Future Science OA

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/ifso20

CD4+ T-cell transcription factors predict phenoconversion in idiopathic rapid eye movement sleep behavior disorder

Monica Pinoli, Michele Terzaghi, Franca Marino, Cristoforo Comi, Maurizio Versino & Marco Cosentino

To cite this article: Monica Pinoli, Michele Terzaghi, Franca Marino, Cristoforo Comi, Maurizio Versino & Marco Cosentino (2024) CD4+ T-cell transcription factors predict phenoconversion in idiopathic rapid eye movement sleep behavior disorder, Future Science OA, 10:1, 2418821, DOI: 10.1080/20565623.2024.2418821

To link to this article: https://doi.org/10.1080/20565623.2024.2418821

FUTURE SCIENCE OA
2024, VOL. 10, NO. 1, 2418821
https://doi.org/10.1080/20565623.2024.2418821

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

OPEN ACCESS Check for updates

CD4+ T-cell transcription factors predict phenoconversion in idiopathic rapid eye movement sleep behavior disorder

Monica Pinoli[a], Michele Terzaghi[b], Franca Marino[a], Cristoforo Comi[a,c], Maurizio Versino[d] and Marco Cosentino[a]

[a]Center of Research in Medical Pharmacology, University of Insubria, Varese (I), Italy; [b]Unit of Sleep Medicine & Epilepsy, IRCCS Mondino Foundation, Pavia, Italy; [c]Movement Disorders Centre, Neurology Unit, Department of Translational Medicine, University of Piemonte Orientale, Novara, Italy; [d]Department of Medicine & Surgery, University of Insubria, Varese, Italy

ABSTRACT

Aim: Early biomarkers of phenoconversion to neurodegeneration are crucial to identify individuals at high risk. In patients with idiopathic REM sleep behavior disorder (iRBD), the strongest risk factor for neurodegeneration, $CD4^+$ T cells exhibit a peculiar transcription factor pattern.
Objective: To assess transcription factor mRNA levels in $CD4^+$ T cells as predictive biomarkers of phenoconversion in iRBD patients.
Methods: iRBD patients were followed prospectively. ROC curve analysis and Kaplan-Meier curves were used to assess the discrimination between converters and non-converters.
Results: $CD4^+$ T cells from converters had higher *STAT1*, and lower *GATA3* and *FOXP3* mRNA levels. Hazard ratio was 58.3 (95% CI: 6.2–547.1) for higher *STAT1*, 101.2 (95% CI: 16.8–609.4) for lower *GATA3* and 15.7 (2.7–91.4) for lower *FOXP3*.
Conclusion: *STAT1*, *GATA3* and *FOXP3* mRNA levels in $CD4^+$ T cells are promising predictive biomarkers of phenoconversion in iRBD patients.

PLAIN LANGUAGE SUMMARY

Idiopathic REM sleep behavior disorder (iRBD) can lead to neurodegeneration, and the early identification of patients at risk would be crucial. Our research now shows that mRNA levels of some transcription factors in $CD4^+$ T cells may represent early biomarkers predicting phenoconversion to established neurodegenerative conditions on average up to 3.5 years earlier.

ARTICLE HISTORY
Received 10 January 2024
Accepted 9 October 2024

KEYWORDS
biomarkers; CD4+ T lymphocytes; gene expression; Parkinson's disease; phenoconversion; transcription factors

Rapid eye movement (REM) sleep behavior disorder (RBD) is classified as REM-phase parasomnia, in which undesirable physical events are predominant during sleep [1]. RBD can occur without any coexisting neurologic disorders (so-called idiopathic RBD or iRBD) but it is typically associated with neurodegenerative disorders (38–75%) [2]. Although with different times and modalities in the various patients, RBD may be a prodromal phase for the development of Parkinson's disease (PD) [3]. Indeed, it has been shown that the RBD-synucleinopathy association was particularly high when RBD preceded the onset of other neurodegenerative syndrome features [4].

People with iRBD have an estimated risk to convert to established neurodegenerative conditions of about 33% at 5 years, which however may rise to more than 96% at 14 years, and most subjects convert to PD (43%) or to dementia with Lewy body (DLB; 25%) [5]. Identifying predictors of phenoconversion from iRBD to PD and other neurodegenerative conditions would be of paramount importance for the timely identification of people at increased risk of conversion, who could therefore undergo earlier and possibly more aggressive to therapeutic approaches [6].

