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Original Article

Abnormal secretion of melatonin and cortisol in relation to sleep disturbances in children with Williams syndrome

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ABSTRACT

Objective: A high rate of sleep disturbances has been reported in individuals with Williams syndrome (WS) but the underlying aetiology has yet to be identified. Melatonin and cortisol levels display circadian rhythmicity and are known to affect and regulate sleep/wake patterns. The current study examined the levels of these two endocrine markers and explored a possible relationship with sleep patterns in children with WS.

Methods: Twenty-five children with WS and 27 typically developing age- and gender-matched comparison children were recruited. Saliva was collected from each child at three time points: 4–6 pm, before natural bedtime, and after awakening. The levels of salivary melatonin and cortisol were analysed by specific enzyme-linked immunoassays. Sleep patterns were examined using actigraphy and the Children's Sleep Habit Questionnaire.

Results: The WS group had shallower drops in cortisol and less pronounced increase in melatonin at bedtime compared to the controls. Furthermore, they also had significantly higher levels of cortisol before bedtime.

Conclusions: Increased bedtime cortisol and less pronounced rise in melatonin levels before sleep may play a role in the occurrence of sleep disturbances, such as delayed sleep onset, observed in children with WS. As both markers play a significant role in our circadian rhythm and sleep/wake cycle, it is necessary to examine sleep using multi-system analysis.

1. Introduction

Sleep involves a finely tuned multidimensional interaction of biochemistry, genetic and psychological processes. It has a modulatory effect on many components of the endocrine system, and, reciprocally, many hormones affect sleep and display circadian rhythmicity [1]. Melatonin and cortisol secretion are amongst hormones that affect the human daily cycle including sleep [1,2]. Melatonin is a neurohormone secreted by the pineal gland, while cortisol is one of the major glucocorticoid hormones secreted by the adrenal cortex [3]. Both play a role in the regulation of the sleep–wake cycle and their levels tend to run opposite to each other. The melatonin levels in plasma begin to increase before night-time sleep and reach a maximum between 3:00 and 4:00 am [4]. On the other hand, the cortisol levels rise before dawn, rapidly increase after awakening, and decrease over the course of the day [5] with a nadir early in the sleep period [6]. Developmentally, this process has been shown to be stable in the first year of life [7]. Moreover, no gender difference in the secretion of melatonin and cortisol has been found in prepubertal children [4].

Melatonin supports a nocturnal decrease in the core body temperature and facilitates sleep [8]; as such, its secretion coincides with sleepiness and the greatest decline in body temperature over a 24-h period [4]. By contrast, cortisol is often referred to as a stress hormone, as its secretion elevates in response to stress and anxiety. It is also responsible for increasing the heart rate, blood pressure, and glucose being released to the blood stream [9]. Thus, it has been suggested that the rise of cortisol before dawn stimulates the brain and diverts energy to the muscles, which in turn facilitates awakening [10]. The pattern of secretion of both of these endocrine markers, melatonin and cortisol, has proved to be stable across days and weeks among individuals of a wide age range [5,11,12]. Thus, although the levels of both these hormones show a high degree of variability between individuals [13], a given individual tends to have

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a consistent rhythm [11]. Hence, both melatonin and cortisol are regarded to be amongst the most robust markers of the sleep–wake circadian rhythm.

The aim of the current study was to examine endocrine sleep markers, namely melatonin and cortisol, in relation to sleep patterns in children with Williams syndrome (WS). To our knowledge, no previously published study has examined the causality of sleep problems via an analysis of endocrine indicators of sleep and endocrine rhythm in the saliva of children with WS.

