

Systematic Review

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Rab proteins as diagnostic biomarkers for Parkinson's disease: a systematic review

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ABSTRACT

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**Purpose:** Parkinson's disease (PD) is a neurodegenerative disorder characterized by a progressive loss of dopaminergic neurons. PD is typically diagnosed at late-stage, concurrent with loss of up to 70% of these neurons so patient care could be improved by diagnosis at prodromal stages. The occurrence of Parkinsonian-like diseases also understores the importance of accurate differential diagnosis, yet no biomarkers are currently used in clinical settings to minimize diagnostic errors. The Rab family of GTPases has been implicated in the PD pathogenesis, with protein levels elevated in pre-clinical models. This review evaluates the utility of Rab proteins as a biomarker for PD.

**Methods:** A systematic search was performed following PRISMA guidelines using PubMed, Scopus, Cochrane, SAGE, OVID and Web of Science (references up to November 2025). Search keywords included: "Parkinson", "Rab" and "biomarker".

**Results:** Out of 1404 records identified, 27 articles were sought for retrieval after duplicate removal and title/abstract screening. The search yielded 13 articles that fit the inclusion criteria. 8 different Rab proteins of interest and/or their phosphorylated forms were reported as differentially expressed in PD patients in these papers. Of the 8 Rab proteins, Rab35 had a consistent elevation. However, this was observable from a single research group, presenting the need for independent validation of Rab35 as a diagnostic biomarker for idiopathic PD (iPD). In contrast, though preliminary, pRab10 may have relevance for patient stratification and monitoring drug response in PD patients. However, with the small evidence base, further research with larger sample sizes, patient sub-types and other contributing factors will be vital before integration into clinical practice.

**Conclusion:** Overall, the evidence for Rab proteins as diagnostic biomarkers is emerging rather than established. Inconsistent reporting, small and frequently overlapping samples prevented quantitative synthesis.

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## 1.0 Introduction

PD affects about 2% of the global aging population. In the last 30 years, the prevalence has doubled, and this trend is projected to continue over the next 30 years. Furthermore, the burden of PD as measured by the years lived with disability (YLDs) has increased globally, impacting the health systems in many countries unable to cope with the increased cost.<sup>1</sup> The substantial growth in the burden of disease highlights the necessity of the development of health strategies in managing PD. Namely, timely and accurate diagnosis of PD can improve individual health-related quality of life (HRQL) and reduce clinical challenges such as long-term healthcare costs and medical resource utilization caused by misclassification.

Resting tremor, bradykinesia, stiffness, and postural instability make up the cardinal symptoms of PD critical in diagnosis. In addition, the disease presents several other motor and non-motor symptoms such as dystonia, sleep disturbances, gastrointestinal dysfunction and behavioral changes.<sup>2-4</sup> Post-mortem pathological features, which confirms the diagnosis PD, include the degeneration of dopaminergic neurons and axonal projections in the substantia nigra and the expansion of Lewy bodies in surviving neurons, whose primary component is alpha-synuclein ( $\alpha$ -Syn).<sup>5</sup>

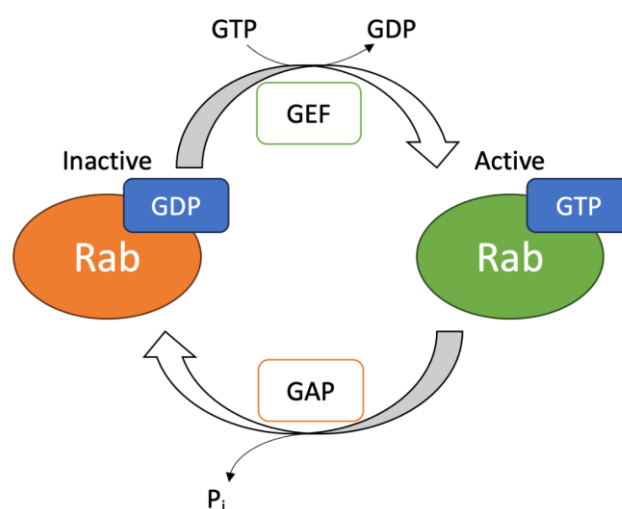
The long prodromal phase of PD during which only non-motor symptoms are evident, makes early diagnosis of PD challenging. This latent phase can last up to 20 years and when the motor symptoms of PD manifest, 50-70% of dopaminergic neurons have degenerated.<sup>6</sup> Accurate diagnosis of PD is an additional challenge due to the similarities of other neurodegenerative diseases, specifically atypical degenerative parkinsonism syndromes. Patients with atypical parkinsonism do not respond to PD drug treatment or dopamine based therapy.<sup>7</sup> The use of imaging techniques such as the Dopamine Transporter Scan (DaT) which identifies the loss of dopaminergic neurons can aid improving the accuracy of PD diagnosis, though it is not definitive and can be costly.<sup>8</sup> Therefore, there is a need for biomarkers to increase diagnostic accuracy.

Blood-based biomarkers have been widely reported in a variety of diseases as reliable indicators of disease diagnosis, progression prediction, and therapeutic development. However, no one biomarker is sufficiently specific for regular use in the diagnosis or prognosis of PD patients. Genetic biomarkers, on the other hand, are mutations in specific genes or susceptibility loci that have only proved beneficial in determining the familial nature of PD. Mutations in genes such as Leucine-rich-repeat kinase 2 (LRRK2), PTEN-induced kinase 1 (PINK1) and Vacuolar protein sorting 35 (VPS35) have been found to be causative in familial PD but these patients only make up ~5-10% of PD cases.<sup>9</sup> As  $\alpha$ -Syn is a primary component of lewy bodies in PD and can be detected in the blood, the use of  $\alpha$ -Syn as a biomarker for PD has been investigated.<sup>10</sup> A recent study has demonstrated that though  $\alpha$ -Syn is a potential biomarker candidate, its specificity as a single diagnostic or prognostic biomarker of PD in bodily fluids or peripheral tissues has yet to be shown.<sup>11</sup> Thus, the development of an effective clinical biomarker alternative to  $\alpha$ -Syn is critical for early identification and management of PD.

Pre-clinical studies have identified proteins within the Rab GTPase family that have a pertinent role in the pathogenesis of both familial and idiopathic PD as they are downstream targets of LRRK2 and PINK1, mutant forms of the genes associated with the development of PD.<sup>12-13</sup> Studies have implicated mutations VPS35 to influence the phosphorylation of Rab GTPases by influencing LRRK2 recruitment, indicating an interaction between genetic components of PD and highlighting the commonality of Rab GTPases in different forms of familial PD.<sup>14</sup>

Rab GTPases are small G-proteins, that are members of the RAS superfamily. They are known to regulate membrane trafficking events such as SNARE-mediated membrane fusion, vesicle budding, tethering, docking and transport.<sup>15</sup> Rab proteins cycle between a GDP-bound inactive and a GTP-bound active form during vesicular

transport and membrane trafficking, acting as molecular switches as seen in Figure 1.<sup>16</sup> Rab1 and Rab2 are regulators between the ER and the Golgi apparatus, and both have additional roles in autophagy.<sup>17-19</sup> Rab7a is involved in late endosome-lysosome trafficking for protein degradation and the maturation of autophagosomes, an essential process for autophagy.<sup>20</sup> Rab8 regulates the transport of vesicles from the Golgi apparatus to the plasma membrane.<sup>21</sup> Rab10 has a role in the endocytic pathway and is involved in the recycling of cell surface receptors from endosomes to plasma membrane.<sup>22</sup> Rab35 is similar to Rab10, due to its role in endocytic recycling but recycles different cargoes such as integrins.<sup>23</sup> Both Rab3 and Rab43 are involved in synaptic vesicle trafficking. Rab43 is involved in trafficking related to neuronal transmission, specifically post-synaptic signaling whereas Rab3 is linked to neurotransmitter release.<sup>24-27</sup>



**Figure 1** Rab proteins regulate vesicle trafficking events by GTP hydrolysis. The conversion from GDP-bound to GTP-bound catalyzed by Guanine nucleotide exchange factor (GEF) allows the activation of Rab proteins. The GTPase activity of Rabs coupled with the effect of GTPase-activating protein (GAP) leads to Rab inactivation.

