The UP study confirms the neuroprotective potential of ursodeoxycholic acid in Parkinson's disease

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Abstract

Mitochondrial dysfunction is a key pathogenic mechanism for Parkinson's disease. We previously undertook the first screen of an entire compound library in Parkinson's disease patient tissue and identified the naturally occurring bile acid ursodeoxycholic acid as a powerful mitochondrial rescue compound. We have now undertaken a "proof of concept" clinical trial to determine safety and tolerability of ursodeoxycholic acid in Parkinson's disease, determine its effect on motor progression and assess midbrain target engagement.

The UP (Ursodeoxycholic acid in Parkinson's disease) study is a phase IIa, randomised, double-blind, two centre, placebo-controlled trial of high dose ursodeoxycholic acid (30mg/kg) in 30 participants with early Parkinson's disease for 48 weeks followed by an 8-week washout period (EudraCT 2018-001887-46, ClinicalTrials.gov: NCT03840005). Randomisation was 2:1 drug to placebo. Primary outcome was safety and tolerability. Secondary outcomes combined subjective clinical rating scales with objective, motion sensor-based quantification of gait impairment. Target engagement was explored using midbrain ^{31}phosphorus magnetic resonance spectroscopy.

Ursodeoxycholic acid was safe and extremely well tolerated without any serious adverse events or drug-related abnormalities in safety bloods in the treatment group throughout the trial (primary outcome). Only mild, transient gastrointestinal adverse events were observed more frequently in patients treated with ursodeoxycholic acid compared to placebo, compliance was excellent (mean ± SD; 97.6±5.4% in ursodeoxycholic acid vs 95.2±8.4% in placebo). Bile acid analysis confirmed a marked, stable increase of ursodeoxycholic acid and its key conjugates throughout the treatment period. Objective quantification of motor impairment demonstrated improvement of several gait parameters such as cadence (steps per minute, p=0.019), stride time (p=0.031), stride time variability (p=0.031), stance time (p=0.031) and stance time

variability (0.024) in the ursodeoxycholic acid treatment group compared to placebo (secondary outcome). In contrast, subjective clinical assessment applying the standard clinical Movement Disorders Society Unified Parkinson's Disease Rating Scale, part III failed to detect a difference, highlighting the possible superiority of objective motor assessment. Midbrain ^{31}phosphorus magnetic resonance spectroscopy revealed an increase in Gibb's free energy, indicating improved ATP hydrolysis in the ursodeoxycholic acid treatment group compared to placebo (*p=0.024,* exploratory outcome).

The results of our UP study establish safety and tolerability of ursodeoxycholic acid in early Parkinson's disease, demonstrate the potential of sensor-based, objective quantification of motor impairment in small phase IIa "proof of concept" studies, confirm target engagement of ursodeoxycholic acid in the midbrain and provide justification for subsequent larger trials to further evaluate the disease-modifying effect of ursodeoxycholic acid in PD.

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Abbreviations: ^{31}P-MRS = ^{31}Phosphorus Magnetic Resonance Spectroscopy; ADP = adenosine diphosphate; AE = Adverse event; AMARES = Advanced Method for Accurate, Robust and Efficient Spectral fitting; AR = adverse reaction; CI = confidence interval; CSI = chemical shift imaging; EDTA = Ethylenediaminetetraacetic acid; ΔG_{ATP} = Gibbs free energy of ATP hydrolysis; GUDCA = glycoursodeoxycholic acid; IDMC = Independent Data Monitoring Committee; IMP = Investigational Medicinal Product; LED = levodopa equivalent dosage; MADRS = Montgomery-Asberg depression rating scale; MDS-UPDRS = Movement Disorder Society Parkinson's Disease Rating Scale; MoCA = Montreal cognitive Assessment; NIHR = National Institute for Health-related Research; NMS-QUEST = Non-motor Symptom Questionnaire; OPLS-DA = orthogonal projection least squares discriminant analysis; PCr = phosphocreatine; PD= Parkinson's disease; PDQ-39 = Parkinson's disease 39 item quality of life questionnaire; Pi = inorganic phosphate; QD = quality control; SAE = Serious Adverse Event; STH = Sheffield Teaching Hospitals NHS Foundation Trust; TUDCA = tauroursodeoxycholic acid; UCLH = University College London Hospitals NHS Foundation Trust; UDCA = Ursodeoxycholic acid; UPLC-MS = ultra-performance liquid chromatography linked to mass spectrometry.

Introduction

Parkinson's disease (PD) remains incurable and relentlessly progressive. Mitochondrial dysfunction was first identified in sporadic PD brains and has also been implicated in all forms of familial PD.[1,2] Rescue of mitochondrial function has therefore long been proposed as a promising neuroprotective strategy.[3,4] However, previous clinical trials assessing putative mitochondrial rescue compounds yielded disappointing results.[5-9]

The selected compounds for these previous negative trials were typically chosen for their promising beneficial effect in toxin-induced model systems of PD, only some of these trials assessed the selected compounds for target engagement and efficacy was solely judged on the outcome of clinical rating scales, in particular the motor examination (part III) of the Movement Disorders Society Unified Parkinson's Disease Rating Scale (MDS-UDPRS). Our group undertook the first screen of an entire compound library in genetically stratified PD patient tissue which led to the identification of the naturally occurring bile acid ursodeoxycholic acid (UDCA) as a promising mitochondrial rescue compound for PD.[10,11] We subsequently confirmed the mitochondrial rescue effect of UDCA in mechanistically stratified sporadic PD patient tissue.[12] Other groups had independently reported a beneficial effect of UDCA or its taurine conjugate tauroursodeoxycholic acid (TUDCA) in classical MPTP- or rotenone-induced rodent models of PD.[13-17] UDCA has been licensed to treat primary biliary cholangitis (PBC) at the dose of 15mg/kg for > 30 years. Its excellent safety and tolerability profile makes it ideally suited for the drug repurposing strategy.[18,19]

Pharmacokinetic studies in patients with amyotrophic lateral sclerosis (ALS) confirmed blood-brain-barrier penetrance of UDCA, especially at higher doses.[20] In 2015, the international Linked Clinical Trials Initiative (iLCT) named UDCA as its most highly prioritized

neuroprotective compound for investigation in clinical trials to further validate its neuroprotective potential in PD.

Here we present the results of a phase II, double-blind, randomised, placebo-controlled trial of 30 mg/kg of UDCA in early PD, the UP study. The primary outcome of our study was safety and tolerability of this comparatively high dose of UDCA in PD, chosen to balance CNS penetration and side-effect profile.[20] Relative abundance of UDCA and its key metabolites in serum was assessed throughout the trial. A key part of this "proof of concept" study was also the evaluation of novel secondary outcome measures to address limitations of traditional PD neuroprotection trial designs. Clinical assessment with validated, "gold standard" clinical rating scales, widely used in previous neuroprotective trials, was complemented by in-depth objective quantification of motor impairment, utilising supervised sensor-based gait analysis before and after treatment.[21,22] ^{31}Phosphorus magnetic resonance spectroscopy (^{31}P-MRS) is an MRI-based technique which allows non-invasive spectroscopic quantification of key energy metabolites such as ATP and phosphocreatine (PCr) and has recently been used to confirm target engagement of the mitochondrial rescue compounds terazosin in the entire brain and nicotinamide riboside in in the occipital cortex of PD patients.[23,24] We further refined ^{31}P-MRS to confirm mechanistic target engagement of UDCA in the midbrain, including the substantia nigra as the predominant site of PD pathology.[25]

Methods

Design

A comprehensive protocol for this trial has previously been published.[26] In brief, this was a phase II, two-centre, double-blind, randomised, placebo-controlled trial of 30 mg/kg of UDCA in recent-onset PD (≤3 years since diagnosis) who demonstrated a clear subjective, sustained (>3 months) motor response to dopaminergic medication which was confirmed by the treating physician. UDCA was administered orally for 48 weeks with a subsequent 8-week washout phase to 31 participants with a 2:1 randomisation of drug vs placebo. The trial was conducted at two sites, Sheffield Teaching Hospitals (STH) and University College London Hospitals (UCLH).

Following a screening visit to confirm eligibility, participants attended six further visits: baseline (start of treatment period), week 12, week 24, week 36, week 48 (end of treatment period) and week 56 (end of washout period). Treatment with either UDCA or placebo was commenced at a dose of 250mg per day and increased by 250mg every three days until the target weight-dependant dose of 30mg/kg was achieved. All patients were advised to completely stop taking the Investigational Medicinal Product (IMP) the evening prior to the week 48 visit.

