TEMPORAL ASSOCIATIONS OF CGRP-RELATED INFLAMMATORY PATHWAY BIOMARKERS IN SYNUCLEIN-ASSOCIATED NEURODEGENERATIVE DISORDERS (SAND)

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ABSTRACT

Aim: This study investigates the role of calcitonin gene-related peptide (CGRP) and its related inflammatory biomarkers in individuals with Synuclein-Associated Neurodegenerative Disorders (SAND), encompassing conditions like Parkinson's disease (PD) and Multiple System Atrophy (MSA). Given CGRP's known anti-inflammatory and neuroprotective properties demonstrated in vitro and in animal models, we aimed to explore its association with inflammatory pathways in humans across different circadian stages. **Material and Methods:** We analysed plasma levels of biomarkers such as CGRP, NLRP3 inflammasome components, IL-1β, and IL-18, along with hair cortisol, in 15 participants (7 with SAND, 8 controls). Blood samples were collected before and after overnight sleep studies, and correlations between these markers were assessed using non-parametric tests. **Results:** Across groups, all plasma biomarkers showed significant correlations before and after sleep. In controls, strong temporal associations within the CGRP pathway were observed, particularly between pre-sleep IL18, post-sleep CGRP, and hair cortisol, driven primarily by control group variance. These biomarkers also related to clinical features such as cognition and motor function, with notable associations between inflammatory markers and cognitive scores. In the SAND group, fewer biomarker correlations were found, with pre-sleep NLRP3 correlating with hair cortisol. Only pre-sleep CGRP and IL18 levels significantly differed between groups, suggesting disease-specific alterations in circadian biomarker patterns. **Conclusions:** The above highlights that temporal fluctuations of inflammatory biomarkers differ in patients with SAND compared to controls. By extension, the lack of association of CGRP to these inflammatory markers in patients, but its association in controls suggests that under healthy conditions CGRP exerts some control over the inflammatory cascade, but its absence allows for stronger inflammatory biomarker co-expressions at 4-5 years of disease onset.

