DR MICHELLE MAGEE (Orcid ID : 0000-0001-6642-0882)
PROFESSOR DAVID J KENNAWAY (Orcid ID : 0000-0002-5864-3514)
DR STEVEN W LOCKLEY (Orcid ID : 0000-0001-5209-2881)

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TITLE

A PERIOD3 variable-number-tandem-repeat polymorphism modulates melatonin treatment response in Delayed Sleep-Wake Phase Disorder

RUNNING TITLE

PER3 VNTR variants in melatonin treatment of DSWPD

AUTHORS

Michelle Magee[1,2,3], Tracey L Sletten[1,2], Jade M Murray[1,2], Christopher J Gordon[2,4,5], Nicole Lovato[2,6], Delwyn J Bartlett[2,4], David J Kennaway[7], Steven W Lockley[1,2,8,9], Leon C Lack[6], Ronald R Grunstein[2,4], Simon N Archer[10] and Shantha MW Rajaratnam[1,2,8,9] for the Delayed Sleep on Melatonin (DelSoM) Study Group.

CONTACT INFORMATION

1. Turner Institute for Brain and Mental Health and School of Psychological Sciences, Monash University, Clayton, Victoria, Australia.
2. Cooperative Research Centre for Alertness, Safety and Productivity, Clayton, Victoria, Australia.
3. Centre for Neuroscience of Speech, Department of Audiology and Speech Pathology, University of Melbourne, Parkville, Victoria, Australia.
4. Woolcock Institute of Medical Research, Sydney, NSW, Australia.
5. Sydney Nursing School, The University of Sydney, NSW, Australia.

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6. Adelaide Institute for Sleep Health: A Flinders Centre of Research Excellence, School of Medicine, Flinders University, Adelaide, South Australia, Australia
7. Robinson Research Institute, Adelaide School of Medicine, University of Adelaide, Adelaide, South Australia, Australia.
8. Division of Sleep and Circadian Disorders, Departments of Medicine and Neurology, Brigham and Women's Hospital, Boston, Massachusetts, USA.
9. Division of Sleep Medicine, Harvard Medical School, Boston, Massachusetts, USA
10. Surrey Sleep Research Centre, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, UK.

CORRESPONDING AUTHOR

Shantha MW Rajaratnam Ph.D.
Turner Institute for Brain and Mental Health
Sleep and Circadian Rhythms Program
School of Psychological Sciences
Monash University
18 Innovation Walk
Clayton Campus, Clayton VIC 3800
Australia
Phone: + 61 3 9905 3934
Email: shantha.rajaratnam@monash.edu

ABSTRACT

We examined whether a polymorphism of the *PERIOD3* gene (*PER3;* rs57875989*)* modulated the sleep promoting effects of melatonin in Delayed Sleep-Wake Phase Disorder (DSWPD). One hundred and four individuals (53 males; 29.4±10.0 years) with DSWPD and a delayed dim light melatonin onset (DLMO) collected buccal swabs for genotyping *(PER3$^{4/4}$* n=43; *PER3* 5 allele [heterozygous and homozygous] n=60). Participants were randomised to placebo or 0.5mg melatonin taken 1 hour before desired bedtime (or ~ 1.45 h before DLMO), with sleep attempted at desired bedtime (4 weeks; 5-7 nights/week). We assessed sleep (diary and actigraphy), Pittsburgh Sleep Quality Index (PSQI), Insomnia Severity Index (ISI), Patient-Reported Outcomes Measurement Information System (PROMIS: Sleep Disturbance, Sleep-Related Impairment), Sheehan Disability Scale (SDS), and Patient- and Clinician-Global Improvement (PGI-C, CGI-C). Melatonin treatment response on actigraphic sleep onset time did not differ between genotypes. For *PER3$^{4/4}$* carriers, self-reported sleep onset time was advanced by a larger amount and sleep onset latency (SOL) was shorter in melatonin-treated patients compared to those receiving placebo (P=0.008), while actigraphic sleep efficiency in the first third of the sleep episode (SE T1) did not differ. For *PER3* 5 carriers, actigraphic SOL and SE T1 showed a larger improvement with melatonin (P<0.001). Melatonin improved ISI (P=0.005), PROMIS Sleep Disturbance (P<0.001) and Sleep-Related Impairment (P=0.017), SDS (P=0.019), PGI-C (P=0.028), and CGI-C (P=0.016) in *PER3$^{4/4}$* individuals only. Melatonin did not advance circadian phase. Overall, *PER3*$^{4/4}$ DSWPD patients have a greater response to melatonin treatment. *PER3* genotyping may therefore improve DSWPD patient outcomes.

KEY WORDS

Melatonin, Delayed Sleep-Wake Phase Disorder, PERIOD3, Polymorphism, Variable Number Tandem Repeats, Circadian Rhythm Sleep Disorders.

INTRODUCTION

Delayed Sleep-Wake Phase Disorder (DSWPD) is a common sleep disorder characterised by a difficulty initiating sleep and waking at conventional times[1,2]. Delayed endogenous circadian timing is thought to be the primary mechanism underlying the disorder[2,3], resulting in misalignment of the circadian clock relative to the behavioural sleep-wake cycle and chronic sleep restriction[3]. It is associated with adverse mental health and poor academic, occupational, and social outcomes[4-9].

Recently, the Delayed Sleep on Melatonin (DelSoM) study group demonstrated that melatonin (0.5 mg, 1 hour prior to an individual's desired bedtime), coupled with behavioural sleep-wake scheduling, improves sleep outcomes in DSWPD patients as indicated by earlier sleep onset, increased sleep efficiency, and decreased sleep disturbance and sleep-related impairments[10]. While these results contribute to the growing evidence for use of melatonin as a treatment for DSWPD[11-16], treatment efficacy can vary between patients; for example, results from the DelSoM study group showed 22.2% of individuals who received melatonin demonstrated no change or worsening of symptoms[10]. Inter-individual factors that modulate melatonin treatment efficacy should therefore be examined.

Circadian rhythms are regulated and maintained by a number of core clock genes[17] including those from the Period (PER) family. *PER3* is one such gene involved in the core mammalian molecular circadian system[18]. A biallelic variable-number-tandem-repeat (VNTR) polymorphism of the *PER3* gene exists where a 54-nucleotide coding region encoding a putative phosphorylation domain[19-21], results in a short (*PER3*$^{4/4}$), intermediate (*PER3*$^{4/5}$) and long (*PER3*$^{5/5}$) genetic variant. Several studies have shown associations between the *PER3* VNTR polymorphism and diurnal preference[21-24], DSWPD[21,25-27], sleep timing[24], blue-enriched light sensitivity[28,29], vulnerability to sleep loss[30-32], and decrements in waking performance[33,34]. Together, these reports highlight an important link between the *PER3* VNTR and dysregulation of the sleep-wake cycle.