Recently, it has been described that the prodromic markers of phenoconversion range from clinical assessments such as smell and motor function, to blood tests, cerebrospinal fluid analysis, tissue biopsies, neuroimaging and genetics [7]. Sumi and colleagues showed in a recent paper that minor hallucinations in iRBD patients may represent an early sign of phenoconversion to neurodegeneration, since in their sample of ten subjects with iRBD and hallucinations, two patients developed PD and three DLB [8]. Park and colleagues (2023), looking for predictors of phenoconversion in iRBD patients, found out that low level of cardiac metaiodobenzylguanidine

CONTACT Marco Cosentino marco.cosentino@uninsubria.it

Supplemental data for this article can be accessed at https://doi.org/10.1080/20565623.2024.2418821

Article highlights

- Patients with idiopathic REM sleep behavior disorder (iRBD) are at risk of progression toward established neurodegeneration, and early biomarkers of phenoconversion would be crucial to identify individuals at higher risk.
- This study shows that, in iRBD subjects progressing to neurodegenerative conditions, $CD4^+$ T cells have higher expression of the transcription factors *STAT1*, and lower levels of *GATA3* and *FOXP3* on average 3.5 years before progression.
- *STAT1*, *GATA3* and *FOXP3* mRNA levels in $CD4^+$ T cells are therefore promising predictive biomarkers of phenoconversion in iRBD patients.

(MIBG) may predict phenoconversion to DLB and elevated plasma neurofilament light chain (NfL) level may predict phenoconversion to multiple system atrophy (MSA) [9,10].

As part of our research focusing on peripheral immunity in PD development and progression, we recently characterized in $CD4^+$ T lymphocytes of PD patients a peculiar pattern of expression of transcription factor genes [11], and in a subsequent study we assessed transcription factor genes expression in $CD4^+$ T cells from subjects with iRBD, in comparison to cells from healthy subjects (HS) and from PD patients [12]. Our main results showed that, compared with HS, iRBD subjects and PD patients had lower *TBX21*, *STAT3* and *STAT4* levels and higher *FOXP3* expression. Interestingly, *TBX21* expression discriminated healthy subjects from iRBD subjects and PD patients. We concluded that peripheral immunity may be an early player in the chain of events ultimately leading to neurodegeneration. Whether the peculiar $CD4^+$ T cells transcription factor profile in iRBD subjects may predict subsequent conversion to clinically defined neurodegenerative conditions remained to be established.

The present study now consists of a follow-up of the same iRBD subjects enrolled in the previous research [12], with the aim to identify 3 years later who was eventually phenoconverted toward a frank neurodegenerative condition. The transcription factor genes expression profile in $CD4^+$ T cells originally characterized has been now compared in converted and not converted iRBD subjects, to identify candidate biomarkers to be tested as predictors of phenoconversion to PD as well as to other neurodegenerative conditions.

1. Methods

1.1. Subjects

The present study includes patients with iRBD, originally enrolled in a study aimed at investigating transcription factor genes expression in $CD4^+$ T cells from subjects with iRBD, in comparison to cells from HS and from PD patients [12]. In the original study, the inclusion criterion was iRBD diagnosis according to the International Classification of Sleep Disorders, third edition [13], and exclusion criteria were any history of autoimmune or inflammatory disorders and chronic immunosuppressive treatment, any sign of neurodegenerative disease or dementia, any neurological comorbidity, posttraumatic stress disorder, use of substance or alcohol abuse, or severe sleep apnea. All the subjects were enrolled between May and June 2019. The Ethics Committee of the Neurological Institute "C. Mondino" of Pavia (I) approved the protocol (number 2021008499, 10/02/2021) and all the participants signed a written informed consent before enrollment. The study was performed according to the Declaration of Helsinki and to the relevant ethical guidelines for research on humans.

1.2. Clinical & laboratory assessment

Patients were followed at the Sleep Center of the Neurological Institute "C. Mondino" of Pavia (I). The clinical evaluation included: motor symptoms, assessed by means of the Unified Parkinson's Disease Rating Scale (UPDRS) part III [14]; cognitive function, by the Mini Mental State Examination (MMSE) [15]; constipation and orthostatic hypotension by means of the Scale for Outcomes in Parkinson's disease□Autonomic (SCOPA□AUT) [16]; depressive symptoms, by means of the Hamilton Rating Scale for Depression (HAM-D) [17].