Further association of Rab GTPases and PD was established when phosphoproteomic analysis in murine embryonic fibroblasts and in immune-stimulated peripheral blood mononuclear cells (PBMC) identified a subset of Rab GTPases, particularly Rab10, as endogenous LRRK2 kinase substrates. These subsets of Rabs are phosphorylated by LRRK2, and in disease-causing mutations of LRRK2, the hyperphosphorylation of these downstream Rabs are induced.<sup>15</sup> These downstream Rab GTPases, play a role in LRRK2-related PD pathology in a kinase-dependent manner. Rab GTPases contributed to pathological outcomes through a-Syn neurotoxicity, the downregulation of endosomal formation and Clathrin-mediated endocytosis (CME), as well as lysosomal stress and impaired autolysosome formation.<sup>28</sup> As Rab GTPase activity is upstream these pathological mechanisms of PD, it may be promising as a clinical biomarker.

The use of Rab GTPases as clinical biomarkers has been proposed in the context of breast, endometrial, and colorectal cancer whereby the expression levels are altered.<sup>29-31</sup> Within neurological diseases, PD, Alzheimer's disease (AD) and Huntington's disease (HD) have known to be associated with Rab GTPases, but these diseases present different symptoms. Rabs might be considered potential indicators or therapeutic targets before the clinical presentation of PD. This systematic review aims to determine which Rab proteins can act as a potential predictive or response biomarker for PD based on present clinical and pre-clinical studies.

## 2.0 Research Methodology

This review was conducted following the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) statement.

### 2.1 Search Strategy

Publications preceding and including **November 2025** (first retrieved in September 2023, updated in November 2025) were extracted from electronic databases, PubMed, Scopus, Cochrane, SAGE, OVID and Web of Science databases, and reviewed. The search was based on using different combinations of the following keywords: “Parkinson’s Disease”, “Biomarker”, “Rab” and associated synonyms as shown in Table 1. These terms were searched for in the titles, abstracts, and full texts where applicable. Only articles published in English were included. Table S1 presents our search strategy for PubMed.

**Table 1** Terms used for literature search

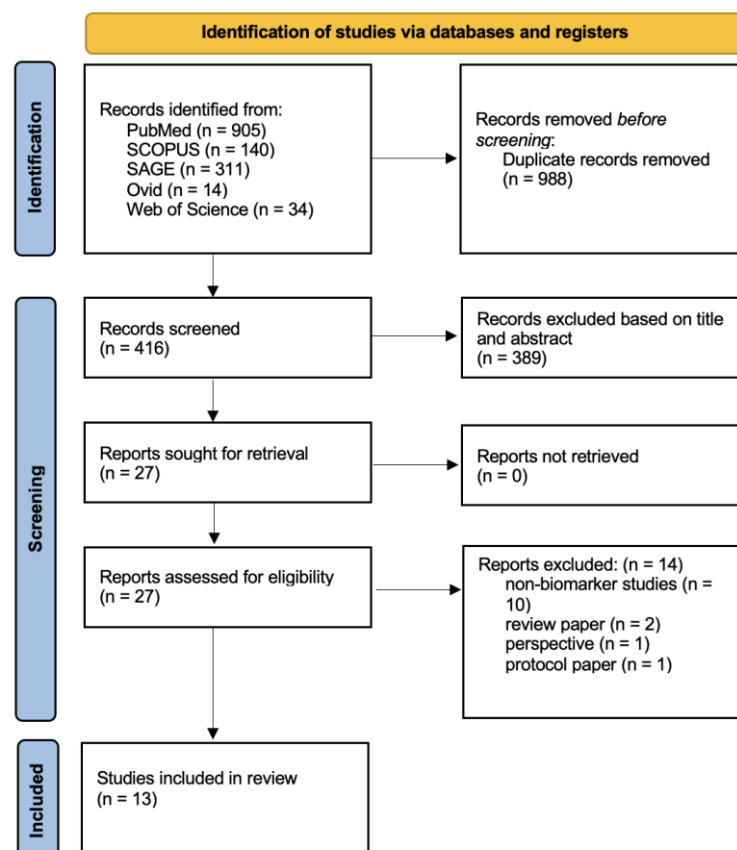
| Concept  | Parkinson’s Disease                    | Biomarker                                                    | Rab                        |
|----------|----------------------------------------|--------------------------------------------------------------|----------------------------|
| Synonyms | Parkinson’s<br>Paralysis Agitans<br>PD | Predictive molecule<br>Molecule marker<br>Signature molecule | Rab GTPases<br>Rab protein |

### 2.2 Study Selection

Two authors independently extracted and proofread the titles and abstracts of each article. The inclusion criteria were studies with human samples related to Rab protein as a biomarker for PD. The authors excluded articles based on the following criteria: review articles, abstracts, editorials/letters, case reports, content not written in English or not related to PD as well as biomarkers studies not involving Rab protein. Details of the inclusion and exclusion criteria are shown in Table 2. Differences of opinion between two authors were determined independently by the third author. Following the first screening, all 27 articles sought for retrieval were also screened by a third author. The flow of the literature search is presented via a PRISM flow diagram as shown in Figure 2.

**Table 2** Inclusion and exclusion criteria

| Inclusion criteria                                                                                                                                                                                                    | Exclusion criteria                                                                                                                                      |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------|
| Human (male and female) participants<br>Original research articles focused on Rab proteins in the context of PD<br>Included participants diagnosed with PD and/or healthy controls<br>Written in the English language | Solely non-human samples or animal subjects<br>Biomarkers that are not Rab proteins<br>Review papers<br>Abstracts<br>Editorials/letters<br>Case reports |



**Figure 2** Prisma flow chart of the literature search

### 2.3 Quality Assessment

Two authors independently performed a quality appraisal of included studies and reached a consensus after discussion. The Quality Assessment and Diagnostic Accuracy Studies-2 (QUADAS-2) tool was adopted for this purpose.<sup>32</sup> QUADAS-2 has 4 domains for risk of bias: (1) patient selection, (2) index test, (3) reference standard, and (4) flow and timing. Domains (1) to (3) were also used to evaluate applicability concerns. Graphical representations were generated through the Risk-of-bias VISualization (robvis) tool.<sup>33</sup>

### 2.4 Statistical assessment

To assess feasibility of quantitative synthesis by random effects meta-analysis, every reported finding from the included studies was extracted and classified according to the availability of poolable data. Poolable data was defined as a mean with either standard deviation (SD) reported explicitly, SD converted from standard error of mean (SEM), 95% confidence interval, or median and interquartile range. This will permit calculation of the standardized mean difference as the summary effect for each biomarker.