Of particular interest, *PER3* genotype-dependencies exist in the modulation of sleep-wake homeostasis. When compared to the shorter 4-repeat allele, homozygosity for the *PER3*$^{5/5}$ allele is associated with greater sleep propensity, characterised by an increase in slow wave sleep and slow

wave activity during non-rapid eye movement (NREM) sleep, and increased alpha and theta activity during wakefulness and REM[34,35]. Changes in sleep homeostasis are believed to mediate decrements in early morning cognitive performance[36] following total sleep deprivation[34], specifically early morning executive function in those homozygous for the *PER3* 5-repeat allele.

Given the genetic interaction between *PER3,* diurnal preference, and differential effects on the sleep-wake homeostat and its associations with DSWPD, the contribution of *PER3* VNTR polymorphisms in modulating the sleep promoting effects of melatonin in DSWPD warrants investigation. The aim of this study was to retrospectively assess the role of *PER3* genetic variants in the efficacy of 0.5 mg melatonin, in individuals diagnosed with DSWPD with confirmed delay of the endogenous melatonin rhythm relative to their desired bedtime. Based on previous findings that homeostatic sleep pressure is accumulated less rapidly in *PER3*$^{4/4}$, we expected a larger degree of sleep disturbance in these individuals when the sleep-wake cycle is shifted to an earlier time, and thus greater opportunity for improvement with melatonin treatment. We therefore tested the *a priori* hypothesis that, compared to those with the *PER3* 5 allele (*PER3* $^{4/5}$ and PER3$^{5/5}$ combined), individuals who are homozygous for the *PER3*$^{4/4}$ allele will display greater response to 0.5 mg melatonin treatment, measured by the following: (1) earlier actigraphic sleep onset time; (2) increased sleep efficiency in the first third of time in bed; (3) decreased reports of sleep disturbance and sleep-related daytime dysfunction; (4) greater proportion of patient and clinician-rated improvements; and, as an exploratory aim, (5) advanced circadian phase measured by dim light melatonin onset (DLMO).

METHODS

Data presented here are part of the Delayed Sleep on Melatonin (DelSoM) Study, a multicentre, double-blind randomised controlled trial (Australian and New Zealand Clinical Trials Registry number ACTRN12612000425897)[10,37]. This study was conducted at three sites in Australia: Monash University (Melbourne), Woolcock Institute of Medical Research (Sydney) and Flinders University (Adelaide). Data collection occurred between September 2012 and September 2014. The study protocol was approved by the Monash University Human Research Ethics Committee, The University of Sydney Human Research Ethics Committee and Southern Adelaide Clinical Human Research Ethics Committee and was performed according to the principles outlined by the Helsinki Declaration.

Participants

One hundred and four participants (53 males, 51 females) aged 29.4 ± 10.0 (mean ± SD) years were recruited via radio, newspaper, television and poster advertisements, and referrals from sleep physicians, general practitioners, and psychologists. Participants were identified as at risk of DSWPD via online questionnaire and were telephone interviewed to ensure they met the eligibility criteria[10,37]. Participants had a body mass index (BMI) between 18.0 and 35kg/m^2, consumed <300mg/day of caffeine or alcohol (<14units/week), and reported not taking illicit drugs for at least 12 months. Any history of psychiatric disorders in the past 12 months (other than depression), regular night shift work or travelling across more than two time zones in the previous 2 months were exclusionary. Current use of medications likely to effect sleep including benzodiazepines, psychostimulants, antiepileptics, antipsychotics, oral steroids and beta blockers were also exclusionary. All participants provided written informed consent prior to commencement of the study and were reimbursed for study-related expenses. Participants were those of the 116 participants in the intention to treat sample from our DelSoM study[10] who completed treatment per-protocol and provided buccal swab samples (89.7% response rate).

Baseline Assessments

Participants were assessed by a sleep medicine physician through clinical interview to confirm they met the diagnostic criteria for DSWPD[38]. Physicians completed the Clinical Global Impression Scale of Change (CGI-C)[39] to assess severity of DSWPD at baseline. Participants

completed the Pittsburgh Sleep Quality Index (PSQI), Epworth Sleepiness Scale (ESS), Insomnia Severity Index (ISI) and the Sheehan Disability Scale (SDS) to assess sleep disturbance and daytime functioning.

Participants were asked to complete a sleep and work diary, and to wear a wrist actigraph (Actiwatch-L, Actiwatch-2 and Actiwatch Spectrum; Respironics, Bend OR USA; 1-minute epochs at medium sensitivity; 40 activity counts) for 7 consecutive days. After 7 days of monitoring, 1-4 hours after waking, participants completed the Patient Reported Outcomes Measurement Information System (PROMIS) for sleep disturbance and sleep-related daytime impairments[40].

After the baseline monitoring period, participants attended the laboratory to assess salivary dim light melatonin onset (DLMO), using methods previously described[10,37]. Samples were collected every hour under dim light conditions (<10 lux) from 5 hours before to 2 hours after habitual bedtime. Salivary melatonin concentrations were determined by radioimmunoassay within 1 week of collection, as per protocols developed by University of Adelaide and licensed to Buhlmann Laboratories (Allschwil, Switzerland)[41]. The limit of detection for these assays was 1pg/mL and the inter-assay coefficients of variation (CV*)* were 7.4% at 4.41pg/mL and 10.7% at 48.14pg/mL. DLMO for each participant was calculated by linear interpolation and determined as the time that melatonin concentrations crossed and remained above a threshold of 2.3pg/mL[10,41]). Participants were subsequently classified as having a delayed endogenous melatonin rhythm relative to desired bedtime if their DLMO occurred 30 minutes prior to their desired bedtime or later. Desired bedtime was derived from the question, 'On the night before school or work, what time would you need to go to bed to feel fully rested in the morning?'[10]

Treatment Phase

Participants classified as having a delayed endogenous melatonin rhythm relative to desired bedtime were randomised to melatonin treatment (0.5mg immediate release, Pure Encapsulations, Sudbury, MA, USA) or matching placebo in a double-blind design, with local pharmacists responsible for dispensing treatment. Participants were provided with a 4-week supply of melatonin (i.e., 28 capsules) and were instructed to take one capsule 60 minutes prior to their fixed

desired bedtime for at least 5 consecutive nights per week, aligning with daytime commitments. Participants were asked to attempt sleep at their fixed desired bedtime on the nights when melatonin was taken. Sleep-wake activity was monitored throughout the 28-day treatment period using wrist actigraphy and sleep diaries. At the end of each week, 1-4 hours after waking, participants were asked to complete the PROMIS for sleep disturbance and sleep-related daytime impairments. Participants were also contacted via telephone on a weekly basis throughout this period to monitor compliance and adverse events.