At the original enrollment, each patient provided a venous blood sample, which was processed to isolate $CD4^+$ T cells, according to an established procedure [11]. Total RNA was then extracted from $CD4^+$ T cells, and mRNA levels of the transcription factor genes *TBX21*, *STAT1*, *STAT3*, *STAT4*, *STAT6*, *RORC*, *GATA3*, *FOXP3* and *NR4A2* were finally assayed by real-time PCR, as previously detailed [12].

Subjects with iRBD were followed prospectively with periodic in-person evaluations to diagnose phenoconversion, i.e., parkinsonism (according to the MDS clinical diagnostic criteria for Parkinson's disease) [18], DLB (Consortium guidelines) [19] and MSA (according to the consensus criteria) [20]. Each patient with suspected dementia underwent a comprehensive neuropsychological evaluation performed by an experienced neuropsychologist [21].

1.3. Isolation of $CD4^+$ T cells & real-time polymerase chain reaction assay

Peripheral blood mononuclear cells were separated by Ficoll-Paque Plus density gradient centrifugation; then $CD4^+$ T cells were obtained by immunomagnetic sorting,

as described [12]. Cell viability was 95% ± 1% (range, 90–98%), and purity was 98% ± 2% (range, 97–100%). Expression of the TF genes *TBX21, STAT1, STAT3, STAT4, STAT6, RORC, GATA3, FOXP3* and *NR4A2* was measured by real-time polymerase chain reaction.

1.4. Real-time PCR conditions

For real-time PCR assays of $CD4^+$ T cells, at least 50,000 $CD4^+$ T cells were resuspended in PerfectPure RNA lysis buffer (5 Prime GmbH, Hamburg, Germany) and total RNA was extracted by PerfectPure RNA Cell Kit™ (5 Prime GmbH, code 2302340). The amount of extracted RNA was estimated by spectrophotometry at $\lambda = 260$ nm. Total mRNA was then reverse-transcribed using a random primer and a high-capacity cDNA RT kit (Applied Biosystems, code 4368813), and the resulting amount of cDNA was estimated by spectrophotometry at $\lambda = 260$ nm. Real-Time PCR reactions were then started with 1 μM cDNA. At the beginning, we always loaded 1 μg/μl of cDNA per reaction (final reaction mix volume + sample = 20 μl). This means that for each reaction we load 2 μl of aqueous solution containing the cDNA (therefore, 2 μg of cDNA in total). The following thermal protocol was used for each sample: 20 s at 95°C (x 1, hot start); 2-step cycles as follows: 1 s at 95°C, 20 s at 60°C (x 40). All the reagents (probes and mix) were used according to the manufacturer's instructions (www.bio-rad.com).

1.5. Statistical analysis

Data are expressed as means ± standard deviations (SD) or medians with interquartile range, after verifying a normal distribution using the D'Agostino & Pearson normality test. Statistical significance of the differences between groups was assessed by two-tailed Student's t test for unpaired data for continuous variables or by the χ^2 test for categorical variables. A receiver operating characteristic (ROC) curve analysis was used to assess the discrimination of phenoconverted (converters) and not phenoconverted (not-converters) iRBD patients, based on $CD4^+$ T cell expression of transcription factor genes. To this end, we estimated the area under the ROC curve (AUC) using a logistic model as a general measure of accuracy, with values between 0.5 and 0.7 indicating no or low discrimination, between 0.7 to 0.8 acceptable, 0.8 to 0.9, good and >0.9 outstanding [22]. Sensitivity was defined as the fraction of values in the group of subjects with the specific condition that are above the threshold, and specificity as the fraction of values in the control group that are below the threshold. Likelihood ratio (LR) was then calculated as sensitivity/(100 – specificity) and taken as the ratio of the probability of the specific test result in future converters to the probability in not-converters. Phenoconversion was evaluated by Kaplan-Meier analysis and the differences were tested by log-rank test with the hazard ratios (HR) calculated using Mantel–Haenszel method. For Kaplan-Meier estimates, time = 0 was set at original enrolment. Censoring time for not phenoconverted iRBD patients was set on January 31st, 2023, or, in the event of death, on the date of death. Calculations were performed using a commercial software (GraphPad Prism version 10.0.0 for Windows, GraphPad Software, MA, USA, www.graphpad.com).