Placebo and UDCA were provided by PRO.MED.CS Praha a.s. and completely matched with no identifiable differences in taste, appearance, or smell. Each capsule was provided as a hard, clear, gelatine capsule containing white powder and capsules of the active drug contained 250mg of UDCA.

Outcome Measures

Primary Outcome

The primary outcome was to compare the safety and tolerability of UDCA at 30 mg/kg in PD compared to placebo as indicated by the following: number of serious adverse events (SAE's), number of adverse treatment-reactions and number of patients who completed the study.

Secondary and Exploratory Outcomes

Secondary outcomes included the change from baseline to week 48 of treatment in the UDCA versus placebo group with respect to: MDS-UPDRS Part III in the practically defined "OFF" medication state; *in vivo* parameter estimates derived from ^{31}P-MRS (ATP, PCr and inorganic phosphate) in the midbrain; sensor-based objective quantification of motor impairment in supervised instrumented clinical gait assessment (consisting of triaxial OPAL sensors, APDM Inc., Portland, OR, USA and OPTOgait 5m system, Microgate Corporation, Bolzano, Italy).[27] The practically defined 'OFF' state was defined as participants not having taken their medication for 8 hours (overnight) in the case of any drug containing Levodopa, or ≥ 36 hours in the case of longer acting agents such as dopamine agonists or enzyme inhibitors.

Exploratory clinical outcomes focused on the changes between baseline and week 48 of: MDS-UPDRS I-IV in the 'ON' state; levodopa equivalent dosage (LED); Montreal cognitive Assessment (MoCA); Montgomery-Asberg depression rating scale (MADRS); Non-motor Symptom Questionnaire (NMS-QUEST); Parkinson's disease 39 item quality of life questionnaire (PDQ-39); calculated Gibbs free energy of ATP hydrolysis (ΔG_{ATP}) and adenosine diphosphate (ADP) concentration in the midbrain.[27-32] If performed at week 56, all measures listed were also assessed for the change between week 48 and week 56 over the washout period.

Assessment Procedures

Safety and clinical assessment

At each visit, adverse events (AE's) were reviewed and assessed for severity and likely relationship to UDCA. Safety monitoring was performed at each visit to capture any adverse events including ECG and blood monitoring (full blood count, urea & electrolytes, liver function tests, blood glucose, Haemoglobin A1c, lipid profile). Compliance was assessed by counting the number of IMP tablets returned by the participant and was expressed as the following percentage: (IMP dispensed - IMP returned)/IMP prescribed. A simplified summary of the safety and clinical assessment study procedures is reported (**Supplementary Figure 1**). At baseline, the predicted risk of rapid disease progression was calculated according to a validated prognostic model, estimating the risk of an unfavourable outcome in PD as defined by the presence of either postural instability or dementia at 5 years.[33]

Genetic analysis

All participants supplied an Ethylenediaminetetraacetic acid (EDTA) blood sample for genetic analysis using the NeuroChip platform.[34] Results were assessed for any known pathogenic mutations in monogenic PD genes (e.g. *PINK1*, *PARK2*, *LRRK2*) and any variants of *GBA1* associated with increased risk of PD. All detected variants were searched in dbSNP (https://www.ncbi.nlm.nih.gov/snp/) and classified according to published guidelines.[35]

Serum bile acid analysis

Serum samples were collected from all participants at each face-to-face visit and stored at -80^{o}C Serum bile acid profiling was performed using ultra-performance liquid chromatography linked to mass spectrometry (UPLC-MS) using a previously-described technique.[36] Quality control (QC) samples were prepared using equal parts of each sample (with some QC samples spiked with known bile acid standards), and were run alongside them. peakPantheR was used

to facilitate identification and relative quantification of bile acids.[37] Further technical detail is included in the **supplementary information**.

Sensor Based Quantification of motor impairment

Sensor-based gait analysis was undertaken at the Clinical Research Facility of the STH study site only. All STH participants were fitted with three tri-axial inertial sensors (OPAL, APDM Inc., Portland, OR, USA) firmly attached to their pelvis and lower legs, which measured acceleration and angular velocity signals. Gait outcomes including temporal metrics and gait quality measures related to intensity and regularity were then extracted from these signals.[38-40]

Participants walked at their comfortable speed at least six times along a walkway of approximately 10m in length to capture around 30 steps per trial. The walkway included an OPTOgait 5m system that triangulated each footfall to calculate several spatial gait parameters with a spatial resolution of 1cm. The experimental design is shown in **Figure 3**.

Temporal measures were computed based on the timings of the foot striking (initial contact) and leaving (final contact) the floor as identified from the angular velocity data. These parameters included: gait speed (meters per second), cadence (steps per minute), stride (timing between initial contacts of same foot), step (timing between initial contacts of contralateral foot), stance (time each foot spent in contact with the floor), swing (time each foot spent of the floor), double support durations (time both feet were on the floor), and stance percentage (percentage of the stride where the foot was on the floor). Spatial measures such as step and stride length and step width were measured by the OPTOgait system. The variability in stride, step, stance, and swing durations and step width were calculated using data from at least 50 steps.[41] Intensity, step regularity, and stride regularity were computed from the lumbar sensor acceleration signals. Further technical detail is included in the **supplementary information**.

^{31}Phosphorus Magnetic Resonance Spectroscopy

All participants were invited for ^{31}P-MRS scans both at baseline and week 48 at the STH site, using a Philips Ingenia 3 Tesla system (Philips Healthcare, Best, Netherlands) and a transmit-receive dual-tuned ^{1}H/^{31}P birdcage quadrature head-coil (Rapid Biomedical, Würzburg, Germany). Two-dimensional chemical shift imaging (CSI) with image-selected in vivo spectroscopy was used for spectral spatial localisation capturing the midbrain, with a reconstruction matrix of 14x14 and voxel size of 15x15x20mm^3.[42,43] Spectra were processed in the time domain using jMRUI software V5.2 (http://www.jmrui.eu) and manually pre-processed using zero and first-order phasing for purely absorptive line shapes. No apodisation was performed. Signal fitting was performed using the Advanced Method for Accurate, Robust and Efficient Spectral fitting (AMARES) algorithm to determine relative amplitudes for ATP, inorganic phosphate (Pi) and PCr.[44,45] All amplitudes were normalised to total phosphorus signal detected within the spectra.

Voxel localisation and exemplar spectra are shown in **Figure 4**. Full details of technical acquisition, analysis and calculation of both ADP and ΔG_{ATP} are reported in the **supplementary information**.

Statistical Analysis

Statistical analyses was by intention-to-treat. As explained above, several assessments were unavoidably delayed due to the COVID pandemic; sensitivity analyses were performed for each analysis excluding data collected outside of the planned assessment window. All results presented are using the full analysis dataset unless stated otherwise. SAE's and adverse treatment reactions are presented descriptively, in summaries individual AE's (by preferred term) are counted once per participant at the worst severity. We considered the rate of SAE's reported in the exenatide trial in Parkinson's disease to be tolerable and acceptable (i.e., 20%).

If no SAE's were found in the group receiving UDCA (n=20) then the likelihood that the true SAE rate is less than 20% is 0.990778 (i.e., there is a less than 1% chance that the true SAE rate is >=20%).[46] The study was not powered to detect differences in the secondary or exploratory endpoints and therefore, the interpretation of observed differences and confidence intervals (CI's) will take priority over statistical significance conferred by *p*-values and no adjustment was made for multiple testing.

Demographic and clinical assessment data were assessed for normality using QQ plots and summarised using relevant summary statistics. Between-group differences in demographics, clinical parameters at baseline, and changes in both clinical parameters from baseline to week 48, baseline to week 56, or week 48 to week 56 were assessed using t-tests for continuous data and chi-squared tests for categorical data. Between-group differences in gait analysis parameters from baseline to week 48 were compared using Mann-Whitney U tests as data were not normally distributed.

The change in ^{31}P-MRS parameters from baseline to week 48 was compared between groups using t-tests for each metabolite in turn. As a further exploratory analysis ^{31}P-MRS was also analysed using linear regression with the change from baseline to week 48 as the response variable and the baseline value of the parameter, treatment group, age and sex as predictors. To ensure the number of covariates in linear regression was appropriate to sample size and prevent over-fitting of linear regression models the correlations between midbrain voxel total brain (grey and white matter) content and ^{31}P-MRS parameters were assessed separately using Pearson's correlation coefficient. In the event of any significant correlations, partial volume effects were added to the model.