## 3.0 Results

### 3.1 Results of literature search

A total of 1404 records were identified, 905 found via PubMed, 140 from SCOPUS, 311 from SAGE, 14 from OVID Medline, 34 from Web of Science. 416 articles remained after duplicate removal within and between databases. Following the screening of the titles and abstracts, 389 articles were removed due to lack of relevance to the subject matter of the review. Hence, 27 articles were sought for retrieval. In total, the full texts of 27 articles

were assessed for eligibility based on the inclusion and exclusion criteria. Of the 27 articles assessed, 14 articles were removed as they did not meet our inclusion criteria. Finally, 13 studies were included in this review as shown in Figure 2.

### 3.2 Characteristics of included studies

Table 3 shows the findings of human studies with iPD patients, familial PD patients and healthy controls. Familial PD patients include carriers of mutations in LRRK2 and VPS35.

**Table 3** Summary of Rab protein findings across included studies

| Rab protein                                | Sample source <sup>a</sup> | Comparison <sup>b</sup> | n (per arm) <sup>c</sup> | Method <sup>d</sup> | Direction <sup>e</sup> | Fold-change <sup>f</sup> | Significance and p-value <sup>g</sup> |
|--------------------------------------------|----------------------------|-------------------------|--------------------------|---------------------|------------------------|--------------------------|---------------------------------------|
| <b>Chiu et al., 2016<sup>34</sup></b>      |                            |                         |                          |                     |                        |                          |                                       |
| Rab35                                      | Serum                      | iPD vs HC               | 213/177                  | ELISA               | Increase               | 1.86                     | SI (<0.0001)                          |
| Rab35                                      | Serum                      | iPD vs PSP              | 213/46                   | ELISA               | Increase               | 1.84                     | SI (<0.01)                            |
| Rab35                                      | Serum                      | iPD vs MSA              | 213/80                   | ELISA               | Increase               | 2.25                     | SI (<0.0001)                          |
| <b>Fan et al., 2018<sup>35</sup></b>       |                            |                         |                          |                     |                        |                          |                                       |
| Rab10                                      | Neutrophils                | iPD vs HC               | 7/13                     | Immunoblot          | No change              | -                        | NS                                    |
| Rab10                                      | Neutrophils                | LRRK2 G2019S PD vs HC   | 5/13                     | Immunoblot          | No change              | -                        | NS                                    |
| pRab10/<br>GAPDH                           | Neutrophils                | LRRK2 G2019S PD vs HC   | 5/13                     | Immunoblot          | Increase               | -                        | SI (<0.005)                           |
| pRab10/<br>total Rab10                     | Neutrophils                | iPD vs HC               | 7/13                     | Immunoblot          | Increase               | -                        | SI (<0.05)                            |
| ΔpRab10/<br>GAPDH                          | Neutrophils                | LRRK2 G2019S PD vs HC   | 5/13                     | Immunoblot          | Increase               | -                        | SI (<0.005)                           |
| ΔpRab10/<br>total Rab10                    | Neutrophils                | iPD vs HC               | 7/13                     | Immunoblot          | Increase               | -                        | SI (<0.005)                           |
| ΔpRab10/<br>total Rab10                    | Neutrophils                | iPD vs LRRK2 G2019S     | 7/5                      | Immunoblot          | Increase               | -                        | SI (<0.05)                            |
| <b>Atashrazm et al., 2019<sup>36</sup></b> |                            |                         |                          |                     |                        |                          |                                       |
| Rab10/actin                                | Neutrophils                | iPD vs HC               | 47/47                    | Immunoblot          | No change              | -                        | NS                                    |
| pRab10/actin                               | Neutrophils                | iPD vs HC               | 47/47                    | Immunoblot          | No change              | -                        | NS                                    |
| pRab10/<br>total Rab10                     | Neutrophils                | iPD vs HC               | 47/47                    | Immunoblot          | No change              | -                        | NS                                    |
| Rab10/actin                                | PBMCs                      | iPD vs HC               | 47/47                    | Immunoblot          | No change              | -                        | NS                                    |
| pRab10/actin                               | PBMCs                      | iPD vs HC               | 47/47                    | Immunoblot          | No change              | -                        | NS                                    |
| pRab10/<br>total Rab10                     | PBMCs                      | iPD vs HC               | 47/47                    | Immunoblot          | No change              | -                        | NS                                    |
| <b>Wang et al., 2019a<sup>37</sup></b>     |                            |                         |                          |                     |                        |                          |                                       |
| Rab7                                       | uEVs                       | iPD vs HC               | 28/22                    | MS                  | Increase               | -                        | NS (>0.05)                            |
| Rab7                                       | uEVs                       | iPD vs HC               | 57/51                    | MS                  | Increase               | -                        | NS (>0.05)                            |
| Rab10                                      | uEVs                       | iPD vs HC               | 28/22                    | MS                  | No change              | -                        | NS                                    |
| Rab10                                      | uEVs                       | iPD vs HC               | 57/51                    | MS                  | No change              | -                        | NS                                    |
| <b>Wang et al., 2019b<sup>38</sup></b>     |                            |                         |                          |                     |                        |                          |                                       |
| Rab35                                      | Serum                      | iPD vs HC               | 59/60                    | ELISA               | Increase               | 3.40                     | SI (<0.001)                           |
| Rab35                                      | Serum                      | iPD vs PSP              | 59/19                    | ELISA               | Increase               | -                        | SI (<0.001)                           |
| Rab35                                      | Serum                      | iPD vs MSA              | 59/20                    | ELISA               | Increase               | -                        | SI (<0.001)                           |
| <b>Karayel et al., 2020<sup>39</sup></b>   |                            |                         |                          |                     |                        |                          |                                       |
| pRab10/<br>GAPDH                           | Neutrophils                | LRRK2 G2019S PD vs HC   | 4/4                      | Immunoblot          | Increase               | -                        | NS                                    |
| pRab10/<br>total Rab10                     | Neutrophils                | LRRK2 G2019S PD vs HC   | 4/4                      | Immunoblot          | No change              | -                        | NS                                    |
| pRab10                                     | Neutrophils                | LRRK2 G2019S PD vs HC   | 4/4                      | MS                  | Increase               | 1.9                      | SI (<0.02)                            |

