ABSTRACT
The field of prediagnostic Parkinson’s disease (PD) is fast moving with an expanding range of clinical and laboratory biomarkers, and multiple strategies seeking to discover those in the earliest stages or those ‘at risk’. It is widely believed that the highest likelihood of securing neuroprotective benefit from drugs will be in these subjects, preceding current point of diagnosis of PD. In this review, we outline current knowledge of the prediagnostic phase of PD, including an up-to-date review of risk factors (genetic and environmental), their relative influence, and clinical features that occur prior to diagnosis. We discuss imaging markers across a range of modalities, and the emerging literature on fluid and peripheral tissue biomarkers. We then explore current initiatives to identify individuals at risk or in the earliest stages that might be candidates for future clinical trials, what we are learning from these initiatives, and how these studies will bring the field closer to realistically commencing primary or secondary preventive trials for PD. Further progress in this field hinges on greater clinical and biological description, and understanding of the prediagnostic, peridagnostic and immediate postdiagnostic stages of PD. Identifying subjects 3–5 years before they are currently diagnosed may be an ideal group for neuroprotective trials. At the very least, these initiatives will help clarify the stage before and around diagnosis, enabling the field to push into unchartered territory at the earliest stages of disease.

INTRODUCTION
The motor features of Parkinson’s disease (PD) (tremor, rigidity, slowness and balance problems) are identified relatively late in the pathological process when approximately 50% of dopaminergic neurons have been lost in the substantia nigra. Symptomatic treatment is efficacious, but there are currently no drugs that demonstrably slow the disease course. It is believed, albeit not proven, that pathology may be too far advanced at the point of clinical diagnosis to be affected by potentially neuroprotective treatments (assuming that these are available). Identifying individuals at the earliest stages of disease would pave the way for clinical trials of emerging and repurposed drugs to prevent/ delay progression to clinically manifest PD (see figure 1). However, modifying risk in those that do not yet have a diagnosis represents a challenge. The terms ‘early disease’ or ‘at-risk’ are frequently used synonymously due to uncertainty about the point at which the pathological process starts, but clarification will be important since it will determine whether prevention is attempted on a primary or secondary basis, and factors that initiate pathology may not necessarily be the same as those that subsequently drive progression. Inability to identify disease activity before diagnosis precludes distinction of the two, but this limitation may be overcome, given current momentum in the field of biomarkers.

In this review, we describe current knowledge and emerging findings in the prediagnostic phase of PD, including early features, genetic and environmental risk and protective factors, discuss current strategies to identify individuals at earliest disease stages to include in future clinical trials, and highlight how the knowledge gleaned from these studies might bridge the gap into preventive or protective drug trials for PD.

IDENTIFICATION OF INDIVIDUALS AT THE EARLIEST STAGES
Genetic factors
Having a family history of PD increases the odds of PD by 3–4.5-fold, and up to 10% of patients report a family history of PD. Studies into the genetic basis of PD implicate lysosomal and mitochondrial dysfunction, and inflammation in pathogenesis. 3 Of the confirmed monogenic forms of PD, most result in abnormalities of one or more of these processes, but most are exceedingly rare and do not account for elevated risk at a population level (see figure 2). A central player in the disease is α-synuclein and mutations in the SNCA gene, which encodes this protein, are a cause of familial PD. Intraneuronal accumulation of α-synuclein is the pathological hallmark of PD, and mounting evidence suggests that fibrillar and oligomeric forms of the protein may be neurotoxic. 4, 5 The full picture of how these complex processes combine to result in neurodegeneration remains incomplete, but current theories include the possibility of prion-like cell-to-cell propagation. 6