2. Results

2.1. Subjects

The original study [12] included 33 iRBD subjects. Two of them however were lost at follow-up, therefore the present study includes 31 subjects (Table 1). Subjects were followed for a mean ± SD of 1277 ± 195.4 days (median: 1333; interquartile range: 1312–1347).

2.2. Phenoconversion during follow-up

During follow-up, 8 patients (24.2%) converted to a neurodegenerative disorder: 5 to parkinsonism (4 PD and 1 MSA) and 3 to dementia (probable DLB) (Supplementary Table S1). At enrolment, future converters did not differ from not-converters according to either gender, age, disease duration, UPDRS Part III, or MMSE (Table 1), constipation [present in 2 (25%) of total converters and in 6 (30%) of total not-converters], orthostatic hypotension [present in 1 converter (12.5%) and 4 not-converters (20%)] and hyposmia [present in 2 converters (12.5%) and no not-converters]. During follow-up, 2 subjects died among converters (1 MSA and 1 DLB) and one among not-converters.

2.3. Transcription factors mRNA levels in $CD4^+$ T cells & phenoconversion

In comparison to not-converters, converters had higher expression levels of *STAT1*, and lower levels of *GATA3* and *FOXP3* (Figure 1). According to ROC curve analysis, on average *STAT1* levels provided a good discrimination (mean AUC = 0.886, with 95% CI = 0.765–1.000), *GATA3* levels an excellent discrimination (mean AUC = 0.918, with 95% CI = 0.775–1.000) of future converters and not-converters, while discrimination provided by *FOXP3* levels was acceptable (mean AUC = 0.799, with 95% CI = 0.598–0.999) (Figure 2). Highest likelihood ratios were 11,500 for *STAT1*, 20,130 for *GATA3* and 11,500 for *FOXP3*. Using the respective corresponding values of mRNA level as thresholds, Kaplan-Meier survival analysis provided a HR of 58.3 (95% CI: 6.2–547.1) for subjects

Table 1. Characteristics of iRBD patients.

	All	Phenoconversion		P no vs yes	Lost at follow-up
		no	yes		
N	33	23	8		2
Gender (M/F)	29/4	20/3	7/1	1.000	2/0
Age (years)	70.4 ± 6.8	70.1 ± 7,3	71.9 ± 5.9	0.501	68 ± 4.2
Disease duration (years)	7.7 ± 7.5	5.9 ± 4.3	9.2 ± 5.3	0.141	21 ± 26.9
UPDRS Part III (score)	1.9 ± 2.7	1.4 ± 2.7	3 ± 2	0.095	3 ± 4.2
0 (n)	20	17	2		1
1–10 (n)	13	6	6		1
11–20 (n)	0	0	0		0
>20 (n)	0	0	0		0
MMSE	28.6 ± 2.1	28.7 ± 2.3	28.4 ± 1.6	0.719	28 ± 1.4
<26	1	1	0		0
≥26	32	22	8		2

Data are means ± SD unless otherwise indicated and refer to the time of recruitment in the study.

with high expression levels of *STAT1*, 101.2 (95% CI: 16.8–609.4) for subjects with low expression levels of *GATA3* and 15.7 (2.7–91.4) for subjects with low expression levels of *FOXP3*. Remarkably, *GATA3* mRNA levels threshold discriminated 7 out of 8 converters, while *STAT1* and *FOXP3* mRNA levels thresholds only 4 and 5, respectively (Figure 2).

2.4. Blood count & phenoconversion

Complete blood counts of iRBD subjects were all within normal limits (Table 2). There was no difference between converters and not-converters, except for the percentage of monocytes, which was on average 23.5% higher in converters in comparison to not-converters. ROC curve analysis of percentage monocytes provided a on average a good discrimination of future converters and not-converters (Supplementary Figure S1), with a mean AUC of 0.864 (95% CI: 0.737–0.991). The highest likelihood ratio (5.556) was obtained taking 9.6% as the threshold value. Using this value, Kaplan-Meier survival analysis provided a HR of 6.0 (95% CI: 1.1–32.2). Percentage monocytes threshold discriminated 5 out of 8 converters (Supplementary Figure S1).

