# Identification of metabolites reproducibly associated with Parkinson's Disease via meta-analysis and computational modelling

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# Identification of metabolites reproducibly associated with Parkinson's Disease via meta-analysis and computational modelling

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**Abstract**

Many studies have reported metabolomic analysis of different bio-specimens from Parkinson's disease patients. However, inconsistencies in reported metabolite concentration changes make it difficult to draw conclusions as to the role of metabolism in the occurrence or development of Parkinson's disease. We reviewed the literature on metabolomic analysis of Parkinson's disease patients. From 74 studies that passed quality control metrics, perturbations to 928 metabolites were reported to be associated with PD diagnosis, but only 190 were replicated with the same changing trends in more than one study. Of these metabolites, 60 exclusively increased, such as 3-methoxytyrosine and glycine, 54 exclusively decreased, such as pantothenic acid and caffeine, and 76 inconsistently changed in concentration in PD versus control subjects, such as ornithine and tyrosine. A novel genome-scale metabolic model of PD and corresponding metabolic map linking most of the replicated metabolites enabled a better understanding of the dysfunctional pathways of PD and prediction of additional potential metabolic markers from pathways with consistent metabolite changes to target in future studies.

Keywords: Parkinson's disease; metabolites; diagnosis; progression; genome-scale metabolic modelling

## Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder. Primarily as a result of an increasing elderly population, from 1990 to 2019, the number of patients with PD increased to almost 4 million globally [1, 2]. As PD is a multifactorial disease with various molecular mechanisms, a series of biomarkers derived from clinical factors, neuroimaging, genomics, and biomolecules have been proposed for PD diagnosis and progression [3, 4].

Metabolites are small endogenous molecules generated by various enzymes in metabolic reactions; they join in essential cellular functions, such as energy metabolism, signal transduction and apoptosis [5]. Evidence has indicated that metabolites may provide insight into the mechanisms of various pathophysiological processes through dysfunctional metabolic pathways [6]. Therefore, an increasing focus of research identifies metabolites as significant biomarkers to distinguish PD patients from asymptomatic controls, and to predict the disease progression or prognosis of PD patients [7].

Several studies have reviewed metabolic biomarkers of PD. These studies briefly summarised significant metabolites of PD from clinical and experimental studies [8], reported advancements of analytical platforms used in metabolomic studies [9], and concisely discussed current PD metabolic biomarker discovery and validation methods [10]. However, currently identified metabolites are highly heterogeneous and segregated, these studies seem not enough to comprehensively understand the significant metabolites and dysfunctional pathways of PD.

Human genome-scale metabolic modelling is increasingly used to understand normal cellular functions and disease states [11, 12, 13, 14]. It provides molecular mechanistic framework for integrative analysis of segregated molecular data, and quantitative prediction of phenotypic states [12]. Such models can be used to simulate whole-body metabolism and perform functional predictions based on cell-specific metabolic models combined with omics data [15, 14, 16]. To

comprehensively understand dysfunctional metabolic pathways of PD, it is necessary to effectively integrate reliable PD metabolites with genome-scale computational modelling.

In this study, we systematically reviewed the literature on the metabolomic studies of Parkinson's Disease patients. Then, we summarised the reported diagnosis and progression associated metabolites to reveal consistent and inconsistent metabolomic changes and analyzed possible reasons for inconsistency. Next, replicated metabolites were identified to explore the dysfunctional pathways of PD and generate a genome-scale metabolic model that is enriched with metabolic pathways containing PD-associated metabolites in order to put the metabolic perturbations associated with PD into a systems biochemical context. Consistently changed pathways and potential metabolic markers of PD were explored through the core map of the metabolic model.

# Results

## Study characteristics

A total of 87 metabolomic studies of PD patients were selected from the databases, as shown in Figure 1. Among these studies, 84 studies contained diagnosis-related metabolites, and 20 studies contained progression-related metabolites, the detailed study information were shown in Supplementary Table 1.

All the selected studies were published from 2003 to March 2022, involving patients from 17 countries across the world (Figure 2). These metabolomic studies utilized various bio-specimens, with plasma used in 39 studies, serum in 22 studies, cerebrospinal fluid (CSF) in 17 studies, brain tissue in six studies, urine in six studies, fecal in six studies, and other samples including sebum, saliva and human appendix in four studies.

The analytical platforms employed for metabolite measurement comprised liquid chromatography combined with mass spectrometry (LC-MS) in 29 studies, LC-tandem MS in 23 studies, gas chromatography combined with mass spectrometry (GC-MS) in 15 studies, GC-tandem MS in three studies, nuclear magnetic resonance (NMR) in 12 studies, only MS in seven studies, electrochemical detection coupled with high-performance liquid chromatography (HPLC-ED) in three studies, liquid chromatography electrochemical array (LCECA) in two studies, Magnetic Resonance Spectroscopy (MRS) in two studies, and other platforms including immunoassay, electrode array, fluorescence detection and enzyme test in six studies. The number of studies on each specimen and platform is shown in Figure 3, most of these metabolomic studies chose to detect metabolomic profiles on blood samples, especially plasma, using liquid chromatography combined with mass spectrometry or tandem mass spectrometry platforms.

## Quality assessment

The quality assessment results of QUADOMICS and Newcastle-Ottawa Scale (NOS) performed on 84 diagnosis-related studies are shown in Supplementary Table 2. Three independent progression-related studies were not performed quality assessment because they were not qualified for the assessment of QUADOMICS. According to the QUADOMICS results, the scores of 76 studies were higher than 7, and eight studies had scores below 8. According to the NOS results, the scores of 79 studies were higher than 6. Five studies had scores below 7, three of which belonged to low quality studies both in the QUADOMICS and NOS assessments. Combining the results of two tools, a subset of 74 diagnosis-related studies were deemed high-quality studies. The metabolites from these high-quality diagnosis-related studies and three independent progression-related studies were used for further analysis.

## Consistency of metabolites

As shown in Figure 4a, a total of 928 diagnosis-related metabolites were identified, with 370 metabolites that were exclusively increased, 442 metabolites that were exclusively decreased, and 116 metabolites that were identified with inconsistently changing trends in different studies. A total of 190 diagnosis-related metabolites were identified as replicated metabolites, with 60 exclusively increased, 54 exclusively decreased, and 76 inconsistently changed metabolites. All diagnosis-related metabolites are displayed in Supplementary Table 3, and the number of involved studies and bio-specimen of replicated diagnosis-related metabolites are given in Supplementary Table 8. Most of the replicated metabolites only appeared in two studies (Figure 4b). The ClassyFire classification indicated that the diagnosis-related metabolites of PD consisted of three inorganic compounds, including iodine, phosphate and potassium chloride, and 925 organic compounds. As shown in Figure 5a, these organic compounds included 499 lipids, 69 of which were replicable; 189 organic acids, 55 of which were replicable; 66 organoheterocyclic compounds, 25 of which were replicable; 63 organic oxygen compounds, 16 of which were replicable; 45 benzenoids, 10 of which were replicable; 28 organic nitrogen compounds, eight of which were replicable; 35 other small class metabolites and seven of which were replicable.

The progression-related metabolites of PD are shown in Supplementary Table 4. A total of 214 progression-related metabolites were identified, with 112 exclusively increased, 90 exclusively decreased and 12 inconsistently changed metabol-

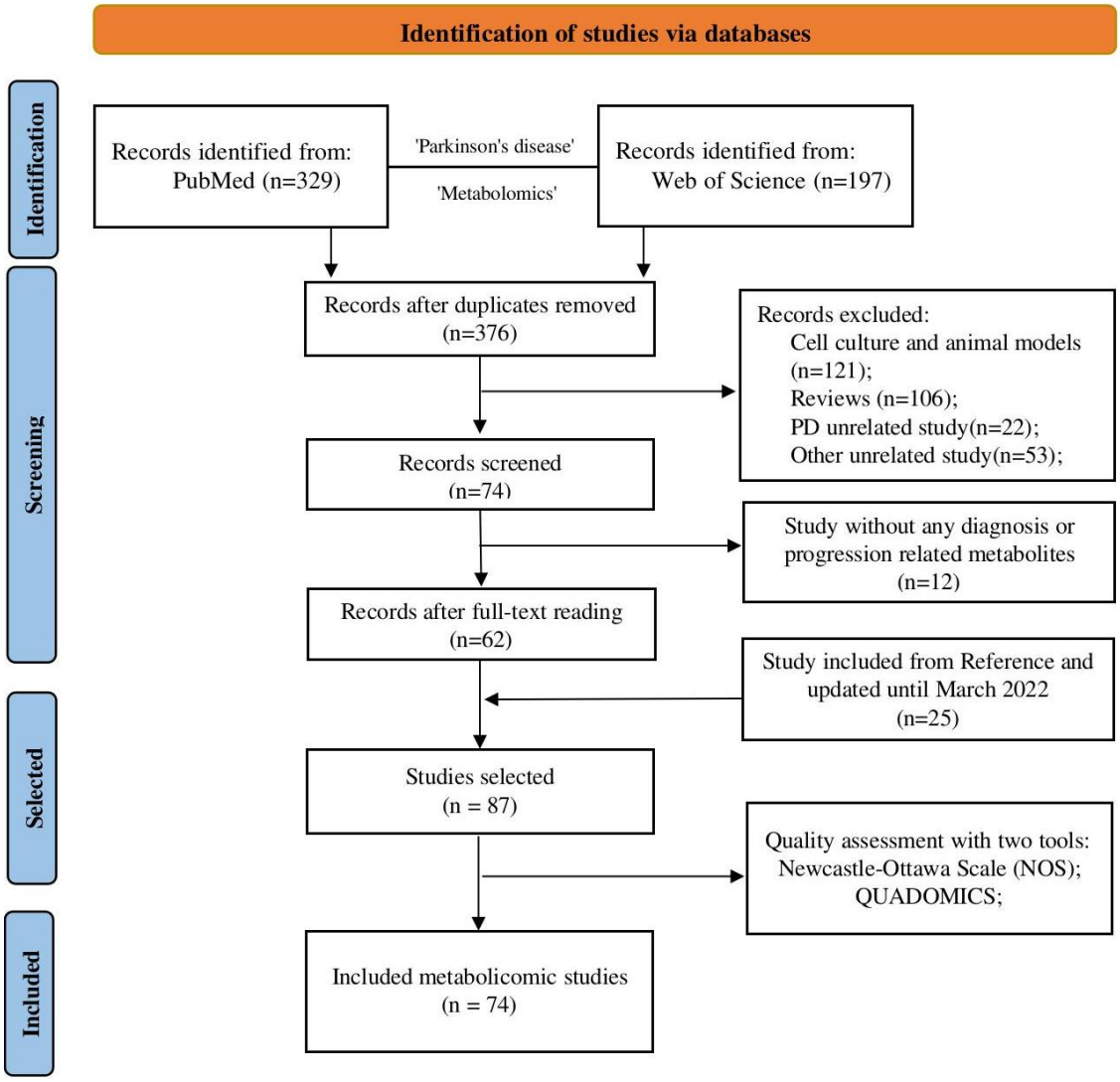


Figure 1: Study selection diagram. The target metabolomic studies of PD were selected from databases until March 2021 and updated until March 2022. In this study, the diagnosis-related metabolites were considered as the metabolites with different abundances between PD patients and asymptomatic controls; the progression-related metabolites were the metabolites associated with PD severity, motor score, disease duration, or different abundances in follow-up cohorts.