Mutations in the LRRK2 gene are the commonest known genetic cause for PD, and the G2019S mutation occurs in 4% of hereditary and 1% of sporadic PD. 7 LRRK2-related disease has age-dependent penetrance (28% at 59 years, 51% at 69 years and 74% at 79 years), meaning that only a proportion of carriers will develop PD during life. 7 LRRK2 mutation carriers have been shown to have subclinical dopaminergic abnormalities, measured with functional imaging, and higher rates of non-motor features of PD than non-carriers. 7, 9 In patients with PD and LRRK2 mutations, the motor picture may be similar to idiopathic PD, but wider manifestations may not be. 7, 9

Heterozygous mutations in the glucocerebrosidase (GBA) gene are associated with an increased risk of PD. Large studies have shown that GBA mutations are common in Ashkenazi Jews,
occurring in 15% of patients and 3% of controls.\textsuperscript{10} In unse-
selected patients with PD, 3.5% carry disease-associated GBA
mutations compared with <1% of controls.\textsuperscript{10, 11} Clinical fea-
tures of patients with PD and GBA mutations may be similar to
sporadic PD and generally respond to levodopa, but onset of
parkinsonism can be earlier, and carriers tend to have higher
rates of cognitive problems and rapidly developing sleep
behaviour disorder (RBD).\textsuperscript{11} Furthermore, early non-motor fea-
tures of PD such as depression, subjective RBD and olfactory
dysfunction have been observed in carriers of GBA mutations
without PD, when compared with healthy controls.\textsuperscript{1, 12}

The study of manifesting and non-manifesting carriers of
LRRK2 and GBA mutations is important for understanding the
prodomal phase of PD, and for studies of drugs targeting spe-
cific pathways. Cohorts of these subjects have been assembled to
fulfil these aims, but with greater study, further differences may
emerge between PD related to these mutations and sporadic

disease. Other examples of monogenic PD include mutations,
duplications and triplications of the SNCA gene causing domi-
nantly inherited PD, and mutations in PARK2, PINK1 and DJ1
causing recessively inherited forms of PD (for review see ref. \textsuperscript{3),}
with more emerging gradually. These monogenic causes of PD
are too rare to base predictive studies on, but they give impor-
tant insight into disease mechanisms and therapeutic targets.

Mutations in single genes do not account for all the heritable
risk apparent in complex diseases, and genome-wide association
studies, in which large numbers of unrelated cases are compared
with unrelated controls, have yielded informative results. There
are at least 28 independent risk variants associated with PD that
increase or decrease risk in a small but potentially additive
way.\textsuperscript{13} This enables the construction polygenic risk profiles,
pooling the combined effect of multiple variants to estimate risk
of PD or indeed age of disease onset.\textsuperscript{14, 15} However, the heri-
table component of PD is estimated to be greater still, at around
30\%, and identified risk loci and monogenic forms explain only
about 5–10\%.\textsuperscript{16} Over time, with increasing numbers of studied
cases and controls, along with deep resequencing and precision
phenotyping, a greater proportion of the heritability of PD will
be uncovered. The influence that genetic variation has on PD is
not limited to risk of getting disease alone, and specific variants
are likely to contribute additionally to age of disease onset,
progression and phenotype, with a number of indicative studies
reported thus far.\textsuperscript{11, 16, 17} Furthermore, these and additional genetic
factors may dictate therapeutic choices in the future in the clin-
ical setting and in terms of recruitment to clinical trials. The
genetic architecture of PD is continually expanding and increas-
ingly complex. It has implications for multiple aspects of the
disease, but other factors are also important in determining risk.

Environmental risk factors
There is a large body of evidence demonstrating a small but sig-
nificantly elevated risk of PD associated with a number of envi-
ronmental risk factors (see \textit{figure 2}). Some of the strongest
environmental evidence exists for exposure to pesticide and
proxies for this, including farming occupation, rural living and
drinking well water.\textsuperscript{1} Specific implicated pesticides include rote-
none and parquat (structurally related to 1-methyl-4-phenyl-
1,2,3,6-tetrahydropyridine (MPTP), which has also been linked
to parkinsonism in users of illicit drugs\textsuperscript{18}), and both these che-

cicals are used to create animal models of PD. Other potential
toxins include heavy metals such as manganese, with exposure
arising through occupations such as welding and in recreational
ephedrine users.\textsuperscript{19} It seems unlikely that environmental toxins
play more than a minor role in PD risk overall, with meta-analysis suggesting ORs in the region of 1.2–2.0.\textsuperscript{1}