WS is an autosomal dominant disorder caused by a microdeletion of ~28 genes on chromosome 7q11.23, which includes a region encoding elastin [14]. WS is characterised by an uneven cognitive profile and a unique personality profile, which involves not only high sociability and empathy [15] but also a high level of anxiety related to social situations. One aspect of the WS phenotype that has yet to be adequately characterised is sleep. Parents of children with WS often report significant sleep-related symptoms that include difficulty in settling down at bedtime/falling asleep, prolonged awakenings from sleep and restless sleep [16–18]. Thus far, studies examining sleep in WS have used questionnaires (such as Children's Sleep Habit Questionnaire – CSHQ), actigraphy, and/or polysomnography (e.g., Refs [17,18]). Based on the CSHQ scores, Goldman and colleagues [16] have shown that >36% of individuals with WS ($n = 23$) have trouble sleeping [15]. A much larger study of 64 children with WS using the CSHQ questionnaire reported sleep problems such as bedtime resistance, sleep anxiety, and night waking [17]. Studies using polysomnography have also shown atypical sleep patterns such as decreased sleep efficiency, higher level of restlessness, and arousals from sleep [19,20]. The propensity to anxiety and sleep disturbances reported in children with WS could be linked by increased bedtime cortisol and in turn circadian disruption.

While associations between sleep disturbances and behavioural and cognitive functioning are well recognised, the underlying factors of sleep disturbances are yet to be determined. The current study thus examines two endocrine sleep markers, namely, cortisol and melatonin in children with WS.

2. Materials and methods

2.1. Participants

From a database provided by the Williams Syndrome Foundation, UK, a total of 35 parents of children with WS were contacted and 25 agreed to take part in the studies (71% response rate). Parental informed consent and the child's verbal assent were obtained prior to participation. All children with WS were diagnosed clinically and the molecular diagnosis for haplo-insufficiency for the *ELASTIN* gene was determined by fluorescence in situ hybridisation (FISH). Children with WS were between 4 and 11 years of age. The age range was chosen in order to minimise individual variability such as schooling. All children with WS were chronologically age- and gender-matched to 27 typically developing (TD) children (12 male, 15 female) recruited from mainstream schools in south-east UK. Each participant was further assessed on Tanner's pubertal scale.

Children were not included in the study if they had co-morbid medical or psychiatric disorders such as attention-deficit/hyperactivity disorder (ADHD) or autism as well as conditions that could affect sleep such as epilepsy, problems with tonsils/adenoids, frequent sinus infection and poorly controlled asthma or eczema, and if they were taking any medication affecting sleep and/or levels of melatonin and cortisol.

Demographic data were obtained for each individual and a series of statistical tests were conducted to determine whether groups differed in age, body mass index (BMI), gender, parents' socio-economic status, and pubertal stage. Several environmental factors were also included: whether there was a TV in the child's bedroom, whether children watched TV 30 min before sleep, whether they shared a bedroom, and also whether the child's bedroom was on a main road where the traffic can be heard at night. These factors were included in the statistical analyses; no statistically significant difference was observed between groups in any of the environmental parameters (all $P > 0.05$).

Ethical approval was granted by Middlesex University, London Natural Sciences Ethics sub-Committee, Institute of Education, University of London and the Williams Syndrome Foundation, UK, prior to recruitment of participants, and all experiments were performed in accordance with its guidelines and regulations.

2.1.1. Cortisol and Melatonin sampling method

In the current study, the levels of cortisol and melatonin were measured in the saliva. It has been established that the salivary levels closely parallel those found in the corresponding blood samples [21,22]. The parents of all the participants underwent training in the collection of saliva samples and a written collection protocol was given to each parent. The parents of all children were also asked for their children to avoid caffeinated drinks as these could affect the levels of melatonin and cortisol [23]. Due to the fact that light exposure suppresses melatonin levels, bedtime samples were collected in dimly lit conditions.