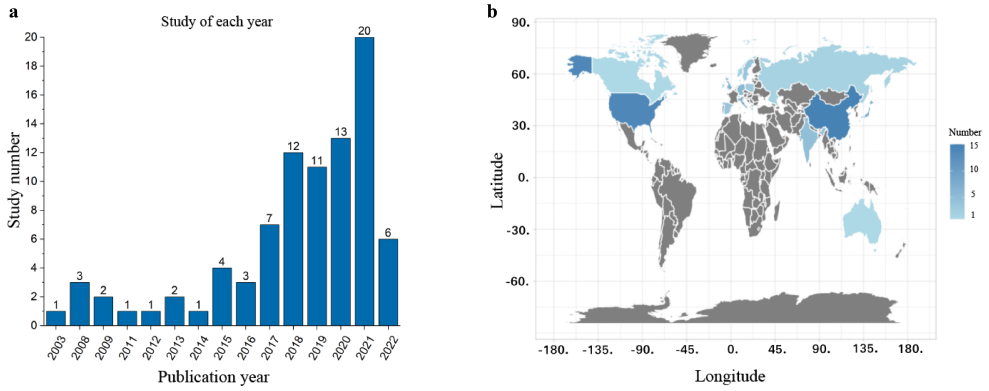


Figure 2: Study characteristics.
**a** The number of studies of each year. **b** The involved countries of PD clinical studies.

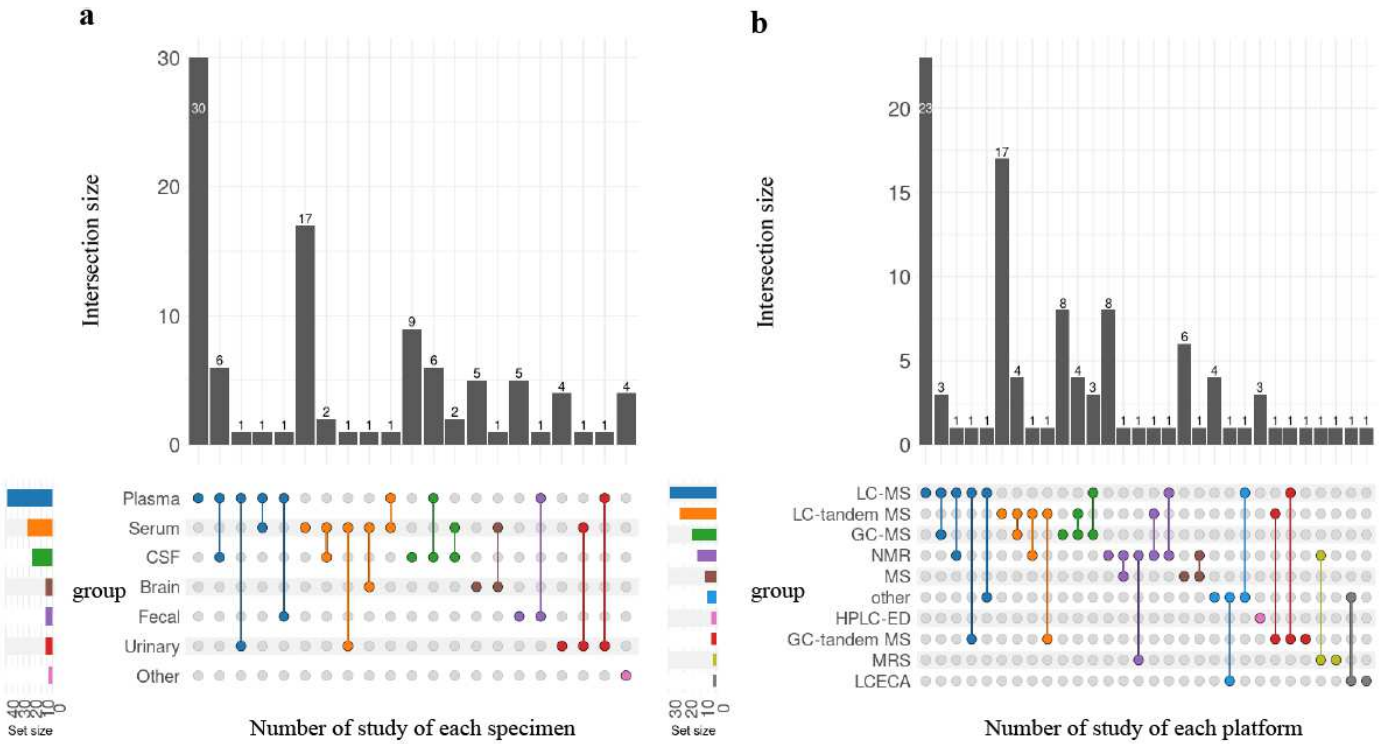


Figure 3: Detailed information on specimens or platforms.
**a** Number of studies of each specimen. **b** Number of studies of each platform. LC-MS: liquid chromatography combined with mass spectrometry; LC-tandem MS: liquid chromatography combined with tandem mass spectrometry; GS-MS: gas chromatography combined with mass spectrometry; GC-tandem MS: gas chromatography combined with tandem mass spectrometry; NMR: nuclear magnetic resonance; HPLC-ED: electrochemical detection coupled with high-performance liquid chromatography; LCECA: liquid chromatography electrochemical array; MRS: magnetic resonance spectroscopy; other: immunoassay, electrode array, fluorescence detection and enzyme test.

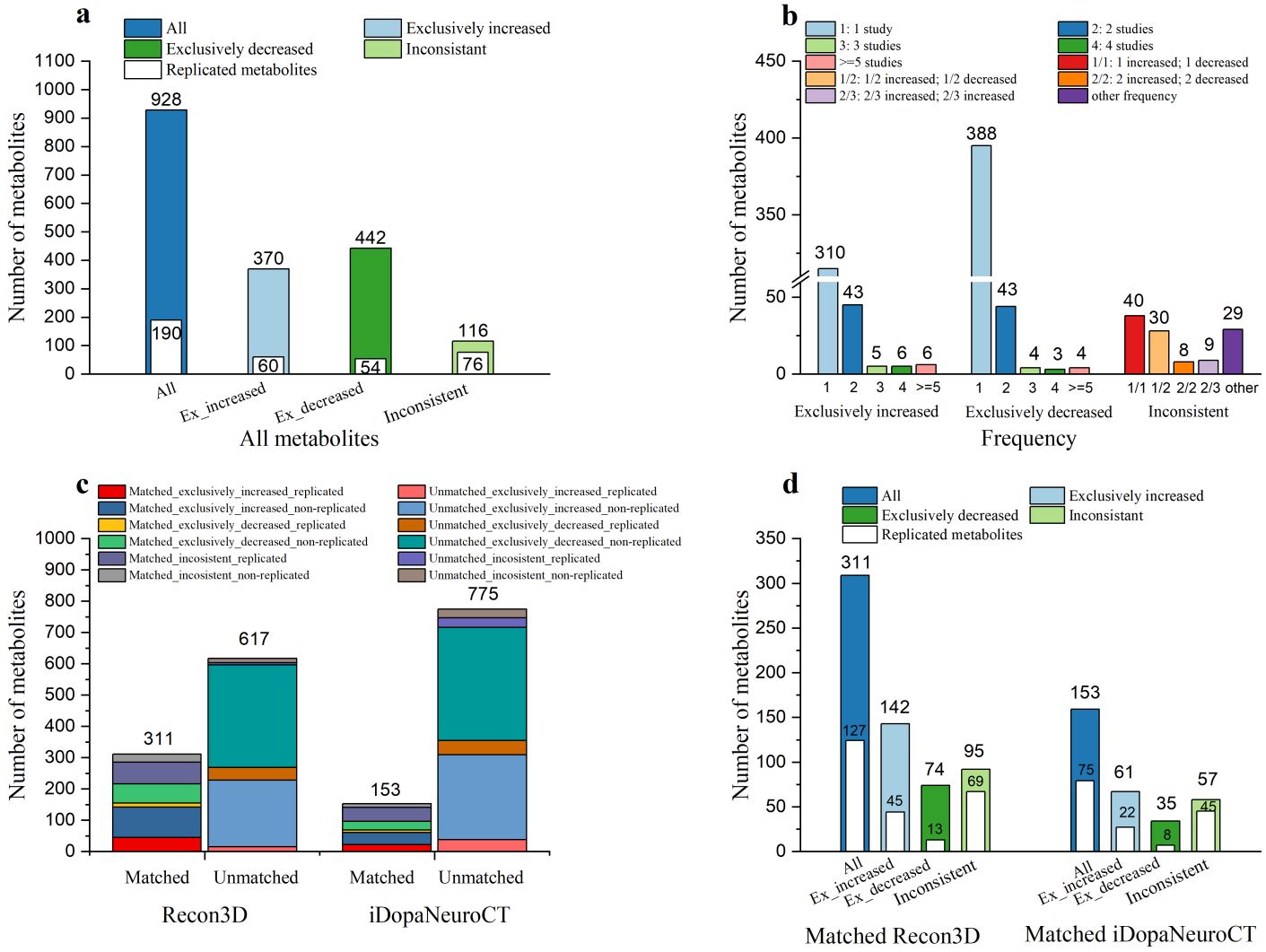


Figure 4: Diagnosis-related metabolites of PD.
**a** All diagnosis-related metabolites with changing trends. **b** The number of studies of each frequency in different changing trends. **c** Cross-matched results with Recon3D and the dopaminergic neuronal cell-type metabolism model (iDopaNeuroCT). **d** The number of studies of matched results in different changing trends.

ites. Only 14 metabolites were replicable, with eight exclusively increased, five exclusively decreased, and one inconsistently changed metabolites.

## Cross-matching with metabolic models

**Diagnosis-related metabolites** The results of matching diagnosis-related metabolites with two metabolic models are shown in Figure 4(c and d).