Keywords: CGRP, biomarkers, neuroinflammation, Parkinson disease, sleep

ΧΡΟΝΙΚΕΣ ΣΥΣΧΕΤΙΣΕΙΣ ΤΩΝ ΒΙΟΔΕΙΚΤΩΝ ΦΛΕΓΜΟΝΗΣ ΣΧΕΤΙΖΟΜΕΝΩΝ ΜΕ ΤΟ ΜΟΝΟΠΑΤΙ CGRP ΣΤΙΣ ΣΥΝΟΥΚΛΕΪΝΟΠΑΘΕΙΕΣ

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ΠΕΡΙΛΗΨΗ

Στόχος: Αυτή η μελέτη διερευνά τον ρόλο του πεπτιδίου CGRP και των σχετιζόμενων με αυτό βιοδεικτών φλεγμονής σε άτομα με Συνουκλεϊνοπάθειες (SAND), όπως η νόσος Πάρκινσον και η ατροφία πολλαπλών συστημάτων. Δεδομένου ότι το CGRP έχει γνωστές αντιφλεγμονώδεις και νευροπροστατευτικές ιδιότητες που έχουν αποδειχθεί in vitro και σε ζωικά μοντέλα, στόχος μας ήταν να διερευνήσουμε τη σύνδεσή της με φλεγμονώδη μονοπάτια σε ανθρώπους κατά την διάρκεια κιρκάδιων ρυθμών. **Υλικά και Μέθοδοι:** Αναλύσαμε τα επίπεδα βιοδεικτών στο πλάσμα, όπως το CGRP, τα συστατικά του ινφλαμμασώματος NLRP3, IL-1β και IL-18, καθώς και κορτιζόλης μαλλιών, σε 15 συμμετέχοντες (7 με SAND, 8 υγιείς μάρτυρες). Τα δείγματα αίματος συλλέχθηκαν πριν και μετά από ολονύκτια μελέτη ύπνου, και οι συσχετίσεις μεταξύ αυτών των βιοδεικτών αξιολογήθηκαν με μη παραμετρικές μεθόδους. Αποτελέσματα: Σε όλες τις ομάδες, όλοι οι βιοδείκτες στο πλάσμα έδειξαν σημαντικές συσχετίσεις πριν και μετά τον ύπνο. Στους υγιείς μάρτυρες, παρατηρήθηκαν ισχυρές χρονικές συσχετίσεις στο μονοπάτι του CGRP, ιδίως μεταξύ προ-ύπνου IL-18, μετά τον ύπνο CGRP και κορτιζόλης μαλλιών, κυρίως λόγω της διαφοράς στη variance της ομάδας ελέγχου. Αυτοί οι βιοδείκτες συνδέονταν επίσης με κλινικά χαρακτηριστικά όπως η γνωστική λειτουργία και η κινητική ικανότητα, με σημανικές συσχετίσεις μεταξύ φλεγμονωδών δεικτών και γνωστικών λειτουργιών. Στην ομάδα SAND, βρέθηκαν λιγότερες συσχετίσεις βιοδεικτών, με το προ-ύπνου NLRP3 να συσχετίζεται με την κορτιζόλη των μαλλιών. Μόνο τα επίπεδα προ-ύπνου της CGRP και IL-18 διαφέρουν σημαντικά μεταξύ των δύο ομάδων, υποδεικνύοντας διαταραχές στα κιρκάδια μοτίβα των βιοδεικτών. **Συμπεράσματα:** Τα ανωτέρω υπογραμμίζουν ότι οι χρονικές διακυμάνσεις των φλεγμονωδών βιοδεικτών διαφέρουν σε ασθενείς με SAND σε σύγκριση με τους υγιείς. Η απουσία συσχέτισης του CGRP με αυτούς τους βιοδείκτες στους ασθενείς, σε αντίθεση με τους υγιείς, υποδηλώνει ότι υπό φυσιολογικές συνθήκες το CGRP ασκεί κάποιον έλεγχο στην φλεγμονώδη αλυσίδα, ενώ η απουσία του επιτρέπει ισχυρότερες συν-εκφράσεις φλεγμονωδών βιοδεικτών 4-5 χρόνια μετά από την έναρξη της νόσου.

Λέξεις-κλειδιά: CGRP, βιοδείκτες, νευροφλεγμονή, νόσος Πάρκινσον, ύπνος

INTRODUCTION

Parkinson's Disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease (AD), and it is associated with significant morbidity and mortality—carrying a 1.75 to 3.86 times higher risk compared to the general population.[1] In 2017, approximately one million individuals were diagnosed with PD in the United States, with direct healthcare costs reaching $51.9 billion.[2] A key pathological feature of PD is the aggregation and propagation of α-synuclein (α-syn), which correlates with disease severity and prognosis. Recent research also links α-syn with inflammasome-induced inflammation.[3] Inflammasomes are multiprotein complexes within the cytosol of immune cells that play a crucial role in disease development.[4-6] Chronic activation of inflammasomes — triggered by pathogenic microorganisms, mitochondrial oxidative stress, endogenous cytokines, and protein aggregates — is proposed as a mechanism underlying neurodegeneration.[3] In PD, fibrillar α-syn released into the extracellular space due to neuronal degeneration activates microglia and amplifies inflammatory responses.[7,8] Studies have shown that NLRP3 inflammasome activation and elevated inflammatory cytokines are involved in PD onset.[9,10]

A key protein involved in the regulation of inflammatory and other pathways is the Calcitonin gene-related peptide (CGRP), which is part of the calcitonin family of peptides and is extensively expressed in neuronal tissues.[11-13] Binding CGRP to its receptor activates multiple signalling pathways that can influence neurodegeneration, with disruptions potentially contributing to disease progression.[14,15] Emerging evidence indicates that CGRP exerts neuroprotective effects across various neuronal populations by engaging multi-kinase signalling pathways.[16] Specifically, CGRP appears to diminish anti-apoptotic signalling while enhancing proliferative pathways in an Akt-dependent manner.[17] Additionally, in vitro and animal studies suggest CGRP possesses anti-inflammatory properties, possibly through modulation of macrophages and inhibition of the NLRP3 inflammasome.[18,19] Long-acting CGRP analogues have shown promise as therapeutic agents for type 2 diabetes due to their favourable metabolic effects and promotion of GLP-1 secretion.[20] Activation of the GLP-1 receptor can increase gene expression of peptides involved in energy regulation, such as CGRP and IL-6, within the parabrachial nucleus.[21] Conversely, the use of DPP4 inhibitors and GLP-1 mimetics has been associated with a reduced risk of developing PD compared to other oral antidiabetic agents. These findings suggest that anti-inflammatory strategies could delay PD progression.[22,23] Furthermore, exogenous CGRP administration has been shown to inhibit macrophage infiltration and reduce inflammatory mediator expression, thereby