Saliva was collected using saliva collection devices, which involves placing an inert polymer swab under the tongue absorbing saliva. The swab was placed in a centrifuge case and tube (Salimetrics Europe, Suffolk, UK). Samples were collected at three time points: 4–6 pm, at bedtime, and immediately after awakening. In order to ensure that the time between first collection and bedtime sample did not vary strongly across study participants, the parents were instructed that the time difference between these collection points should fall within 3–4 h. In order to avoid environmentally triggered stress before collection times, the parents of all the participants were asked to maintain a normal home routine and to note if there was anything atypical on a day of testing. Labelled sample swabs were stored in the household fridge until collection (maximum 24 h) and were transported on dry ice to the laboratory. The samples were further centrifuged for 10 min at 1500 *g*. Saliva extracted from the swab collected at the bottom of the centrifuge tubes were subsequently divided into two to three aliquots and stored at –20 °C until assayed. It has been reported that melatonin and cortisol are stable for months (probably years) when kept frozen (at –20 °C or lower) [24,25]. Several studies have reported high stability and intraday reproducibility of melatonin [26] and cortisol [5] in the same individual; thus, in the current study, samples were collected over the period of 1 day.

2.2. Determination of melatonin and cortisol levels

The levels of melatonin in saliva samples (100 µl) were measured in duplicate by a competitive enzyme-linked immunosorbent assay (ELISA) kit from IBL International (Hamburg, Germany). Melatonin in the saliva samples and standards competed with biotinylated melatonin, for solid-phase antibody coating 96-well assay plates. Streptavidin-conjugated enzyme was added for detection and substrate chromophore was formed in inverse proportion to the amount of melatonin in the sample/standard. Absorbance at 450 nm was measured on a FLUOstar OPTIMA plate reader (BMG LABTECH, Ortenberg, Germany) and sample concentration was read against the standard curve; the sensitivity of the assay was 0.3 pg/mL. The levels of cortisol in the saliva samples (25 µl) were measured in duplicate using a competitive enzyme immunoassay kit from Salimetrics Europe (Suffolk, UK). Ninety-six-well assay plates were utilised and optical absorbance at 450 nm was measured on a FLUOstar OPTIMA plate reader (BMG LABTECH). The sample concentration was read against the standard curve; the sensitivity of

the assay was 30 pg/mL. Both assays were carried out according to manufacturer's instructions [27,28].

2.3. Sleep measures

2.3.1. Actigraphy

Each child wore an Actiwatch Mini (CamNTech, Cambridge, UK) to objectively assess her/his sleep patterns. This was worn around the non-dominant wrist continuously for three consecutive days and nights as if wearing a watch. Data were downloaded to a computer and were analysed using Sleep Analysis 7. The actigraphy parameters used in this study were time in bed, sleep latency, actual wake percentage, sleep efficiency, moving, and sleep fragmentation index. In addition, parents completed a sleep diary recording their child's bedtime and awakening time for the duration of the study. These diary parameters were used to support the calculation and analyses of actigraphy data.

2.3.2. Children's sleep habit questionnaire [29]

The parents of all participants were asked to complete the standardised 45-item parent questionnaire to examine sleep behaviour in school-age children. The parents answered on three-point scale: 'usually' if the sleep behaviour occurred five to seven times per week, 'sometimes' for two to four times per week and 'rarely' for zero to one time per week. Thirty-three items from the questionnaire were used to calculate a total sleep disturbance score, as well as scores in eight sub-scales: bedtime resistance, sleep onset delay, sleep duration, sleep anxiety, night waking, parasomnias, sleep-disordered breathing, and daytime sleepiness.

Actigraphy, CSHQ, and saliva samples were all obtained within the same time and during the weekdays to ensure normal routine. Actigraphy sleep measurements were taken over the three consecutive days, and saliva samples were collected on the last day of the child participation in the study. In order to estimate the participant child's pubertal stage, the parents were asked to indicate the estimated stage on the Tanner's scale [30,31].