3. Discussion

In the present study, we followed over about 3.5 years the cohort of iRBD subjects enrolled in our previous research [12], with the aim to identify who was eventually phenoconverted toward a frank neurodegenerative condition and assess whether any of the transcription factor genes in $CD4^+$ T cells originally characterized could now work as a biomarker predicting phenoconversion to neurodegenerative conditions. We originally enrolled 33 iRBD subjects, however 2 were lost during follow-up, thus only 31 could be finally evaluated. As a whole, 8 patients (24.2%) converted to a neurodegenerative disorder: 4 to PD, 1 to MSA and 3 to DLB (Supplementary Table S1). In iRBD subjects, the rate of phenoconversion over 5 years is about 35%, while the 10- to 25-year risk is about 41% to 90.9% [23]. In a recent study, of 281 patients who were free of parkinsonism or dementia, the overall phenoconversion rate was 24.2% after 3 years, 44.8% after 6 years and 67.5% after 10 years [24]. In our cohort of iRBD subjects, at the time of the original enrolment average iRBD duration was 7.7 years, therefore now it is about 11.2 years. A phenoconversion rate of 24.2% between 7.7 and 11.2 years of disease duration appears in good agreement with published evidence.

Our results show that, in comparison to not-converters, converters had higher expression levels of *STAT1* and lower levels of *GATA3* and *FOXP3* (Figure 1), which according to ROC curve analysis provided overall a good discrimination of future converters and not-converters. Remarkably, *GATA3* mRNA levels performed best, discriminating 7 out of 8 converters, while *STAT1* and *FOXP3* mRNA levels only 4 and 5, respectively (Figure 2). A good discrimination of future converters and not-converters was also provided by the percentage of monocytes, which was higher in converters in comparison to not-converters. Percentage monocytes threshold discriminated 5 out of 8 converters (Supplementary Figure S1).

Transcription factor genes expression in $CD4^+$ T cells likely affects the differentiation process toward the different $CD4^+$ T cell lineages/phenotypes [11]. In particular, *STAT1* contributes to driving Th1 differentiation [25], while *GATA3*, regulates Th2 development [26] and *FOXP3* is centrally involved in the development and maintenance of the Treg phenotype [27]. $CD4^+$ T cells Whether the peculiar $CD4^+$ T regarded as key players in the complex chain of events finally leading to neuroinflammation and neurodegeneration in the central nervous system [28], and in particular increased Th1 and decreased Th2 and Treg have been shown in

Table 2. Complete blood count in iRBD patients.

	Units	Range	All	Phenoconversion noyes		P no vs yes	Lost at follow-up
RBC	1012/l	4.70–6.10	4.9 ± 0.5	4.9 ± 0.4	5.1 ± 0.3	0.278	4.7 ± 0.1
Hemoglobin	g/dl	14.0–18.0	14.4 ± 1.4	14.1 ± 1,5	15.2 ± 1.4	0.080	14.0 ± 1.0
hematocrit	%	42.0–52.0	44.8 ± 4.0	44.2 ± 4.1	47.0 ± 3.7	0.091	43.7 ± 2.3
MCV	Fl	80.0–94.0	91.0 ± 9.3	90.4 ± 8.6	92.5 ± 9.4	0.588	92.0 ± 3.2
MCH	Pg	27.0–31.0	29.2 ± 3.4	28.9 ± 3.2	30.0 ± 3.4	0.474	29.5 ± 1.4
MCHC	g/dl	32.0–36.0	32.0 ± 0.7	31.9 ± 0.8	32.2 ± 0.6	0.381	32.0 ± 0.5
platelets	10^9/l	130–400	215.3 ± 56.6	216.3 ± 62.1	216.3 ± 48.5	0.996	201.0 ± 0.0
WBC	10^9/l	4.8–10.8	6.1 ± 1.4	6.1 ± 1.3	6.1 ± 1.0	0.967	7.5 ± 1.1
lymphocytes	10^9/l	1.3–2.9	1.7 ± 0.5	1.8 ± 0.5	1.7 ± 0.2	0.599	1.7 ± 0.1
	%	20.5–45.5	28.9 ± 8.3	29.9 ± 9.1	27.8 ± 7.0	0.551	22.3 ± 1.3
monocytes	10^9/l	0.3–0.8	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.080	0.6 ± 0.1
	%	5.5–11.7	8.6 ± 1.5	8.1 ± 1.4	10.0 ± 1.7	0.002	8.3 ± 1.8
neutrophils	10^9/l	2.2–4.8	3.7 ± 1.2	3.6 ± 1.2	3.7 ± 1.0	0.949	4.9 ± 0.7
	%	43.0–65.0	59.1 ± 8.8	58.8 ± 9.4	58.9 ± 8.8	0.979	65.1 ± 0.5
eosinophils	10^9/l	0.0–0.2	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.974	0.2 ± 0.2
	%	0.9–2.9	2.7 ± 1.2	2.6 ± 1.2	2.7 ± 1.3	0.841	3.5 ± 2.3
basophils	10^9/l	0.0–0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.584	0.0 ± 0.1
	%	0.2–1.0	0.5 ± 0.2	0.5 ± 0.3	0.6 ± 0.2	0.811	0.7 ± 0.3