mitigating inflammation-related damage in AD. Consequently, targeting CGRP receptor pathways may offer a novel therapeutic approach for both AD and PD.[24]

In the present study, we explored the associations of CGRP related plasma biomarkers in individuals who underwent detailed deep phenotyping of SAND and matched controls before and after sleep. Specifically, we examined the relationship between CGRP and inflammatory biomarkers in relation to detailed phenotypic features of people with SAND, and more specifically, whether NLRP3, IL-1β, IL-18, and hair and blood cortisol were associated with CGRP levels, and whether this association differed between groups and before and after sleep. We hypothesised that CGRP has a neuroprotective role; therefore, we expected CGRP related patterns to reveal increased expression early in the disease process, and conversely, we anticipated that their expression would decline as the disease progresses. We further hypothesised that pre- to post- sleep fluctuations of CGRP-related biomarkers would be more closely associated with disease severity than their absolute levels, and further mediated by sleep disturbances and executive dysfunction rather than to motor symptoms.

MATERIAL AND METHODS

Participants

We recruited 18 participants with SAND (age 48-80; F:M 9:9; 16 PD and 2 MSA; years of disease 4.73; UPDRSIII 29.89; H&Y 2.29; S&E 87.65) and 13 non-impaired controls (age 42-78; F:M 8:5) from the Movement Disorders and Sleep & Memory Centres at the Neurological Institute of Athens (NIA) and after obtaining informed consent post Institutional Review Board approval (**Tables 1 and 2**). Participants with SAND were older than 18 years old, had a clinical diagnosis of SAND, Hoehn and Yahr ≤ 3, and MMSE ≥ 24. Participants were excluded if they presented with major vascular brain disease, and/or history of major psychiatric disease or medication use affecting movement, cognition, or sleep regulation, features suggesting atypical parkinsonism, and features of prodromal PD. The control group included people with features of prodromal PD (i.e., idiopathic REM behaviour disorder, constipation).

In the present study, we present the preliminary results from the plasma analysis on 15 participants (7 with SAND and 8 controls).

Clinical Protocol

All participants underwent multisource-multidisciplinary clinical assessments of structured interviews and questionnaires on history & physical information, including motor assessments (e.g., UPDRS-III, Schwab and England), and neuropsychological testing. Structured questionnaires included the Neurological Institute of Athens Cognitive Behavioural Symptoms Questionnaire (Q-CBS) for patients and caregivers, a 51-item questionnaire, of which the first three items capture gross impressions of cognition (Q-CBS-1), movement (Q-CBS-2), and sleep (Q-CBS-3), respectively. Plasma biomarker levels (CGRP, NLRP3, IL1β, IL18, cortisol, NFL, GFAP, insulin) were collected before and after an overnight in-lab sleep study. Hair cortisol, representing average cortisol levels over the previous three months, was collected from hair post-sleep (**Figure 1**).

Biosample processing: Blood biomarker quantification, including CGRP, NLRP3, IL-1β and IL-18 Western Blot and ELISA were pursued at the National Centre for Scientific Research (NCSR) "Demokritos" and blood cortisol, NFL, GFAP, and insulin levels were pursued in at Uppsala and Gothenburg Universities in Sweden.

Statistical Analysis

We performed non-parametric tests (Spearman and Mann Whitney test) between markers across and between the two groups to assess associations within and between biomarkers and groups across circadian stages.

Table 1. Demographics of control and SAND participants.