3. Results

3.1. Participants

Data were analysed using SPSS for Windows, Version 19 (SPSS Inc., Chicago, IL, USA). There were no significant group differences in chronological age ($P = 0.861$) and BMI ($P = 0.376$). Chi-squared analyses showed that children in both groups did not differ in gender ($P = 0.785$), ethnicity ($P = 0.220$) and parental occupation (mother's occupation: $P = 0.385$; father's $P = 0.441$; see Table 1). Based on Tanner's rating scale, 88% of children with WS and 94% of TD controls were in the prepubertal stage and hence no significant group difference was found ($P = 0.266$).

Table 1
Detail characteristics of children with Williams syndrome (WS) and Typically developing (TD) control group including mean age, age range and gender distribution as well as percentage of ethnicity and parents' occupation in both groups.

	Williams syndrome ($n = 21$)	Typically developing controls ($n = 27$)
Age, years (SD)	7.30 (1.87)	7.47 (2.00)
Age range	4.48–11.00	4.04–10.80
Gender (M/F %)	48/52	44/56
Ethnicity (%White)	92	78
Ethnicity (% Black)	0	0
Ethnicity (% Other)	8	22
Parents' occupation:		
Professional (%)	33	46
Clerical (%)	20	22
Manual and other (%)	47	32

Table 2
Comparison of normalised levels of melatonin and cortisol in Williams syndrome (WS) and typically developing (TD) children using the Mann–Whitney test. The *U* value and *P* value are also shown for determination of significance. The *N* value accounts for the differences from $n = 27$, as some children failed to provide all three saliva samples.

	Groups	Median	*U* value	*P* value
Normalised melatonin afternoon (%)	TD ($n = 25$)	40.37	153.00	0.127
	WS ($n = 17$)	72.22		
Normalised melatonin bedtime (%)	TD ($n = 21$)	80.48	190.00	0.797
	WS ($n = 19$)	61.03		
Normalised cortisol afternoon (%)	TD ($n = 25$)	22.22	232.50	0.689
	WS ($n = 20$)	27.17		
Normalised cortisol bedtime (%)	TD ($n = 22$)	11.71	142.50	0.031
	WS ($n = 21$)	18.52		

3.2. Laboratory analysis

In order to compensate for individual variation and to observe the changes of melatonin and cortisol before night-time sleep, the morning level of both markers was set to 100% and the afternoon and bedtime levels were normalised as a percentage of the morning value [32,33]. In addition, as expected when dealing with young children and those with developmental disorders, the melatonin and cortisol data were not normally distributed, as revealed by the Shapiro–Wilk tests of normality. Thus, the non-parametric statistical Mann–Whitney U test was used for further group comparisons.

3.2.1. Melatonin

High individual variability in the amount of melatonin secreted was observed in both groups. For the WS group, the afternoon level ranged from <0.3 to 17.40 pg/mL, bedtime from <0.3 to 26.47 pg/mL, and morning from <0.3 to 14.71 pg/mL. In the TD group, these values were <0.3–20.60, <0.3–46.95 and <0.3–51.03 pg/mL, respectively. There was no significant difference between the groups in samples collected in the afternoon ($P = 0.127$) and bedtime ($P = 0.797$) (Table 2, Fig. 1). In order to investigate changes in the level of melatonin before bedtime, the ratio between bedtime and afternoon samples was calculated for each participant. There was a median-fold increase of 1.83 in the TD group; no such increase was observed in the WS ($P = 0.038$) (Table 3 and Fig. 1).

3.2.2. Cortisol

The median level of cortisol was found to be at the maximum in the morning and the lowest at bedtime in both groups, indicating the circadian rhythmicity of salivary cortisol levels. In the TD control, the cortisol ranges were found to be 200–1600, 100–1200, and 900–11,100 pg/mL in the afternoon, bedtime and morning samples, respectively. By contrast, the levels of salivary cortisol in children with WS ranged from 100 to 2100 pg/mL, from 100 to 1400 pg/mL and from 400 to 7500 pg/mL. There was no significant difference in the levels of normalised cortisol in the afternoon between the WS and TD groups ($P = 0.689$), but a significantly higher normalised value was observed in the evening before bedtime in the WS group ($P = 0.031$) (Table 2 and Fig. 1). Further analyses calculating the ratio of cortisol levels between the afternoon and bedtime samples showed a median-fold decrease of 1.37 in the WS group and a drop of 2.25 in the TD group ($P = 0.007$) (see Table 3 and Fig. 1).