A total of 311 metabolites were matched with metabolites in Recon3D, with 142 metabolites were exclusively increased, 74 metabolites were exclusively decreased, 95 matched metabolites with inconsistent changes. A total of 127 matched metabolites were replicated, with 45 exclusively increased, 13 exclusively decreased, and 69 inconsistently changed metabolites. The classification results of matched and unmatched with Recon3D are shown in Figure 5b. A total of two inorganic compounds, iodine and phosphate, and 309 organic compounds can be matched. The matched organic compounds included 102 lipids, 32 of which were replicable; 96 organic acids, 47 of which were replicable; 34 organoheterocyclic compounds, 14 of which were replicable; 28 organic oxygen compounds, 13 of which were replicable; 20 benzenoids, 9 of which were replicable; 17 organic nitrogen compounds, 7 of which were replicable; 8 nucleosides and 4 phenylpropanoids. The matched results with the blood-brain barrier (BBB) metabolites shows that 34 of 43 BBB crossing metabolites and 13 of 240 BBB noncrossing metabolites could be matched. Of these matched metabolites, 27/34 BBB crossing metabolites were replicable and 3/13 noncrossing metabolites were replicable; the detail was shown in Supplementary Table 5. The remaining 617 metabolites could not be matched with Recon3D, with 228 exclusively increased, 368 exclusively decreased,

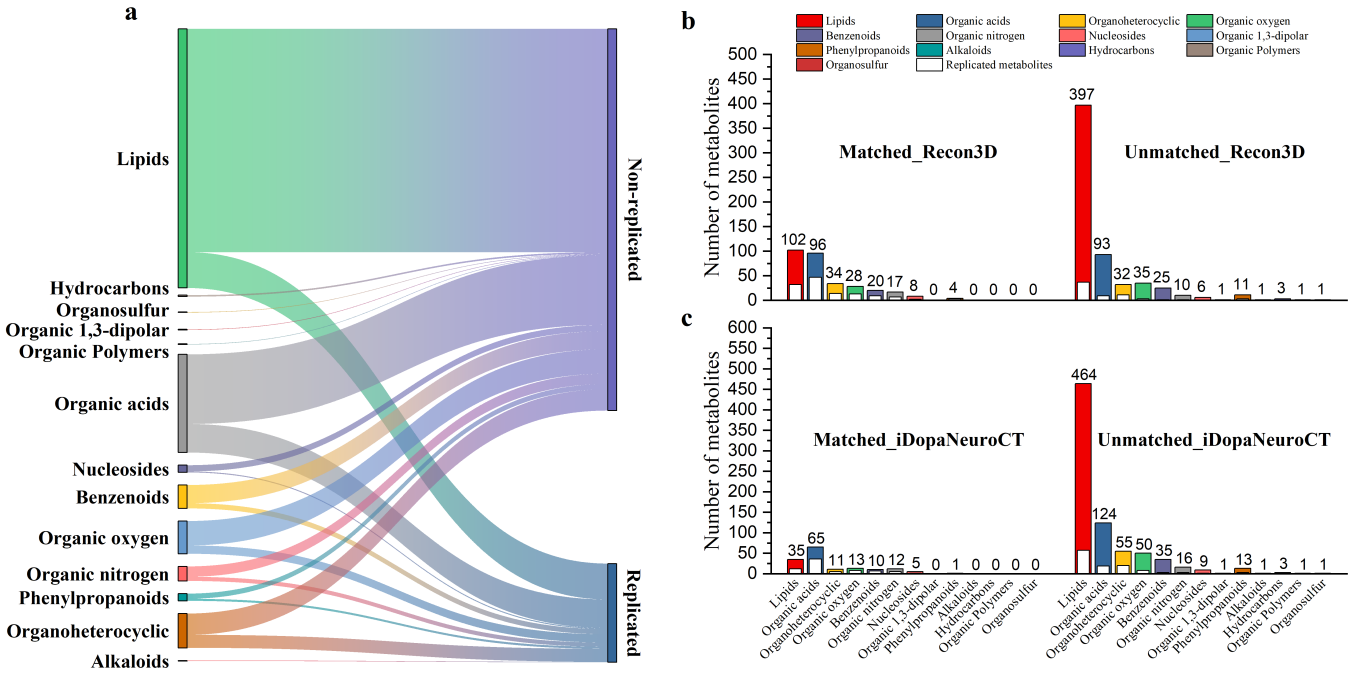


Figure 5: Organic compounds classification of PD diagnosis-related metabolites.
**a** An overview of all organic compounds of PD diagnosis-related metabolites. **b** The organic compounds classification of the matched results with Recon3D. **c** The organic compounds classification of the matched results with the dopaminergic neuronal model (iDopaNeuroCT).

and 21 inconsistently changed metabolites. Manual searching based on the unmatched metabolites indicated that of these 617 metabolites, 37 replicated and 26 non-replicated metabolites could be found within at least one metabolic reaction, as shown in Supplementary Figure 1a. However, only the reactions of caffeine metabolites can form a specific pathway of PD, which is caffeine metabolism.

Metabolites were cross-matched with the dopaminergic neuronal metabolic model (iDopaNeuroCT model), 153 metabolites were matched involving 65 organic acids and 35 lipids, with 61 exclusively increased, 35 exclusively decreased, and 57 inconsistently changed metabolites. A total of 75 matched metabolites were replicated metabolites, 22 of which were exclusively increased, 8 of which were exclusively decreased, and 45 replicated matched metabolites were inconsistently changed. All of these 153 metabolites could be found in the matched results with Recon3D.

**Progression-related metabolites:** As shown in Supplementary Figure 1b, only 11 progression-related replicated metabolites were matched with Recon3D and eight replicated metabolites were matched with the dopaminergic neuronal cell-type metabolic model (iDopaNeuroCT model). Since only a few progression-related metabolites were replicable, more progression-related metabolic studies of PD are needed. Therefore, further analysis was only based on diagnosis-related metabolites of PD.

## Literature synthesis

### Significant pathways of matched metabolites

Several dysfunctional pathways of PD were reviewed based on the classification of matched replicated metabolites, including lipid metabolism, amino acid metabolism, purine metabolism and organic oxygen metabolism.

**Lipid metabolism** Lipids are a large class of organic compounds that are insoluble in water. Evidence has established that lipids function as structural components, energy molecules and signal messengers in living cells [17]. Several replicated metabolites are lipids; these lipids played important roles in many aspects of the pathogenesis of PD, ranging from oligomerisation and aggregation of α-Synuclein (α-Syn) to mitochondrial dysfunction [18]. Below, we summarised several lipid pathways of PD, including fatty acid metabolism, sphingolipid pathway and bile acid metabolism.

**Fatty acid metabolism** Accumulating evidence indicates that fatty acid metabolism is dysfunctional in PD patients [19, 20, 21]. Monounsaturated fatty acids, such as oleic acid, and polyunsaturated fatty acids including linoleic acid,

arachidonic acid, and docosahexaenoic acid, are essential for maintaining neuronal membrane permeability and regulating brain inflammation [22]. In four studies, decreased levels of oleic acid in the blood of PD patients [20, 23, 21, 24] may reflect the reduced neuroprotective effects in PD patients. Increased levels of polyunsaturated fatty acids [25, 26, 27, 28, 29, 20, 30], especially arachidonic acid [20, 27, 28, 24, 29], were identified in the plasma and CSF of PD patients. The weaker C-H bond at the bis-allylic position makes these fatty acids vulnerable to lipid peroxidation, and newly generated deleterious lipid peroxidation end products further influence cellular functions by altering membrane structure and circulating lipids [31]. For example, arachidonic acid metabolism is strictly regulated in a healthy brain; therefore, any dysfunction resulting from neuroinflammation or oxidative stress may increase levels of arachidonic acid and form multiple pro-inflammatory metabolites in the brain, and further lead to neurodegenerative disorders [27, 28].

Evidence indicates that α-Syn may react with the lipids in membranes [22]. The structure of α-Syn can immediately change to a α-helical conformation in the presence of both arachidonic acid and docosahexaenoic acid, which accelerates the aggregation of α-Syn and the formation of the oligomers that are harmful to neurons [22]. In addition, it was reported that α-Syn could connect with lipids to form lipid-protein conjugates to cross the blood-brain barrier or transport in the blood [19].

Dysfunctional mitochondria may also affect fatty acid metabolism. One of the reasons for mitochondrial dysfunction is impaired mitochondrial membrane fluidity caused by increased oxidative stress and reduced ATP levels [32]. Dysfunctional mitochondria may lead to neuronal degeneration in PD patients through impairment of the mitochondrial carnitine transport system and the respiratory chain [33, 34]. The increase of these acyl-carnitine metabolites [30, 20, 35, 36] in PD patients may result from the defects in the carnitine transport system [37, 38]. Fatty acid metabolism dysregulation may be directly related to the carnitine metabolic abnormalities in PD patients [34]. For example, long-chain fatty acids (LCFAs) are required to transport into the mitochondrial matrix; this transport is carnitine-dependent and involves active translocation machinery [37]. Therefore, the accumulation of long-chain fatty acids in the blood of PD patients, such as hexadecanoic acid and octadecanoic acid [37, 30, 39], may result from decreased fatty acid beta-oxidation due to the dysfunctional mitochondria. However, the lower levels of such fatty acids in the blood of PD patients may relate to dysfunctional energy metabolism, particularly components of the pentose phosphate pathway [24, 20]. Besides, in a PD cohort in Sweden, reduced levels of several long-chain fatty acids in CSF were identified in the PD patients with GBA mutations, which may indicate these lipids are related to GBA mutations since there is an increased rate of Gaucher's disease in northern Sweden [23].

The metabolism of short-chain fatty acids (SCFAs), such as valeric acid [34, 40, 41] and butyric acid [39, 42, 40, 41], has been reported to be changed in the plasma and faeces of PD patients. SCFAs are the primary metabolites produced from dietary fibre by the fermentation of gut bacteria; they have part of the effects on maintaining intestinal barrier integrity and gut mucosal immunity [40]. Butyric acid was identified with increased levels in the plasma but decreased levels in the faecal of PD patients [40], which may associate with gut microbiota changes in PD. The decreased levels of SCFAs in the faeces, especially butyric acid, may cause non-motor symptoms of PD, such as a leaky gut and increased intestinal inflammation, and further aggravate the PD disease process [43]. Besides, SCFAs, also involved in the gut-brain crosstalk, could regulate the expression of tight junction proteins and have remote effects on neurons through stimulation of G protein-coupled receptors, may associate with the clinical severity of PD [40, 44].

**Sphingolipid pathway** Perturbations of sphingolipid pathways have been identified in several studies of PD [25, 26, 45, 46, 47]. Sphingolipids are a kind of lipid containing a backbone of a sphingoid bases and aliphatic amino alcohols, including ceramides, sphingomyelins, gangliosides, and cerebrosides; they are an integral part of cell signaling and regulation [19]. It has been reported that the perturbation of the sphingolipid pathway was an actual neurotoxic process following the aggregation of α-Syn [48, 25]. The misfolding and accumulation of α-Syn may disrupt ceramide-sphingolipid homeostasis in the endoplasmic reticulum [49].

Sphingomyelin (SM) is a major myelin component that contains acyl chains with fatty acids; it is one of the constituents of the cellular membrane, and plays a role in inflammation, autophagy and cell death [45, 47]. Several sphingomyelin molecules have been identified with increased [19, 38, 45, 50] or decreased levels [25, 26, 51, 49, 38, 47, 52] in the CSF and plasma of PD patients. The accumulated sphingomyelin in the CSF of PD patients may associate with neurodegeneration in the brain [45]. Besides, the genetic variants of PD, especially SMPD1, a sphingomyelin phosphodiesterase encoding gene, which results in sphingomyelin accumulation, have been identified as associated with increased SM 26:0 blood levels [53]. However, three studies reported decreased levels of SM 26:0 in the CSF [38] or plasma [47, 46] of PD patients, so the potential role of SM 26:0 in the occurrence and development of PD needs to be further elucidated. Moreover, sphingomyelin could be hydrolyzed by sphingomyelinase to produce phosphocholine and ceramide [54]. Therefore, up-regulated sphingomyelinase expression and activity, may result in a reduction in sphingomyelin and an increase in ceramide [55]. Elevated levels of ceramides may lead to neuronal apoptosis and astrocyte activation, playing a pro-inflammatory role [45].