Data are means ± SD unless otherwise indicated and refer to the time of recruitment in the study.
MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; MCV: Mean corpuscular volume; RBC: Red blood cell; WBC: White blood cell.

the peripheral blood of PD patients [11]. One would be therefore tempted to speculate that higher expression levels of *STAT1*, and lower levels of *GATA3* and *FOXP3*, which in the present study predict iRBD phenoconversion to established neurodegeneration, may correspond to increased Th1 differentiation and decreased Th2 and Treg differentiation of $CD4^+$ T cells, eventually favoring the development of a proinflammatory milieu similar to the one that may occur later in PD [11].

As for increased monocytes, the finding is in strong agreement with recent evidence showing correlation of monocyte markers with immune and neuronal brain changes in RBD subjects [29], and with data pointing to a key role of monocytes in aging and neurodegeneration [30].

Which could be the real-world usefulness of the biomarkers identified in the present study? Let's take as a case study *GATA3* levels, which was the best predictor, discriminating 7 out of 8 converters in our cohort. *GATA3* levels had on average 87.5% sensitivity and 95.6% specificity. Taking as a reference the phenoconversion rate reported by Zhang, et al. [24], which was 24.2% after 3 years, and 67.5% after 10 years, we obtain for phenoconversion after 3 years a positive predictive value (PPV, the probability that the disease is present when the test is positive) of 92%, and a negative predictive value (NPV, the probability that the disease is not present when the test is negative) of 96%. After 10 years, the PPV is 98.7% and the NPV is 79%. This means that in case testing iRBD subjects at the time of diagnosis provides a positive result, the risk of phenoconversion is 92% after 3 years and nearly 99% after 10 years. In other words, the test could be extremely useful to identifiy with precision subjects at high and low risk of phenoconversion, resulting of major benefit in both planning individualized monitoring strategies for the follow-up of iRBD subjects as well as for organizing and running clinical trials of potential neuroprotective and phenoconversion-preventing therapeutics, preferentially targeting subjects at high risk of phenoconversion.

4. Conclusion

We have identified some promising biomarkers, possibly predictive of phenoconversion in iRBD subjects. These markers include the expression levels of *STAT1*, *GATA3* and *FOXP3* mRNA in $CD4^+$ T cells, as well as the percentage of circulating monocytes. Confirmatory studies are now needed to validate these markers for routine clinical use.

The present findings moreover provide further support to the notion that early engagement of peripheral immunity provides a major contribution to the processes ultimately leading to neurodegeneration and emphasize the potential of the peripheral immune system as target for novel therapeutic strategies in RBD and in related neurodegenerative conditions [31].

Acknowledgments

The authors wish to thank all the patients and their relatives and caregivers who accepted to be enrolled in the study, as well as all the nurses and colleagues who helped in recruitment, clinical assessment and collection of demographic and clinical data. The authors wish to express their gratefulness to G Malomo, Department of Brain and Behavioral Sciences, University of Pavia, Pavia (I), who is responsible for updating and maintaining the iRBD patients archive, and to G Veronesi, Department of Medicine and Surgery, University of Insubria, Varese (I), who kindly supervised in part the statistical analysis.

Author contributions

(1) Research project: A. Conception, B. Organization, C. Execution; (2) Statistical Analysis: A. Design, B. Execution, C. Review and Critique; (3) Manuscript: A. Writing of the first draft, B. Review and Critique.

Monica Pinoli: execution of research project, review and critique of statistical analysis, writing of the first draft of the manuscript, review and critique.

Michele Terzaghi: organization and execution of the research project, review and critique of the manuscript.

Franca Marino: conception of research project, review and critique of statistical analysis, review and critique of the manuscript.

Cristoforo Comi: conception of research project, review and critique of statistical analysis, review and critique of the manuscript.

Maurizio Versino: conception of research project, review and critique of statistical analysis, review and critique of the manuscript.