Pooled observational study data also implicates head injury as
a minor but significant risk factor for PD.\textsuperscript{1, 20–23} There is increasing
evidence that individuals who suffer recurrent head injury, par-

cularly sportspersons such as boxers, jockeys, American foot-
ball and rugby players, are at risk of developing a range of
degenerative neurological conditions including parkinsonism,
dementia and motor neuron disease, although pathological
examination of these subjects tends to reveal alternative path-
ology than that typically associated with PD.\textsuperscript{21}

By stark contrast with other common chronic diseases, there
exist a number of intriguing but consistent negative associations
with PD and lifestyle factors such as smoking, caffeine and
alcohol.\textsuperscript{1} Additionally, there are a number of medications for
which negative associations with PD have been reported in
observational studies, including calcium channel blockers,
non-steroidal anti-inflammatories and statins.\textsuperscript{1} Some of these are

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{A schematic depicting normal (black solid line) and
Parkinson’s disease-related (grey solid line) nigral cell loss over time,
including the point at diagnosis typically occurs (horizontal black
hashed line) and the potential for modifying the trajectory of
degeneration, if identified earlier (hashed grey line). NB. For colour
version grey lines appear red, black lines remain black.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Risk factors and early features of Parkinson’s disease
associated with increased (or decreased) risk of subsequent
disease. Estimated magnitude of effect is plotted against estimated frequency.
SN+ is hyerechohgenicity in the region of the substantia nigra using
transcranial sonography. Genetic and environmental risk factors are
shown in lighter grey, and pre-diagnostic features and heprechohgenicity
in darker grey. NB. For colour version genetic factors are red,
environmental factors are orange, pre-diagnostic features are green and
imaging features are blue.}
\end{figure}
currently the focus of clinical trials examining their potential use in PD.

Whether these exposures offer true neuroprotective properties or whether negative association, at least with lifestyle factors, arises due to a common feature (eg, avoidance as part of an early PD personality change) is yet to be determined. The former possibility is supported by clinical studies reporting improvement of motor function in a clinical trial of caffeine to treat excessive daytime somnolence in PD and PD animal models that show protective effects of nicotine on nigrostriatal damage.22 23 Spurious association may arise as a result of reverse causality, a potential problem with observational studies. This may be plausible even in prospective studies that exclude cases of incident PD in the first few years of follow-up because the prodromes of PD are likely to be very long, during which time pathology is present, but the classical features are not yet overt.

Another consistent negative association exists between levels of serum urate and PD, with a number of studies demonstrating a ‘protective’ effect of elevated serum urate.1 The most recent of these studies is an example of Mendelian randomisation (MR), which is a powerful technique to assess causal relationships between risk factors and disease. In MR studies, a gene variant is used as a proxy for an environmental exposure (or intermediate phenotype), to determine the effect of this on a disease outcome. Simon and colleagues used single nucleotide polymorphisms in the SLC2A9 gene (which explain a proportion of the genetically specified variability in serum urate) to test whether these polymorphisms predicted rate of progression in early PD. They demonstrated that SLC2A9 status was associated with elevated serum urate and was associated with slower disease progression.24 The MR approach is protected against biases that can arise in traditional observational studies assessing causation; if, for example, reduction in serum urate occurred as an early consequence of PD, a spurious negative association between the exposure and outcome might arise. MR on the other hand operates more like a randomised controlled trial in which the exposure (in this case a gene variant) is randomly allocated at baseline (conception) along with known and unknown confounding factors. Given the consistency of this relationship in the MR study with previous observational studies, therapeutic modulation of urate levels is a strong target for clinical trials.