Multi-variate statistical analysis of serum bile acid profiling data was performed using SIMCA 17.0 (MKS Umetrics AB). Both unsupervised and supervised models (orthogonal projection

least squares discriminant analysis, OPLS-DA) were performed using Pareto-scaled, log-transformed data.

Study Approval

The trial was approved by the East of England – Cambridgeshire and Hertford Shire Research Ethics committee (UK, Protocol ID: 18/EE0280). STH acted as the sponsor of the study (local sponsor study number STH18493). The trial was registered on European Union Drug Regulating Authorities Clinical Trials Database (EudraCT no. 2018-001887-46). All participants provided written informed consent prior to any study related activates being performed in accordance with the Declaration of Helsinki.

Data availability

Raw data are available from the corresponding author on reasonable request.

Results

Patient characteristics

A total of 33 participants were assessed for eligibility from January 2019 to October 2019, with 22 participants assessed at STH and 11 assessed at UCLH.[26] Two participants were excluded due to a Montreal Cognitive Assessment (MoCA) score < 25. Full details of cohort enrollment are shown in **Figure 1**. Demographic and clinical characteristics are summarized in **Table 1**. Treatment groups were well matched for age ($P > 0.05$), sex ($P > 0.05$), disease duration ($P > 0.05$), family history of PD ($P > 0.05$) and predicted risk of rapid disease progression ($P > 0.05$). None of the trial participants carried pathogenic mutations in monogenic PD genes (e.g., *LRRK2*, *SNCA*) or pathogenic risk variants of *GBA*. In total 31 patients were randomised, 11 to placebo and 20 to 30mg/kg of UDCA daily, titrated to target dose over approximately 8 weeks.

Serum bile acid analysis

Serum samples were available for all 30 participants (UDCA n=19, placebo n=11) at both baseline and week 12, due to COVID-19 restrictions reduced numbers of samples were available for subsequent timepoints (see **supplementary information** for further information). Following commencement of treatment with UDCA, changes in the overall serum bile acid profile compared to baseline were found at all visits during the treatment period (weeks 12, 24, 36 and 48, **Fig. 2A**). Bile acid profiles returned to levels comparable to baseline values at week 56 following the 8-week washout. No changes compared to baseline were seen at any time point in placebo-treated patients. Supervised multivariate modelling of serum bile acid profiles of UDCA- *vs* placebo-treated patients at week 12 (chosen for data completeness, using an orthogonal projections to latent structures discriminant analysis; OPLS-DA, **Fig.2B**) revealed a robust model for group separation (R2X=0.721, R2Y=0.964, Q2=0.929, CV-ANOVA: $P=$

4.68 x 10^{-12}). Discriminatory feature analysis derived from this model confirmed a marked enrichment in serum UDCA and its conjugates glycoursodeoxycholic acid (GUDCA) and tauroursodeoxycholic acid (TUDCA) in the serum of UDCA-treated patients compared to controls (**Fig. 2C**). OPLS-DA did not demonstrate any statistical differences in serum bile acid profiles between treatment groups at baseline or week 56. Relative abundance of UDCA and related conjugates across all time points between groups is shown in **Figure 2D,** with further data detailing OPLS-DA modelling of all bile acids in **Supplementary Fig. 2**.

Safety and tolerability

One patient withdrew from the trial after 5 weeks of treatment due to difficulties swallowing the number of IMP capsules in addition to their regular medication, but not pharmacological side effects of the IMP as such. This participant was replaced with a new patient. The remaining 30 participants all completed the trial, resulting in a total intention-to-treat analysis cohort of 31 trial participants. Two participants stopped taking the medication early at 28 weeks (UDCA group) and 30 weeks (placebo group) respectively **(Fig 1)**. Both cited the burden of taking an additional 9-10 tablets in addition to their usual medications. All other trial participants (19/20 in the UDCA group and 9/11 in the placebo group) completed the full treatment period. Compliance was excellent in participants completing the 48-week treatment period (mean ± SD; 97.6±5.4% in UDCA vs 95.2±8.4% in placebo).

Two serious adverse events (SAE's) occurred, both in the same participant, namely retroperitoneal haemorrhage leading to hospital admission and subsequent hospital- acquired pneumonia. Administration of the study drug was withheld during the inpatient admission. The independent data monitoring committee (IDMC) advised that this event was unlikely to be related to study medication, unblinding was not indicated and the trial medication was restarted

after discharge. Unblinding after completion of the trial revealed that this participant was randomised to the placebo group.

Twenty-four adverse reactions (AR's) were observed in 14/31 participants (10 UDCA and 4 placebo, **Table 2**). The most frequent AR's were gastrointestinal symptoms: 5/20 (25.0%) participants on UDCA developed mild diarrhoea (i.e., not requiring any treatment) with three episodes resolving within 48 hours or less; a further two patients had episodes that resolved within 72 hours. In the placebo group, 1/11 (9.1%) developed diarrhoea that resolved within 24 hours. Mild nausea (i.e., not requiring any treatment) occurred in 2/20 (10%) of participants taking UDCA, in one participant this episode resolved within 24 hours, in the second participant the nausea was of unspecified duration due to missing data. No other AR's occurred in the UDCA treatment group at a frequency of more than 1 of the 20 participants. An SAE rate of 20% was reported in the recent Exenatide-2 trial in PD patients [46]. Since we found no SAEs in the UDCA group in the full intention-to-treat population (n = 20), the likelihood that the true SAE rate for UDCA was less than 20% is 0.990778. Blood monitoring performed at all face-to-face visits revealed no clinically significant changes in any blood tests performed other than one incidental finding of asymptomatic hyperkalaemia (5.6mmol/L) in the UDCA group and one isolated increase in alkaline phosphatase (ALP) (194 IU/L) in the placebo group. The hyperkalaemia was already present at baseline prior to commencement of treatment and therefore unrelated to UDCA. The raised ALP in the placebo group was only observed in the participant who had suffered the retroperitoneal haemorrhage at the visit following the associated hospital admission and normalized for all subsequent visits.

Clinical assessment

All secondary and exploratory outcome results are summarised in **Table 3**. All treatment differences are reported as the difference between UDCA and placebo groups. We assessed for

the clinical response in PD to treatment using the MDS-UPDRS in both the practically defined 'OFF' state (where dopaminergic medication was withheld prior to assessment) and the 'ON' state (at least 60 minutes following the administration of a participant's usual dopaminergic medication). Whenever COVID-19 restrictions prevented face-to-face review of participants, clinical assessments were conducted remotely over video, see **supplementary information** for further information.

MDS-UPDRS III scores in the 'OFF' state reduced in severity from baseline to week 48 by a mean of -1.68 points (95% CI -4.90, 1.53) in the UDCA group and by -5.2 points in the placebo group (95% CI -9.82, -0.58) with a mean difference between UDCA and placebo of 3.52 (95% CI -1.83, 8.86, *P = 0.1844*); from week 48 to week 56 (end of treatment to end of washout period) scores reduced by -3.42 points (95% CI -6.48, 0.36) in the UDCA group and by -0.9 points (95% CI -2.96, 1.16) in the placebo group with a mean difference between UDCA and placebo of -2.52 (95% CI -6.05, 1.01 *P = 0.1543*); from baseline to week 56 the MDS-UPDRS III scores reduced by -5.11 points (95% CI -9.33, -0.89) in the UDCA group and by -5.55 points (95% CI -9.36, -1.73) in the placebo group with a mean difference between UDCA and placebo of 0.44 (95% CI -4.97, 5.85 *P = 0.8688*). There was therefore no significant treatment effect at any time point between the two treatment groups.

The mean MDS-UPDRS III scores in the 'ON' state showed similar trends from baseline to week 48, week 48 to week 56 and baseline to week 56 to those seen in the 'OFF' state, again with no significant treatment effect between groups seen at any time point (see **Table 3**). There were no significant differences between groups in the changes in MDS-UPDRS part I or II scores in the 'ON' state from baseline to week 48. MDS-UPDRS part IV scores reduced from baseline to week 48 in in the UDCA group by -1.26 (95% CI -2.74, 0.22) compared to a mild increase in the placebo group (0.55, 95% CI -0.21, 1.3) with a mean difference between UDCA

and placebo of -1.81 (95% CI 0.20 3.42, *P = 0.0293*). However, scores at each visit remained low overall (see **Table 3**).