Glycosphingolipids, one of the products in the sphingolipid pathway, have been shown to regulate fundamental cell properties and biological functions such as cell adhesion, cell growth, cell proliferation, autophagy, apoptosis and senescence [56]. Due to the bioactive role in cell membranes, glycosphingolipids can regulate blood-brain barrier permeability

through the ordered regions in biological membranes, such as lipid rafts, which can serve as a potent inflammatory process regulator [22]. The decreased levels of glycosphingolipids in the plasma of PD patients [25, 26] may reflect increased neuroinflammation.

**Bile acid metabolism** Bile acids are mainly synthesised from cholesterol in the liver, and participate in cholesterol homeostasis [34]. These lipids play critical roles in the digestion and absorption of other lipids in the small intestine; they are closely associated with intestinal hormones, microbiota, and energy balance [38]. The increase in the level of unconjugated bile acids in the plasma of PD patients [9, 34, 57] may reflect the increased bacterial degradation of conjugated bile acids, which may cause irreversible damage to the nervous system through the gut-brain axis, or reflect the decreased removal efficiency of unconjugated bile acids from the peripheral circulation [58]. Besides, the increased levels of secondary bile acids in the plasma of PD patients, such as lithocholic acid and deoxycholic acid [59, 30], may cause pro-inflammatory and direct cytotoxic effects through affecting the gut-brain axis, and accelerate the aggregation of pathological α-Syn. Moreover, ursodeoxycholic acid and its derivatives, such as tauroursodeoxycholic acid and glycoursodeoxycholic acid, have been reported with lower levels in PD animal models [60, 61, 62]; increase their levels could rescue mitochondrial dysfunction and prevent the losses of striatal dopamine neurons [61]. However, only one study reported decreased levels of glycoursodeoxycholic acid in the plasma of PD patients [34], while another study reported increased levels of tauroursodeoxycholic acid in the plasma of PD patients [26]. Therefore, more studies are needed to characterise the changes of ursodeoxycholic acid.

**Amino acid metabolism** Numerous amino acids significantly changed in different biospecimens of PD patients, and the most commonly changed amino acids are discussed.

**Ornithine metabolism** Significantly increased levels of ornithine were observed in ten PD studies with different biofluids, including blood, urine and CSF [20, 63, 47, 42, 64, 65, 66, 52, 46, 67]. Ornithine is derived from glutamine, synthesised from arginine and involved in the formation of urea [54]. Increased levels of ornithine may relate to the increased levels of urea [37, 68] through the urea cycle, and cause hyperosmolarity in multiple brain regions [67]. However, dysfunctional mitochondria may have an effect on the urea cycle, resulting in the decreased urea levels [69, 70, 71, 63] and citrulline levels [72, 65, 73] in PD patients. Besides, proline metabolism is also related to the changes in ornithine and glutamate; the increased levels of proline were identified in the blood and urine in six studies [63, 35, 42, 65, 51, 72]. The involved enzymes in proline metabolism, such as P5C reductases (PYCRs) and nicotinamide adenine dinucleotide (NAD)-dependent P5C dehydrogenase (P5CDH), have been reported to play important roles in neuronal function and brain structure, and to be associated with neurodegeneration disorders in several human and animal models [74].

Besides, the dysfunctional metabolism of ornithine may indicate the dysfunction of nitrogen-metabolizing pathways and polyamine synthesis in PD patients [65]. Polyamines, including putrescine, spermidine, N1-acetylspermidine and spermine, participate in various biological processes such as cell proliferation, differentiation and division, and could act as anti-oxidant agents [75]. Increased metabolism of polyamines has been proposed to be due to high levels of oxidative stress in PD patients. Ornithine can be converted to putrescine by ornithine decarboxylase, and further affect the metabolism of polyamines in PD patients [76]. The increased ornithine may result in an increased level of putrescine [67, 38] and spermidine [58, 33, 67]. Moreover, the dysfunctional ornithine may also reflect impairment of small bowel movement function or gastroparesis, which may relate to nonmotor symptoms of PD, including constipation and dysphagia [42].

**Glutamine metabolism** Glutamine is the most abundant free amino acid; it has a critical role in mitochondria energy production, DNA damage response, apoptosis, and autophagy [77]. Evidence indicates that glutamine can maintain the supply of neurotransmitter glutamate and gamma-aminobutyric acid (GABA) through the glutamine-glutamate-GABA metabolic cycle in the brain [78, 79]. In PD patients, eight studies reported the increased levels of glutamine in the blood, urine and CSF [20, 79, 38, 63, 47, 26, 80, 42], while one reported decreased levels of glutamine in the CSF. Six studies identified decreased levels of glutamate in the blood and faeces [20, 81, 80, 82, 83, 84], while one identified increased levels of glutamate in the brain tissue of PD patients. Glutamate and GABA are the major excitatory and inhibitory neurotransmitters expressed in the brain. The released glutamate and GABA are taken up by astrocytes and quickly aminated to glutamine, then transported to the extracellular space and taken up by glutamatergic and GABAergic neurons again to generate glutamate and GABA [85]. The altered levels of glutamine and glutamate in the brain may act as a protection mechanism for neurons from glutamate excitotoxic injury after striatal dopamine depletion [79]. However, the increased levels of GABA were only found in the plasma, urine and saliva [81, 35, 33, 86]. Normally, GABA is generated from glutamate by the enzyme glutamic acid decarboxylase (GAD) in the neuron, but this enzyme is also found in the insulin-secreting β cells in the pancreatic islets and further activating GABA receptors on the endocrine cells or immune cells [87]]. Therefore, the increased levels of GABA in peripheral circulation may relate to inflammatory cell activation and immune dysregulation in PD patients [88].

Since the glutamate in the gut normally comes from the degradation of dietary proteins and free glutamate contained in food additives, the reduced faecal levels of glutamate may indicate dietary changes in PD patients or gut microbiota-host interactions [83]. Besides, glutamate is a precursor of glutathione (gamma-glutamyl-cysteinyl-glycine). Glutathione plays an important role in antioxidant activity and maintains redox homeostasis in neurons [89]. It is common to find GSH depletion in patients with neurodegenerative diseases, therefore, a reduction of glutamate may reflect increased oxidative stress in the progression of neurological disorders [84].

**Glycine metabolism** Six studies reported increased levels of glycine in the plasma, urine and CSF of PD patients [20, 33, 35, 86, 81, 47]. As one of the inhibitory neurotransmitter amino acids [81], glycine has been hypothesised to modulate the release of dopamine and glutamate [35, 90]. Higher levels of glycine may indicate a neurotransmitter imbalance between dopaminergic and muscarinic cholinergic neurons [86], and dopamine could induce glycine release from astrocytes to regulate neuronal excitability by reversing the function of astrocytic glycine transporter [90]. Besides, glycine was associated with an anti-oxidant effect against neurodegeneration; the increased levels of glycine and may suggest higher oxidative stress in PD [47]. Moreover, glycine is involved in the synthesis of glutathione when combined with cysteine and glutamate, increased glycine, glutamate and cysteine levels [20, 63] may indicate the reduction of glutathione synthesis in PD [86]. Furthermore, glycine derivatives, such as hexanoylglycine [35, 33, 30], phenylacetylglycine [35, 33], tiglylglycine [35, 33], furoylglycine [35, 33], are fatty acid oxidation products. Therefore, the increased levels of such glycine derivatives may associate with dysfunctional fatty acid beta-oxidation in the mitochondria of PD patients [35].

**Tryptophan metabolism** Four studies reported decreased levels of tryptophan in the blood, faeces and CSF of PD patients [91, 82, 23, 92], while one study reported that the level of urinary tryptophan was significantly increased in early-stage PD patients [35]. Tryptophan was reported to be involved in the generation of NAD+ and can induce increased oxidative stress in preclinical PD studies [23]. Dysfunctional tryptophan metabolism is related to mitochondrial disturbances and the impairment of brain energy metabolism, which may further contribute to the psychiatric symptoms of PD [93, 35]. The changes in tryptophan may result from the increased degradation and urine excretion of tryptophan, and further result in the increased levels of tryptophan catabolites, including kynurenine [35, 47, 33].

Several studies have indicated that the imbalance of kynurenine metabolism plays an essential role in PD pathogenesis [47, 73, 94]. Kynurenine is a downstream metabolite of tryptophan, which can be further converted to 3-hydroxykynurenine or kynurenic acid [91]. Increased levels of kynurenic acid [91] and 3-hydroxykynurenine [73, 94] have been identified in the blood of PD patients, indicating increased kynurenine degradation. 3-hydroxykynurenine is a neurotoxic compound that causes neuronal death [73], so that it could be a potential excitotoxic mechanism in PD. Besides, based on the unmatched metabolites of PD, the products of kynurenine, 3-hydroxy-l-kynurenine [66] and aminobenzoic acid [35], were identified with increased levels, while the other products of tryptophan, indoleacetylglutamine [30] and indolelactic acid [51], were identified with decreased levels, which further emphasized the dysfunction of the kynurenine metabolism from tryptophan. However, a few studies showed a lower level of kynurenine in the blood of PD patients, which may be associated with the excessive kynurenine released in urine [91, 51, 82]. Besides, tryptophan can also be degraded to 5-hydroxytryptophan by tryptophan hydroxylase [8]. Both 5-hydroxytryptophan and acetyl-serotonin are involved in serotonin pathways, which synthesize the monoamine neurotransmitters serotonin and melatonin [33].

**Tyrosine metabolism** Perturbation of the phenylalanine/tyrosine/levodopa pathway has been identified in several PD metabolic studies. Tyrosine is derived from phenylalanine and converted to levodopa through phenylalanine hydroxylase and tyrosine hydroxylase, and then synthesised dopamine by aromatic amino acid decarboxylase [42]. Ten studies identified increased levels of tyrosine in the blood, saliva, urine, faecal and CSF of PD patients [20, 51, 80, 42, 35, 86, 47, 95, 66, 67], while three studies identified decreased levels of tyrosine in the faeces and plasma of PD patients [83, 96, 92]. Eight studies reported increased levels of phenylalanine in the blood, saliva and urine of PD patients [80, 97, 42, 35, 86, 52, 34], while three studies reported decreased levels of phenylalanine in the faeces and serum of PD patients [82, 20, 92]. Both phenylalanine and tyrosine are precursors of dopamine [35]. Since phenylalanine and tyrosine can cross the blood-brain barrier, the increased levels of phenylalanine and tyrosine may indicate impairment in dopamine synthesis [42], reflecting dopaminergic cells of the PD brain attempt to increase the dopamine synthesis due to dopaminergic neuron depletion [67], resulting in increased lateral metabolism to tyramine [19, 95]. Besides, increased levels of levodopa [8] and its metabolite, 3-methoxytyrosine [51, 97, 30, 68, 64, 20, 24] and dopamine [47, 38], were affected by antiparkinsonian medications, such as levodopa [51]. However, only one study showed significantly changed levels of tyrosine in the cerebrospinal fluid of patients with PD [67], which may result from the interaction between drugs and such amino acids. Therefore, more comprehensive studies of antiparkinsonian drugs are needed to explore the association with amino acids in the brain of PD patients.