Due to the number of samples, the melatonin and cortisol assays were performed over several days (10 days for the melatonin assay and 5 days for the cortisol assay). The inter-assay coefficients of variation ranged between 7.6% and 13% for melatonin and between 3.75%

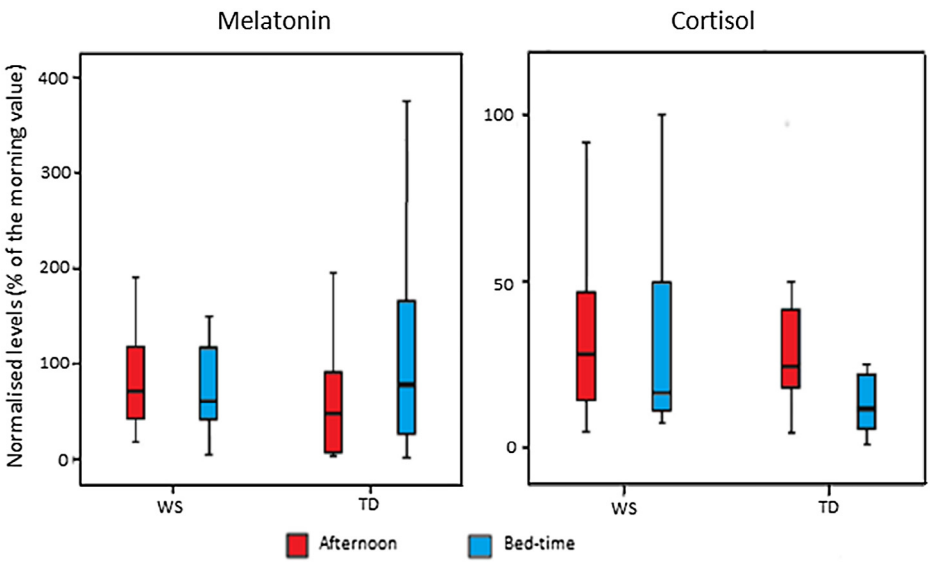

Fig. 1. Normalised levels of salivary melatonin and cortisol in the afternoon and bedtime in children with Williams syndrome (WS) and typically developing (TD) control group. Data are shown as a median and quartiles.

and 6.41% for cortisol, while the intra-assay coefficients of variation were 6.1–10.8% and 3.35–3.65% for melatonin and cortisol, respectively.

3.2.3. Age and gender effects

The levels of melatonin and cortisol were also analysed by carrying out an analysis of covariance (ANCOVA) with a chronological age covariate. There was no effect of age on the levels of melatonin and cortisol in the afternoon and bedtime samples in both groups (P value ranged between 0.259 and 0.962 for the WS group and between 0.408 and 0.983 for the TD group). In order to further investigate the effect of age, children were divided into two age groups: 4–8 and 8–11 years. No significant difference in the levels of melatonin and cortisol was observed between both age groups in WS and TD children (P values ranged between 0.235 and 0.906).

Interestingly, it was noted that girls had higher afternoon cortisol levels than boys in the WS group ($P = 0.027$) and TD group ($P = 0.086$). There were no gender differences in cortisol levels in both groups (P values ranged between 0.110 and 0.990).

There were no differences in the time taken to collect saliva samples from the moment the child woke up in both groups ($P = 0.260$), as well as the time taken from saliva collection to sleep start ($P = 0.461$). In addition, melatonin and cortisol were analysed using ANCOVA with collection times (time taken to collect saliva samples from the moment the child woke up and time taken from saliva collection to sleep start) as covariates. No effect of collection times was found in both study groups (P values ranged between 0.132 and 0.857 for the WS group and between 0.301 and 0.960 for TD children).