Dopaminergic medication regimes were expressed as total levodopa equivalent daily dosage (LED). LED increased in 9/19 (47%) in the UDCA group and in 4/11 (36%) in the placebo group. LED reduced in 1/19 participants in the UDCA group. The range of change was from a reduction of 100mg to an increase of 264mg in UDCA group and 0 mg – 240 mg in the placebo group. The remaining 16/30 participants completed the study at the same LED as they started.

Depressive symptoms were assessed using the MADRS. Mean MADRS scores increased slightly in the UDCA group from baseline to week 48 by 1.7 (95% CI 0.1, 3.3), but decreased in the placebo group (-0.4, 95% CI -1.5, 0.8), with a mean difference between UDCA and placebo of 2.05 (95% CI 0.15, 3.94, *P = 0.0353*). Overall, MADRS scores remained relatively low across groups throughout the study (**Table 3**).

There were no differences between UDCA and placebo in the mean change from baseline to week 48 for cognitive function as assessed by MoCA (treatment difference 0.7, 95% CI -0.3, 1.7, *p=0.1758*), autonomic function as assessed by NMS-QUEST, (treatment difference 1.1, 95% CI -0.4, 2.6, *p=0.1479*) or quality of life as assessed by PDQ-39 (treatment difference -0.9, 95% CI -5.5, 3.7, *p=0.6983*).

Sensor Based Quantification of Motor Impairment

To complement clinical assessment applying subjective clinical rating scales, we also objectively measured changes in motor impairment using a quantitative supervised, sensor-based gait analysis approach at STH only. Data before and after treatment was available for 12/19 in the UDCA group and 6/11 in the placebo group (**Table 3** and **Figure 3)**. All *P* values reported in this section are for group differences tested by the Mann-Whitney U method. Between baseline and 48 weeks, cadence (steps per minute) increased in the UDCA group

(median change +1.14 step/min) but decreased in the placebo group (median change -4.58 step/min, group-difference $P = 0.019$, **Fig. 3C**). Stride time was slightly reduced in the UDCA group (median change -0.01s) but increased in the placebo group (median change +0.03s, group-difference $P = 0.031$, **Fig. 3D**). Stride time became less variable in the UDCA group (median change -2.43s), but more variable in the placebo group (median change +6.16s, group-difference $P = 0.031$, **Fig. 3E**). Similarly, stance time decreased in the UDCA group (median change -0.02s) and increased in the placebo group (median change +0.02s, group-difference $P = 0.024$, **Fig. 3F**). A similar difference was observed for stance time variability with a decrease in the UDCA group (median change -2.81SD), but an increase in the placebo group (median change +6.48SD, $P = 0.039$, **Fig. 3G**). Taken together, these results indicate less deterioration in core bradykinetic gait features such as overall speed and time taken standing on each lower limb and stride time in the UDCA treated group compared to the placebo. Of note there was no clear relationship between the change in gait parameters and the change in MDS-UPDRS III scores. No significant differences were found between groups for gait speed, any of the spatial parameters (step length, stride length or step width) or the intensity or regularity measures. Proportionate change across all gait parameters for is shown in **Supplementary Fig. 3**.

^{31}Phosphorus Magnetic Resonance Spectroscopy

In total, 25 participants underwent ^{31}P-MRS at both visits (UDCA n=16, placebo n=9). Follow-up scans were delayed in 5 participants from week 48 to 56 due to COVID-19 and a further participant was unable to attend for a second ^{31}P-MRS scan at all. One participant had their ^{31}P-MRS follow-up scan repeated at week 56 due to poor technical acquisition at week 48. One data point from one follow-up scan quantifying magnesium had to be removed from the analysis for technical issues prior to unblinding.

Evidence of target engagement was assessed using ^{31}P-MRS to determine the effect of the IMP on the bioenergetic profile in the midbrain (including the substantia nigra). Twenty-six of the 30 participants completing the study had ^{31}P-MRS at the baseline visit, twenty-five of these (96%) had follow-up scans after completion of treatment. There was no correlation between total brain volume in each midbrain voxel and any ^{31}P-MRS parameter (data not shown); therefore the reported linear regression did not include these measures as additional covariates to prevent overfitting.[47,48] There was no correlation between age or disease duration and any 31P-MRS parameter at baseline (data not shown). Reported *P* values and confidence intervals are for treatment estimates of UDCA as assessed by linear regression. The age and sex covariates included in the models were not significant in any models other than when assessing mean midbrain pH, for which sex was significant but had minimal effect on the overall observed treatment effect of UDCA on mean midbrain pH, which remained non-significant. Reported *p* values and confidence intervals are for treatment estimates of UDCA as assessed by linear regression.

ΔG_{ATP} reflects the amount of energy released from the hydrolysis of ATP to ADP and Pi. As this reaction is exergonic, the value is negative, with more negative values representing greater amounts of energy released to the tissue examined. Mean midbrain ΔG_{ATP} reduced by -0.672 kJ/mole (95% CI, -1.62, 0.277) in the UDCA group, but increased by +2.145 kJ/mole (95% CI -0.491, 4.781) in the placebo group from baseline to week 48 (treatment estimate -1.929, 95% CI -3.472, -0.385, *P = 0.024*; **Fig. 4D**). This reduction was accompanied by a non-significant trend towards decreased calculated ADP in the UDCA group (-18.6µmol,95% CI -52.3, 15.17) and an increase of calculated ADP by 33.7µmol (95% CI -14.1, 81.5) in the placebo group (treatment estimate -36.3, 95% CI -72.3, -0.3 *P = 0.062;* **Fig. 4E**). Mean midbrain Pi increased by +0.02 (95% CI 0.00, 0.04) in the UDCA group and reduced by -0.006 (95% CI -0.032, 0.02) in the placebo group (treatment estimate 0.032, 95% CI 0.013, 0.051, *P* = 0.004, **Fig. 4G**).

There were no significant treatment effects observed between groups with respect to any changes between the initial ^{31}P-MRS scan at baseline visit and the subsequent follow-up scan (typically week 48) for pH (**Fig. 4H**), ATP or PCr (**Table 3**).

Discussion

The UP study has confirmed that UDCA at a dose of 30mg/kg is safe and extremely well tolerated in PD with no SAE's and only mild, transient side effects reported in the UDCA treatment group (primary outcome). Additionally, we report a beneficial effect of UDCA on the progression of motor impairment (as assessed by objective sensor-based gait analysis, secondary outcome) and provide additional tentative, ^{31}P-MRS based evidence of mechanistic mitochondrial target engagement (exploratory outcome). The latter part of our UP study was compromised by the COVID-19 pandemic and consequent necessary changes to remote study visits and/or delayed assessments such as repeat ^{31}P-MRS imaging. However, our sensitivity analysis of data only collected at the correct time points suggests that this did not have a significant effect on the overall outcome of the trial (data not shown).

We only recruited patients with recent onset PD (arbitrarily defined as ≤ 3 yr. since diagnosis) to increase the homogeneity of the study cohort which may be of particular importance in a small proof of concept study. In our view, it is also plausible to assume that the likelihood of any compound exerting a neuroprotective effect on the remaining dopaminergic neurons is considerably greater in PD patients with comparatively short disease duration.[49]

The excellent safety and tolerability of UDCA is reflected by an extremely high compliance rate (mean of 97.6%) of those in the UDCA treated group completing the full treatment duration. Early treatment cessation was due to high pill burden in combination with regularly prescribed medications or, in the case of one participant who withdrew after 5 weeks, difficulties in swallowing the IMP and not due to the presence of side-effects. The lack of clinically significant changes in blood monitoring throughout the trial related to UDCA is also extremely reassuring. This safety profile contrasts with the side effect profile of other recently explored putative neuroprotective compounds.[50,51] The increase in MADRS scores in the

UDCA treatment group continued throughout the washout period following cessation of UDCA and may therefore not be related to the IMP. No patient had depression levels requiring pharmacological intervention.