Increasing research activity in exploring the overlap between genes and the environment will further our understanding of the causal basis of disease. As for many diseases, the total picture of risk remains incomplete due to apparent and substantial randomness of the onset, the obscuration of risk factors either because of rarity, ubiquity or poor measurement, or the fact that disease tends to strike those at moderate risk, simply because those at ‘highest risk’ are far fewer.

Early clinical features
Recognition of the importance of non-motor features of PD has been building for several years.25 26 In established PD, patients regard non-motor symptoms at least as troublesome as motor features, and treatment is often difficult.27 Non-motor symptoms are experienced early and there is substantial evidence which suggests that they also predate diagnosis by several years (see figure 2). A number of studies have demonstrated the association of PD with earlier diagnoses such as depression, anxiety, constipation and erectile dysfunction.28 29 The best characterised early non-motor features are idiopathic anosmia and RBD. Anosmia is relatively common in the ageing population and is non-specific, with only a proportion going on to develop neurodegenerative disease.30 RBD on the other hand, is relatively specific for neurodegeneration, with high conversion rates, but clinical and pathological heterogeneity in that it can precede PD, dementia with Lewy bodies and multiple system atrophy.31 An important distinction lies between subjective RBD (on clinical history or questionnaire-based diagnosis) and that which is formally diagnosed with an overnight sleep study and polysomnography (PSG). PSG-confirmed RBD is rare in the general population, and the largest observational study amassed just over 300 subjects despite international collaborative efforts.32 Nonetheless study of PSG-confirmed RBD has resulted in positive strides forward given that time during which a proportion will convert to neurodegenerative disease is known (25% at 3 years and 40% at 5 years).33 The emergence of anosmia or subtle motor signs, in particular, in those with RBD appears to further refine estimates of those that are likely to convert.34

Characterisation of non-motor features is potentially valuable for early identification of PD and the Movement Disorders Society (MDS) has recently released Research Criteria for Prodromal PD.35 The literature initially described the early phase of PD as being the ‘pre-motor’ phase, but more recently this has fallen out of favour with the recognition that subtle motor features can be present before diagnosis.28 36 The clinical diagnosis of PD requires multiple motor features to be established, and while subtle motor signs may be present, a clinical diagnosis of PD cannot be made until they become more definite.4 Given that subtle or single motor abnormalities occur prior to diagnosis and alongside early non-motor features, this period is better referred to as the prediagnostic phase. The MDS recommends the following terminology:1

1. Preclinical PD—presence of neurodegenerative synucleinopathy without clinical symptoms (this stage will be defined by disease biomarkers when available).
2. Prodromal PD—presence of early symptoms and signs before PD diagnosis is possible.
3. Clinical PD—diagnosis of PD has been made based on the presence of classical motor signs.

The emergence of large longitudinal primary care datasets has allowed detailed exploration of the full range of early motor and non-motor symptoms that predate PD, while being free from the biases that many traditional observational studies have suffered from.38 Alongside, advances in wearable technology, and the availability of remote testing, will aid objective measurement of emerging motor dysfunction in those at risk of PD.39 A variety of measuring devices have been developed, including software applications which harness information on activity and motion (and in some cases speech) captured by smart phones and tablet-devices, custom-built sensors that measure gait, bradykinesia, dyskinesia and nocturnal movements, keyboard-based speed and accuracy tests, as well as custom-built home-testing devices (for examples of these, see online supplementary material). Many of these devices demonstrate high sensitivity and specificity in differentiating patients with PD from controls, and are likely to see increasing use in clinical practice to guide decision-making. Despite the indication that objective motor dysfunction occurs prior to diagnosis in PD, currently there are few examples of the application of remote or wearable devices in prediagnostic PD. A major foreseeable hurdle is ensuring that validation and regulatory approval for software and devices will keep abreast of rapid and continuous change in available technology.38
Imaging markers
Radiotracer imaging with single photon emission computed tomography (SPECT) or positron emission tomography (PET), and transcranial sonography (TCS) have repeatedly demonstrated the ability to differentiate patients with PD from healthy individuals with adequate sensitivity and specificity (see Table 1). These modalities may also have the potential to identify subclinical PD prior to diagnosis, and early support for this notion came from studies showing that SPECT and PET were typically abnormal bilaterally in patients with unilateral PD, and could identify unaffected twins of patients with PD who later went on to develop PD. Using SPECT, the clinical diagnosis of PD tends to be made once there has been 40–50% reduction in tracer binding and an average 11.2% decline in striatal binding has been observed each year after diagnosis. Multitracer PET has also shown deterioration over time in patients with established PD, but is expensive when considering use on a large scale.