Final Clinical Assessment

Participants attended the laboratory within 7 days of treatment ending. During this visit, a sleep physician assessed treatment outcome through completion of the CGI-C. Patients were asked to complete the Patient Global Impression of Change (PGI-C), as well as repeat the PSQI, ESS, ISI and the SDS. Of the n=104 participants, n=49 performed a follow-up DLMO assessment in their home 24 hours after completion of treatment[10].

Genotyping *PER3* Polymorphisms

Buccal swabs were collected prior to treatment for post hoc genetic analysis, using procedures previously described [34]. Genomic DNA was extracted via the QuickExtract system (Epicentre Biotechnologies, Madison, Wisconsin) and genotyping for *PER3* polymorphisms was performed using polymerase chain reaction (PCR) (Viola et al., 2007) with some modifications[24,42]. PCR fragments representing the amplified repeat alleles were resolved on a 2% agarose gel and digitally imaged with ethidium bromide staining under UV light. Of the 104 participants genotyped for *PER3* VTNR polymorphisms, n=1 was unable to be genotyped. Participants were grouped based on their VTNR polymorphisms, with all participants genotyped as heterozygote (n=51, 85.0%) or homozygote (n=9, 15.0%) for the 5-allele collapsed. The genotype distribution was in Hardy-Weinberg equilibrium (χ=1.28, p>0.05).

Outcome Measures and Data Analysis

As per the original report of this randomised controlled trial (RCT)[10], the primary outcome was actigraphic sleep onset time. Secondary outcomes included sleep efficiency in the first third of

time in bed (SE T1) on treatment nights, sleep disturbance, sleep-related daytime impairment, patient- and clinician-rated improvements and DLMO time.

SE T1 was determined as a secondary outcome as we expected that the phase advance of the sleep-wake cycle would result in sleep being initiated close to the time of the wake maintenance zone, resulting in a decrease in sleep efficiency for the total sleep opportunity. We hypothesized that 0.5mg melatonin would alleviate the disruption in sleep in the first third of the sleep episode, during the wake maintenance zone, as seen in our previous study[43].

SPSS Statistics Version 24.0 (IBM, Armonk, New York) was used for all data analyses. No missing value imputation or substitution was performed. Continuous data are presented as mean ± SD and categorical data are summarised as frequency and percentages. An alpha of < 0.05 was accepted as statistically significant.

Subjective reports of bedtime (BT), waketime (WT), sleep onset latency (SOL), and wake after sleep onset (WASO) were extracted from sleep diaries. From these variables, sleep onset, time in bed (TIB), total sleep time (TST), and sleep efficiency (SE) were calculated. Objective variables of sleep onset, WT, SOL, SE T1[10] and WASO were extracted from Actiware 5 software (Philips Respironics, Bend, OR) where subjective reports of bedtime and wake time were used to identify each sleep episode[10]. Discrepancies between sleep diaries and actigraphy of 60 min or more were corrected to match objective actigraphy[10]. The criteria used were as follows: 1) If the reported subjective bedtime was ≥60 min prior to a sustained substantial reduction in activity and light levels, bedtime was adjusted to the time of activity and light reduction. 2) If reported wake time was ≥60 min after a sustained substantial increase in activity and light levels, wake time was adjusted to match the time of increased activity and light. Two researchers visually reviewed alignment between diary and actigraphic bed and wake times and discrepancies were resolved by discussion before unblinding[10].

To assess the individual role of *PER3* VNTR polymorphisms in melatonin treatment response in DSWPD, *PER3* genotypes (*PER3*$^{4/4}$ and *PER3* 5 alleles) were stratified and assessed independently. For continuous outcomes, a random-effects mixed-model analysis using restricted maximum likelihood estimation (participant: random variable) was applied to the model with as

a linear function of *time* (T_0 baseline, T_1 treatment) by *treatment* (placebo, 0.5mg melatonin) interaction. In cases where data were categorical, binomial logistic regression was performed with the addition of a *time by treatment* interaction term. Bonferroni correction was applied to account for multiple post hoc comparisons.

RESULTS

Demographics

Demographic information, diurnal preference and circadian phase measures are reported in Table 1. At baseline, participants did not differ on demographic characteristics (age, sex, and BMI), diurnal preference (Composite Morningness Eveningness Questionnaire; cMEQ), DSWPD severity (CGI-S) or circadian phase (DLMO) based on *PER3* genotype. We compared baseline characteristics (Supplementary Table 1; $PER3^{4/4}$, $PER3^{4/5}$ and $PER3^{5/5}$) and self-reported and actigraphic sleep parameters (Supplementary Table 2; $PER3^{4/4}$, *PER3* 5 alleles) between genotypes and found no significant differences.

Insert Table 1

Primary outcome stratified by *PER3* genotype

Sleep Onset

In the ***PER3*$^{4/4}$ allele group**, no *time by treatment* interaction effects were noted for actigraphic sleep onset (Table 2). There was, however, a *time by treatment* interaction for self-reported sleep onset ($F_{1,39.9}$=4.6, P=0.04, β=-44.0 mins, 95% CI -84 to -4 mins]) where post hoc analysis revealed that compared to baseline (T_0), those randomised to placebo treatment reported a 78 minute advance in sleep onset ([95% CI -108 to -47 mins]; P<0.001, d=0.95) compared to those randomised to melatonin reporting a 122 minute advance ([95% CI -149 to -94 mins]; P<0.001, d=2.33). Post hoc analysis confirmed that the change in sleep onset from T_0 to T_1 was significantly larger in melatonin treated participants compared to placebo (t(38) = 2.11, P= 0.042, d=0.66). There were, however, no significant differences between melatonin treatment group and placebo post-treatment (T_1) (P=0.92, d=0.49).

For the ***PER3 5* allele group**, there were no *time by treatment* interaction effects for both actigraphic and self-reported measures of sleep onset time (Table 2).