**Other significant amino acids** Elevated levels of alanine were observed in the plasma and CSF of PD patients in five clinical studies of PD [79, 63, 23, 35, 86]. Alanine metabolism may be an alternative pathway to compensate for an inadequate energy supply [79]. Alanine can be synthesised from protein breakdown under fasting states, then transformed

into pyruvate to synthesise glucose through gluconeogenesis in the liver [86]. The increased levels of alanine in PD patients may result from the dysfunction of the glucose-alanine cycle [63], and affect the metabolism of Cori cycle [23, 96]. The glucose-alanine cycle and the Cori cycle are crucial to gluconeogenesis, could increase glucose bioavailability through gluconeogenesis in PD patients [96]. Besides, alanine may be released by neurons and taken up by astrocytes; it could act as a nitrogen carrier from neurons to astrocytes, further affecting other amino acids metabolism [79]. However, three studies identified the decreased levels of alanine in the plasma and CSF of PD patients [30, 98, 96], two of which involved genetic PD patients, including PARK2 [30] and LRRK2 [96] PD patients. Further studies are needed to explore the specific metabolomic pattern in genetic PD patients.

Increased levels of the branched-chain amino acids, such as histidine [33, 38, 79, 63, 86] and isoleucine [21, 33, 38, 79, 86, 42, 35, 63] were identified in several studies of PD. Branched-chain amino acids play an important role in synthesising neurotransmitters and maintaining the balance of nitrogen between astrocytes and neurons. Increased levels of histidine were related to neuronal damage in PD since it plays an integral part in neuronal transmission [79]. Metabolism of isoleucine was important in energy production and synthesis of the neurotransmitter glutamate, acetyl-CoA and proteins [79, 42]. Increased levels of isoleucine may relate to the motor symptoms of PD patients through the increased synthesis of excitatory neurotransmitters [35].

Four studies indicated decreased levels of pipecolic acid in the blood and CSF of PD patients [99, 73, 94, 24]. The decreased levels of pipecolic acid may associate with peroxisomal dysfunction, and influence several metabolic pathways, including reactive oxygen species (ROS) metabolism, fatty acid oxidation, and bile acid synthesis [100]. It has been reported that pipecolic acid could act as a neurotransmitter to modulate gamma-aminobutyric acid transmission, and could be taken up by cerebral mitochondria and induce neuronal apoptosis [101]. Besides, pipecolic acid is also involved in normal brain functions through the gut-brain axis [102]. Therefore, decreased levels of pipecolic acid may indicate the dysfunction of the gut-brain axis [24]. Besides, the increased levels of trimethylamine N-oxide (TMAO) [103, 35, 33, 86, 104] and p-Cresol sulfate [26, 51, 105, 20, 27, 28] were identified in the blood, urine or CSF in PD patients; these metabolites were generated by gut microbiota, which may relate to the progression of neurodegenerative disorders by increasing oxidative stress and neuroinflammation in the brain through the gut-brain axis [103, 106].

**Purine metabolism** Metabolites involved in purine metabolism were identified with significant changes in different PD studies, including hypoxanthine, xanthine and uric acid. Hypoxanthine [57, 35, 33] and xanthine [57, 30, 93] are precursors of uric acid, and were identified with increased levels when the uric acid level was decreased in PD patients [93, 107]. It was reported that uric acid is an endogenous antioxidant and end product of purine metabolism [107, 108], playing a role in protecting DNA from single-strand breaks caused by free radicals in alleviating oxidative damage in neurodegenerative diseases [109]. Decreased levels of uric acid in the blood of PD patients may reflect an antioxidant defence process in PD patients, indicating an increasing trend with disease progression [109].

Perturbed xanthine concentrations in brain tissues and CSF of PD patients may lead to several physiological influences, including hypoxia, ischaemia, excitotoxicity, and neurodegenerative inflammation [110]. Increased levels of xanthine in PD may result from the chronic impairment of mitochondrial function, leading to oxidative stress and energy metabolism dysfunction in PD patients. Meanwhile, oxidative stress could enhance the release and degradation of adenosine and ATP, which may result in increased levels of xanthine [110]. Dopamine depletion in the brain of PD patients might also influence purine metabolism, resulting in increased levels of hypoxanthine and xanthine [111]. These findings suggest a potential role of the purine pathways in the occurrence and development of PD [111, 93]. Besides, it has been reported that individuals consuming diets that increase serum uric acid have a lower risk of PD [112]; this association need to be further investigated. The inconsistent changes of purine metabolites may reflect the combined effects of endogenous and dietary factors [93, 38].

**Organic oxygen metabolism** Several organic oxygen metabolites showed consistent changes across different metabolic studies. The decreased levels of pantothenic acid in PD patients have been found in the plasma, serum, faecal and brain tissues of PD patients [51, 64, 73, 21, 113]. Pantothenic acid could be produced by gut bacteria, and result in protective responses such as anti-inflammatory and antioxidant activity [21]. Decreased pantothenic acid may weaken protective responses from neurodegenerative inflammation and oxidative stress through the brain-gut axis. However, metabolic modelling of the human gut microbiome predicted increased secretion of pantothenic acid by microbiota in the human gut [114], which is the converse of the changes in clinical studies of PD. Besides, pantothenic acid is a necessary precursor for coenzyme A (CoA) synthesis. CoA is a carbon transporter, required for pyruvate to be converted to acetyl-CoA before the citric acid cycle [21]. Therefore, the decreased levels of pantothenic acid may relate to the dysfunction of glycolysis and glucose metabolism through reduced production of CoA in PD patients [113].

Energy dysfunction is highly related to the alterations in glucose metabolism in the pathogenesis of PD [115]. Increased levels of glucose [79, 71, 96] were identified in the blood and CSF of PD patients in three studies, while decreased levels [64, 94] were identified in the plasma and CSF of PD patients in two studies. The energy requirements for metabolism in the brain are mainly glucose-dependent using NAD+, while cellular NAD+ level declines during the process of ageing [116], resulting in the decreased glucose utilization. Besides, increased glucose levels in blood are highly connected with

type 2 diabetes mellitus (T2DM) in PD patients, where the dysfunctional production and secretion of insulin may be caused by dysautonomia [117]. Moreover, the treatment with dopaminergic medication, such as levodopa, may relate to glucose tolerance, hyperglycaemia and hyperinsulinaemia but this association still remains controversial [117, 118].

The changed glucose levels may also result from the influence of food intake or gluconeogenesis [86]. Perturbations in glucose metabolism have been further identified through several significant metabolites in PD, including mannitol, glucitol, lactate, acetone, and pyruvate. The dysfunctional glucose levels may relate to the increased [119, 35] or decreased [63, 83] pyruvate levels through glycolysis, and the increased [79, 20, 38] or decreased [96] levels of lactate through the Cori cycle. Besides, the increased levels of mannitol [64, 63] and glucitol [119, 63] may imply the dysfunction of polyol metabolic pathways. The polyol pathway is a minor metabolic pathway of glucose running parallel to glycolysis, whose activity is altered in mitochondrial dysfunction [119]. Moreover, four studies found increased levels of acetone in serum, CSF and brain tissue of PD patients [80, 79, 38, 52]. Acetone is a product formed from acetoacetic acid during fatty acid beta-oxidation [79]. The brain could increase lipid metabolism using the fatty acid beta-oxidation pathway for producing energy due to the dysfunctional glucose metabolism in PD [80]. Furthermore, the increased levels of acetone may lead to spontaneous neurotransmitter release, and death of the surrounding neurons caused by excitotoxicity [80].

### Significant pathways of unmatched metabolites

**Caffeine metabolism** Based on the unmapped replicated metabolites, caffeine metabolism was identified as dysfunctional in several studies. Caffeine is a natural chemical compound with stimulant effects [120]. It is contained in coffee, tea, and cocoa, and is widely used in various energy drinks and medical products. Once the caffeine has been ingested, it is quickly absorbed by the gastrointestinal tract, and the blood concentrations of caffeine are highest 30 minutes after ingestion [121]. A similar trend in caffeine concentration is found in the brain, which means that caffeine can be transferred from blood to the brain through the rapid crossing of the blood-brain barrier due to its hydrophobic characteristics [120]. Evidence indicates that caffeine may act against oxidative damage caused by reactive oxygen or nitrogen species through a purinergic signalling pathway [122, 123, 124].

Several studies have reported decreased levels of caffeine and its downstream metabolites [30, 64, 108, 125, 123] in the blood of PD patients. One study [108] indicated that reduced levels of caffeine in PD patients may partly result from PD patients consuming significantly less caffeine compared to healthy controls. However, in other studies [64, 73, 111, 123], the lower levels of caffeine and its decomposition products in PD patients were identified with no association with caffeine consumption in participants after adjusting for coffee intake; this may indicate that endogenously perturbed caffeine metabolism in PD patients is unrelated to exogenous caffeine intake [64, 111].

Caffeine is a known antagonist of adenosine A2A receptors[123]. Adenosine A2A receptors are highly expressed in the basal ganglia and the limbic brain region, which tightly interacts structurally and functionally with the dopamine D2 receptor to modulate the function of movement [126]. The activity of Adenosine A2A receptors may associate with pathogenic processes in neurodegenerative illnesses, such as excessive glutamate release, the aggregation of toxic protein species, activation of the indirect pathway of the cortico-striato-thalamo-cortical loop and disrupting the redox homeostasis [127, 124]. The effects of caffeine in the substantia nigra could be competitive inhibition with adenosine binding to adenosine A2A receptors. The blockade of adenosine A2A receptors by caffeine could modify motor function by changing the metabolism of glutamate and dopamine, and decreasing the activation of the indirect pathway [125]. Besides, caffeine may alleviate dopaminergic neuronal degeneration by preventing adenosine-mediated neuroinflammatory actions [123].

Moreover, the Cytochrome P450 enzymes, such as Cytochrome P450 Family 1 Subfamily A Member 2 (CYP1A2) and Cytochrome P450 Family 1 Subfamily E Member 1 (CYP2E1), are involved in the caffeine metabolic pathway, which can affect the efficiency of caffeine metabolism [30]. However, the prevalence of single-nucleotide polymorphism of CYP1A2 and CYP2E1 has no difference between PARK2 patients and control subjects [30]. Besides, it was difficult to exclude the possible influence of drug therapy, such as levodopa or other antiparkinson drugs that influence the collateral hypermetabolism of caffeine [125]. More independent studies are needed to explore the therapeutic potential of caffeine for PD treatment.