Evidence of demonstrable longitudinal change using DaT (dopamine transporter) SPECT in early PD is now being sought with serial studies performed as part of the Parkinson’s Progression Markers Initiative (PPMI) study. Furthermore, progressive change in SPECT imaging has been reported in a study of patients with RBD, and in the Parkinson’s At Risk Study (PARS), which includes subjects with idiopathic anosmia (and other prodromal markers).

By contrast, TCS demonstrating hyperechogenicity of the substantia nigra (SN) appears to be a static rather than changing marker. A study in individuals with mild parkinsonian features, found a sensitivity of 91%, specificity of 82% and positive predictive value (PPV) of 93% for SN hyperechogenicity and PD diagnosis after follow-up, despite blinding those performing sonoography to clinical details at baseline. Current efforts to improve standardisation and quantitative analysis for TCS and SPECT may increase their utility in the prediagnostic phase of disease, with SPECT more likely to demonstrate sensitivity to change.

High-field and novel sequences of MRI also provide opportunities to address the challenge of imaging disease progression in the prediagnostic phase of PD. Correlations of MRI microstructural imaging abnormalities have been reported with postmortem findings and quantitative differences between patients with PD and healthy subjects in terms of iron deposition, loss of neuromelanin and alterations in nigrosome 1, in mainly single studies (for examples see online supplementary material). In addition to subtle structural abnormalities, application of functional MRI methods have shown differences in functional connectivity with the basal ganglia network, the default-mode network and the sensorimotor resting state network, between patients with PD and healthy controls, some of which are influenced by dopaminergic medication (see online supplementary material for examples of studies).

Table 1 Imaging modalities and markers for Parkinson’s disease

<table>
<thead>
<tr>
<th>Modality</th>
<th>Example tracers/sequence</th>
<th>Observation(s)</th>
<th>Analysis</th>
<th>Accessibility</th>
<th>Cost</th>
<th>Suitability for screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPECT</td>
<td>123I-β-CIT, 123I-FP-CIT</td>
<td>Loss of binding in striatum</td>
<td>Visual inspection, quantification</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>TCS</td>
<td>2–3.5 Hz transducer</td>
<td>Hyperechogenicity in the region of the substantia nigra</td>
<td>Visual inspection, quantification</td>
<td>++++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>PET</td>
<td>18F-dopa, 18F-FDG</td>
<td>Loss of binding in striatum</td>
<td>Visual inspection, quantification</td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>MRI</td>
<td>Traditional (T1 &amp; T2), T2/T2* (gradient echo), DTI, spin echo, fMRI</td>
<td>Numerous reported, none established</td>
<td>Visual inspection, quantification</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>MIBG</td>
<td>123I-metaiodobenzylguanidine</td>
<td>Low heart-to-mediastinum ratio</td>
<td>Visual inspection, quantification</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

Accessibility, cost and suitability for screening are estimated semiquantitatively on a four-point relative scale with + lowest and ++++ highest. DTI, diffusion tensor imaging; FDG, fludeoxyglucose; fMRI, functional MRI; Hz, hertz; MIBG, metaiodobenzylguanidine; PET, positron emission tomography; SPECT, single photon emission computed tomography; TCS, transcranial sonography.
Current studies mapping the prediagnostic and per diag nostic phases of PD