Insert Table 2

Secondary outcomes stratified by *PER3* genotype

Sleep parameters

In the ***PER3$^{4/4}$*** **allele group,** there was a *time by treatment* interaction for both self-reported ($F_{1,38.7}$=6.8, *P*=0.01, β=23.1 mins, [95% CI 5.8 to 40.4 mins]) and actigraphic SOL ($F_{1,36.65}$=8.1, *P*=0.007, β=19.7 mins, [95% CI 6.1 to 33.4 mins]) (Table 2). Post hoc analysis showed that those randomised to placebo showed further impairment of sleep initiation when compared to T_0 (19.5 min longer self-reported SOL; [95% CI 6.2 to 32.7 mins]; *P*=0.03, *d*=0.39 and 25.1 min longer actigraphic SOL; [95% CI 14.6 to 35.6 mins]; *P*<0.001, *d*=1.00). In contrast, there was no further impairment to sleep initiation for those randomised to melatonin when compared to T_0 (self-reported; *P*=1.00, *d*=0.15 and actigraphic; *P*=1.00, *d*=0.32). Further to this, those randomized to melatonin displayed a shorter SOL when compared to placebo treatment at T_1 (33.4 min shorter self-reported SOL; [95% CI 7.36 to 59.4 mins]; *P*=0.008, *d*=0.84 and 24.0 min shorter actigraphic SOL; [95% CI 10.7 to 37.4 mins]; *P*=0.004, *d*=0.93). No *time by treatment* interaction effects were noted for self-reported or actigraphic wake time, total sleep time, SE and WASO or actigraphic SE in the first third of time in bed (Table 2).

In the ***PER3 5*** **allele group**, there was a *time by treatment* interaction for actigraphic SOL ($F_{1,49.1}$=7.5, *P*=0.009, β=18.7 mins, [95% CI 5.3 to 32.2 mins]), SE T1 ($F_{1,48.1}$=8.4, *P*=0.006, β=7.3%, [95% CI 2.4 to 12.1%]) and WASO ($F_{1,43.2}$=6.7, *P*=0.013, β=10.2 mins, [95% CI - 2.5 to 17.9 mins]). Post hoc analysis showed that those randomised to placebo experienced further impairment of sleep initiation (23 min longer actigraphic SOL; [95% CI 13.7 to 33.0 mins]; *P*<0.001, *d*=1.08) resulting in a 7.5 % decrease in SE first third of time in bed ([95% CI 4.0 to 11.0 %]; *P*<0.001, *d*=0.98) and 13.8 minute increase in wake after sleep onset ([95% CI 3.2, 27.2]; *P*<0.001, *d*=0.71) when compared to T_0. In contrast, no differences were found in the melatonin group when compared to T_0 or when compared to placebo at T_1, respectively. No *time by treatment* interaction effects were noted for actigraphic or self-reported measures of WT and TST or self-reported measures of SOL, SE and WASO in the *PER3* 5 allele group (Table 2).

Circadian phase markers

There was no significant *time by treatment* interaction found for DLMO, DBT phase angle (DBT-DLMO) or bedtime phase angle (BT-DLMO) when stratified by *PER3* genotypes (Table 2).

Sleep disturbance and sleep-rated daytime dysfunction

The ***PER3$^{4/4}$*** **allele group** displayed significant improvements in self-reported sleep disturbances and associated sleep-related impairments with melatonin treatment (Table 3). A significant *time by treatment* interaction was found for ISI total score ($F_{1,39.3}$=13.4, *P*=0.005, β=-4.5 points, [95% CI -7.5 to -1.5]), PROMIS Sleep Disturbance ($F_{1,41}$=13.4, *P*<0.001, β=-6.4 points, [95% CI -9.8 to 3.0]), SDS total score ($F_{1,39.2}$=7.4, *P*<0.01, β=-4.9 points, [95% CI -8.5 to -1.4]) and PROMIS Sleep-Related Impairment ($F_{1,41}$=6.3, *P*=0.02, β=-4.7 points, [95% CI -8.4 to -1.0]). Post hoc analyses showed that *PER3$^{4/4}$* individuals randomised to melatonin displayed a 6.3-point reduction on the ISI ([95% CI -8.4 to -4.2]; *P*<0.001, *d*=1.51), a 5.0-point reduction on the PROMIS Sleep Disturbance scale ([95% CI -7.3 to 2.7]; *P*<0.001, *d*=0.93), a 6.1-point reduction on the SDS ([95% CI -8.5 to -3.7]; *P*<0.001, *d*=1.33) and a 8.6-point reduction on the PROMIS Sleep-Related Impairment scale ([95% CI-11.2 to -6.1]; *P*<0.001, *d*=1.45) when compared to T_0. Additionally, *PER3$^{4/4}$* individuals randomised to melatonin displayed greater reductions on the ISI (mean diff= -3.8 points, [95% CI -6.2 to -1.4]; *P*=0.002, *d*=1.04), PROMIS Sleep Disturbance scale (mean diff= -5.5-points, [95% CI -8.9 to -2.2]; *P*=0.002, *d*=1.03), SDS (mean diff= -4.5-points, [95% CI -7.2 to -1.7]; *P*=0.002, *d*=1.06) PROMIS Sleep-Related Impairment scale (mean diff= -4.9-points, [95% CI-8.5 to -1.3]; *P*=0.009, *d*=0.88) when compared to placebo at T_1. While changes were noted on the ISI and PROMIS sleep disturbance, melatonin treatment did not improve PSQI scores in individuals genotyped with *PER3$^{4/4}$* (Table 3).

Cut-off scores for measures of sleep disturbance and sleep-related impairment were also used to examine the clinical significance of these findings (Table 3). There was a significant *time by treatment* interaction for *PER3$^{4/4}$* individuals on measures of ISI (OR= -2.74, [95% CI -5.17 to -0.43]; *P*=0.02) where 72.7% of *PER3$^{4/4}$* individuals randomised to melatonin, reported an absence of insomnia symptoms compared to 17.6% who were allocated placebo (*P*=0.009).

In the ***PER3*** **5 allele group**, melatonin treatment did not improve self-reported measures of sleep disturbance or sleep-related impairment compared to T_0 or placebo group at T_1, respectively (Table 3).

Insert Table 3

Clinician and patient-rated measures of melatonin treatment efficacy

Following melatonin treatment, *PER3*$^{4/4}$ individuals demonstrated greater improvements on both Clinical and Patient Global Index scales (Table 4) compared to placebo while illness severity (CGI-C *Severity* categories: normal-mildly ill *vs*. moderate-severe) did not differ between treatment groups.

For the ***PER3***$^{4/4}$ **allele group**, ratings of improvement (CGI-C *Global Improvement*) were significantly different between placebo and melatonin treatment groups; 95.8 % were classified as very much/much improved following melatonin treatment compared to 68.4% on placebo (OR= 0.09, [95% CI 0.01 to 0.87]; P=0.02). Treatment efficacy, as measured by the CGI-C *Efficacy Index*, was lower following melatonin treatment compared to placebo (β= -4.18, [95% CI -6.13 to -2.23]; P<0.001, d=1.33), indicating a larger therapeutic effect (greater efficacy, reduced adverse effects). PGI-C scores were also significantly different between placebo and melatonin groups (OR= 4.13, [95% CI 1.13 to 15.10]; P=0.03), with 75.0% of those on melatonin classified as very much/much improved compared to 57.8% for those randomised to placebo.