Marco Cosentino: conception and organization of research project, design and execution of statistical analysis, writing of the first draft of the manuscript, review and critique.

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved and declare to have confidence in the integrity of the contributions of their co-authors.

Financial disclosure

All authors have funding from their institutions. The authors have no financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Ethical conduct of research

The study was performed according to the Declaration of Helsinki and to the relevant ethical guidelines for research on humans.

Funding agencies

The study was supported in part by a contribution from the AGING Project, Department of Excellence, University of Piemonte Orientale, Novara (I).

ORCID

Monica Pinoli https://orcid.org/0009-0006-2764-0472
Michele Terzaghi https://orcid.org/0000-0001-9692-2218
Franca Marino https://orcid.org/0000-0003-1837-7810
Cristoforo Comi https://orcid.org/0000-0002-6862-9468
Maurizio Versino https://orcid.org/0000-0003-1813-9492
Marco Cosentino https://orcid.org/0000-0002-6978-7775

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. Gagnon JF, Postuma RB, Mazza S, et al. Rapid-eye-movement sleep behavior disorder and neurodegenerative diseases. Lancet Neurol. 2006;005:424–432. doi:10.1016/S1474-4422(06)70441-0
2. Sforza E, Krieger J, Petiau C. REM sleep behavior disorder: clinical and physiopathological findings. Sleep Med Rev. 1997;1:57–69. doi:10.1016/s1087-0792(97)90006-x
3. Shrestha N, Abe RAM, Masroor A, et al. The correlation between Parkinson's disease and rapid eye movement sleep behavior disorder: a systematic review. Cureus. 2021;13:e17026. doi:10.7759/cureus.17026
4. Boeve BF, Silber MH, Ferman TJ, et al. Clinicopathologic correlations in 172 cases of rapid eye movement sleep behavior disorder with or without a coexisting neurologic disorder. Sleep Med. 2013;14:754–762. doi:10.1016/j.sleep.2012.10.015
5. Galbiati A, Verga L, Giora E, et al. The risk of neurodegeneration in REM sleep behavior disorder: a systematic review and meta-analysis of longitudinal studies. Sleep Med Rev. 2019;43:37–46. doi:10.1016/j.smrv.2018.09.008
6. Postuma RB, Iranzo A, Hu M, et al. Risk and predictors of dementia and parkinsonism in idiopathic REM sleep behavior disorder: a multicentre study. Brain. 2019;142:744–759. doi:10.1093/brain/awz030.
• Useful to substantiate the background.
7. Figorilli M, Meloni M, Lanza G, et al. Considering REM sleep behavior disorder in the management of Parkinson's disease. Nat Sci Sleep. 2023;15:333–352. doi:10.2147/NSS.S266071
8. Sumi Y, Ubara A, Ozeki Y, et al. Share minor hallucinations in isolated rapid eye movement sleep behavior disorder indicative of early phenoconversion: a preliminary study. Acta Neurol Scand. 2022;145:348–359. doi:10.1111/ane.13555
9. Park DG, Kim JY, Kim MS, et al. Neurofilament light chain and cardiac MIBG uptake as predictors for phenoconversion in isolated REM sleep behavior disorder. J Neurol. 2023;270:4393–4402. doi:10.1007/s00415-023-11785-0.
•• Interesting because they consider new biomarkers related to early disease identification.
10. Zhang X, Ma L, Liang D, et al. Neurofilament Light Protein Predicts Disease Progression in Idiopathic REM Sleep Behavior Disorder. J Parkinsons Dis. 2023;13:485–499. doi:10.3233/JPD-223519.
•• Interesting because they consider new biomarkers related to early disease identification.
11. Kustrimovic N, Comi C, Magistrelli L, et al. Parkinson's disease patients have a complex phenotypic and functional Th1 bias: cross-sectional studies of CD4+ Th1/Th2/T17 and Treg in drug-naïve and drug-treated patients. J Neuroinflammation. 2018;15:205. doi:10.1186/s12974-018-1248-8.
•• Very useful to deepener the importance of immunity in PD.
12. De Francesco E, Terzaghi M, Storelli E, et al. CD4+ T-cell transcription factors in idiopathic REM sleep behavior disorder and Parkinson's disease. Mov Disord. 2021;36:225–229. doi:10.1002/mds.28137