|                                                            |             |                              |       |            |           |                         |              |
|------------------------------------------------------------|-------------|------------------------------|-------|------------|-----------|-------------------------|--------------|
| <b>pRab10/<br/>GAPDH</b>                                   | Neutrophils | VPS35<br>D620N PD vs<br>HC   | 3/3   | Immunoblot | Increase  | -                       | SI           |
| <b>pRab10/<br/>GAPDH</b>                                   | Neutrophils | iPD vs HC                    | 3/3   | Immunoblot | No change | -                       | UC           |
| <b>pRab10/<br/>total Rab10</b>                             | Neutrophils | VPS35<br>D620N PD vs<br>HC   | 3/3   | Immunoblot | Increase  | -                       | SI           |
| <b>pRab10/<br/>total Rab10</b>                             | Neutrophils | iPD vs HC                    | 3/3   | Immunoblot | No change | -                       | UC           |
| <b>pRab10</b>                                              | Neutrophils | VPS35<br>D620N PD vs<br>HC   | 3/3   | MS         | Increase  | 3.7                     | SI (<0.005)  |
| <b>pRab10</b>                                              | Neutrophils | VPS35<br>D620N PD vs<br>iPD  | 3/3   | MS         | Increase  | -                       | SI (<0.05)   |
| <b>pRab10</b><br><b>Nirujogi et al., 2021<sup>40</sup></b> | Neutrophils | iPD vs HC                    | 3/3   | MS         | No change | -                       | NS           |
| <b>pRab10/<br/>total Rab10</b>                             | Neutrophils | VPS35<br>D620N PD vs<br>HC   | 3/3   | MS         | Increase  | 3.1                     | SI (<0.05)   |
| <b>pRab10</b>                                              | Neutrophils | VPS35<br>D620N PD vs<br>HC   | 3/3   | MS         | Increase  | 5.4                     | SI (<0.05)   |
| <b>pRab8</b>                                               | Neutrophils | VPS35<br>D620N PD vs<br>HC   | 3/3   | MS         | Increase  | 4.8                     | SI (<0.05)   |
| <b>pRab43</b>                                              | Neutrophils | VPS35<br>D620N PD vs<br>HC   | 3/3   | MS         | Increase  | 6.6                     | SI (<0.05)   |
| <b>pRab1</b>                                               | Neutrophils | VPS35<br>D620N PD vs<br>HC   | 3/3   | MS         | Increase  | 1.5                     | SI (<0.05)   |
| <b>pRab3</b>                                               | Neutrophils | VPS35<br>D620N PD vs<br>HC   | 3/3   | MS         | Increase  | 1.9                     | SI (<0.05)   |
| <b>pRab35</b>                                              | Neutrophils | VPS35<br>D620N PD vs<br>HC   | 3/3   | MS         | Increase  | 1.4                     | NS           |
| <b>Fan et al., 2021<sup>41</sup></b>                       |             |                              |       |            |           |                         |              |
| <b>pRab10/<br/>total Rab10</b>                             | Neutrophils | LRRK2<br>G2019S PD<br>vs HC  | 12/32 | Immunoblot | No change | -                       | NS           |
| <b>pRab10/<br/>total Rab10</b>                             | Neutrophils | LRRK2<br>G2019S NMC<br>vs HC | 9/32  | Immunoblot | No change | -                       | NS           |
| <b>pRab10/<br/>total Rab10</b>                             | Neutrophils | LRRK2<br>G2019S NMC<br>vs HC | 13/32 | Immunoblot | Increase  | 4                       | SI (=0.0007) |
| <b>pRab10/<br/>total Rab10</b>                             | Neutrophils | LRRK2<br>R1441G<br>NMC vs HC | 8/32  | Immunoblot | Increase  | 4                       | SI (=0.0007) |
| <b>pRab10/<br/>total Rab10</b>                             | Neutrophils | iPD vs HC                    | 27/32 | Immunoblot | No change | -                       | NS           |
| <b>Wang et al., 2021<sup>42</sup></b>                      |             |                              |       |            |           |                         |              |
| <b>pRab10</b>                                              | PBMCs       | iPD vs HC                    | 46/22 | MSD assay  | No change | -                       | NS           |
| <b>pRab10</b>                                              | PBMCs       | LRRK2<br>G2019S NMC<br>vs HC | 16/22 | MSD assay  | No change | -                       | NS           |
| <b>pRab10</b>                                              | PBMCs       | LRRK2<br>G2019S PD<br>vs HC  | 33/22 | MSD assay  | No change | -                       | NS           |
| <b>pRab10/<br/>LRRK2</b>                                   | PBMCs       | LRRK2<br>G2019S PD<br>vs iPD | 33/46 | MSD assay  | Increase  | RGM 1.44<br>(1.07–1.95) | SI (<0.05)   |
| <b>pRab10/<br/>LRRK2</b>                                   | PBMCs       | LRRK2<br>G2019S NMC<br>vs HC | 16/22 | MSD assay  | Increase  | RGM 1.44<br>(1.07–1.95) | SI (<0.05)   |

|                                                                          |       |                              |         |                       |           |     |             |
|--------------------------------------------------------------------------|-------|------------------------------|---------|-----------------------|-----------|-----|-------------|
| <b>pRab10/<br/>LRRK2</b>                                                 | PBMCs | iPD vs HC                    | 46/22   | MSD assay             | No change | -   | NS          |
| <b>Wang et al., 2022<sup>43</sup></b>                                    |       |                              |         |                       |           |     |             |
| <b>pRab10/<br/>total Rab10</b>                                           | uEVs  | iPD vs HC                    | 17/12   | Immunoblot            | No change | -   | NS          |
| <b>pRab10/<br/>total Rab10<br/>(fold of<br/>baseline at<br/>month 0)</b> | uEVs  | iPD vs HC                    | 66/19   | Immunoblot            | Increase  | -   | SI (=0.046) |
| <b>pRab10/<br/>total Rab10</b>                                           | uEVs  | LRRK2<br>G2019S PD<br>vs HC  | 16/12   | Immunoblot            | No change | -   | NS          |
| <b>Taymans et al., 2023<sup>44</sup></b>                                 |       |                              |         |                       |           |     |             |
| <b>Rab8a</b>                                                             | uEVs  | iPD vs HC                    | 60/53   | Immunoblot            | Increase  | -   | SI (<0.05)  |
| <b>Rab8a</b>                                                             | uEVs  | LRRK2<br>G2019S PD<br>vs HC  | 7/53    | Immunoblot            | No change | -   | NS          |
| <b>pRab8a</b>                                                            | uEVs  | iPD vs HC                    | 60/53   | Immunoblot            | No change | -   | NS          |
| <b>pRab8a</b>                                                            | uEVs  | LRRK2<br>G2019S PD<br>vs HC  | 7/53    | Immunoblot            | No change | -   | NS          |
| <b>Yuan et al., 2024<sup>45</sup></b>                                    |       |                              |         |                       |           |     |             |
| <b>pRab10/<br/>total Rab10</b>                                           | Serum | iPD vs HC                    | 276/246 | Transcriptomi<br>cs   | No change | -   | NS          |
| <b>Cortes et al., 2025<sup>46</sup></b>                                  |       |                              |         |                       |           |     |             |
| <b>pRab10</b>                                                            | PBMCs | iPD vs HC                    | 20/25   | MS                    | No change | -   | NS          |
| <b>pRab10</b>                                                            | PBMCs | LRRK2<br>G2019S PD<br>vs HC  | 16/25   | MS                    | Increase  | -   | NS          |
| <b>pRab10</b>                                                            | PBMCs | LRRK2<br>G2019S NMC<br>vs HC | 11/25   | MS                    | Increase  | -   | NS          |
| <b>pRab10</b>                                                            | PBMCs | LRRK2<br>R1441G PD<br>vs HC  | 1/25    | MS                    | No change | -   | NS          |
| <b>pRab10</b>                                                            | PBMCs | LRRK2<br>R1441G<br>NMC vs HC | 3/25    | MS                    | No change | -   | NS          |
| <b>pRab12</b>                                                            | PBMCs | LRRK2<br>G2019S PD<br>vs HC  | 37/45   | Phosphoprote<br>omics | Increase  | 1.9 | SI (<0.05)  |