In comparison, the ***PER3 5*** **allele group** demonstrated no significant differences following melatonin treatment on CGI-C Global Improvement, CGI-C Efficacy Index, or PGI-C when compared to placebo.

Insert Table 4

DISCUSSION

This is the first study to investigate the role of the *PER3* VNTR polymorphism in melatonin treatment response for DSWPD patients. When stratified by *PER3* genotype, we showed that individuals with the $PER3^{4/4}$ shorter repeat allele displayed melatonin treatment effects on self-reported sleep disturbance, sleep-related impairments, patient- and clinician-rated improvement in DSWPD symptomology and overall treatment efficacy. Irrespective of *PER3* genotype variant, melatonin treatment response did not differ on actigraphic sleep onset time. A larger advance in self-reported sleep onset time was, however, observed in individuals with the $PER3^{4/4}$ shorter repeat allele receiving melatonin compared to those receiving placebo. For latency to sleep onset, a melatonin treatment effect was observed in individuals homozygous to $PER3^{4/4}$, whereas no such effect was found for *PER3 5* allele carriers. Overall, this study demonstrated in a clinically diagnosed sample of DSWPD, with a confirmed delayed melatonin rhythm, homozygosity for the $PER3^{4/4}$ allele, is associated with greater efficacy to melatonin treatment (combined with behavioural sleep-wake scheduling).

In this pragmatic treatment protocol, all participants were required to attempt sleep at their self-selected *desired bedtime*. This imposed an earlier bedtime, on average 2 hours prior to their habitual bedtime (measured during 7-day baseline). Consequently, enforcing earlier bedtimes resulted in longer latency to sleep onset, independent of treatment and genotype. Melatonin treatment attenuated the deleterious effect of scheduling sleep at the desired bedtime by lessening the increase in sleep onset latency. This was particularly apparent when stratifying by *PER3* genotype; individuals homozygous to the $PER3^{4/4}$ allele treated with melatonin had a shorter self-reported and actigraphic sleep onset latency when compared to the matching placebo group. In comparison, *PER3* 5 allele carriers on placebo displayed significant increases in sleep onset latency, corresponding to a reduction in sleep efficiency in the first third of the sleep episode.

These findings may be explained using the proposed conceptual model for *PER3* VNTR polymorphism in the regulation of sleep homeostasis[44]. According to this model, the *PER3* VNTR polymorphism produces differential regulation of the sleep-wake homeostat, such that there is a more rapid accumulation of homeostatic sleep pressure in those homozygous for the $PER3^{5/5}$ allele compared to $PER3^{4/4}$, the shorter repeat allele[44]. Furthermore, $PER3^{5/5}$ homozygotes have increased theta and alpha activity during prolonged wakefulness and spent more time in slow wave

sleep after prolonged wakefulness compared to *PER3*$^{4/4}$ homozygotes[34,35]. In the present study, the treatment included a sleep-wake schedule based on an individual's desired bedtime, whereby participants were asked to attempt sleep at an earlier time. If there is more rapid accumulation of homeostatic sleep pressure in *PER3* 5 allele carriers, one would expect that shifting bedtime earlier would result in less sleep disruption, and a greater propensity to sleep in *PER3* 5 allele carriers. Treatment effects of melatonin could, therefore, be more apparent in *PER3*$^{4/4}$ individuals given the magnitude for improvement in sleep onset latency is larger. Our findings are generally consistent with this hypothesised mechanism.

Limitations of the present study should be noted. First, individual homozygous to the *PER3*$^{5/5}$ allele exists with relatively low frequency in humans[20] and in our DSWPD cohort, only 8.7% were genotyped with the longer allele. To avoid a small sample size, we grouped those with the *PER3*$^{5/5}$ and *PER3*$^{4/5}$ alleles, as in other studies[45-47]. Ideally, comparing *PER3*$^{4/4}$ homozygotes, *PER3*$^{4/5}$ heterozygotes, and *PER3*$^{5/5}$ homozygotes independently may show larger effect sizes. Furthermore, DSWPD patients recruited for this study were required to not only meet current diagnostic criteria for DSWPD[38] but also, present with a delayed circadian phase relative to desired bedtime. For participants to be included in our study, DLMO had to occur 30 minutes prior to desired bedtime or later. We acknowledge, therefore, that the generalisability of our findings is limited to those DSWPD patients who show misalignment between the circadian pacemaker and the desired sleep-wake cycle, which accounted for 57% of our sample. It is not known whether *PER3* VNTR polymorphisms will still contribute to treatment efficacy when diagnosed according to the standard diagnostic criteria[1].

The present study assessed circadian phase using DLMO before and after treatment in a subset of participants (n=18 *PER3*$^{4/4}$ carriers and n=26 PER3 5 allele carriers). We found no significant time by treatment interaction effects on circadian phase. This was not surprising given that we designed the protocol to primarily test the soporific effects of melatonin, and the small sample size for examining circadian phase. The results should, therefore, be interpreted with caution. The current study did not administer melatonin at the optimal time for circadian phase advancing effects. Previous studies have demonstrated with phase response curves that maximal phase advances occur when 0.5mg melatonin is administered between 2-4 hours prior to DLMO[48] We administered melatonin on average 1.45 hours prior to DLMO, which may not have been optimal

for inducing phase-advancing effects. Finally, participants were instructed to take melatonin before their fixed desired bedtime for at least 5 consecutive nights per week, aligning with daytime commitments (e.g., school, work). If participants reverted back to delayed bedtimes on free days, the treatment effects of melatonin may have been over-ridden by the light-dark effects, as discussed previously[10]. Future investigations are warranted to determine whether the circadian effects of melatonin vary depending on *PER3* genotype.

Previous reports show that individuals homozygous for the *PER3*$^{5/5}$ allele are particularly sensitive to blue-enriched light, demonstrated through the suppression of endogenous melatonin and attenuated waking theta activity[28]. In line with this, functional knockout of *PER3*[49] and melanopsin[50] in mice reduced non-visual responses to light, demonstrating that *PER3* polymorphisms may play a vital role in the light input to the circadian pacemaker. Ambient light exposure and light sensitivity should, therefore, be investigated in *PER3* genotypes to explore phase delaying and advancing effects of light and the impact on melatonin treatment.

In contrast to our findings that improvements in self-reported sleep-wake characteristics following melatonin treatment were significantly larger in *PER3*$^{4/4}$ individuals, these differences were not consistently observed in actigraphy-derived measures. This discrepancy should not detract from the key findings, however. The American Academy of Sleep Medicine (AASM) recently released a clinical practice guideline noting actigraphy measures may differ from patient-reported sleep logs for some sleep parameters in adult patients with circadian rhythm sleep-wake disorders[51]. Self-report assessment of sleep-wake outcomes are commonly used in clinical practice, and are important when evaluating the patient experience of sleep-wake disturbances.