The relative abundance of serum bile acids following treatment with UDCA showed a marked enrichment of detectable UDCA (increasing from ~1% of the serum bile acid profile at baseline to ~30% across the period of treatment) as expected but also of UDCA-related conjugates; including TUDCA, which has also separately been linked with neuroprotection in rodent models of PD [13]. Although not specifically studied in PD, GUDCA has also shown to exert protective effects in *in vitro* models of oxidative stress which is intrinsically linked to mitochondrial dysfunction.[52,53] UDCA administration resulted in marked changes in bile acids after only 12 weeks treatment (which also included a titration period to target dose). These changes were persistent and remained stable at weeks 24 and 36. The fall in mean UDCA and related conjugates at week 48 compared to other visits in the treatment period is due to ~75% of participants stopping to take their last UDCA dose the evening prior to their week 48 visit (rather than on the morning of the study visit day itself) together with all other PD medication to enable repeat clinical examination in the practically defined OFF. Serum peak concentrations for 30 mg/kg UDCA are achieved 1hour after administration.[20]

Non-significant improvements of MDS-UPDRS III "OFF" scores between baseline visit and after treatment (week 48) were observed in both the UDCA and the placebo group, but more marked in the placebo group (approximately 18% mean improvement in the placebo group). This is unlikely to be due to changes in dopaminergic medication as the increases in LED over the course of the trial were generally small in both treatment arms. Previous clinical trials investigating putative neuroprotective compounds for their beneficial effect in PD frequently relied on clinical outcomes only, in particular, favourable changes on the MDS-UPDRS III

[5,6,8,54], and whether this represents the optimum primary outcome remains debated.[55] Prominent placebo effects have been noted in other PD neuroprotection treatment trials.[56]

To address the inherent shortcomings of this approach, we included sensor-based, objective quantification of motor impairment as a secondary trial outcome. The supervised, sensor-based gait analysis showed a change suggestive of a degenerative pattern for several gait variables in the placebo treatment group. In contrast, we observed either an improvement in the UDCA treatment group or comparatively less worsening in gait over the treatment period. The longitudinal deterioration in the placebo group is comparable with previous studies of similar PD cohorts that identified stride time variability, irregularity and increased step time variability as potential progression markers.[57,58] Decreases in gait speed have also been identified to closely correlate with disease progression.[59,60] A greater burden of axial features has consistently been associated with poorer adverse clinical phenotypes and increased risk of rapid progression PD.[33,61] Therefore, the changes observed appear consistent with a disease-modifying effect of UDCA, through a reduction of the natural progression of gait impairment in PD, but this awaits confirmation in a subsequent, larger trial. Changes in MDS-UPDRS III scores did not correlate with any gait parameters (data not shown) which is not unexpected as only a small proportion of MDS-UPDRS III is comprised of gait-related assessments. Supervised sensor-based gait analysis therefore offers promise as an alternative or complementary endpoint in future neuroprotective trials in PD and may be more sensitive to detecting disease progression, in particular over comparatively short periods of time than the MDS-UPDRS.

Conceptually, the proof of target engagement is a key aspect of early, proof of concept studies for any IMP, but has been lacking for many PD neuroprotection studies. Elevated (i.e. less negative) ^{31}P-MRS measured ΔG_{ATP} has previously been observed in mitochondrial cytopathies and is therefore consistent with mitochondrial dysfunction.[62] More recently, using

a similar ^{31}P-MRS protocol, our group has demonstrated differences in ΔG_{ATP} in the midbrain of patients with amyotrophic lateral sclerosis, a further neurodegenerative disorder with growing evidence of mitochondrial dysfunction.[25] In the context of otherwise stable ATP levels, a more negative value in ΔG_{ATP} (as observed in the UDCA treatment arm) implies that a relatively greater amount of energy was released by ATP hydrolysis. In mitochondrial cytopathies the administration of coenzyme Q10 resulted in an improvement (lowering) of both ΔG_{ATP} and ADP, approaching the values found in healthy controls and providing evidence of possible target engagement.[63] Similarly, the observed lowering of ΔG_{ATP} in the UDCA treatment arm of our study is in keeping with the assumption of mechanistic target engagement for UDCA, resulting in improved mitochondrial function. Sathe and co-workers also reported ^{31}P-MRS based evidence of target engagement for UDCA in PD in a small open-label pilot-study.[64] However, a different imaging protocol focussing on the occipital cortex was applied, ΔG_{ATP} was not calculated and only three PD patients had ^{31}P-MRS imaging before and after a 6-week course of UDCA at a dose of 50 mg/kg. Notably, ^{31}P-MRS is also being applied in other completed or on-going proof-of-concept studies for mitochondrial rescue compounds in PD.[23,24,65]

^{31}P-MRS has been used previously to identify bioenergetic dysfunction in PD, with deficits of PCr and ATP in the midbrain compared to healthy controls.[66] We did not observe any increases in ATP or PCr, this may be due to methodological differences as we have not attempted absolute quantification of the concentration of ^{31}P-MRS metabolites. Alternatively, rather than directly increasing the overall amount of ATP, UDCA may be reducing the reliance upon alternative pathways to ATP production such as glycolysis by improving the efficiency of oxidative phosphorylation.

Conceptually, the trial design of our UP study is similar to other recent early clinical trials such as the AiM-PD trial, an open-label trial of ambroxol, and a recent randomised controlled-trial

assessing niacin (a vitamin B3 derivative) which both focused on determining the mechanistic effect of the respective trial compound in human PD patients.[67,68] As stated above, the UP study was not formally powered to confirm or refute a neuroprotective effect of UDCA. Subsequent, considerably larger and therefore more costly phase IIb/III studies will be required to confirm or refute such a neuroprotective effect for UDCA. However, the excellent safety profile of UDCA at 30 mg/kg, combined with the ^{31}P-MRS-based evidence of target engagement and the promising results from the gait analysis provide strong rationale for such future trials of UDCA in PD.

The action of UDCA might be pleiotropic and is yet to be fully elucidated. For instance, there is growing evidence supporting the role of the microbiome and gut-brain axis in PD.[69,70] Changes in the PD gut microbiome are associated with alterations in the bile acid pool.[71] Intriguingly, a marked reduction of UDCA and its taurine conjugate TUDCA has been reported in an experimental model of prodromal PD; in addition, UDCA treatment partially restores the gut microbial profile in other conditions.[72-74] A beneficial effect of UDCA in PD may therefore not be limited to a restoration of cerebral mitochondrial function but also relate to an additional, but as yet speculative beneficial effect on the PD microbiome and the gut-brain axis.

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Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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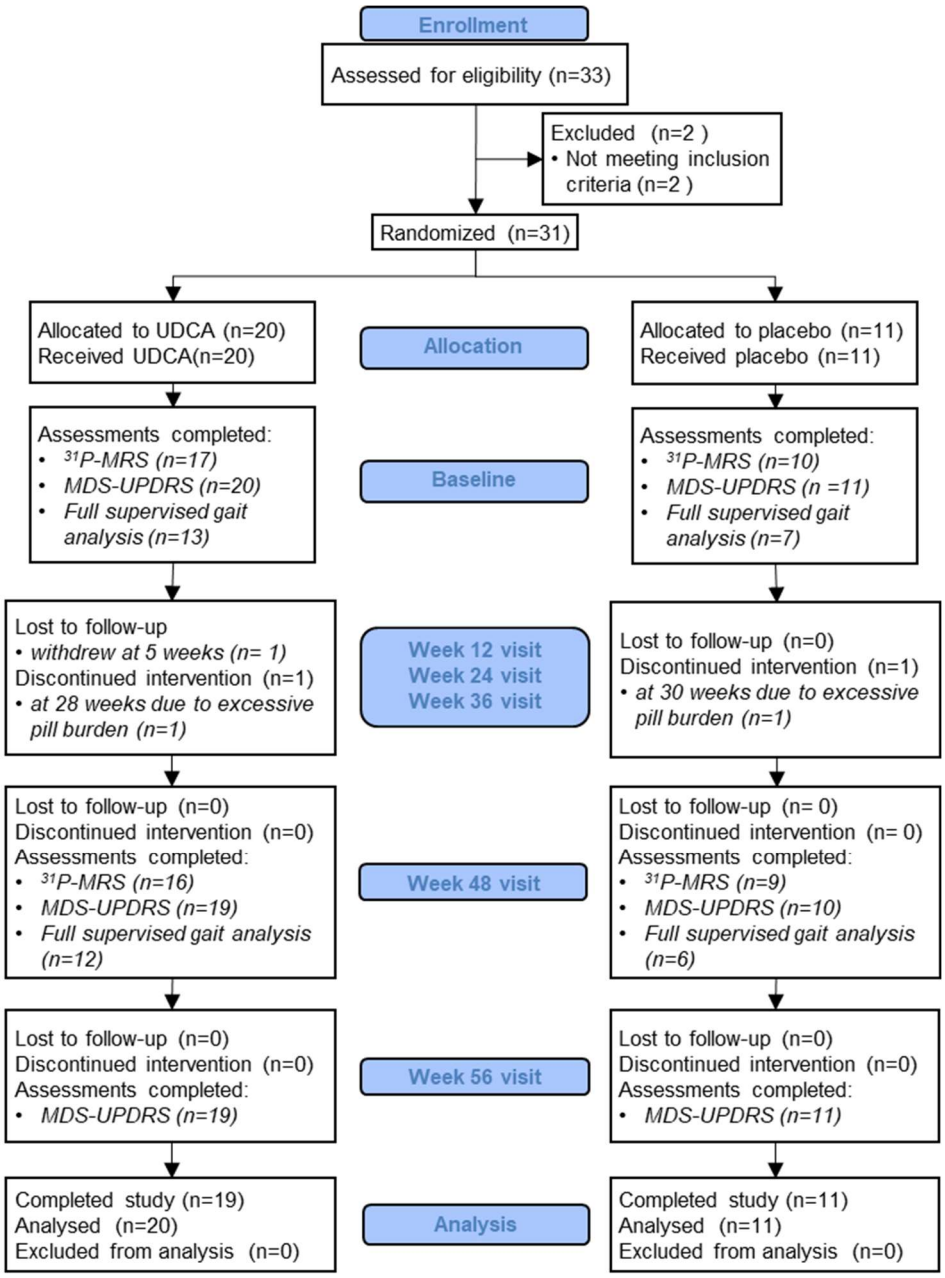