**Other replicated metabolites** Except for lipids, other unmatched diagnosis-related metabolites indicated that the metabolic changes of PD were also involved in drug metabolism, food metabolism, protein degradation, and gut microbiota metabolism in PD patients.

Four metabolites, including trigonelline, catechol sulfate, pyroglutamine and homovanillate sulfate, were identified as replicable in different clinical studies of PD. Trigonelline or N-methyl nicotinic acid, a non-purine constituent in coffee that serves to produce specific aroma compounds, was identified with lower levels of concentration in the plasma of PD [51, 30, 68, 108]. The decreased levels of trigonelline indicated that there was a difference in coffee intake between PD patients and healthy controls. It was reported that trigonelline could be partially transformed into nicotinic acid during the roasting process, form into vitamins and be involved in human metabolism [128]. Besides, the pharmacological activities of trigonelline have been explored in central nervous system disease, especially its neuroprotective effects [129, 130]. The biochemical reactions of trigonelline in humans need to be further explored. Catechol sulfate is an end product of the

metabolism of benzoate metabolism [131]. The decreased levels of catechol sulfate may be associated with abnormal gut microbiota in PD patients [30, 64, 131]; it has been reported to involve the combined activity of gut microbial metabolism and liver and kidney functions such as constipation and dysphagia [64]. The pathway of catechol sulfate is currently unclear.

Pyroglutamine, a cyclic derivative of glutamine involving glutamine metabolism [132], belongs to the class of organic compounds known as alpha amino acids and derivatives. Two studies reported increased levels of pyroglutamine in the blood of PD patients [64, 20], which may relate to the dysfunctional glutamine metabolism of PD. Besides, the research on the association between antihypertensive and lipid-lowering drugs with human metabolism indicated that the increased levels of pyroglutamine were positively associated with the concentration of beta-blockers, but negatively associated with fibre intake [133]. More research on this metabolite is needed to explore its physiological role. Increased levels of homovanillate sulfate were reported in two studies [30, 64]. Homovanillate sulfate or homovanillic acid sulfate, a primary metabolite of catecholamine metabolism, is located downstream of the levodopa metabolite, which was associated with levodopa intake and affected by the metabolism of levodopa medication [64]. Besides, some phenolic acids, such as caffeic acid, and vanillic acid, are found in the outer bran layer of wheat grains [134]. The increased levels of homovanillate sulfate may associate with the metabolic conversion of wheat grains dietary by microbial enzymes or endogenous enzymes in the liver [134].

### PD model

The new global model, ReconX, contained 4,213 metabolites and 13,950 reactions, including all reactions from Recon3D, 277 new reactions from Human1, 14 reactions from a dopaminergic neuronal model, 73 fatty acid oxidation reactions and 43 caffeine metabolism reactions, with all the additional metabolites and reactions listed in Supplementary Table 6 and 7. Then, the de-compartmentalized thermodynamically flux-consistent global subset was generated, which contained 2,828 metabolites and 5,602 reactions. A total of 137 replicated metabolites were used as core metabolites, 8 of which were excluded from the global subset as they did not correspond to any thermodynamically consistent network flux. These excluded metabolites belonged to gut microbiota metabolites, such as trimethylamine N-oxide (TMAO), p-Cresol sulfate, p-Cresol, glutaric acid and indoxyl sulfate.

An ensemble of models was generated containing 1,689 shared reactions (except for newly added exchange reactions) and 129 core metabolites. The heat maps for overlapped metabolites, reactions and genes between the randomly generated 10 models are shown in Supplementary Figure 2; the overlapped proportion for metabolites, reactions and genes between each pair of models can reach ˜90%. Then, the genome-scale metabolic PD model was extracted using the shared reactions, which contained 1689 reactions, 910 metabolites, and 808 metabolic genes, involving 82 subsystems. We ordered the subsystems according to the proportion of the core metabolites out of all 137 core metabolites contained in each subsystem, and the top 20 subsystems are shown in Figure 6. Most of the significant pathways related to the replicated metabolites were visualised in a planar map ignoring the exchange reactions, as shown in the Supplementary Figure 3.

Since most of the metabolites were performed with inconsistent changing trends, the pathways with consistently changed metabolites were identified through the map and highlighted in the Figure 7, including dopamine metabolism (tyrosine metabolism), polyanime metabolism (urea cycle), steroid metabolism and caffeine metabolism.

## Discussion

### Study assessment

Of 84 studies reviewed in total, 10 were omitted from further consideration as they scored below thresholds following externally specified quality control assessments, specifically QUADOMICS [135, 136] and the Newcastle-Ottawa Scale [137]. In the QUADOMICS results, most below-threshold studies did not provide a detailed description of sample inclusion and exclusion criteria; they failed to control potential factors affecting the metabolomic profiles during sample collection or they were unable to perform validation in an independent cohort. In the Newcastle-Ottawa Scale results, below-threshold studies were undermined by sample selection bias, such as hospital admission rate bias, failure to describe the source of control samples, or failure to control additional factors besides age and sex between cases and controls.

### Consistency and inconsistency

Metabolites reported to be changing in PD were matched with the content of an established computational model of human metabolism [138]. However, it was difficult to match many of the reported lipids due to differences between the level of resolution and annotation of lipids in the human metabolic model and in the lipidomics data [139]. For example, triglycerides (TGs) are a lipid class that is composed of a large number of lipid molecules with different structures. For lipidomic data, classes such as TG(52:2), or species such as TG(17:0_17:2_18:0) or TG(17:1_17:1_18:0), could only be mapped to an entity in the model ('tag_hs') that represents a lipid class rather than a lipid with a uniquely specified

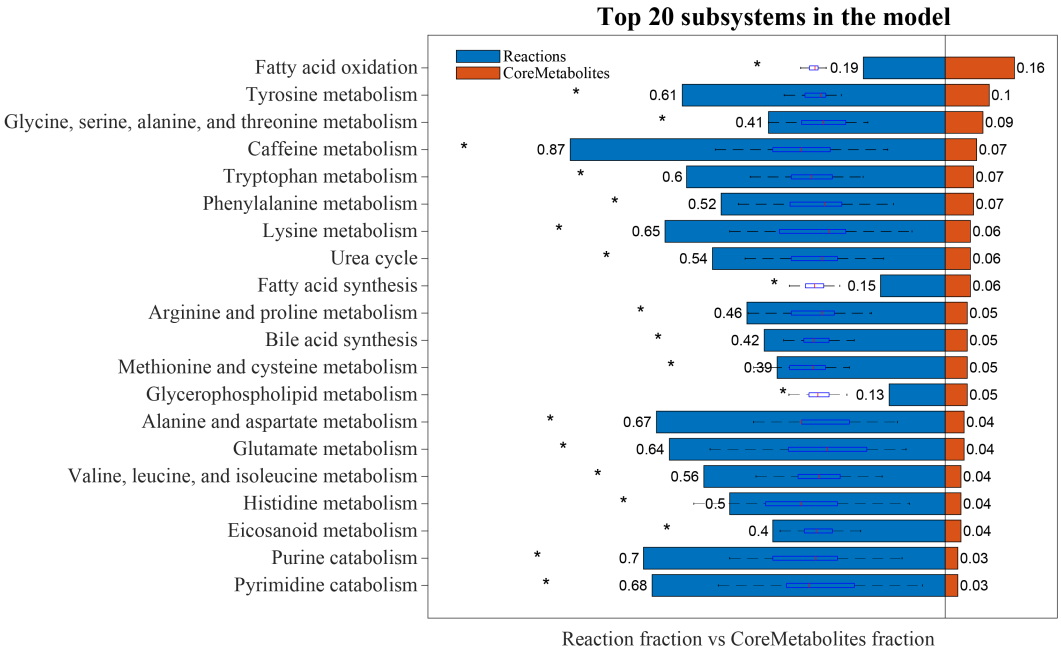


Figure 6: The top 20 subsystems in the PD model, ordered by the proportion of core metabolites involved in each subsystem. The decimal number on the reaction bars of each subsystem represents the fraction between the reaction number in the PD model and in the de-compartmentalized thermodynamically flux-consistent global subset; the decimal number on the core metabolite bars represents the proportion of the core metabolites involved in each subsystem out of the 137 core metabolites. The box plots represent the random distribution of reaction fraction in each subsystem , * means P-value<0.001.

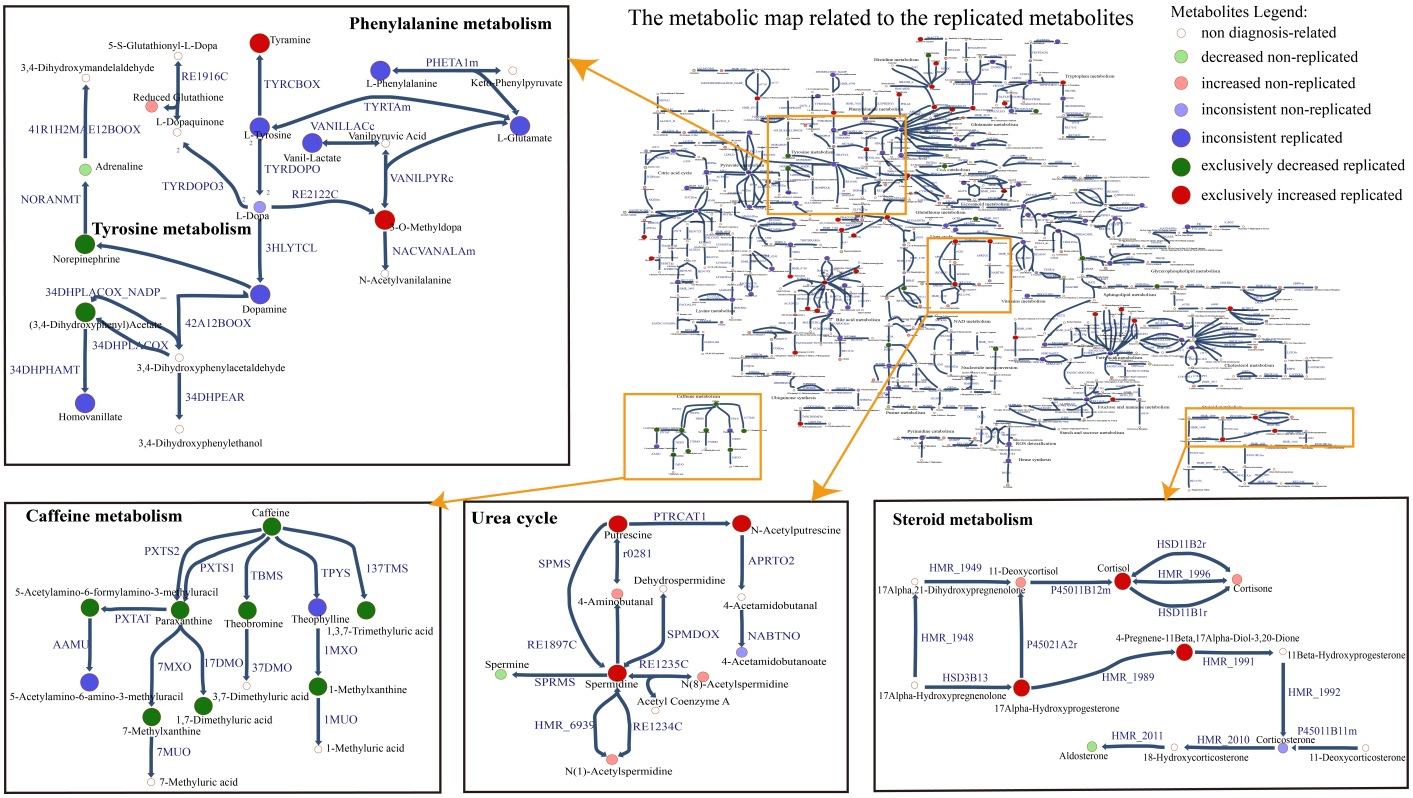


Figure 7: The overview map related to the replicated metabolites and four consistent pathways. Each disc represents a metabolite with non-white colours representing metabolites associated with PD diagnosis, with different trends and degree of replication, while white discs represent metabolites that have not been reported to be associated with PD diagnosis.

structure. Therefore, further refinement of lipid metabolism in the metabolic model and more detailed specifications of the lipids measured in clinical samples is necessary before computational modelling can be used to interpret PD associated lipidomic changes.