3.2.4. Actigraphy

Data were recorded from 47 children (90%) of the 52 involved in the studies. Three children with WS and two TD children either refused to wear or removed the actigraphs during the night. In order to investigate if there were any differences between the WS and TD groups on each actigraphy, variable data were analysed using analysis of variance (ANOVA) tests. Children in the WS group showed significantly increased sleep latency, increased wake time, moving time, and sleep fragmentation in comparison with TD controls (Table 4). The outliers of over 3 SD from the mean were excluded.

3.2.5. CSHQ

TD and WS group comparisons of scores on the CSHQ were made using one-way between-group ANOVAs. See Table 5. Parents indicated that 15% of the children with WS had sleep problems in the past and 65% had current sleep issues from which 23% demonstrate this problem occasionally. None of the parents of TD children responded that their child has/had sleep problems.

3.2.6. Correlation between sleep measures and levels of melatonin and cortisol

Pearson's product moment correlations were used to investigate whether the levels of melatonin and cortisol in the WS and TD groups were related with sleep parameters measured by CSHQ and actigraphy. For the TD group, higher levels of bedtime melatonin were linked with shorter sleep onset time ($r = -0.494$, $P = 0.042$) and conversely lower levels of cortisol also meant shorter sleep onset ($r = 0.450$, $P = 0.039$). The higher levels of cortisol were also related to the reduced sleep efficiency in the TD group ($r = -0.577$, $P = 0.010$).

Table 3
Change in melatonin and cortisol levels between afternoon and bedtime saliva samples collected from children with Williams syndrome (WS) and typically developing (TD) controls. The table includes minimum and maximum value, as well as median of the ratio of melatonin and cortisol between afternoon and bedtime samples. The U value and P value are also shown for determination of significance. The N value accounts for the differences from $n = 27$, as some children failed to provide all three saliva samples.

	Groups	Minimum	Maximum	Median	U value	P value
Changes in melatonin levels from afternoon to bedtime	TD ($n = 20$)	0.36	10.73	1.83	102.00	0.038
	WS ($n = 17$)	0.19	2.72	0.96		
Changes in cortisol levels from afternoon to bedtime	TD ($n = 21$)	0.21	10.00	2.25	99.50	0.007
	WS ($n = 19$)	0.09	3.50	1.37		

Table 4
Comparison of actigraphic scores in Williams syndrome (WS) and typically developing (TD) children using ANOVA. The table includes mean and standard deviation (SD) corresponding to categories of actigraphic scores on the left-hand column; the *F* value and *P* value for determination of significance at 95% confidence interval ($P < 0.05$) are also shown.

Category of actigraphic scores	TD ($n = 22$) Mean (SD)	WS ($n = 21$) Mean (SD)	*F*	*P*
Time in bed (hh:mm)	10:24 (00:31)	10:27 (00:50)	0.24	0.62
Sleep latency (hh:mm)	00:34 (00:25)	00:53 (00:29)	5.02	0.03
Actual wake time (%)	10.65 (3.07)	13.10 (4.37)	4.28	0.04
Night waking	29.02 (6.51)	28.52 (8.73)	0.04	0.84
Sleep efficiency (%)	82.55 (5.60)	78.92 (6.40)	3.63	0.06
Moving time (%)	13.59 (2.98)	17.45 (5.71)	6.92	0.01
Sleep fragmentation index	30.05 (6.71)	39.75 (13.59)	7.97	0.01