13. Sateia M. International classification of sleep disorders-third edition: highlights and modifications. Chest. 2014;146:1387–1394. doi:10.1378/chest.14-0970
14. Goetz CG, Tilley BC, Shaftman SR, et al. Movement Disorder Society UPDRS Revision Task Force. Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): scale presentation and clinimetric testing results. Mov Disord. 2008;23:2129–2170. doi:10.1002/mds.22340
15. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12:189–198. doi:10.1016/0022-3956(75)90026-6
16. Visser M, Marinus J, Stiggelbout AM, et al. Assessment of autonomic dysfunction in Parkinson's disease: the SCOPA-AUT. Mov Disord. 2004;19:1306–1312. doi:10.1002/mds.20153
17. Hamilton M. A rating scale for depression. J Neurol Neurosurg Psychiatry. 1960;23:56–62. doi:10.1136/jnnp.23.1.56
18. Postuma RB, Gagnon JF, Bertrand JA, et al. Parkinson risk in idiopathic REM sleep behavior disorder: preparing for neuroprotective trials. Neurology. 2015;84:1104–1113. doi:10.1212/WNL.0000000000001364
19. McKeith IG, Boeve BF, Dickson DW, et al. Diagnosis and management of dementia with Lewy bodies: fourth consensus report of the DLB Consortium. Neurology. 2017;89:88–100. doi:10.1212/WNL.0000000000004058
20. Gilman S, Wenning GK, Low PA, et al. Second consensus statement on the diagnosis of multiple system atrophy. Neurology. 2008;71:670–676. doi:10.1212/01.wnl.0000324625.00404.15
21. Terzaghi M, Toscano G, Casoni F, et al. Assessment of cognitive profile as a prodromal marker of the evolution of rapid eye movement sleep behavior disorder. Sleep. 2019;42:zsz103. doi:10.1093/sleep/zsz103
22. Mandrekar JN. Receiver operating characteristic curve in diagnostic test assessment. J Thorac Oncol. 2010;5:1315–1316. doi:10.1097/JTO.0b013e3181ec173d
23. Schenck CH, Boeve BF, Mahowald MW. Delayed emergence of a parkinsonian disorder or dementia in 81% of older men initially diagnosed with idiopathic rapid eye movement sleep behavior disorder: a 16-year update on a previously reported series. Sleep Med. 2013;14:744–748. doi:10.1016/j.sleep.2012.10.009
24. Zhang H, Iranzo A, Högl B, et al. Risk factors for phenoconversion in rapid eye movement sleep behavior disorder. Ann Neurol. 2022;91:404–416. doi:10.1002/ana.26298.
• Interesting to understand in which direction the scientific research is evolving.
25. Oestreich KJ, Mohn SE, Weinmann AS. Molecular mechanisms that control the expression and activity of Bcl-6 in TH1 cells to regulate flexibility with a TFH-like gene profile. Nat Immunol. 2013;13:405–411. doi:10.1038/ni.2242
26. Maier E, Duschl A, Horejs-Hoeck J. STAT6-dependent and -independent mechanisms in Th2 polarization. Eur J Immunol. 2012;42:2827–2833. doi:10.1002/eji.201242433
27. Lu L, Barbi J, Pan F. The regulation of immune tolerance by FOXP3. Nat Rev Immunol. 2017;17:703–717. doi:10.1038/nri.2017.75
28. Kustrimovic N, Marino F, Cosentino M. Peripheral immunity, immunoaging and neuroinflammation in Parkinson's disease. Curr Med Chem. 2019;26:3719–3753. doi:10.2174/0929867325666181009161048
•• Very useful to understand how peripheral immune challenges can exacerbate the process of neuroinflammation, that is a key mediator to the progression of PD.
29. Farmen K, Nissen SK, Stokholm MG, et al. Monocyte markers correlate with immune and neuronal brain changes in REM sleep behavior disorder. Proc Natl Acad Sci USA. 2021;118:e2020858118. doi:10.1073/pnas.2020858118.
• Useful because we also considered and analyzed monocyte in our paper.
30. Silvin A, Uderhardt S, Piot C, et al. Dual ontogeny of disease-associated microglia and disease inflammatory macrophages in aging and neurodegeneration. Immunity. 2022;55:1448–1465; e6. doi:10.1016/j.immuni.2022.07.004
31. Schwab AD, Thurston MJ, Machhi J, et al. Immunotherapy for Parkinson's disease. Neurobiol Dis. 2020;137:104760. doi:10.1016/j.nbd.2020.104760