While the role of the *PER3* VNTR polymorphism in melatonin treatment response to actigraphy-derived sleep-wake measures remains equivocal, improvements in patient and clinician reported sleep disturbances and sleep-related impairments were consistently more pronounced in homozygous *PER3*$^{4/4}$ individuals. These findings support the hypothesis that melatonin is more efficacious in DSWPD patients with the *PER3*$^{4/4}$ genotype, and therefore these individuals may show increased benefit from melatonin treatment.

In summary, we have demonstrated for the first time that *PER3* VNTR polymorphisms modulate the efficacy of a melatonin treatment combined with behaviourally scheduled sleep-wake, in a

DSWPD population. These findings provide a basis for a personalised treatment approach for DSWPD, and novel insights into the pathophysiology of the condition and inter-individual differences in response to melatonin treatment. *PER3* genotype-dependencies to melatonin treatment response should be further explored to understand the potential to predict and improve patient outcomes in DSWPD, rather than the current 'one size fits all' approach.

Table One. Participant characteristics in DSWPD patients stratified by *PER3* VNTR.

	***PER3*$^{4/4}$**				***PER3* 5 alleles**			
	Placebo	**0.5mg Melatonin**	**Diff (95% CI)**	***p***	**Placebo**	**0.5mg Melatonin**	**Diff (95% CI)**	***p***
N	19	24			30	30		
Sex (n)	7 M 12 F	12 M 12 F			17 M 13 F	16 M 14 F		
Age (years)	25.11 ± 8.11	29.25 ± 8.93	4.14 (-1.80, 10.09)	0.17	30.77 ± 11.10	30.50 ± 10.28	-0.27 (5.26, 4.73)	0.92
Range	17-52	17-53			19-60	18-64		
BMI (kg/m^2)	24.74 ± 4.82	25.30 ± 4.74	0.56 (-2.10, 3.22)	0.68	24.46 ± 4.08	25.64 ± 4.23	1.18 (-1.06, 3.42)	0.30
Ancestry[a]								
Caucasian	14 (73.7)	14 (58.3)		0.56	23 (76.7)	25 (83.3)		0.42
Asian	0 (0.0)	5 (20.3)			1 (3.3)	1 (3.3)		
Hispanic	1 (5.2)	3 (12.5)			2 (6.7)	2 (6.7)		
Unidentified	4 (21.0)	2 (8.3)			3 (10.0)	3 (10.0)		
CGI-C Severity								

[a] Determined by Ancestry Informative Markers (AIMS) analysis

n (%)	Normal - Mildly Ill (1-3)	5 (26.3)	10 (41.7)	-0.69	0.30	14 (46.7)	9 (30.0)	0.71	0.19
	Moderate - Severe (4-7)	14 (73.8)	14 (58.3)	(-2.00, 0.61)		16 (53.3)	21 (70.0)	(-0.35, 1.77)	
cMEQ		23.68 ± 3.89	24.46 ± 4.01	0.77 (-2.10, 3.65)	0.60	24.20 ± 4.62	24.67 ± 5.88	0.47 (-1.95, 2.89)	0.71
n (%)	Evening (0-22)	9 (47.3)	9 (36.5)	0.41	0.52	15 (50)	16 (53.3)	-0.13	0.80
	Intermediate (23-43)	10 (52.7)	15 (62.5)	(-0.82, 1.63)		15 (50)	14 (47.7)	(-1.15, 0.68)	
DBT (hh:mm)		22:24 ± 0:59	22:28 ± 0:50	00:04 (-00:30, 00:39)	0.81	22:30 ± 0:54	22:23 ± 1:05	-00:07 (-00:37, 00:22)	0.62
DLMO (hh:mm)		22:35 ± 1:02	22:49 ± 1:15	00:32 (-00:34, 01:02)	0.53	22:56 ± 1:15	22:54 ± 1:35	00:20 (-00:42, 00:39)	0.94
DLMO –Melatonin$_{admin}$ (dec h)		1.12 ± 0.69	1.34 ± 1.02	0.17 (-0.42, 0.75)	0.58	1.44 ± 0.93	1.54 ± 1.10	0.10 (-0.39, 0.59)	0.69

Abbreviations: BMI: body mass index, cMEQ: composite morningness-eveningness questionnaire, CGI-C severity: Clinical Global Impression of Change Severity, DLMO: dim light melatonin onset, dec h: decimal hours, Melatonin$_{admin}$; time melatonin was taken. Data are presented as mean ± SD unless otherwise specified.

Table Two. Melatonin treatment response to sleep-wake characteristics in DSWPD patients stratified by *PER3* VNTR.

	***PER3*$^{4/4}$**						***PER3* 5 alleles**					
	T_0 Baseline		**T_1 Treatment Period**		**Timepoint x Treatment**		**T_0 Baseline**		**T_1 Treatment Period**		**Timepoint x Treatment**	
	Placebo	**0.5mg Melatonin**	**Placebo**	**0.5mg Melatonin**	**β (95% CI)**	***p***	**Placebo**	**0.5mg Melatonin**	**Placebo**	**0.5mg Melatonin**	**β (95% CI)**	***p***
Actigraphy												
N	17	23	17	20			28	27	24	23		
Sleep Onset (hh:mm)	01:01 ± 1:23	01:05 ± 1:15	23:35 ± 1:09	23:15 ± 0:51	-00:23 (-1.14, 0.37)	0.321	01:03 ± 1:19	01:15 ± 1:31	23:27 ± 1:05	23:29 ± 1:11	-00:22 (-0:57, 00:13)	0.214
WT (hh:mm)	08:28 ± 1:07	08:40 ± 2:03	08:03 ± 0:57	07:42 ± 1:03	-0,39 (-1.08, 0.30)	0.278	08:20 ± 1:18	08:40 ± 1:39	08:10 ± 1:25	07:46 ± 1:10	-0.76 (-1.55, 0.03)	0.066
TST (hours)	6.92 ± 1.40	6.84 ± 1.99	7.80 ± 1.07	8.20 ± 1.22	36.11 (-6.63, 78.85)	0.106	6.78 ± 1.21	6.82 ± 1.03	7.98 ± 1.18	7.71 ± 1.71	-15.23 (-58.78, 28.41)	0.497
SOL (min)	22.60 ± 14.86	17.41 ± 13.79	**46.89 ± 30.84***	**22.81 ± 19.98$^{\#}$**	**-19.74 (-33.35, -6.13)**	**0.007**	16.99 ± 12.51	26.54 ± 20.49	**39.63 ± 26.97***	31.61 ± 16.75	**-18.71 (-32.15, -5.28)**	**0.009**
SE T1 (%)	82.24 ± 5.94	84.53 ± 5.96	75.34 ± 10.32	82.46 ± 7.53	5.01 (-0.77, 10.79)	0.098	84.85 ± 5.86	81.06 ± 9.16	**77.63 ± 8.67***	80.64 ± 6.03	**7.23 (2.35, 12.11)**	**0.006**
WASO (min)	49.62 ± 17.04	40.18 ± 14.80	58.27 ± 21.44	48.98 ± 19.13	0.35 (-11.98, 12.68)	0.956	45.01 ± 22.63	46.99 ± 21.31	**60.24 ± 20.05***	52.6 ± 22.45	**10.19 (-17.91, -2.47)**	**0.013**
Sleep Diary												
N	17	23	17	22			29	28	25	25		