This typical relationship was observed in WS individuals for cortisol levels and sleep latency ($r = 0.514$, $P = 0.042$); however, no relation between the levels of bedtime melatonin and sleep onset ($r = 0.058$, $P = 0.832$) as well as bedtime cortisol and sleep efficiency ($r = –0.087$, $P = 0.749$) were found in children with WS. Melatonin and the parasomnias score from CSHQ had different patterns in both groups ($P < 0.01$). In the WS group, the relationship between parasomnias and afternoon levels of melatonin was directly proportional ($r = 0.631$, $P = 0.007$). In TD controls, the higher the level of melatonin, the lower the parasomnias scored; however, this relationship did not prove to be significant ($r = –0.268$, $P = 0.195$). For the CSHQ total scores, a significant negative correlation was observed with bedtime melatonin levels in TD children ($r = –0.500$, $P = 0.021$), indicating that the higher the melatonin concentration, the lower the sleep disturbance level in children. Again, this relationship was not observed for individuals with WS ($r = 0.079$, $P = 0.746$). Lastly, a correlation between afternoon cortisol levels and sleep onset delay was found in the WS group ($r = 0.399$, $P = 0.090$), but not the TD group ($r = –0.045$, $P = 0.831$).

4. Discussion

Melatonin and cortisol are known to affect and regulate sleep/wake patterns. Yet, to date, very few studies have been carried out to analyse endocrine sleep markers in children with developmental disorders and only two of them included adults with WS [34–39]. A study by Lense *et al.* [37] reported elevated cortisol levels in response to the novel settings (residential summer camp) [37], while a study by Tordjman *et al.* reported low melatonin production in two WS patients diagnosed with WS and autism [39]. In this study, atypical secretion of both endocrine markers in WS was found.

High individual variability in the amount of melatonin secreted was observed in children in the WS and TD groups. Similar observation to the current data was reported by Burgess and Fogg who determined that the peak value of salivary melatonin ranges between 2 and 84 pg/mL [40].

The nocturnal secretion of melatonin facilitates sleep; thus, the lack of a marked rise in its concentrations before bedtime may play an underlying and/or contributory role in continued arousal and reported problems with settling down and falling asleep. In the current study, a median increase in the level of melatonin in the TD group was 1.83-fold between afternoon and bedtime samples, whereas for the WS group no such increase was observed. Similar findings of abnormal circadian rhythm of melatonin were found by Potocki *et al.*, albeit the authors analysed this indole in Smith–Magenis syndrome [34].

Cortisol is often described as a stress hormone; hence, high levels of this hormone before bedtime may potentially cause sleep problems such as difficulty with relaxing and falling asleep [41]. The lack of decrease of salivary levels of cortisol before bedtime in children with WS seen in the current study may explain their difficulty falling asleep.

The current study used a natural home environment as opposed to sleep laboratory or hospital settings. This is an important factor when examining children with developmental disorders and young children, in order to eliminate alteration in sleep quality and increased anxiety due to exposure to novel environments (see Refs. [36,42]). In addition, although the bedtime varies among children, melatonin and cortisol patterns are dependent on the habitual sleep routine of the individual. Melatonin levels rise approximately 2 h before habitual night-time sleep [43]; thus, a slightly different timing should not cause inconsistency, and in turn alter the results, as long as samples are collected at bedtime, as it was done in the current work. Moreover, cortisol levels rise rapidly during the first 30 min after awakening [10]; hence, it is necessary to collect the samples as soon as the child is awake.

Similar to the current study, Lense *et al.* analysed the diurnal cortisol profile in adults with WS [37]. However, the objective of that study was to examine cortisol as a biomarker of stress in both novel (during a residential summer camp) and familiar settings (at home), so any association with sleep disturbance was not explored. Nevertheless, participants with WS demonstrated elevated cortisol levels late in the day in the novel setting when social demands were higher. The current study used a natural home environment in order to avoid an increase in anxiety levels, which could potentially lead to increased levels of cortisol. In addition, the parents were trained how to collect samples; thus, the children did not have to deal with an unfamiliar person.