	SAND	CONTROLS	P-value
F:M	9:9	8:5	0.394
AGE Mean (SD); Range	66.06 (9.53); 47-80	61.23 (9.51); 42-78	0.063

Table 2. Baseline demographics and rating scales' scores of SAND group.

Metric	SAND	
	Mean (SD)	**range**
AGE OF ONSET	59.00 (9.87)	44-77
YEARS OF DISEASE	4.73 (3.41)	1-11
UPDRS III	29.89 (14.74)	12-63
Hoehn **&Y**ahr	2.29 (0.71)	2-4
Schwab **& E**ngland	87.65 (16.02)	50-100

RESULTS

Across groups, all plasma biomarkers correlated to themselves before and after sleep Within the CGRP-related pathway across circadian rhythms, in control's group temporal associations were strongest between pre-sleep IL18 and to post-sleep CGRP, IL-18 and hair cortisol. IL-18 and CGRP were correlated both within times and across circadian rhythms (r = 0.97 – 0.99; p

< 0.05). CGRP before sleep was only associated with pre- and post-sleep IL18, an observation driven by variance in the control group. Post-sleep IL-18 and CGRP were correlated with hair cortisol (**Figure 1**). These plasma associations were primarily driven by biomarker variance in the control group rather than the SAND group (**Figure 2**). Actually, only pre-sleep NLRP3 was correlated with hair cortisol in people with SAND.

Figure 1. Cross-sectional and longitudinal biomarker associations in Controls.

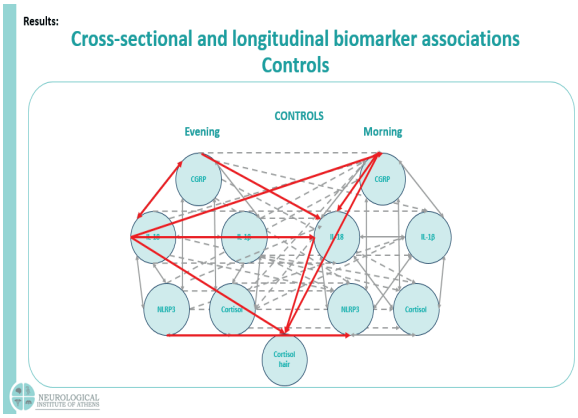
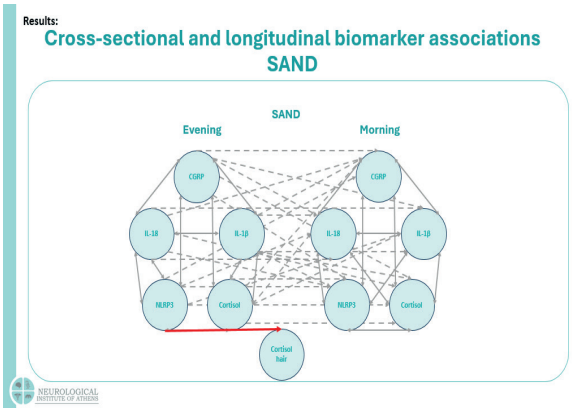


Figure 2. Cross-sectional and longitudinal biomarker associations in SAND.



The CGRP-related pathway biomarkers across circadian rhythms were also associated with clinical features. In the control group, pre-sleep CGRP correlated with phonemic fluency, while post-sleep CGRP was linked to MMSE scores. A significant relationship was observed between evening NLRP3 and phonemic fluency and Q-CBS-1 (cognitive), and morning NLRP3 with Q-CBS-3 (sleep). Pre-sleep IL18 was associated with MMSE, and post-sleep IL18 with Q-CBS-1 (cognitive) and MMSE. Cortisol levels in hair showed a significant correlation with recall. Additionally, morning NFL levels were related to phonemic fluency (**Table 3a**). In the SAND group, post-sleep CGRP was associated with phonemic fluency. Evening IL18 and IL1β levels were linked to recall. Pre-sleep NFL correlated with UPDRS III scores. Both pre- and post-sleep GFAP levels were associated with CBS 1, with post-sleep GFAP also showing a significant relationship with phonemic fluency. Lastly, morning cortisol and insulin levels were associated with Schwab and England and digit span backward tasks, respectively (**Table 3b**).