<sup>a</sup> Sample sources include whole blood serum, neutrophils, Peripheral Blood Mononuclear Cells (PBMCs) and urinary extracellular vesicles (uEVs)

<sup>b</sup> Comparison column reports the groups included in the analysis. Groups included: Healthy controls (HC), idiopathic PD (iPD), (PSP), (MSA), asymptomatic carriers/non-manifesting carrier (NMC), LRRK2 G2019S PD, VPS35 D620N PD, LRRK2 R1441G PD.

<sup>c</sup> n (per arm) gives the number of participants per group in the order named in the Comparison column

<sup>d</sup> Quantification methods of Rab proteins. Abbreviations: enzyme-linked immunosorbent assay (ELISA), mass spectrometry (MS), Meso Scale Discovery (MSD) assay

<sup>e</sup> Direction is relative to the first-named group in the Comparison column

<sup>f</sup> Fold-change is the value reported by the original study where available; a dash (-) indicates that no fold-change was reported or that the original data were presented in a form (figure, normalized ratio, or geometric-mean ratio) where simple fold-change cannot be stated. RGM, relative geometric mean (with 95% confidence interval).

<sup>g</sup> Significance designation is as follows: Significantly increased (SI) = elevated in the first-named group at  $p < 0.05$ ; Not significant (NS) = no statistically significant difference; Data not reported / unclear (UC) = significance not reported or not interpretable as stated. p-value if provided, will be stated in brackets

Five of 13 studies included asymptomatic carriers of LRRK2 PD-associated genes, G2019S and R1441G. Two studies included other parkinsonian diseases such as Progressive supranuclear palsy (PSP) and Multiple system atrophy (MSA). Of those two studies, one study quantified Rab levels of the following non-parkinsonian neurodegenerative diseases: AD, spinocerebellar ataxia (SCA) and HD. The source of biomarkers from patient and healthy controls includes blood serum, PBMCs, neutrophils and urinary extracellular vesicles (uEVs). The first three are blood-based biomarkers and uEVs are sourced from urine.

As seen in Table 3, there are a total of 8 different Rabs investigated in studies exploring the utility of Rabs as a biomarker. These studies report both total Rab levels and/or levels of the phosphorylated forms of Rab proteins (pRab) as well as Rab levels normalized to other readouts. Collectively, the Rabs reported were Rab1, Rab3, Rab7a, Rab8, Rab10, Rab12, Rab35 and Rab43.

A critical finding from the studies was the observed difference of pRab or Rab levels in PD patients compared to healthy controls.

Rab1, Rab3, Rab7a, Rab8 and Rab43 were assessed in  $\leq$  two separate studies. In one study, pRab1 and pRab3 were found to be elevated in neutrophils isolated from three VPS35 D620N PD patients by 1.5-fold compared to healthy controls.<sup>40</sup> The same study reported that pRab8 and pRab43 levels in neutrophils of VPS35 D620N PD patients were increased by 4.8 and 6.6 fold respectively.<sup>40</sup> In a latter study, the significant elevation of total Rab8 levels was observed in uEVs of iPD patients.<sup>44</sup> One study involving two separate cohorts reported an increase in total Rab7a levels in iPD patient uEVs compared to controls but was not significant after multiple testing correction.<sup>37</sup>

For comparison between patients and healthy-controls, Rab10 or pRab10 levels was quantified in 11 of the 13 included studies.<sup>35-37,39-46</sup> 8 out of the 11 studies reported no significant differences in the Rab10 or pRab10 levels of PD patients (whether iPD or familial PD) compared to healthy controls.<sup>35-37,42-46</sup> 10 of 11 studies included iPD patients in their cohort and found that pRab10 and Rab10 levels derived from neutrophils, PBMCs and uEVs had no significant difference compared to the healthy controls.<sup>35-37,39,41-46</sup> Although no significant elevation was reported between iPD and healthy controls, two of these studies using blood-based biomarkers observed a significant correlation between pRab10 levels (normalized to total Rab10) and disease severity as measured by motor UPDRS scores.<sup>36,45</sup> One study with a longitudinal cohort reported that pRab10 levels (normalized with total Rab10) increased over time in iPD patient uEVs.<sup>43</sup>

In addition, when comparing PD carriers of LRRK2 mutations and healthy controls, pRab10 or Rab10 levels do not report a significant change. 7 of 11 studies involving Rab10 included LRRK2 PD patients.<sup>35,39,41-44,46</sup> Amongst the 7 studies, one study observed a 1.9-fold increase in pRab10 levels in neutrophils derived from LRRK2 G2019S PD patients.<sup>39</sup> Another study observed a 4-fold increase in pRab10 levels in neutrophils derived from PD patients with the R1441G variant of LRRK2.<sup>41</sup> In one study, pRab10 levels in PBMCs were only significantly different after normalization for variations in total LRRK2 levels.<sup>42</sup> An increase of pRab10 in LRRK2 G2019S PD compared to sporadic PD patients and in asymptomatic LRRK2 G2019S carriers compared to healthy controls was observed.<sup>42</sup>