Several studies (table 3) have been initiated: to identify those in the prediagnostic and prodromal phases of PD; identify clinical and biological markers to track progression of disease before diagnosis; create platforms to recruit individuals with a single strong risk factor such as carrier genotypes at common risk loci; establish PD-related pathology in those with idiopathic RBD; and determine the role of LRRK2 or GBA mutations, or idiopathic RBD or Parkinson disease with dementia (PDD), in disease ontogenesis at baseline. The aforementioned PARS study uses objective smell testing to identify subjects with idiopathic anosmia at stage 1, followed by DaT SPECT at stage 2 to identify subclinical presynaptic denervation. The study has reported early results which demonstrated reductions in nigrostriatal DaT binding in subjects with hyposmia compared to those with normal smell, as well as associations between a number of prodromal features of PD within the study cohort. The Tübingen evaluation of Risk factors for the Early detec tion of Neuropiregulation (TREND) study examines subjects who are over 50 years of age, with a limited combination of risk factors (anosmia or depression or RBD), using serial studies of movement, laboratory tests and imaging, with follow-up to incident PD. Baseline data from this cohort have been reported showing associations between selected prodromal markers and other early associated features of PD.

Two large multicentre studies, one coordinated from the USA (the Parkinson’s Progression Markers Initiative, PPMI) and one based in the UK (the Tracking Parkinson’s or PReBaND study), recruit patients immediately after the clinical diagnosis of PD and undertake detailed clinical, imaging and biomarker studies longitudinally. While not strictly looking at prediagnostic PD, the PPMI and PReBaND studies will help define the role of clinical markers (motor and non-motor) in the early measurement of PD, and the identification of novel imaging and laboratory biomarkers, as well as giving insight into what might be

<table>
<thead>
<tr>
<th>Source</th>
<th>Medium</th>
<th>Main techniques</th>
<th>Candidate markers</th>
<th>Invasiveness</th>
<th>Cost</th>
<th>Suitability for screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebrospinal fluid</td>
<td>Fluid</td>
<td>Assay, electrophoresis, mass-spect</td>
<td>t-α-syn, p-α-syn, t-tau, p-tau, NFL</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Blood</td>
<td>Fluid</td>
<td>Assay, electrophoresis, mass-spect</td>
<td>Uric acid, inflammatory markers, (GF-1, ApoA1, DJ-1)</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Saliva</td>
<td>Fluid</td>
<td>Assay, electrophoresis, mass-spect</td>
<td>α-syn, DJ-1</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Gut</td>
<td>Tissue biopsy</td>
<td>Immunohistochemistry</td>
<td>t-α-syn, p-α-syn</td>
<td>++++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Salivary gland</td>
<td>Tissue biopsy</td>
<td>Immunohistochemistry</td>
<td>t-α-syn, p-α-syn</td>
<td>++++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Skin</td>
<td>Tissue biopsy</td>
<td>Immunohistochemistry</td>
<td>t-α-syn, p-α-syn</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

Invasiveness, cost and suitability for screening are estimated semiquantitatively on a four-point relative scale with + lowest and ++++ highest.

ApoA1, apolipoprotein A1; GF-1, insulin like growth factor 1; NFL, neurofilament light chain; p-α-syn, phosphorylated α-synuclein; t-α-syn, total α-synuclein.

Movement disorders


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apparent through back-extrapolation to the prediagnostic phase. The PPMI study also includes a prodromal arm (P-PPMI) in which subjects with RBD, anosmia or a mutation (LRRK2, GBA or SNCA), will be assessed and followed in the same way as PD subjects, allowing for a seamless examination of the prediagnostic and early disease stages of PD. Separately, as part of a large study aimed at understanding the biological basis of disease in patients with established PD, the Oxford Parkinson's Disease Centre (OPDC) includes smaller 'high-risk groups' with a family history or RBD. Clinical assessments, laboratory and imaging biomarker studies are being undertaken and early results are emerging.