When comparison of biomarkers was carried out, only pre-sleep CGRP and IL18 levels were significantly different between the two groups (z = -2.78; p = 0.004) (**Table 4**).

Table 3. Longitudinal biomarker and clinical manifestation associations in a. Controls, b. SAND.

PS, pre-sleep; AS, post-sleep. Q-CBS, Structured questionnaires included the Neurological Institute of Athens Cognitive Behavioural Symptoms Questionnaire; Q-CBS-1, Cognition, Q-CBS-2, Movement; Q-CBS-3, Sleep.

a.

P	Q-CBS- 1	N	Q-CBS- 2	N	Q-CBS- 3	N	MMSE	N	Digit backwards	N	recall_PS	N	phonemic_fluency PS	N
CGRP_PS	0.8	4	0.058	5	0.604	5	0.854	6	0.305	6	0.084	6	*0.037	5
CGRP_AS	0.499	6	0.854	6	0.381	6	*0.039	8	0.974	8	0.326	8	0.432	7
NLRP3_PS	*0	4	0.638	5	0.306	5	0.573	6	0.512	6	0.913	6	*0.037	5
NLRP3_AS	0.288	6	0.573	6	*0.02	6	0.689	8	0.446	8	0.583	8	0.294	7
IL18_PS	0.6	4	0.638	5	0.361	5	*0.021	6	0.749	6	0.173	6	0.505	5
IL18_AS	*0.036	6	0.854	6	0.956	6	*0.008	8	0.821	8	0.563	8	0.432	7

Cortisol_ hair	0.211	9	0.774	10	0.653	10	0.385	11	0.221	10	*0.043	11	0.608	8
NFL_nl_AS	0.08	6	0.067	7	0.658	7	1	8	0.717	8	0.204	8	*0.049	7

b.

p	**UPDR-SIII**	**N**	**Schwab & England**	**N**	**CBS 1**	**N**	**CBS2**	**N**	**CBS 3**	**N**	**MMSE**	**N**	**Digit backwards**	**N**	**recall_ PS**	**N**	**phonemic_fluency PS**	**N**
CGRP_AS	0.913	6	0.32	6	0.14	6	0.518	6	*0.041	5	0.816	6	0.117	6	0.468	6	0.104	5
IL18_PS	0.935	5	0.638	5	1	5	0.269	5	0.684	4	0.638	5	0.434	5	*0.037	5	0.8	4
IL1β_PS	0.741	5	0.638	5	1	5	0.614	5	0.684	4	0.308	5	0.434	5	*0.037	5	0.6	4
cortisol_ng/ml_AS	0.452	11	*0.038	11	0.759	11	0.462	11	0.833	11	1	11	0.639	11	0.6	11	0.623	9
NFL_nl_PS	*0.032	9	0.384	9	0.168	9	0.067	9	0.325	8	0.717	9	0.041	9	0.827	9	0.957	6
GFAP_nl_PS	0.795	9	0.197	9	*0.045	9	0.948	9	0.833	8	0.331	9	0.576	9	0.695	9	0.072	6
GFAP_nl_AS	0.965	9	0.284	9	*0.005	9	0.965	9	0.563	8	0.097	9	0.414	9	0.347	9	*0.019	6
Insulin_pg/ml_AS	0.531	9	0.98	9	0.78	9	0.449	9	0.281	8	0.768	9	*0.031	9	0.703	9	0.439	6

p<0,5

Table 4. Biomarker associations between two groups (Controls and SAND).