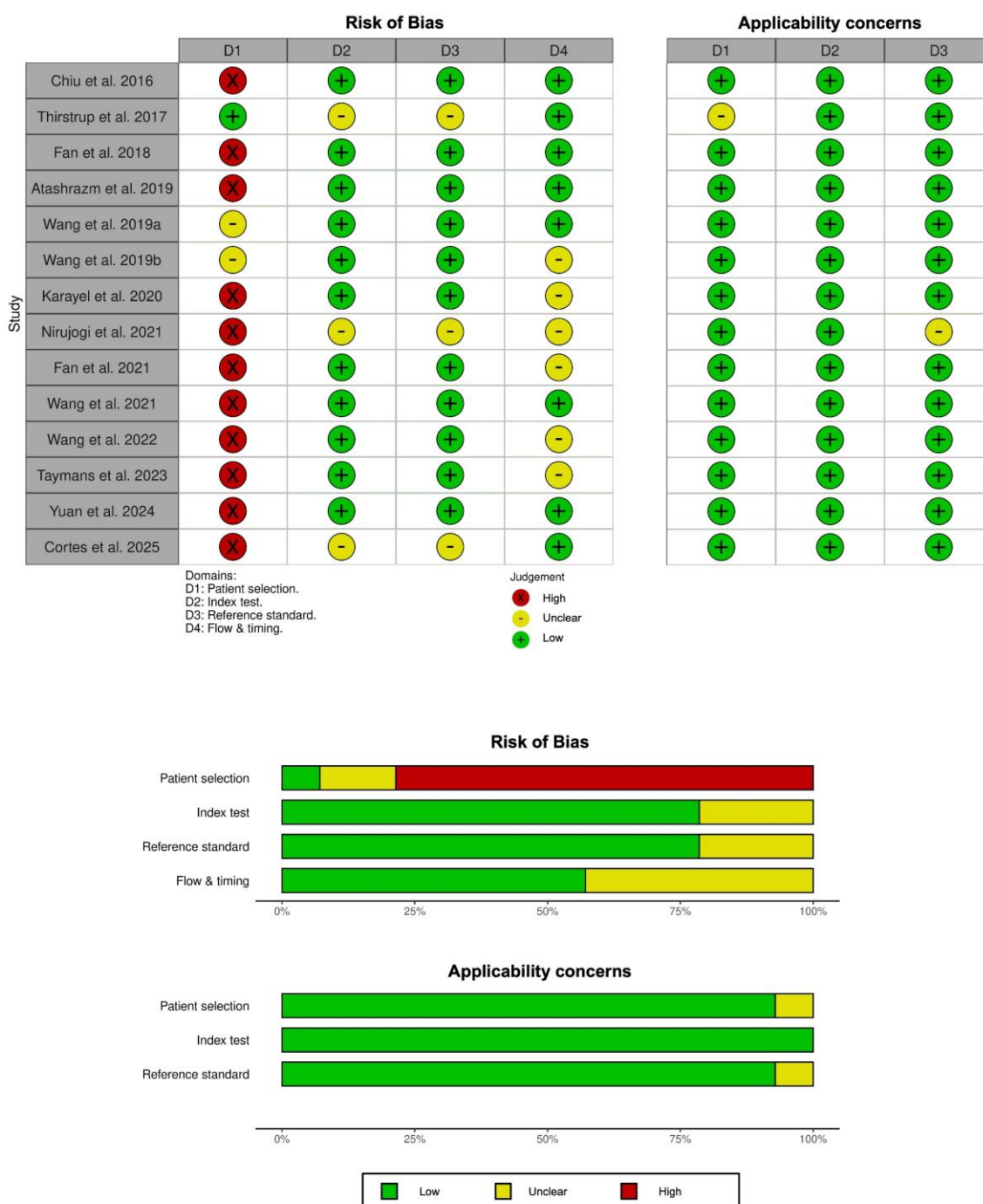
Rab10 levels were also assessed in VPS35 D620N PD patients.<sup>39-40</sup> In neutrophils isolated from VPS35 D620N PD patients, pRab10 levels were elevated by 3.7-fold in one study and increased by 5.4-fold in another study.<sup>39-40</sup> The latest study captured in the updated literature search of this review reported pRab12 levels in PBMCs of LRRK2 G2019S PD patients.<sup>46</sup> Briefly, a significant fold change of 1.9 was observed in LRRK2 G2019S PD patients compared to healthy controls.<sup>46</sup>

Blood serum levels of Rab35 were elevated in iPD patients compared to healthy controls and patients with PSP, MSA, AD, SCA, and HD.<sup>34,38</sup> pRab35 levels in neutrophils of VPS35 D620N PD patients were elevated though not to a significant degree.<sup>40</sup> included information of Rab35 levels corresponding to age of onset and disease severity. Elevated Rab35 in blood serum was associated with a younger age of onset of iPD, longer disease duration and Levodopa equivalent daily dose.<sup>34</sup> The same study found that levels of Rab35 was higher in non-tremor dominant patients with predominant akinetic-rigid syndrome compared to those with tremor-dominant PD. In another study, the serum level of Rab35 was positively correlated with  $\alpha$ -Syn levels in iPD patients so assessing

both Rab35 and  $\alpha$ -Syn could be used to discriminate PD patients from other parkinsonian disorders.<sup>38</sup> The assessment of Rab35 and  $\alpha$ -Syn as a biomarker was more effective in identifying younger iPD patients.<sup>38</sup>

### 3.3 Results of quality assessment

The quality of included studies was assessed using the QUADAS-2 tool. Overall, the applicability concerns were low in most studies for all the relevant domains. In terms of risk of bias, the findings in each domain varied. Majority of studies had a high risk of bias in the patient selection domain. In the index test and reference standard domain, almost all studies had low risks of bias. For the flow and timing domain, the number of studies with low and unclear risk of bias were almost equivalent. The summary of findings of the appraisal is shown in Figure 3.



**Figure 3** QUADAS-2 tool reviews authors' judgements about each risk of bias item and applicability concerns in all included studies

### 3.4 Results of statistical assessment

Findings specific to PD were extracted from included studies to determine the feasibility of a random-effects meta-analysis. From 13 studies, 61 findings were extracted and classified according to sample type, type of Rab and quantification method. Of 61 extracted findings from 13 different studies, only 11 findings were in a poolable format. The remainder were presented as figures without tabulated values (34 out of 61 findings), reported fold-change without SD nor SEM (13 out of 61 findings), or expressed as adjusted ratios of geometric means (3 of 61 findings). When poolable findings were restricted to those sharing an identical biomarker, sample type, and comparison across independent cohorts, no combination was supported by three or more independent studies. The only two studies comparing pRab10 normalized to total Rab10 in neutrophils was derived from a single cohort reported across genotype subgroups. Two studies of serum levels of Rab35 originated from the same research group and patient samples originated from the same geographical location. Quantitative synthesis was determined unfeasible, as pooling the data would falsely represent evidence that is sparse, heterogeneous and in some instances derived from overlapping samples. Findings were instead synthesized narratively to provide an unambiguous summary of the current evidence. File S1 presents the extracted findings of all studies.

## 4.0 Discussion

### 4.1 Rab biomarkers as a diagnostic tool for idiopathic PD

In patient blood serum, Rab35 was the only Rab quantified.<sup>34,38</sup> Elevated levels of Rab35 were consistently observed in iPD patients (total of 272 patients) across both investigations conducted.<sup>34,38</sup> Importantly, these studies ensured proportional representation by matching the age of healthy controls to that of the PD patients. However, while the ethnicity of patients was undisclosed, both studies were conducted in Taiwan, which may limit the applicability of findings globally. Regardless, the assessments demonstrated that Rab35 quantification holds discriminatory potential, enabling differentiation between iPD and other parkinsonian disorders such as PSP and MSA, as well as neurodegenerative conditions like AD, SCA and HD. Given the challenges in distinguishing PD from disorders with similar clinical presentation, incorporating Rab35 assessment as a supplementary diagnostic tool alongside the Movement Disorder Society (MDS) Clinical Diagnostic Criteria could enhance the accuracy of PD diagnosis.

Wang et al<sup>38</sup> suggested that the correlation of Rab35 with  $\alpha$ Syn levels as a combined assessment could be used for increased accuracy in discrimination. This is an interesting finding as pre-clinical studies have found that rather than Rab35, Rab10 is associated with  $\alpha$ Syn pathology.<sup>47-48</sup> The significance of  $\alpha$ Syn levels in blood serum as potential indicators of Lewy body pathology in iPD brains was also highlighted through post-mortem analyses, adding a layer of complexity to the relationship between Rab35 and  $\alpha$ Syn.<sup>49</sup> Based on these findings, Rab35 could be a candidate for the diagnosis of iPD. This finding should be regarded as promising but preliminary: both supporting studies originate from a single research group in one geographical setting, and the result has not yet been independently replicated or validated in an external cohort. Independent confirmation in demographically diverse populations is required before serum Rab35 can be considered a validated diagnostic marker.