Figure 1: Consort flowchart of enrollment, allocation and follow-up assessments performed. Analysis used an intention-to-treat population therefore all patients randomised were included in the analysis dataset. Details of key secondary outcome assessments are included to demonstrate data completeness.

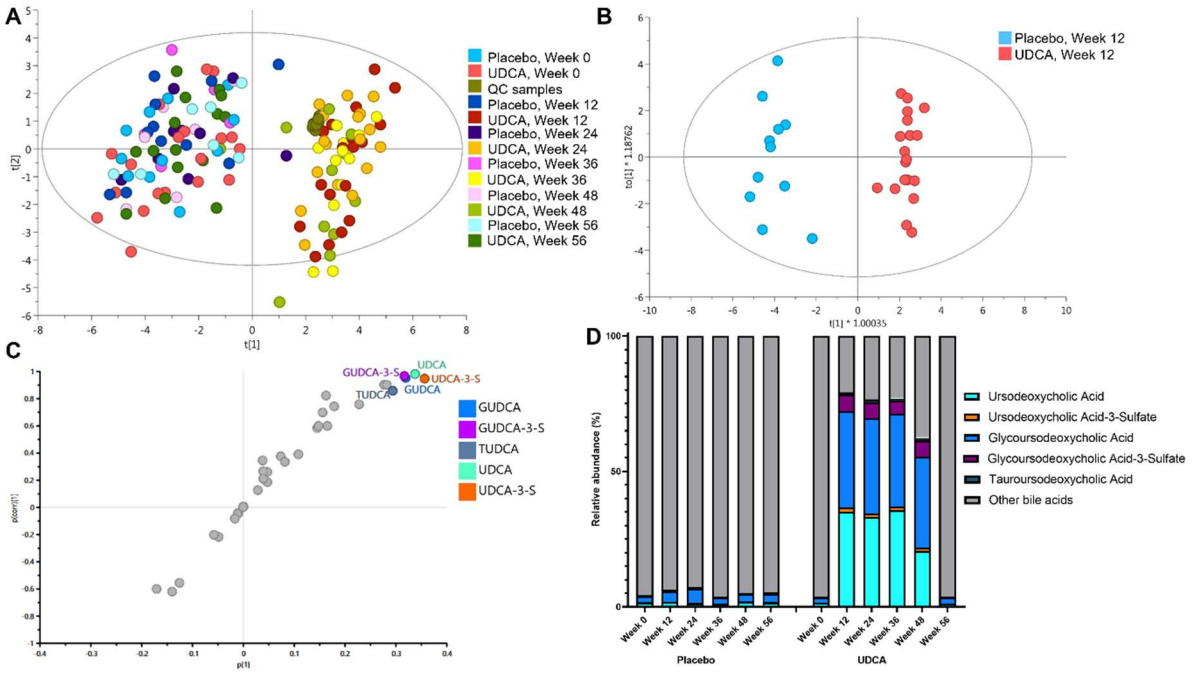

Figure 2: Impact of UDCA upon serum bile acid profiles. As assessed using analysis of ultra-performance liquid chromatography linked to mass spectrometry serum bile acid profiling data. (**A**) PCA scores plot, all participants at all time points. (**B**) OPLS-DA scores plot, placebo *vs* UDCA-treated patients at week 12. (**C**) Discriminatory metabolomic analysis via S-plot, placebo *vs* UDCA-treated patients at week 12; metabolites at top right of plot are those enriched in UDCA vs placebo week 12 serum samples. Other detected bile acids are in grey with further detail on these bile acids shown in the supplementary information (**D**) Relative abundance plot of key bile acids of interest. Features in top right of S-plot are bile acids elevated in serum of UDCA *vs* placebo participants at week 12; features in bottom left of S-plot are bile acids elevated in serum of placebo *vs* UDCA participants at week 12. For (**B**) and (**C**), placebo, *n*=11; UDCA, *n*=19. Abbreviations: GUDCA; glycoursodeoxycholic acid; GUDCA-3-S; glycoursodeoxycholic acid-3-sulfate; OPLS-DA: orthogonal projections to latent structures discriminant analysis; PCA: principal component analysis; TUDCA: tauroursodeoxycholic acid; UDCA: ursodeoxycholic acid; UDCA-3-S: ursodeoxycholic acid-3-sulfate; QC: Quality Control.