Marker		**SAND**			**CONTROLS**		
	N	**Mean (SD)**	**range**	**N**	**Mean (SD)**	**range**	**P-value**
MMSE	17	28.33 (1.85)	24-30	13	29.23 (0.83)	27-30	0.157
p-tau 217 AS	9	0.34 (0.24)	0.00-0.72	8	0.14 (0.14)	0.00-0.30	0.057
p-tau 217 PS	9	0.33 (0.36)	0.00-0.85	8	0.18 (0.12)	0.00-0.29	0.176
Cortisol hair	11	13.87 (14.32)	4.64-52.20	11	10.11 (8.83)	2.77-26.43	0.311
Cortisol blood AS	9	174.11 (53.29)	90.18-254.72	8	212.50 (63.54)	128.86-311.19	0.377
Cortisol blood PS	10	107.15 (49.61)	46.39-200.92	8	64.73 (34.70	28.13-130.08	0.126
CGRP AS	6	67.57 (68.47)	4-186	6	41.07 (18.30)	18-67	*0.030
CGRP PS	6	95.35 (73.65)	6-191	8	61.70 (39.85)	23-140	0.801
NLRP3 AS	5	20.13 (14.89)	10-50	6	17.03 (4.13)	12-22	0.362
NLRP3 PS	6	21.51 (19.48)	13-66	8	17.86 (4.12)	13-25	0.119
IL18 AS	5	98.76 (36.08)	68-157	6	74.73 (20.72)	53-106	0.133
IL18 PS	6	105.33 (28.56)	74-154	8	74.83 (14.25)	53-90	*0.009
IL 1β AS	5	7.03 (4.06)	2-12	6	12.36 (12.8)	2-29	0.563
IL 1β PS	6	17.64 (19.05)	2-45	8	12.29 (8.5)	3-30	0.837

p<0,5

DISCUSSION

The relationship between CGRP and inflammatory biomarkers in relation to detailed phenotypic features of individuals with synuclein-associated neurodegenerative disorders has not been investigated yet. We examined whether inflammatory markers — such as NLRP3 inflammasome components, IL-1β, IL-18, and also hair and blood cortisol were associated with CGRP levels before and after sleep.

In the present study, the primary objective was to explore the relationship between CGRP and inflammatory biomarkers in relation to the detailed phenotypic features of individuals with SAND. Specifically, we aimed to assess whether inflammatory markers—such as NLRP3 inflammasome components, IL-1β, IL-18, as well as hair and blood cortisol—are associated with CGRP levels. Our hypothesis was that CGRP plays a neuroprotective role; thus, we expected to see increased expression of CGRP and

related inflammatory biomarkers in the preclinical stages of the disease. Conversely, we anticipated that their levels would decrease as the disease advances.

The second objective was to investigate the associations and the dynamic fluctuations of CGRP-related pathways, particularly the nighttime-to-morning variations, in relation to clinical and neurophysiological features across motor, cognitive-behavioural, sleep, and circadian domains. We hypothesised that reduced fluctuations in CGRP-related biomarkers would be more strongly linked to disease severity than their absolute levels, with particular connections to sleep disturbances and executive dysfunction, rather than to motor symptoms such as rigidity and bradykinesia.

This comprehensive analysis highlights the dynamic relationships within the CGRP-related pathway across circadian rhythms and their associations with clinical features in both control and SAND groups. In the control group, strong temporal associations were observed between pre-sleep IL18 and post-sleep CGRP, as well as between IL-18 and hair cortisol. The high correlation coefficients ($r = 0.97–0.99$; $p < 0.05$) indicate a tightly coordinated fluctuation of these biomarkers within and across circadian phases. Specifically, pre-sleep CGRP was primarily linked with IL18 at the same time points, whereas post-sleep IL-18 and CGRP correlated with hair cortisol, emphasising the interconnectedness of inflammatory and neuroendocrine pathways in controls. These plasma associations were predominantly driven by variability in the control group, with minimal associations observed in the SAND population, except for pre-sleep NLRP3's correlation with hair cortisol.

The fact that only pre-sleep CGRP and IL18 levels significantly differed between controls and people with SAND, suggests disease-specific alterations in these biomarkers related to sleep or circadian regulation. These findings underline notable differences between healthy controls and people with SAND.

