsychology.online). The battery includes tasks of image encoding, simple reaction time, digit span backwards, spatial working memory and image recall. Within this battery participants are scored on dysfunction in working and episodic memory, disturbances in attention focus and sustainment and memory retrieval rate.

**Hyposmia assessment**

Olfactory function is assessed on a biennial basis via the University of Pennsylvania Smell Identification Test (UP-SIT) [59]. The UPSIT is delivered remotely through the post, and scores are uploaded directly onto the RAPSODI GD portal by participants. The UPSIT utilizes a series of booklets, each containing ten microencapsulated odorant panels. Participants apply mechanical pressure to the panels to release the odorants, and then select which of four options they believe each odorant to represent. The participants are assigned a score of olfaction sensitivity from 0 to 40 depending on the accuracy of their responses.

**Genotyping**

In their baseline year with RAPSODI GD, participants are posted a saliva sample collection kit (DNA Genotek Oragene.DNA OG-500), with biological substance category B compliant return packaging. Genomic DNA is extracted via the protocol provided in the DNA purification handbook published by DNA Genotek, for use with the Oragene.DNA OG-500 kits [60]. The full 8.9 kb amplicon of the \( GBA \) gene is sequenced for all known deleterious variants, on the Oxford Nanopore MinION, via a protocol validated by Leija-Salazar et al. [61]. Due to RAPSODI GD’s active selection of individuals with \( GBA \) mutations, there is a likelihood that individuals from an Ashkenazi Jewish population will be overrepresented within the cohort [23]. As such, genomic DNA samples are also MinION sequenced for the most common \( LRRK2 \) mutation, G2019S, as this is a common, salient and potentially confounding PD associated risk factor for Ashkenazi Jewish individuals [62].

Upon detection of mutation positive cases, DNA samples will be sent for external confirmatory Sanger sequencing in an NHS accredited lab.

**Follow-up**

The self-reporting scales, Bradykinesia akinesia incoordination test, and cognitive testing are repeated once a year from baseline. The UPSIT is repeated once every other year from baseline.

**Conclusion**

The RAPSODI GD study described here aims to define prodromal features of PD in a large cohort of \( GBA \) mutation carriers, to accurately predict clinical diagnosis. The study also looks to stratify the risk of phenoconversion to PD in homozygote and heterozygote \( GBA \) mutation carriers, and to clarify the variable penetrance of the \( GBA \)-associated PD phenotype, while developing a platform that will allow for the assessment of biomarkers of disease. The postulated outcome of the RAPSODI GD study is to allow for the identification and earlier diagnosis of individuals at risk of developing PD, so that neuroprotective interventions can be administered within an efficacious therapeutic timeframe.

**Future perspective**

The development and validation of clearly defined features of prodromal PD for \( GBA \) mutation carriers, could eventually allow for the accurate detection of at-risk individuals. Clinical detection of disease in this latent phase, would lend itself to the identification of earlier therapeutic timeframes to deliver neuroprotective interventions before irreversible neurodegeneration can take place. As the mechanisms of pathogenesis in the GCase-mediated pathway of PD are elucidated, biomarkers and therapeutic targets characterized for \( GBA \) PD may also prove salient in developing preventative measures within sPD as well.
Author contributions
A Higgins drafted the manuscript. M Toffoli, C-Y Lee, S Koletsi, S Mullin, M Avenali, F Blandini and AHV Schapira reviewed and edited the draft.

Financial & competing interests disclosure
This study was funded by the MRC (MR/M006646/1), Cure Parkinson’s Trust, Kattan Trust, an investigator-initiated research grant (IIR-GBR-001110) provided by Shire International GmbH, a member of the Takeda group of companies, and by the joint efforts of The Michael J Fox Foundation for Parkinson’s Research (MIF) and the Aligning Science Across Parkinson’s (ASAP). MJF administers the grant (ASAP-000420) on behalf of ASAP and itself. For the purpose of open access, the author has applied for a CC-BY public copyright license to the Author Accepted Manuscript (AAM) version arising from this submission. AHV Schapira is supported by the UCLH NIHR BRC. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research
RAPSODI has been approved by London – Queen Square Research Ethics Committee (REC reference: 15/LO/1155). Participant consent is gained upon enrolment within the online RAPSODI GD portal (www.rapsodistudy.com), or the PD FRONTLINE portal for participants with idiopathic Parkinson’s disease (www.pdfrontline.com).

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References
Papers of special note have been highlighted as: ● of interest; ●● of considerable interest
● Highlights the upward trend in Parkinson’s disease (PD) global burden.
● Postulates that nonmotor features of PD can emerge years before clinically defined motor deficits.
●● Discusses validated features of the clinical prodom of PD.
Identified heterozygous GBA as the most common risk factor for PD.

Identified enhanced prodromal features of GBA-associated PD.

- Linked the GCase deficiency to both GBA associated PD and sporadic PD.