The potential of Rab35 as a discriminatory and diagnostic biomarker for iPD could be further evaluated if further studies involving Rab35 included familial PD cohorts. Only one study in this review assessed Rab35 levels in neutrophils of patients with familial PD, specifically carriers of VPS35 D620N, where they found the increase in pRab35 to be negligible.<sup>40</sup> The observed minimal alteration could be attributed to the absence of baseline Rab35

levels in neutrophils. The current body of literature lacks studies assessing Rab35 blood serum levels in patients with genetic PD, underscoring the need for further investigation.

#### 4.2 Rab biomarkers as a diagnostic tool for familial PD

In the studies included in this review, the association between Rabs and LRRK2 seems to be controversial especially in consideration of their utility as biomarkers in LRRK2-linked PD. Historically, autophosphorylation of LRRK2 at Ser 1292 has been used as an indicator of LRRK2 activity. However, studies found that it did not accurately reflect the state of kinase activity in the body. Therefore, some studies, both with human and animal cells, suggest that pRab10 can act as an indicator of LRRK2 kinase activity and subsequently reflect the disease state of individuals carrying LRRK2 mutations i.e. identifying familial PD patients.<sup>50</sup> However, in most studies included in this review, pRab10 levels were similar between healthy controls and LRRK2 PD patients. This lack of correlation, however, is not applicable to other PD-related genes, namely VPS35. The elevation of several different phosphorylated Rabs—Rab1, Rab3 Rab8, Rab10 and Rab43 was observed in patients with the VPS35 D620N variant.<sup>40</sup> VPS35 mutations represent the second most common cause of late-onset familial PD after LRRK2 mutations. Therefore, as this combination of pRabs is specific to VPS35 D620N, it can be used to discriminate between these types of familial PD. While there was a significant elevation in patients, the sample size of the study is small so further studies with larger sample sizes need to replicate these findings before statistical validity and clinical relevance can be claimed.

An additional study not captured in this systematic search reported that PBMC pRab10 levels had significant elevation in patients.<sup>52</sup> pRab10 is elevated in LRRK2 G2019S PD patients and not asymptomatic carriers of LRRK2 G2019S.<sup>52</sup> In contrast to the studies included in this review, pRab10 is elevated in iPD patients.<sup>52</sup> The study also compared pRab10 in uEVs and reported no significant changes between patients and healthy controls. Overall, the diagnostic performance of Rab10 was inconsistent across the included studies. For the most frequently examined comparison, pRab10 normalized to total Rab10 in iPD versus healthy controls, most cohorts reported no significant difference, with significant elevation confined to a minority of findings and not reproduced across sample types (neutrophils, PBMCs, uEVs, or serum). This pattern does not support phosphorylated Rab10 as a stand-alone diagnostic marker.

The latest study in the updated literature search of this review reported significant fold changes of pRab12 levels in PBMCs of LRRK2 G2019S PD patients.<sup>46</sup> Furthermore, differences were observed between the pRab12 levels in LRRK2 G2019S PD and iPD patients suggesting discriminatory potential between these forms of PD.<sup>46</sup> However, this is the first study reporting the potential of pRab12 as a familial PD biomarker and requires further evaluation.

A key limitation is that several of these studies have cohorts largely comprising of LRRK2 PD patients and due to the late age of onset of LRRK2 PD, the mean age of the cohort could affect the Rab protein level quantified. It may be not reflective of early-stage patients, which questions the feasibility of using Rabs as predictive biomarkers. Further studies can be done with a younger age of onset cohort to evaluate Rab levels in early-onset PD.

#### 4.3 Rab biomarkers as indicators of disease progression

The studies in this review show that an elevation of Rab levels may be indicative of disease severity.<sup>34,36,38,43,45</sup> The findings of two studies, support the use of Rab35 levels from blood serum as an indicator of disease severity

due to an observed correlation with younger age of onset of iPD and higher Levodopa daily dose.<sup>34,38</sup> Measuring UPDRS scores could strengthen this finding. Despite not having any differences in pRab10 levels compared to the control group, the findings of three studies support the use of pRab10 as an indicator of severity of iPD where correlation with UPDRS motor score was observed.<sup>36,43,45</sup> This indicates that pRab10 may have a utility despite the lack of diagnostic potential.

In the current state of knowledge, pRabs or total protein levels of Rabs remain valuable indicators of treatment effectiveness and offer crucial insights for patient stratification.

The assessment of Rab levels as a diagnostic tool could be used in accordance with the measurement of Braak staging to understand the association between disease progression and biomarkers. Braak staging is a tool used to evaluate disease progression in both PD and AD.<sup>53</sup> A recent study was conducted to understand the relationship between Braak staging and biomarker levels. A PET-based Braak study was conducted to model the trajectories of amyloid- $\beta$ , phosphorylated tau (pTau) in cerebrospinal fluid.<sup>54</sup> Tau pathology observed in a subset of PD patients gives relevance to the monitoring of pTau levels in PD patients. The implementation of Rab quantification in PD patients can also complement other potential clinical biomarkers. One such biomarker is  $\alpha$ Syn aggregates which are currently under investigation to identify the severity or clinical stages of PD.<sup>55</sup> The utilization of  $\alpha$ Syn as a biomarker presents an ante-mortem method of identifying PD and has been found to be present in CSF, skin and blood serum.<sup>56</sup> For specific types of PD, pRab levels may be a better reflection of the disease status as it is dependent on protein activity in the patient. Future studies may be conducted as a framework to model the natural history of PD and monitor PD severity in living humans.

#### *4.4 Rab biomarkers for drug-stratification and drug-response indication*

In addition to their role as potential diagnostic biomarkers, Rab proteins exhibit promising utility in drug-stratification and drug-response indication. Notably, the phosphorylation of Rab10 and Rab12 has been identified to correlate significantly with LRRK2 kinase activity. Several studies using patient samples also reported decreased pRab10 levels after treatment with LRRK2 inhibitors.<sup>36,40</sup> This finding establishes a foundation for considering Rab10 and Rab12 quantification as informative biomarkers for assessing drug responses specifically related to LRRK2 inhibition. As the field advances, the measurement of Rab10 and Rab12 levels could serve as crucial indicators, offering insights into the effectiveness of LRRK2-targeted therapies. Moreover, given the intricate relationship between Rab10 and PINK1, similar investigations could explore the use of Rab biomarkers in the context of PINK1 inhibition, potentially extending their applicability to a broader range of therapeutic interventions.<sup>52</sup> Further investigation of Rab proteins as biomarkers for drug stratification and response indication may clarify their functional relevance and could inform treatment strategies in the management of PD and related neurodegenerative disorders.

Moreover, the capability to diagnose PD at earlier stages prior to extensive neurodegeneration will lay the groundwork for advancing clinical trials. Suboptimal clinical trial outcomes often stem from the enrolment of participants in advanced PD stages, where the progression of the disease reduces the efficacy of disease-modifying agents.