In the UK, the PREDICT-PD study combines risk factors and early non-motor features to devise a risk scoring process for future PD. Risk scores were calculated based on a meta-analysis of the published literature. These, in turn, were used to generate ORs for the effect on risk of PD ascribed by individual early non-motor features and risk factors. Using a priori odds of PD attributed to age, a Bayesian model of risk was constructed to yield combined risk estimates for each subject in the study. The study runs almost entirely via the internet with more detailed laboratory, motor and imaging investigation for groups at the extremes of risk. PREDICT-PD is the first study to try and combine large numbers of risk factors for PD and has the potential to screen a large, community-based population, and aims to facilitate recruitment into clinical trials in the future. Unlike some of the other studies, it seeks to identify individuals spanning the full spectrum of PD, which makes this cohort highly applicable to occurrence of typical PD in clinical settings.

**FURTHER CHALLENGES AND OPPORTUNITIES IN THE PREDIAGNOSTIC PHASE**

The above studies aim to overcome the important challenge of identification of ‘at-risk’ individuals who may develop the disease activity across phases.

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**Table 3** Studies in the prediagnostic phase of Parkinson’s disease

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Study name</th>
<th>Participants</th>
<th>Country</th>
<th>Number recruited</th>
<th>Tests/exposures</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRIPS57</td>
<td>Prospective validation of risk factors for the development of Parkinson syndromes</td>
<td>Subjects over 50 years old</td>
<td>Germany, Austria</td>
<td>1847</td>
<td>TCS, smell, UPDRS</td>
<td>Clinical diagnosis of PD</td>
</tr>
<tr>
<td>TRENDS58</td>
<td>Tübinger evaluation of risk factors for the early detection of neurodegeneration</td>
<td>Subjects over 50 years with anosmia, self-report RBD or depression</td>
<td>Germany</td>
<td>&gt;1200</td>
<td>TCS, smell, UPDRS, quantitative motor, psychometry, blood biomarkers</td>
<td>Clinical diagnosis of PD</td>
</tr>
<tr>
<td>PARS68</td>
<td>Parkinson’s at-risk syndrome study</td>
<td>Subjects over 50 years with hyposmia and DaT deficit on SPECT</td>
<td>USA</td>
<td>4999 (completed baseline smell test)</td>
<td>Smell, DaT SPECT, UPDRS, cognition, blood biomarkers</td>
<td>Clinical diagnosis of PD</td>
</tr>
<tr>
<td>P-PPMI41</td>
<td>Prodromal Parkinson’s Progression Markers Initiative</td>
<td>Subjects with prodromal features or gene mutations</td>
<td>International</td>
<td>Anosmia/RBD=65 Genetic=150</td>
<td>CSF and blood biomarkers, UPDRS, cognition, sleep and autonomic assessments</td>
<td>Clinical diagnosis of PD</td>
</tr>
<tr>
<td>OPDC59</td>
<td>Oxford Parkinson’s Disease Centre study</td>
<td>Subjects with a first-degree relative with PD, or subjects with RBD (PSG confirmed)</td>
<td>UK</td>
<td>~190</td>
<td>UPDRS, non-motor assessments, blood and CSF biomarkers</td>
<td>Clinical diagnosis of PD</td>
</tr>
<tr>
<td>PREDICT-PD</td>
<td>PREDICT-PD</td>
<td>Subjects over 60 years old</td>
<td>UK</td>
<td>1323</td>
<td>In all (online): risk scoring, smell, RBDSQ, quantitative motor (BRAIN test), genetics in extremes of risk: UPDRS, cognitive, TCS</td>
<td>Clinical diagnosis of PD/DaT deficit on SPECT</td>
</tr>
</tbody>
</table>