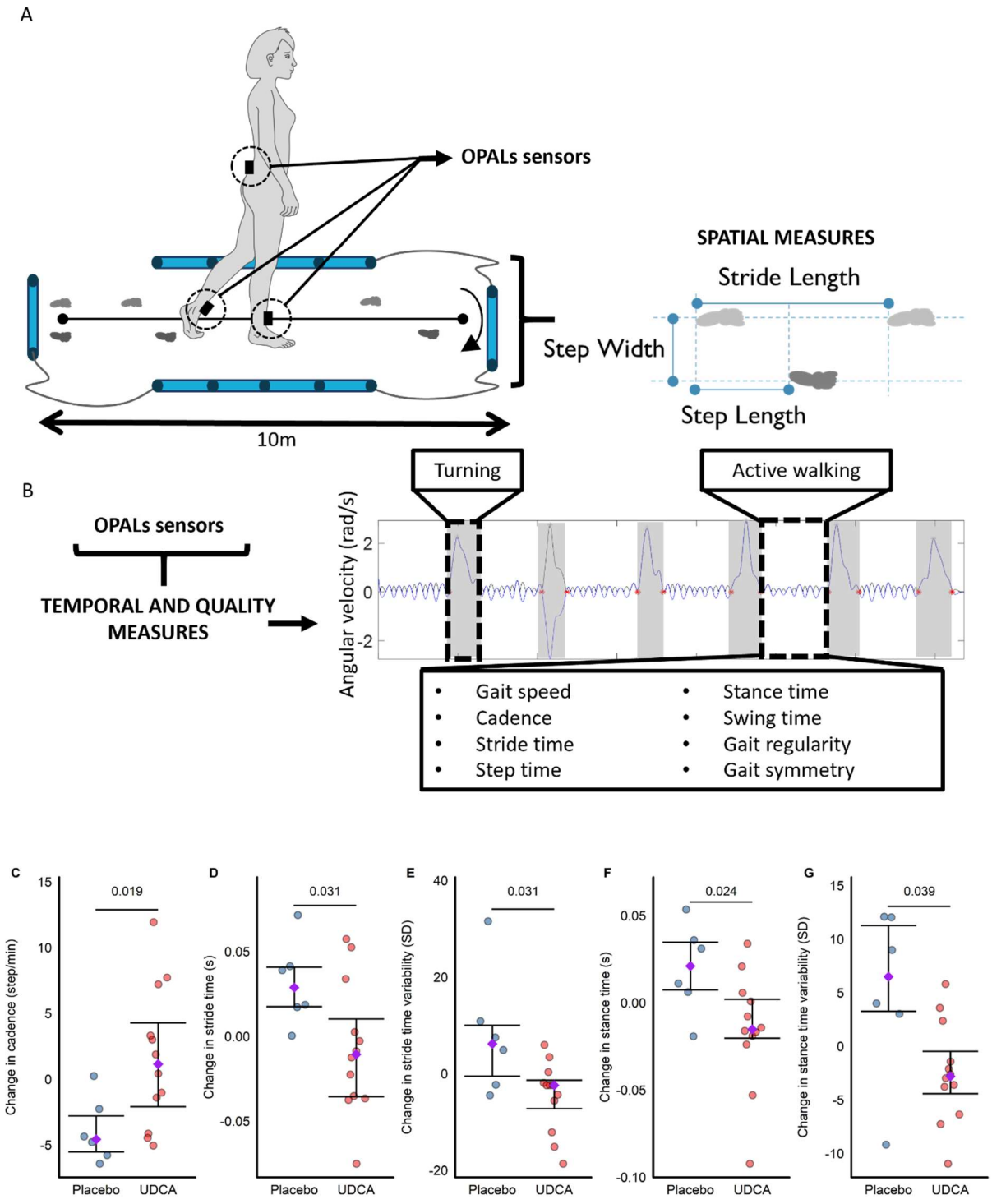

Figure 3: Gait analysis equipment and data processing. (**A**) Schematic of testing procedure with the OPTOgait system in a two-dimensional configuration with a participant wearing OPALs sensors on the lower shins, as well as their lower back (denoted by black boxes). **(B)** Angular velocity signals recorded using the lumbar sensor during the walking test used to

define the turns within the data. Acceleration and angular velocity are then used during active walking to generate temporal, intensity, and regularity quality measures of gait. Comparison of gait parameters from baseline to week 48 in the two treatment groups (12 on UDCA 30 mg/kg vs 6 on placebo) demonstrated that PD patients on UDCA took more steps per minute (increased cadence) (**C**), with reduced amount of time between each heel strike of the same foot (stride time) (**D**) and reduced stride time variability (**E**) as well as reduced stance time (**F**) and stance time variability (**G**). For **C-G** purple diamond and error bars signify median and interquartile range as gait analysis data was not normally distributed, *P* values show significance for group differences as assessed by the Wilcoxon signed rank test.

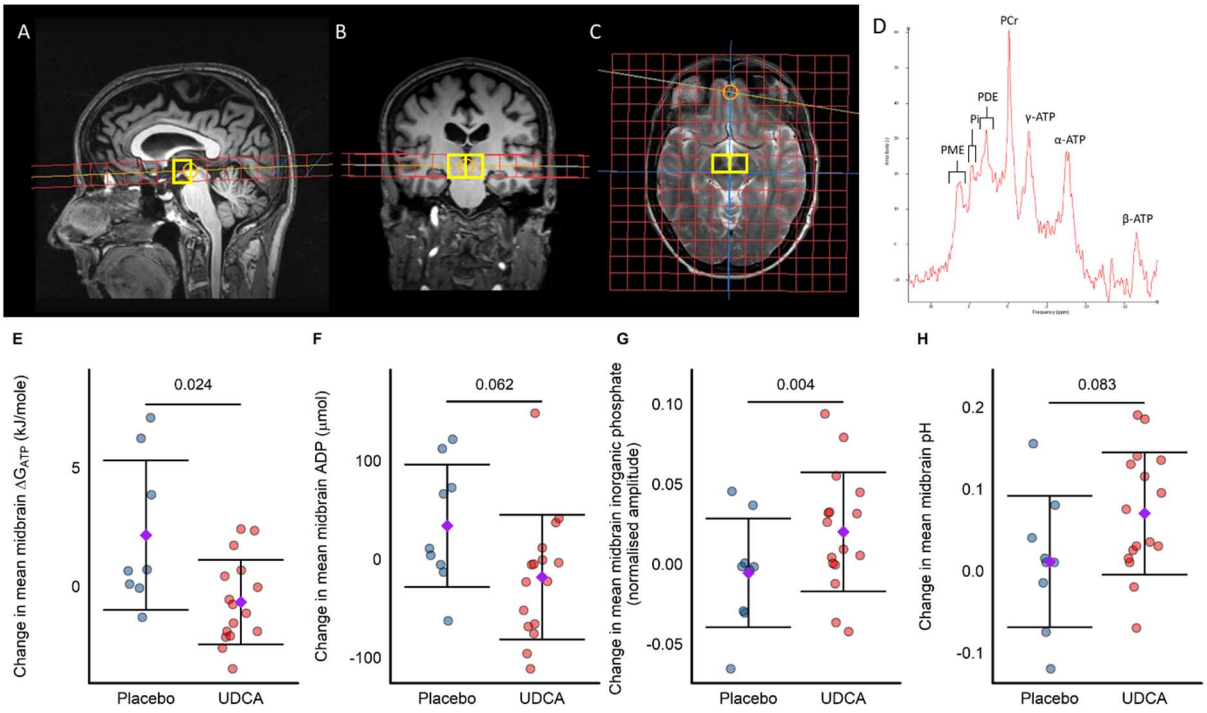

Figure 4: ^{31}Phosphorus Magnetic Resonance voxel localisation, example spectra and results. Sagittal (**A**), coronal (**B**) and axial (**C**) images demonstrating 14x14 CSI spectroscopic grid positioning for the midbrain voxels. Voxel placement ensures the substantia nigra will be included within the voxel of interest. Voxels of interest from each acquisition are highlighted in yellow. (**D**) Example spectrum obtained from the midbrain of a participant in the placebo group. This spectrum has been phased and apodised to aid visualisation with phosphocreatine frequency shifted to 0ppm. Change from baseline to week 48 in key ^{31}P-MRS parameters from the midbrain for; (**E**) ΔG_{ATP}, (**F**) ADP concentration, (**G**) inorganic phosphate and pH (**H**). For **E-H** purple diamond and error bars signify mean ± standard deviation and *P* values are for the significance of the estimated treatment coefficient with UDCA as assessed by linear regression. UDCA *n*=16, placebo *n*=9 except panel (**E**) where placebo *n*=8 due to excluded magnesium value prior to unblinding required for calculation of ΔG_{ATP}. PME=phosphomonoesters, PDE= phosphodiesters, Pi= inorganic phosphate, PCr= phosphocreatine, γ-ATP= gamma adenosine triphosphate, α-ATP= gamma adenosine triphosphate, β-ATP= gamma adenosine triphosphate, ΔG_{ATP} = Gibbs free energy of ATP hydrolysis.

Table 1. Demographic and clinical features

		UDCA (n=20)	**Placebo (n=11)**	***p*-value**
Age (years)	Mean ± SD	56.3 ± 7.6	61.9 ± 8.28	0.0762[a]
	Range	40-74	53-73	
Sex	Male (%)	14 (70)	5 (45.5)	0.3385[b]
(n, %)	Female (%)	6 (30)	6 (54.5)	
Disease Duration (months)	Mean ±SD	16.3 ± 11.7	22.1 ± 7.2	0.0989[a]
	Range	2.3 - 41.5	10.7 – 32.7	
Family History of PD in a first degree relative (n, %)	Present	1 (5)	2 (18.2)	0.5803[b]
	Absent	19 (95)	9 (81.8)	
Modified Hoehn &Yahr	Stage 1	5 (25)	2 (18.2)	
(n, %)	Stage 1.5	2 (10)	2 (18.2)	0.7725[b]
	Stage 2	13 (65)	7 (63.6)	
Predicted Risk of Rapid Disease Progression	Mean ±SD	0.31 ± 0.16	0.28 ± 0.21	0.6902[a]
	Range	0.09 – 0.77	0.10 – 0.69	

[a]tested with two-sample t-test with Welch's correction
[b]tested with Pearson's Chi-squared test

Table 2. Details of Adverse Treatment Reactions

System Organ Class	Adverse Treatment Reaction[a]	UDCA (n=20)	Placebo (n=11)
Gastrointestinal disorders	Abdominal distension	1 (5.0%)	1 (9.1%)
	Abdominal pain	1 (5.0%)	1 (9.1%)
	Constipation	1 (5.0%)	1 (9.1%)
	Diarrhoea	5 (25.0%)	1 (9.1%)
	Dry mouth	0 (0.0%)	1 (9.1%)
	Gastroesophageal reflux disease	0 (0.0%)	1 (9.1%)
	Nausea	2 (10.0%)	0 (0.0%)
	Salivary hypersecretion	1 (5.0%)	0 (0.0%)
Metabolism and nutrition disorders	Abnormal loss of weight	1 (5.0%)	0 (0.0%)
Musculoskeletal disorders	Arthralgia	1 (5.0%)	0 (0.0%)
Nervous system disorders	Parkinson's Disease progression	0 (0.0%)	1 (9.1%)
	Restless legs syndrome	1 (5.0%)	0 (0.0%)
Skin and subcutaneous tissue disorders	Pruritis	1 (5.0%)	0 (0.0%)
	Rash	1 (5.0%)	0 (0.0%)

[a]All patients with at least 28 days exposure to study treatment are listed. Only adverse reactions recorded as having a definite, probable or possible relationship to trial medication which started on or after first dose are included. Patients are counted once per row but may appear in more than one row.