In our study, only about 20% of PD diagnosis-related metabolites were replicated in more than one study. Most non-replicated metabolites were lipids, which may be because lipids have complex structures that are difficult to analytically identify as the same metabolite with different platforms. The low percentage of metabolites replicated in more than one study revealed that reported biomarkers are highly heterogeneous. This heterogeneous picture may result from complex factors affecting metabolite levels, such as different types of PD patients and control groups, different ethnicity, diet, exercise levels and medication intake, different detection platforms with targeted and untargeted measurements, and different metabolite annotation databases and statistical analysis methods used in different metabolic studies. We assumed that replicated metabolites were more reliable biomarkers than other non-replicated metabolite.

Almost one-third of replicated metabolites were inconsistently changed across different studies. One of the reasons for this inconsistency may be caused by various bio-specimens of included metabolomic studies. For example, taurocholic acid was considered a large molecule that can not cross the blood-brain barrier (BBB). The inconsistent changes in taurocholic acid, including increased levels in plasma [26, 51] and decreased levels in CSF [38], may be caused by the natural barrier. Besides, pathological changes in the permeability of the BBB may contribute to changes in metabolite levels, such as several triacylglycerols and derivatives, which are BBB impermeable metabolites but identified with increased levels in both plasma and CSF of PD patients [45, 140, 19].

Inconsistency may also arise due to drug intake, as certain metabolites, such as ornithine and putrescine, can be influenced by antiparkinsonian drugs (AB et al., Lipid pathway dysfunction in Parkinson's Disease, in preparation). Genetic mutations are also known to be a significant contributing factor to this inconsistency, as the concentration of several long-chain fatty acids are known to be influenced by GBA mutations [23]. Use of different analytical platforms or different sample preparation procedures may also lead to reports of inconsistent changes in metabolite levels. Reduced and elevated levels of plasma lysine were identified in the same study using nuclear magnetic resonance and mass spectrometry platforms, respectively [46]. Pre-analytical variation in human plasma and serum may also contribute to inconsistencies [141]. For example, the level of several metabolites including hypoxanthine, guanosine, inosine and eicosanoids may change as a function of time interval between sampling and centrifugation [141].

Disease progression may also result in inconsistencies. For example, methionine and leucine were identified as significantly changed in concentration over time in the plasma of PD patients [97]; their concentrations were higher in PD compared to the controls at baseline but then decreased to be lower than the controls at follow-up [97]. This change may result from the changed levels of the precursor by oxidative stress or gut microbial pathways during PD progression [97].

## Dysfunctional metabolism in the PD model

Using a global human metabolic model and a set of metabolites associated with PD diagnosis, we generated a PD model subnetwork that links diagnosis-related metabolites. The semi-automated model generation process biased for inclusion of pathways that enabled steady state flux involving PD associated metabolites but relaxed this requirement for highly connected metabolites that were not associated with PD. Therefore the PD model focuses on PD associated pathways rather than pathways for synthesis and degradation of non PD related metabolites that are omnipresent in metabolism. As a result, pathways associated with PD were included in the model, while pathways for cofactor synthesis were excluded, unless that cofactor was associated with PD.

The metabolic subsystems enriched in the PD model was broadly reflective of the dysfunctional pathways summarized in the literature synthesis. Metabolic alterations in PD patients are primarily involved in six pathways: 1) Tyrosine metabolism contained 14 replicated metabolites, five of which are highly replicated and appeared in more than three studies with the same changing trends. These highly replicated metabolites include 3-O-Methyldopa, glycine, tyrosine, hydroxyphenylacetic acid and glutamine; 2) Caffeine metabolism contained 10 replicated metabolites, with six highly replicated metabolites (caffeine, paraxanthine, 1-methylxanthine, theophylline, 1,7-dimethyluric acid and 5-acetylamino-6-amino-3-methyluracil); 3) Tryptophan metabolism contained nine replicated metabolites, four of which were highly replicated, including 5-hydroxytryptophan, glutamate, tryptophan and alanine; 4) Phenylalanine metabolism contained nine replicated metabolites, six of which were highly replicated, including glycine, glutamine, phenylacetyl-L-glutamine, hydroxyphenylacetic acid, phenylalanine and glutamate; 5) Lysine metabolism contained contained eight replicated metabolites, five of which were highly replicated, including glycine, glutamate, pipecolic acid, acetate and lysine ; 6) The urea cycle contained eight replicated metabolites, three of which were highly replicated, including ornithine, glycine and acetate. Except for these pathways which involved highly replicated metabolites, other pathways with consistent metabolic changes, including steroid metabolism, polyamine metabolism and dopamine metabolism, may be used to explore potential metabolic markers further.

Dysfunction of steroid metabolism was identified with cortisol and its related metabolites, including cortisone, 11-deoxycortisol, 17-hydroxyprogesterone and 21-Deoxycortisol, increased in PD patients [51, 20, 35, 33]. Stress-sensitive cortisol, produced by the adrenal cortex, may affect motor and non-motor symptoms of PD [142]. Increased stress in the

neurodegenerative process may induce the dysfunctional hypothalamic-pituitary-adrenal (HPA) axis and further influence cortisol levels; the increased cortisol levels may relate to mitochondrial dysfunction and the vulnerability of nigral neurons [143, 144]. Besides, the dysfunction of sphingolipid metabolism may affect steroidogenesis metabolism [48]. Sphingolipids are strongly connected with sterol metabolism through the physical association of the planar ring of sterols with the acyl chains of sphingolipids; they may interact through the cross-talk on metabolic pathways, resulting in increased steroid levels [145]. Therefore, cortisol and its related metabolites could have the potential to be the biomarkers to reflect dysfunctional lipid metabolism and the stress in the brain leading to the disruption of mitochondrial function and neuroinflammation [144].

As shown in the map, putrescine and its downstream metabolites, including spermidine, 4-Aminobutanal [99] and N-acetylputrescine [58, 99], were identified with increased levels in PD patients. Meanwhile, the lateral metabolism from spermidine to N1-acetylspermidine [58], N8-acetylspermidine [58] increased in PD patients, while the metabolism from spermidine to spermine was identified to be decreased [58]. These consistently changed polyamines could be used as potential biomarkers to reflect oxidative stress in PD; and the decreased metabolism from spermidine to spermine and its involved protein activity needs further exploration.

The PD model reflects the classical biosynthesis and degradation pathways of dopamine, where dopamine comes from phenylalanine/tyrosine/L-Dopa, and then degrades to norepinephrine or homovanillic acid [146]. One of the direct dopamine products, 3,4-dihydroxyphenylacetaldehyde (DOPAL), has been reported to accumulate in the brain of PD patients through the increased dopamine conversion to DOPAL by monoamine oxidase and decreased DOPAL degradation by aldehyde dehydrogenases, which may play an important role in the aberrant accumulation of α-Syn [147]. However, our study was limited to analyzing most of the metabolomic studies of PD patients. Hence, other relevant studies, such as the paper to explore the dysfunction of catechols in PD patients [148] were not included, leading to the insignificance of DOPAL in our study. Therefore, more metabolomic studies are needed to focus on the dopamine metabolites in the brain of PD patients. In contrast, the intermediate dihydroxyphenylacetic acid (DOPAC), which is also the product of DOPAL, was consistently reported with decreased changing levels, and plays an important role in several biological processes [149]. Both norepinephrine [81, 95] and dihydroxyphenylacetic acid (DOPAC) [81, 150] were consistently decreased in PD patients no matter whether the dopamine level was increased or decreased. Therefore, norepinephrine and dihydroxyphenylacetic acid could be used as potential biomarkers to identify the loss of dopaminergic neurons [149].

Except for the highly replicated metabolites in caffeine metabolism, other caffeine downstream metabolites, including theobromine, 1,3,7-trimethyluric acid, 7-methylxanthine and 5-acetylamino-6-formylamino-3-methyluracil, may also be considered as possible metabolic biomarkers to reflect the dysfunction of caffeine metabolism as they are consistently decreased in PD patients.

## Limitations and Recommendations

Our analysis was limited metabolic perturbations associated with PD, without distinguishing between genetic and idiopathic, or treated versus untreated PD patients. Additional stratification would be possible with consistent access to raw data for each of the 74 studies that passed quality control assessments. A more detailed analysis of all of the individual raw metabolomic data and metadata from high-quality past and ongoing clinical metabolomic studies will be necessary to enable a paradigm shift from mono to multi-centric clinical metabolomic analysis of PD.

Given that the metabolic biomarkers in PD patients are significantly impacted by various factors, it is necessary to avoid potential biases and control such factors in future PD metabolomic studies. When designing the metabolite detection method, careful consideration is required, as different detection platforms exhibit different sensitivity and separation power for different metabolites. Besides, inherent barriers may lead to inconsistent metabolic changes in PD patients across different bio-specimens, and dynamic metabolite changes may occur before sample centrifugation. Therefore, the adoption of standard analytical techniques and sample preparation operations are needed to be able to more precisely compare metabolomic studies. Additionally, it is essential to consider the different metabolomic changing patterns in various PD patient classifications, such as genetic or idiopathic PD patients, early- or late-stage PD patients, treated and untreated PD patients, due to some metabolites may change over time at different stages or be influenced by antiparkinsonian drugs. Also, the selection of an appropriate control group is crucial in obtaining accurate results, taking into account factors such as age onset or the impact of daily diet. Moreover, several metabolites have highlighted gut microbiota changes in PD, and further research is necessary to elucidate the association between the gut-brain axis and PD.