Sleep Onset (hh:mm)	01:10 ± 1:32	01:23 ± 0:53	**23:51 ± 1:11***	**23:21 ± 0:52****	**-00:47 (-1:24, -0:03)**	**0.039**	01:14 ± 1:12	01:31 ± 1:36	23:47 ± 0:54	23:39 ± 1:06	-00:29 (-1:03, 0:05)	0.101
WT (hh:mm)	08:30 ± 1:08	08:20 ± 1:43	07:49 ± 1:15	07:16 ± 1:22	-0.21 (0.90, 0.48)	0.554	08:18 ±1:17	08:28 ± 1:43	07:45 ± 1:03	07:38 ± 0:58	-0.38 (-0.99, 0.23)	0.224
TST (hours)	6.65 ± 0.88	6.52 ± 0.81	7.25 ± 0.91	7.19 ± 0.82	8.11 (-27.74, 43.97)	0.660	6.43 ± 1.05	6.43 ± 0.85	7.30 ± 0.69	7.27 ± 0.77	3.06 (-29.08, 35.21)	0.853
SOL (min)	52.56 ± 49.13	42.30 ± 38.40	**72.97 ± 56.05***	**37.91 ± 17.80**[#]	**-23.09 (-40.41, -5.78)**	**0.013**	35.59 ± 25.07	48.54 ± 32.34	58.71 ± 35.52	54.69 ± 45.75	-16.96 (-36.29, 2.37)	0.091
SE (%)	84.76 ± 12.17	84.79 ± 17.35	83.16 ± 11.19	89.81 ± 6.05	5.88 (-0.51, 12.26)	0.079	87.95 ± 7.54	86.27 ± 9.08	85.17 ± 9.76	84.86 ± 13.55	1.62 (-2.84, 6.09)	0.479
WASO (min)	21.85 ± 21.99	27.64 ± 46.47	24.38 ± 22.37	17.68 ± 18.12	-9.54 (-26.84, 7.75)	0.287	18.67 ± 20.62	18.55 ± 23.51	24.45 ± 37.45	25.52 ± 35.29	-2.17 (-15.44,11.10)	0.750
Circadian Phase												
N	17	23	11	14			30	30	14	12		
DLMO (hh:mm)	22:35 ± 1:02	22:49 ± 1:15	22:11 ± 1:03	22.07 ± 1:40	-00:23 (-1:20,0:32)	0.42	22:56 ± 1:15	22:54 ± 1:35	22.88 ± 1:46	22.14 ± 1:40	-00:38 (-1:47, 0:30)	0.28
DBT-DLMO (hours)	-0.18 ± 0.69	-0.34 ± 1.02	0.00 ± 1.06	0.28 ± 0.97	-0.27 (-0.50, 1.37)	0.43	-0.44 ± 0.93	-0.54 ± 1.10	-0.56 ± 1.58	0.06 ±1.67	0.69 (-0.37,1.76)	0.21
BT– DLMO (hours)	-2.54 ± 1.25	-2.26 ± 1.51	-1.25 ± 0.94	-1.51 ± 0.72	-0.17 (-1.70, 1.36)	0.829	-2.10 ± 0.78	-2.36 ± 1.06	-0.83 ± 1.01	-0.84 ± 1.74	0.21 (-0.84, 1.25)	0.702

Abbreviations – WT: wake time, TST: total sleep time, SOL: sleep onset latency, SE: sleep efficiency, SE T1: sleep efficiency in the first third of time in bed averaged over treatment nights, WASO: wake after sleep onset. Data are presented as mean ± SD. Underlined variable actigraphic sleep onset was the primary outcome. Genotype stratified to assess time x treatment response. Bolded values indicate significant results. Bonferroni correction applied for post hoc comparisons. * $p < 0.05$ T_0 placebo *vs* T_1 placebo ** $p < 0.05$ T_0 0.5mg melatonin *vs* T_1 0.5mg melatonin [#] $p < 0.05$ T_1 placebo *vs* T_1 0.5mg melatonin.

Table Three. Melatonin treatment response to self-report measures of sleep disturbance and sleep related impairment in DSWPD patients stratified by *PER3* VNTR.