**Notes**

- BRAIN test, BRadykinesia Akinesia INcoordination test; B-SIT, Brief Smell Identification Test; CSF, Cerebrospinal Fluid; DaT, Dopamine Transporter; PD, Parkinson’s disease; PSG, polysomnography; RBD, REM sleep Behaviour Disorder; RBDSQ, RBD Screening Questionnaire; SPECT, single photon emission computed tomography; TCS, Transcranial Sonography; UPDRS, Unified Parkinson’s Disease Rating Scale.
classical clinical syndrome of PD, with the eventual aim of initiating treatment to avoid or delay clinically relevant symptoms. In addition, studies such as PPMI (with P-PPMI), TREND, PARS and PREDICT-PD will document the time immediately before, during and after the emergence of clinically recognisable PD, delineating the clinical and biomarker features of this phase that will be crucial to commencing clinical trials. These studies will help refine the determination of risk status and course of early disease progression (see figure 3).

There are additional hurdles that must be overcome before clinical trials recruiting subjects in this phase of disease can be planned: (1) determination of appropriate study endpoints and duration of trials. Prevention or delaying emergence of classical symptom onset is the ultimate aim, but PD is insidious, with its clinical manifestations emerging over months and years, making many clinical end points unsuitable and such studies difficult to fund for the duration required. A sensitive clinical marker of progression would be valuable in detecting subtle changes at this early phase, however, an imaging or laboratory marker that spanned the prediagnostic and early postdiagnostic phases may offer better sensitivity, specificity, reliability and precision overall (see figure 3). This, in turn, could allow appropriate calculation of sample sizes and trial duration, dependent on anticipated drug effect; (2) another important consideration in trial design is the heterogeneity in clinical manifestation of PD, rate of progression and the presence/absence of other features. Clinical trials designed to show the disease-modifying effect of an agent may initially need to include homogenous samples or samples stratified for presentation and rate of progression in order to show an effect before trials in wider groups can be conducted; (3) other factors are continuity and applicability through the early stages of the disease. Even with an optimised early detection process, there will still be individuals who are ‘undetected’, and first present with overt signs of PD, and potential treatments will need to be assessed for demonstrable effects in these subjects too. Longer-term observational studies that examine risk status could support registries through which subjects indicate their willingness to participate in future clinical trials and biomarker initiatives. Consenting eligible subjects could be offered inclusion into clinical trials with the benefit of extensive available pre-trial data, but issues of selection bias and generalisability of results must be considered; (4) Finally, there are ethical implications of treating at-risk populations. For a repurposed drug, with previous data on safety and tolerance, the implications of undertaking clinical trials in those at-risk are perhaps less than for novel drugs with unknown safety profiles and potential toxicity. Justification for more invasive therapies could probably not be found without clear results in established PD. In addition, disclosure of risk status is likely to be a prerequisite for participation in clinical trials, but has the potential to bias recruitment and poses an ethical barrier in the absence of proven neuroprotective effects. Ultimately, disclosure may be unavoidable in order to make an informed decision about trial participation.

CONCLUSION
Significant progress has been made in the understanding and identification of subjects in the prediagnostic phase of PD and a number of initiatives are underway to further define these groups. These studies may contain subjects that would be candidates for recruitment into clinical trials targeting neuroprotection within a few years. Parallel exploration of peripheral tissue, fluid and multimodal imaging is needed to identify differences between patients and controls across a range of markers. Of major interest is whether these differences can be demonstrated in high-risk/prediagnostic subjects, and whether they change up to the point of diagnosis and immediately beyond. This will enable testing of drug therapies at a time when more neuronal tissue can potentially be preserved, and there is an absence of symptomatic effects of medication with the potential to confound.

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Supplementary file 1 – Additional individual published studies relevant to pre-diagnostic Parkinson’s disease

Quantitative motor testing


Magnetic resonance imaging markers in Parkinson’s disease


Wet biomarker studies in Parkinson's disease