## Conclusions

A comprehensive comparison of 74 high-quality metabolomic studies of PD patients, revealed 928 metabolites that were reported to be associated with PD diagnosis, 137 of which were replicated in more than one study. Integration with computational modelling of metabolism revealed perturbations in tyrosine metabolism, caffeine metabolism, tryptophan metabolism, phenylalanine metabolism, lysine metabolism, and the urea cycle. Furthermore, several potential metabolic markers were identified from the pathways with consistent metabolite changes, including cortisone and its metabolites from

steroid metabolism, putrescine and its metabolites from polyamine metabolism, norepinephrine and dihydroxyphenylacetic acid (DOPAC) from dopamine metabolism, as well as caffeine and its metabolites.

# Methods

## Study search and data collection

Metabolomic studies of PD were selected till March 2021 from two public databases, PubMed and Web of Science, with the combination of search keywords 'Parkinson's disease' and 'Metabolomics' in all fields. The studies based on all PD patients, including genetic and idiopathic, treated and untreated PD patients, were included in this study. No limitations were placed on analytical platforms and bio-specimens used in PD metabolomic studies. The following studies were excluded: 1) studies that only concerned cell culture, animal models and drug therapy; 2) review papers; 3) studies without any diagnosis-related or progression-related metabolites. In this study, the diagnosis-related metabolites were considered as the metabolites with different abundances between PD patients and asymptomatic controls; the progression-related metabolites were the metabolites associated with PD severity, motor score, disease duration, or different abundances in follow-up cohorts. Then, the target metabolomic studies of PD were updated until March 2022.

Basic information, including publication year, country, sample size, analytical platforms of metabolites, type of specimen, and possible diagnosis-related or progression-related metabolites with increased or decreased changing trends compared to controls were collected from each selected study.

## Quality assessment

To assess the reliability of the included results, the quality assessment of selected studies was applied by combining the tools Newcastle-Ottawa Scale (NOS) [137] and QUADOMICS [135]. Newcastle-Ottawa Scale (NOS) is widely used for assessing the quality of non-randomised studies in meta-analyses [137]. It consisted of three parts: sample selection, comparability and outcome with 9 questions. Each question can be awarded 1 score if it is 'yes', and each study can get a maximum of 9 scores. The studies that scored greater than 6 were considered to be relative high-quality [151].

QUADOMICS, a quality assessment panel specified for the "omics"-based diagnostic research, was generated from Quality Assessment tool for Diagnostic Accuracy Studies (QUADAS) [135, 136]. Except for the assessment of study design, this tool was also focused on sample collection, pre-analytical and analytical procedures, and statistical analysis. In this study, the 10/16 questions related to metabolomic were selected to assess the quality of PD metabolomic studies [152]. Each item is quantified as 1 score if it is 'yes', and each study can get a maximum of 10 scores. The studies that scored greater than 7 were considered to be relatively high-quality. Any study that was assessed as high-quality by both tools was deemed a high-quality study. A random subset with eight studies was assessed by an independent clinical research fellow to validate the quality assessment consistency. Any disagreement on these assessment items was discussed to establish a consensus.

## Metabolite classification and matching to human metabolic databases

The Virtual Metabolic Human (VMH, https://www.vmh.life/) is a metabolic database linking genes, reactions and metabolites into a human metabolic model, where each metabolite is identifiable with database dependent and independent identifiers [153]. As collected metabolites were represented by different identifiers in different studies, a Virtual Metabolic Human identifier (VMHID) was used to unify metabolite into a single namespace. Uncertain metabolites, including fragments that could not be identified or annotated accurately, were excluded from this study. Then, the classification of each metabolite was obtained to identify the metabolite characteristics. A computer program, ClassyFire (http://classyfire.wishartlab.com/) [154], which uses chemical structures and structural features to automatically assign all known chemical compounds to taxonomy, was used to obtain the corresponding classification through the InChI string and InChI key that was collected from the VMH database for each metabolite. The metabolites without InChI string and InChI key, especially lipids, were manually classified to the closest parent class.

Statistical analysis for the metabolites with different changing trends was performed to explore the consistency and inconsistency of PD metabolic biomarkers. Replicated metabolites were identified by calculating the number of studies for each metabolite; the metabolites with the same changing trends that appeared in at least two studies were identified as replicated metabolites. As the blood-brain barrier (BBB) can prevent the transportation of some molecules, 43 known metabolites that can cross the BBB and 240 known metabolites that cannot pass the BBB, were matched with the collected metabolites of PD [140, 14]. Besides, all collected metabolites were cross-matched with the metabolites in two computational models, the global human metabolism model (Recon3D) [138] and the dopaminergic neuronal cell-type metabolism model (iDopaNeuroCT model) [16] to explore dysfunctional pathways of PD. The corresponding biochemical metabolic reactions in human beings of the unmatched metabolites, especially non-lipid replicated metabolites, were manually searched in literature and public databases to identify metabolic pathways associated with PD. As the replicated metabolites could

help us better understand the metabolic perturbations in PD patients, the most significantly dysfunctional pathways with their changing trends and possible mechanisms in PD were reviewed based on the replicated metabolites of PD.

## Generating a PD model

### Global model refinement

A global genome-scale metabolic model, Recon3D [138], was used to generate the PD model. Recon3D is a genome-scale model of human metabolism, it contains 4,140 unique metabolites and 13,543 metabolic reactions, representing the activity of 3,697 metabolic genes, and provides information about gene-protein-reaction associations that connected metabolic genes with enzymes and metabolic reactions [138]. To better describe the metabolic perturbations of PD, additional metabolic reactions of human beings were added to refine the global model, including novel human metabolic reactions from the human metabolic model, Human1 [155], and novel neuronal reactions from a dopaminergic neuronal metabolic model [16]. Besides, new reactions of fatty acid oxidation metabolism were added to extend lipidomics of the global model, and new reactions associated with PD pathways missing from Recon3D that were added before PD model generation.

### Modelling

An established context-specific metabolic model extraction pipeline 'XomicsToModel' was used to generate a thermodynamically flux-consistent model [156]. This pipeline has been used to generate the model of dopaminergic neuronal metabolism [16]; it enables the integration of omics data, including genomic, transcriptomic, proteomic, metabolomic, and bibliomic data, and the extraction of a physicochemically consistent mechanistic model from a global metabolic network [156]. Due to the limited input data, we mainly used the 'thermoKernel' algorithm of the pipeline to extract the thermodynamically flux-consistent model, where the solver 'gurobi' was chosen to solve linear optimisation problems, the 'fastcc' algorithm [157] was chosen to check the flux consistency. The parameter epsilon was set as 1e-5 to get the smallest non-zero flux, and other parameters were set as default.

All the compartments were removed from the refined global model to explore the connection of metabolites better, and the reactions of 'Artifical reactions', 'R group synthesis' and 'Pool reactions' subsystems were removed from the global model due to these reactions cannot reflect the connection of specific metabolites. Then, a thermodynamically flux-consistent global subset of the extended Recon3D model, was extracted to shorten the running time before the PD model generation, and the default weight of all the metabolites and reactions in the subset was set to 0.01.

To get a compact model containing most of the pathways related to diagnosis-related metabolites of PD, the replicated metabolites were used as core metabolites. The weight of each core metabolite was calculated using the maximum study number if the metabolite changed in different trends, and then converted the weight to a negative value. The negative value promotes a metabolite or reaction to be present in the model, the positive value promotes a metabolite or reaction to be absent, and zero means ambivalence with respect to presence or absence [156].

Since the PD model was only generated based on a limited input set of core metabolites, alternate optimal models resulted in the variability of replicate generated models given the same input data. Therefore, we used a semi-steady state approach to identify relatively stable dysfunctional pathways of PD. A weight of zero was set for highly connected metabolites, except for the core metabolites, where the highly connected metabolites were defined as the metabolite connected with more than 9 reactions. Exchange reactions of these highly connected metabolites were added to relax them in the model if there were no corresponding exchange, demand or sink reactions in the global model. Then, the reaction weights were heuristically set through iterations: Ten models were generated without specifying reaction weights in the first iteration. Then, we identified overlapping reactions for these ten models and set their weight to -1.1, where newly added exchange reactions of which were excluded to avoid the model becoming too large. Next, the updated reaction weights were added to the next iteration to explore more overlapped reactions until the reaction weight was stable and all overlapped reactions were more than 85% of reactions from any randomly generated model.

The final relatively stable reactions with the weight were used to extract the core PD model, and the most significant pathways of replicated metabolites were visualised in the Escher map (https://escher.github.io/#/) [158] to reflect the connectivity of the dysfunctional metabolites and further explore the possible PD metabolic markers. In the map, the metabolites were highlighted with their corresponding changing trends. To validate that the PD model was highly connected with core metabolites, we randomly generated extra 100 models with the same reaction size as the PD model from the global subset to identify the random distribution of reactions on each subsystem. One sample T-test was used to explore if there is any difference between the reaction proportion of random distribution and the reaction proportion of the PD model on each subsystem.

Constraint-based modelling process was implemented using the latest COBRA Toolbox [12]. The solver and algorithms were compatible with the COBRA Toolbox, and all of these calculations were conducted using MATLAB 2021a (MathWorks Inc.). The figures involved in this study were generated using OriginLab software (OriginLab Corporation, Northampton, MA, USA), MATLAB and R programming language.

## Author contributions

XL played a critical role in this study by collecting, analysing, and interpreting the metabolic data, and made main contributions to writing the manuscript.

YL contributed to the quality assessment validation and provided valuable feedback on the data analysis.

AB participated in the quality assessment process, provided valuable input on the discussion section, and was involved in revising the manuscript.

CK also contributed to the manuscript as a reviser, providing critical feedback on important content.

RF provided guidance on the study design and supervised the entire investigation process to ensure accuracy and integrity, particularly in regard to data analysis and model generation, and also made significant contributions as a major reviewer of the manuscript.

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## Competing interests

Co-author C.K. serve as a medical advisor to Centogene and Retromer Therapeutics and has received a speaker's honoraria from Bial and Desitin. Other authors declare no competing interests.

## Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files.

## Code availability

The analysis codes used in this study is available in COBRA repository and can be accessed via this link: https://github.com/opencob

## Supplementary information

Supplementary Table 1: Selected metabolomic studies of PD.

Supplementary Table 2: Quality assessment results of selected studies using QUADOMICS and NOS tools.

Supplementary Table 3: All 928 diagnosis-related metabolites.

Supplementary Table 4: All 214 progression-related metabolites.

Supplementary Table 5: Blood brain barrier metabolites.

Supplementary Table 6: The newly added metabolites in global model refinement.

Supplementary Table 7: The newly added reactions in global model refinement.

Supplementary Table 8: The replicated diagnosis-related metabolites.

Supplementary Figure 1: Statistics of PD metabolites. **a** Unmatched replicated metabolites of diagnosis-related metabolites. **b** Data statistics of progression-related metabolites of PD.

Supplementary Figure 2: The heat maps for overlapped metabolites, reactions and genes between the randomly generated 10 models. The diagonal label represents the number of metabolites, reactions and genes in each model, respectively. Other labels and the colourbar represent the shared proportion between each pair of models. All shared metabolites, reactions and genes over ten models were listed on the top of the map, respectively.

Supplementary Figure 3: The Escher map related to the replicated metabolites in the PD model.

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