		***PER3*$^{4/4}$**						***PER3* 5 alleles**					
		T_0 Baseline		**T_1 Post Treatment**		**Timepoint x Treatment**		**T_0 Baseline**		**T_1 Post Treatment**		**Timepoint x Treatment**	
		Placebo	**0.5mg Melatonin**	**Placebo**	**0.5mg Melatonin**	**β (95% CI)**	***p***	**Placebo**	**0.5mg Melatonin**	**Placebo**	**0.5mg Melatonin**	**β (95% CI)**	***p***
N		19	24	17	22			30	30	29	26		
Sleep Disturbance													
PSQI[a]		9.40 ± 3.69	8.30 ± 2.77	7.88 ± 2.93	5.09 ± 1.77	-1.46 (-3.44, 0.51)	0.155	7.73 ± 3.26	8.72 ± 3.42	7.69 ± 3.66	7.50 ± 3.68	-1.03 (-2.60, 0.53)	0.202
n (%)	Normal (< 5)	1 (5.2)	4 (17.4)	4 (23.5)	13 (59.1)	-0.21	0.877	9 (30.0)	5 (17.2)	11 (37.9)	8 (30.8)	-0.40	0.636
	Sleep disturbance (≥ 5)	18 (94.8)	19 (82.6)	13 (76.5)	9 (40.9)	(-2.73, 3.04)		21 (70.0)	24 (82.8)	18 (62.1)	18 (69.2)	(-2.11, 1.26)	
ISI		12.37 ± 4.67	13.17 ± 4.81	10.76 ± 4.00	**6.95 ± 3.30**#**	**-4.52 (-7.52, -1.53)**	**0.005**	11.77 ± 4.88	12.93 ± 5.11	10.52 ± 5.87	9.50 ± 6.78	-2.20 (-4.98, 0.58)	0.126
n (%)	Absent	3 (15.8)	3 (13.0)	3 (17.6)	**16 (72.7) **#**	**-2.74**	**0.021**	5 (17.7)	5 (17.9)	10 (34.5)	11 (42.3)	-0.25	0.780
	Subthreshold-severe	16 (84.2)	20 (87.0)	14 (82.4)	**6 (27.3)**	**(-5.17, -0.43)**		25 (83.3)	23 (82.1)	19 (65.5)	15 (57.7)	(-2.01, 1.51)	
PROMIS Sleep Disturbance		22.84 ± 6.27	23.67 ± 5.31	24.21 ± 5.31	**18.67 ± 5.48**#**	-6.37 (-9.78, -2.96)	**<0.001**	22.80 ± 5.68	23.80 ± 5.73	22.37 ± 6.99	21.17 ± 7.75	-2.20 (-5.99, 1.59)	0.259
Sleep Related Impairment													
ESS		6.26 ± 4.31	6.75 ± 4.34	6.12 ± 4.05	5.27 ± 3.24	-1.53 (-3.46, 0.40)	0.129	5.00 ± 3.16	5.80 ± 2.83	4.52 ± 2.20	4.54 ± 2.37	-0.99 (-2.42, 0.44)	0.182
n (%)	Normal-borderline (< 10)	14 (73.7)	19 (79.2)	14 (82.4)	20 (90.9)		0.706	28 (93.3)	26 (86.7)	29 (100.0)	25 (96.2)		
	Excessive daytime sleepiness (≥ 10)	5 (26.3)	5 (20.8)	3 (17.6)	2 (9.1)			2 (7.7)	4 (13.3)	0 (0.0)	1 (3.8)		
SDS[b]		14.58 ± 4.19	15.00 ± 5.08	13.47 ± 4.54	**9.00 ± 3.85**#**	**-4.91**	**0.010**	14.53 ± 5.70	14.55 ± 5.49	11.52 ± 6.11	11.50 ± 7.44	-0.23	0.854

[a] T_0 *PER3*$^{4/4}$ placebo n=19, 0.5mg melatonin n=23; *PER3* 5 alleles placebo n=30, 0.5mg melatonin n=29.

					(-8.45, -1.38)						(-2.66, 2.22)	
Absenteeism[b]	2.89 ± 3.49	2.17 ± 2.90	1.35 ± 2.34	1.77 ± 3.04	1.06	0.307	3.32 ± 4.17	2.36 ± 4.29	2.55 ± 3.75	0.77 ± 2.05	-0.84	0.477
					(-0.94, 3.05)						(-3.14, 1.46)	
Presenteeism[c]	10.05 ± 7.34	7.26 ± 5.07	5.24 ± 6.10	4.00 ± 5.27	1.49	0.547	9.20 ± 6.71	8.64 ± 6.95	6.52 ± 6.42	5.00 ± 6.42	-0.92	0.667
					(-3.31, 6.29)						(-5.11, 3.26)	
PROMIS Sleep-Related Impairment[c]	23.26 ± 7.14	23.08 ± 7.44	19.37 ± 6.82	**14.46 ± 3.89**#**	**-4.71**	**0.017**	22.13 ± 4.94	24.23 ± 7.10	18.03 ± 7.30	16.70 ± 6.54	-3.43	0.068
					(-8.44, -1.02)						(-7.05, 0.19)	

Abbreviations - CI: Confidence intervals, PSQI: Pittsburgh Sleep Quality Index, ISI: Insomnia Severity Index, PROMIS: Patient-Reported Outcomes Measurement Information System, ESS: Epworth Sleepiness Scale, SDS: Sheehan Disability Scale. Data are presented as mean ± SD. Genotype stratified to assess time x treatment response. Bolded values indicate significant results. Bonferoni correction applied for post hoc comparisons. * $p < 0.05$ T_0 placebo *vs* T_1 placebo ** $p < 0.05$ T_0 0.5mg melatonin *vs* T_1 0.5mg melatonin # $p < 0.05$ T_1 placebo *vs* T_1 0.5mg melatonin.

[b] T_0 *PER3*$^{4/4}$ placebo n=19, 0.5mg melatonin n=23; *PER3* 5 alleles placebo n=30, 0.5mg melatonin n=28.
[c] T_0 *PER3*$^{4/4}$ placebo n=19, 0.5mg melatonin n=24; *PER3* 5 alleles placebo n=30, 0.5mg melatonin n=30.

Table Four. Clinician and patient-rated measures of disorder severity and treatment efficacy in DSWPD patients stratified by *PER3* VNTR.

		***PER3*$^{4/4}$**				***PER3* 5 alleles**			
		Placebo	**0.5mg Melatonin**	**OR (95% CI)**	***p***	**Placebo**	**0.5mg Melatonin**	**OR (95% CI)**	***p***
N		19	24			30	30		
Clinician report									
CGI-C									
Severity									
n (%)	Normal - Mildly Ill (1-3)	11 (57.8)	16 (66.7)	0.69	0.555	14 (46.7)	18 (60.0)	0.58	0.301
	Moderate – Severe (4-7)	8 (42.2)	8 (33.3)	(0.20, 2.39)		16 (53.3)	12 (40.0)	(0.21, 1.62)	
Global Improvement									
n (%) (1-3)	Very much - minimally improved	13 (68.4)	23 (95.8)	**0.09**	**0.016**	20 (66.6)	19 (63.3)	1.16	0.787
	No change - very much worse (4-7)	6 (31.6)	1 (4.2)	**(0.01, 0.87)**		10 (33.3)	11 (36.7)	(0.40, 3.35)	
Efficacy Index[a]		7.87 ± 4.83	5.29 ± 3.16	**-4.18**	**<0.001**	9.47 ± 3.13	8.13 ± 4.79	0.27	0.831

[a] Efficacy index calculated using general linear model, β stated.

				(-6.13, -2.23)				(-2.22, 2.75)	
Patient Report									
PGI-C[b]									
n (%)	No change – a little better (1-3)	11 (57.8)	6 (25.0)	**4.13**	**0.028**	15 (51.7)	13 (43.3)	0.82	0.703
	Somewhat better – great deal better (4-7)	8 (42.2)	18 (75.0)	**(1.13, 15.10)**		14 (48.3)	17 (56.7)	(0.29,2.29)	

Abbreviations - CGI-C: Clinical Global Impression Scale of Change, PGI-C: Patient Global Impression Scale of Change, OR: odds ratio.

[b] *PER3* 5 alleles, placebo group n=29.

Author/s:
Magee, M; Sletten, TL; Murray, JM; Gordon, CJ; Lovato, N; Bartlett, DJ; Kennaway, DJ; Lockley, SW; Lack, LC; Grunstein, RR; Archer, SN; Rajaratnam, SMW

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