Actigraphic measurements were adopted here as this is a non-intrusive measure and several studies confirmed its good reliability with sleep laboratory polysomnography [44]. The current actigraphy data are similar to findings previously reported [19,20] providing further evidence of significant sleep disturbances in individuals with WS, such as decreased sleep efficiency and increased wake time after sleep onset as well as a higher level of restlessness and arousals. The CSHQ results in the current study were also in line with the previous studies. For instance, Goldman and colleagues have shown that >36% of young adults and adolescents with WS have trouble sleeping [16] (see also Annaz et al. [17]). This is comparable to the results obtained in this study in which the parents of 42% of children with WS reported that their children have current sleep problems.

The obtained data from actigraphy and CSHQ were further used to investigate the relationship between sleep disturbances and levels of endocrine markers of sleep. As melatonin is a hormone facilitating sleep, it was expected that sleep improves with increasing levels of melatonin. This typical relationship was observed in data obtained from the TD group. However, in WS children, this relationship was positive, indicating that the higher the concentration

Table 5
Comparison of Child's Sleep Habit Questionnaire (CSHQ) scores in Williams syndrome (WS, $n = 25$) and typically developing children (TD, $n = 27$) using ANOVA. Table includes mean, standard deviation (SD) as well as *F* and *P* value for the determination of significance at 95% confidence interval ($P < 0.05$).

Subscale (possible score range)	TD ($n = 22$) Mean (SD)	WS ($n = 21$) Mean (SD)	*F*	*P*
Bedtime resistance (6–18)	6.41 (1.04)	8.02 (2.67)	8.67	0.01
Sleep onset delay (1–3)	1.56 (.80)	2.04 (.84)	4.53	0.04
Sleep duration (3–9)	3.31 (0.61)	5.23 (1.76)	25.62	<0.001
Sleep anxiety (4–12)	4.70 (1.24)	5.96 (1.90)	8.09	0.01
Night wakings (3–9)	3.89 (.85)	4.80 (1.55)	7.03	0.01
Parasomnias (7–21)	8.37 (1.60)	9.92 (1.91)	10.11	0.003
Sleep disordered breathing (3–9)	3.30 (.67)	3.44 (0.71)	0.56	0.46
Daytime sleepiness (8–24)	11.00 (2.50)	11.24 (2.65)	0.11	0.74
Total score (33–99)	40.44 (4.73)	48.08 (7.51)	19.74	<0.001

of melatonin, the higher the parasomnias score in individuals with this syndrome. Furthermore, melatonin levels did not correlate with the time taken to fall asleep by children with WS. Thus, it could be speculated that melatonin does not function properly as a hormone regulating sleep patterns in individuals with WS. However, caution should be taken as high bedtime cortisol levels could be stimulating this atypical pattern. It was also shown that the levels of cortisol in TD children correlated positively with sleep latency and adversely with sleep efficiency. This typical relationship was observed in the WS group for sleep latency only.

In the current study, analyses of salivary cortisol and melatonin have indicated possible contributing and/or underlying factors for sleep problems in WS. However, due to the nature of the current study, we were not able to collect night-time samples; the current study does not provide data regarding the nocturnal levels of sleep hormones. There is a possibility that the patterns of secretion of melatonin and cortisol are shifted, what would correspond to a rhythm desynchronisation and in turn play a major role in sleep problems observed in children with WS.

In summary, this study suggests that abnormalities in the secretion of melatonin and cortisol may contribute to or be an underlying factor of sleep problems observed among individuals with WS. This is an important finding to consider as sleep disturbances have a huge negative impact not only on a developing brain of a child but also on the functioning of a family. Both cortisol and melatonin play a significant role in the circadian rhythm and sleep/wake cycle; therefore, it is necessary to look closely at these endocrine markers in individuals suffering from sleep disorders/problems. Future clinical studies are necessary to examine the modulation of melatonin and cortisol in individuals suffering from abnormalities in the levels of these hormones. It would be valuable to extend the analysis of melatonin and cortisol to other neurodevelopmental disorders and individuals suffering from sleep problems.

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Conflict of interest

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: http://dx.doi.org/10.1016/j.sleep.2014.09.003.

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