#### *4.5 Biological relevance of biomarker sources*

In majority of these studies, blood-based biomarkers such as PBMCs, neutrophils and serum were used to quantify the pRab/Rab levels in patients. In general, only the studies using neutrophils report significant changes of pRab10

levels, indicating that neutrophils may be a better bioresource compared to PBMCs.<sup>39-41</sup> While uEVs present a less invasive alternative, only Rab7a and Rab8 were reported to have significant differences in patients compared to healthy controls, signifying a lack of applicability. Since disease-status, disease severity and drug-response can be assessed through different source of biomarkers, combined assessment may be beneficial to have a broader understanding on PD patients.

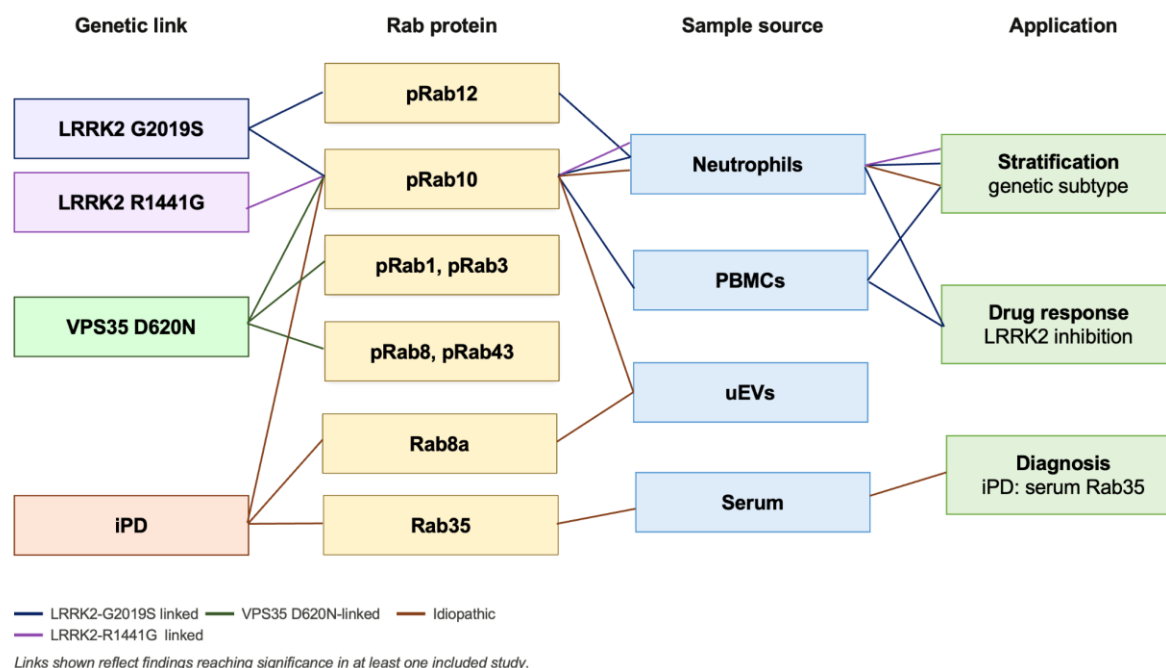
The detectability of specific Rabs across sample types likely reflects their underlying biology. Neutrophils express LRRK2 at high constitutive levels, providing a favorable substrate-to-kinase ratio for detecting phosphorylated Rab10. This could explain why significant pRab10 changes were observed predominantly in neutrophil-derived samples rather than in PBMCs, where LRRK2 expression is comparatively lower.<sup>39-41</sup> In contrast, the increase of total Rab35 in serum does not correlate with LRRK2 kinase activity but rather could be consistent with the role of Rab35 in endocytic recycling of cargoes, supporting its correlation with serum  $\alpha$ -synuclein.<sup>38</sup> The detection of Rab7a and Rab8 in uEVs may reflect the trafficking proteins that are preferentially packaged into and preserved within excreted vesicles. Altogether, these observations suggest that the optimal biomarker source is Rab-specific: pRab10 is best evaluated where LRRK2 kinase activity is abundant, whereas total Rab35 in serum may be more representative of general pathology. The biological relevance of each Rab protein supports the case for matching each Rab to the sample type in which its signal is physiologically expected, rather than surveying all Rabs in a single sample source.

#### 4.6 Study limitations

Our results demonstrate that Rabs have potential but preliminary clinical utility in assessing disease-state of PD patients as well as potential for drug-response and patient stratification. However, there are methodological limitations in this study. Namely, the lack of heterogeneity analysis and small evidence base. The results presented may not be substantive in a clinical context. The QUADAS-2 tool was utilized for quality appraisal of included studies. As seen in Figure 3, majority of the studies had a high or unclear risk of bias in patient selection. This can be attributed to the fact that all studies had a case-control design, which may weaken the findings. A meta-analysis was pre-specified but following formal assessment, it was determined unfeasible: 61 extracted findings only 11 reported poolable data and no biomarker to sample comparison was supported by three or more independent studies with compatible data (see Section 3.4). The heterogeneous and largely figure-based reporting of effect sizes across the field, rather than any single methodological choice, presents a barrier to synthesis.

#### 4.7 Towards clinical translation

Figure 4 synthesizes the findings of this review, mapping specific Rabs to their genetic links, proposed sample sources, and potential clinical applications. The translation of biomarkers from exploratory findings into clinical relevance requires coordination of further work. Firstly, the use of serum-derived Rab35 in idiopathic PD requires independent replication in cohorts that are demographically and ethnically diverse.<sup>34,38</sup> Next, the standardization of assays (quantification methods and normalization strategies) in the evaluation of Rab biomarkers will allow future cross-study comparison and quantitative synthesis. Finally, there is a pressing need for longitudinal studies to determine whether changes in Rab levels reflect disease progression and therapeutic response, especially for pRab10 in the context of LRRK2-directed therapies. At present, Rab proteins remain promising biomarkers for further evaluation before clinical applicability.



**Figure 4** Conceptual synthesis of Rab proteins as candidate biomarkers in Parkinson's disease.

## Conclusion

This systematic review provides insight into the utility of Rabs as biomarkers for PD through the integration and analysis of multiple sources. The existing literature supports further investigation of Rab35 as a biomarker for iPD. There remains a lack of evidence in the use of Rabs for the identification of familial PD. Despite that, pRab10 and Rab35 show differing forms of potential: serum Rab35 is the most consistent candidate for the diagnosis of idiopathic PD, albeit a small evidence base, whereas pRab10 holds potential relevance for patient stratification and the measurement of drug response in PD patients. Further studies are required to evaluate Rab levels at younger age of onset to understand the age-related changes of pRabs in patients. This longitudinal approach could provide invaluable insights into the progression of PD and the dynamic nature of Rab proteins in the disease course. Moreover, a combination of biomarker and pathological classification could be used to better understand the association of PD severity and quantification of biomarkers.

## Author contributions

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All authors have read and agreed to the published version of the manuscript.

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### Conflict of Interest

We declare no conflict of interest.

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