From a clinical perspective, results suggest that biomarkers related to the CGRP pathway exhibit circadian variations that are linked to specific clinical manifestations. In the control group, pre-sleep CGRP influencing phonemic fluency, and post-sleep CGRP being associated with general cognitive function (MMSE. The relationship between evening NLRP3 and both phonemic fluency and cognition, as well as morning NLRP3 and sleep, indicates that inflammasome activity fluctuates across the day and impacts cognitive and sleep-related aspects. IL18 levels before and after sleep are linked to cognitive performance, further emphasising the role of inflammation in cognition. In the SAND group, post-sleep CGRP correlates with phonemic fluency, and evening inflammatory markers (IL18 and IL1β) are associated with recall, suggesting inflammation may influence memory in these patients. The correlation between pre-sleep NFL and motor function (UPDRS III) points to neurodegeneration impacting sleep-related processes. GFAP, a marker of astroglial activity, is associated with cognition, especially after sleep, hinting at glial involvement in neurodegenerative processes. Morning cortisol and insulin levels' links to functional and cognitive assessments imply that stress and metabolic regulation also play roles in symptom expression. Overall, these findings highlight the complex interplay between circadian biomarker fluctuations, inflammatory responses, neurodegeneration, and clinical features, suggesting that timing of biological processes significantly influences disease manifestation and progression.

Pursuing this study, we hypothesised that at late disease stages CGRP expression would be significantly reduced, and, indeed, results from people with SAND indicate that CGRP has limited correlation with other markers of the inflammatory cascade. As already has been discussed, there are five primary pathophysiological mechanisms through which CGRP may influence SAND: (a) neuroinflammatory, (b) anti-apoptotic and proliferative, (c) metabolic, (d) neuromodulatory, and (e) antimicrobial.[25] Among these, most existing data pertain to CGRP-related neuroinflammatory processes in SAND, which also intersect with the other mechanisms. Despite these considerations, although the dynamic regulation of CGRP pathways appears relevant to SAND, there is still a lack of definitive causal evidence and circadian data to confirm whether these pathways are necessary or sufficient drivers of the disease.

PD is generally regarded as a condition that develops with increasing age.[26] Inflammation is a prevalent factor in many age-related diseases, including Parkinson's disease. Prior studies have examined the inflammatory response associated with aging, a process referred to as inflammaging.[27,28] The inflammasome plays a role in inflammaging, a process associated with the early phases of neurodegeneration.[28-31] Neuroinflammation has been linked to the initiation and progression of pathological changes in numerous neurodegenerative diseases, including Parkinson's disease.[32] Central to these inflammatory processes is the overactivation of microglia, particularly via the NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3) inflammasome pathway, which has been detected in tissues from patients with SAND.[33]

The inflammatory response contributes to the progress of age-related macular degeneration (AMD) which is a progressive degenerative disease. In a recent study researchers found that the levels of the NLRP3 component, Apoptosis-Associated Speck-like Protein (ASC) and IL-18 are elevated in patients with AMD, and the protein levels of IL-18 are partially the result of ASC protein expression.[34] CGRP levels can be altered in neurodegeneration. Elevated CGRP

levels were found in cerebrospinal fluid in PD patients compared to people with major depressive disorder according to Svenningsson et al.[35] These findings align with our results, where aside from post-sleep IL-1β, all pre- and post-sleep biomarkers tended to be higher in the SAND participants compared to controls.

The present study also has certain limitations. Besides the small number of participants, the control group had people with REM Sleep Behaviour Disorder (RBD) which is strongly associated with SAND, and people with comorbidities (such as autoimmune or systematic diseases), which can influence inflammatory biomarkers levels. Another point is that no reliable biomarkers exist to define the preclinical and prodromal stages of SAND towards correlating these stages with CGRP levels.

In conclusion, the present study identified that: (a) temporal fluctuations of inflammatory biomarkers differ in patients with SAND compared to controls, and (b) the lack of association of CGRP to these inflammatory markers in patients, but its association in controls suggests that under healthy conditions CGRP exerts some control over the inflammatory cascade, but its absence allows for stronger inflammatory biomarker co-expressions at 4-5 years of disease onset.

Larger multidisciplinary projects with longitudinal design, including biomarker panels, skin biopsy or/and seeding amplification assays are needed to examine the unexplored cross-sectional and dynamic associations of blood and skin biopsy CGRP-related pathway biomarkers to multidimensional real-world data.

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