Table 3. Results of secondary outcomes

		Baseline (mean ± SD)	Week 48 (mean ± SD)	Week 56 (mean ± SD)	Change from baseline to week 48 (mean ± SD)	Change from week 48 to week 56 (mean ± SD)	Change from baseline to Week 56 (mean ± SD)
Clinical Rating Scales							
MDS-UPDRS I	UDCA	6.6 ± 5.1	6.8 ± 6.1	7.4 ± 5.6	0.5 ± 2.9	0.6 ± 3.6	1.3 ± 3.8
	Placebo	6.0 ± 2.8	6.5 ± 2.7	6.1 ± 3.5	0.7 ± 4.4	-0.7 ± 2.0	-0.1 ± 3.6
MDS-UPDRS II	UDCA	5.6 ± 5.5	5.1 ± 5.3	5.5 ± 5.0	-0.4 ± 4.0	0.5 ± 3.7	0.1 ± 4.1
	Placebo	5.1 ± 5.2	5.0 ± 3.1	4.4 ± 3.3	-0.1 ± 2.8	-0.7 ± 3.1	-1.0 ± 3.9
MDS-UPDRS III ("OFF" state)	UDCA	32.5 ± 11.5	29.5 ± 10.8	26.1 ± 10.3	-1.7 ± 6.7	-3.4 ± 6.4	-5.1 ± 8.8
	Placebo	31.2 ± 7.88	26.4 ± 11.1	25.6 ± 9.5	-5.2 ± 6.5	-0.9 ± 2.88	-5.5 ± 5.7
MDS-UPDRS III ("ON" state)	UDCA	24.0 ± 11.2	20.3 ± 9.2	20.3 ± 9.2	-1.7 ± 5.4	-0.9 ± 5.0	-2.8 ± 6.8
	Placebo	22.5 ± 8.0	19.3 ± 9.3	19.5 ± 9.0	-3.3 ± 8.3	0.2 ± 8.2	-3.1 ± 6.2
MDS-UPDRS IV	UDCA	3.1 ± 2.8	1.9 ± 2.3	2.4 ± 3.1	-1.3 ± 3.1 [b*]	0.5 ± 2.3	-0.7 ± 3.4
	Placebo	1.2 ± 1.8	1.7 ± 1.7	2.1 ± 2.1	0.5 ± 1.1 [b*]	0.4 ± 1.3	0.9 ± 1.14
Levodopa Equivalent Daily Dosage (mg)	UDCA	438 ± 198	510 ± 241	512 ± 237	79 ± 124	2 ± 39	81 ± 116
	Placebo	464 ± 123	516 ± 157	541 ± 155	52 ± 82	25 ± 49	77 ± 86
Montreal Cognitive Assessment[a]	UDCA	27.4 ± 1.9	28.3 ± 1.3	28.4 ± 1.6	1.1 ± 1.6	0.1 ± 1.1	1.2 ± 1.7
	Placebo	28.3 ± 1.2	28.6 ± 1.4	29.2 ± 0.8	0.4 ± 1.1	0.6 ± 1.3	0.9 ± 1.2
MADRS[a]	UDCA	2.5 ± 2.8	4.1 ± 4.7	4.6 ± 5.3	1.7 ± 3.4 [b*]	0.5 ± 3.0	2.2 ± 4.9[b**]
	Placebo	2.9 ± 2.8	2.6 ± 2.6	1.2 ± 1.6	-0.4 ± 1.7 [b*]	-1.4 ± 2.1	-1.7 ± 2.6 [b**]
NMS-QUEST	UDCA	5.6 ± 3.5	6.3 ± 4.1	6.5 ± 4.3	0.9 ± 2.3	0.2 ± 2.4	1.1 ± 2.5 [b**]
	Placebo	5.4 ± 3.9	5.2 ± 3.2	4.4 ± 2.7	-0.2 ± 1.6	-0.8 ± 1.5	-1.0 ± 1.9 [b**]
Sensor-based objective quantification of motor impairment (NB. Data shown here is median and interquartile range)							
Cadence (step/min)	UDCA	117.80 (110.23; 122.65)	115.82 (107.90, 126.52)	*NA*	1.14 (-2.10; 4.28)[c*]	*NA*	*NA*
	Placebo	114.75 (111.85; 119.15)	111.43 (107.69-124.74)	*NA*	-4.58 (-5.54; 2.58)[c*]	*NA*	*NA*
Stride time (s)	UDCA	1.04 (0.99; 1.10)	1.04 (0.95, 1.11)	NA	-0.01 (-0.04; 0.01)[c*]	NA	NA
	Placebo	1.05 (1.02; 1.07)	1.08 (1.04, 1.12)	NA	0.03 (0.02; 0.04)[c*]	NA	NA
Stride time variability (SD)	UDCA	26.29 (24.70, 38.32)	22.19 (20.62, 31.95)	*NA*	-2.43 (-7.27; -1.38)[c*]	*NA*	*NA*
	Placebo	23.04 (19.81, 27.98)	27.90 (25.17, 31.74)	*NA*	6.16 (-0.54; -9.99)[c*]	*NA*	*NA*
Stance time (s)	UDCA	0.62 (0.58, 0.66)	0.62 (0.54, 0.66)	*NA*	-0.02 (-0.02; 0) [c*]	NA	NA
	Placebo	0.62 (0.59, 0.65)	0.64 (0.62, 0.65)	*NA*	0.02 (0.01; 0.03) [c*]	NA	NA
Stance time variability (SD)	UDCA	24.90 (18.57; 30.95)	22.33 (17.16; 26.38)	*NA*	-2.81 (-4.43; -0.48) [c*]	*NA*	*NA*
	Placebo	18.49 (15.60; 21.29)	26.44 (19.81, 28.32)	*NA*	6.48 (3.26; 11.26) [c*]	*NA*	*NA*
Midbrain ^{31}Phosphorus Magnetic Resonance Spectroscopy							
Total ATP	UDCA	0.449 ± 0.058	0.418 ± 0.076	*NA*	-0.028 ± 0.089	*NA*	*NA*
	Placebo	0.429 ± 0.050	0.453 ± 0.061	*NA*	0.0268 ± 0.077	*NA*	*NA*
Total Phosphocreatine	UDCA	0.184 ± 0.023	0.192 ± 0.022	*NA*	0.0072 ± 0.031	*NA*	*NA*
	Placebo	0.194 ± 0.033	0.181 ± 0.025	*NA*	-0.0135 ± 0.040	*NA*	*NA*
Total Inorganic Phosphate	UDCA	0.080 ± 0.025	0.101 ± 0.018	*NA*	0.020 ± 0.037[d***]	*NA*	*NA*
	Placebo	0.08 ± 0.023	0.071 ± 0.028	*NA*	-0.006 ± 0.034[d***]	*NA*	*NA*
ΔG_{ATP} (kilojoule/mole)	UDCA	-64.0 ± 2.39	-64.4 ± 2.13	*NA*	-0.672 ± 1.780[b,d‡]	*NA*	*NA*
	Placebo	-65.5 ± 3.35	-63.2 ± 1.42	*NA*	2.145 ± 3.153[b,d*]	*NA*	*NA*
	UDCA	104.4 ± 55.0	88.7 ± 43.5	*NA*	-1.86 ± 63.3	*NA*	*NA*

ADP (micromolar)	Placebo	77.7 ± 68.7	116.0 ± 36.6	*NA*	3.37 ± 62.2	*NA*	*NA*

[a]performed at screening rather than baseline.

[b]tested with two-sample t-test with Welch's correction

[c]tested with Mann Whitney U test

[d]significant treatment effect assessed using linear regression

*indicates significance at the 0.05 level comparing treatment groups

**indicates significance at the 0.01 level comparing treatment groups

*** indicates significance at the <0.001 level comparing treatment groups