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Melatonin Levels in 89 Individuals With Smith Magenis Syndrome

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ABSTRACT

In patients with Smith–Magenis syndrome (SMS), an inverted circadian rhythm of melatonin (MT) contributes to the sleep disturbance. Standard treatment of sleep disturbance with MT often leads to extremely high daytime MT levels, resulting in even more sleep disorders. We therefore retrospectively evaluated the MT data of 89 SMS patients. Mean MT levels in participants on exogenous MT were significantly higher than in participants that did not use MT ($p = < 0.0001$). In 9 participants with very high MT levels, these dropped significantly after discontinuation of exogenous MT ($p = 0.0037$). In 12 participants, MT levels were significantly higher after MT therapy start compared to MT levels before MT start ($p = 0.028$). MT is catabolized principally by the CYP1A2 enzyme, with a half-life of about 40 min. *CYP1A2* genotyping was performed in five participants with high MT levels during MT use. Although clinically suspected to be a poor CYP1A2 metabolizer, all five turned out to have haplotype *CYP1A2*1F*, which is associated with increased enzyme activity. This shows that genotyping of *CYP1A2* is not suitable to estimate the level of CYP1A2 enzyme activity. When prescribing MT, it is strongly recommended to measure a MT level prior to treatment in order to determine the dose to be given. Checks of the MT level during treatment are necessary due to the frequent occurrence of poor CYP1A2 metabolism. Since *CYP1A2* genotyping does not provide adequate information about the level of CYP1A2 enzyme activity, we propose a simple way to determine *CYP1A2* phenotype.

1 | Introduction

Smith-Magenis syndrome (SMS) is a complex neurodevelopmental disorder most commonly caused by a microdeletion in chromosome 17p11.2 that includes the *RAI1* gene; in other cases the syndrome is caused by a pathogenic variant in the *RAI1* gene itself, even without the deletion. The proportion of SMS patients due to a pathogenic RAI1 variant is now felt to be > 20%, higher than originally estimated (10% RAI1; 90% deletion) (Smith et al. 2001; Boot et al. 2021). This likely reflects the recent decade of advances in genetic diagnostic tools that now include next generation sequencing (NGS).

Estimated to occur in 1/15,000 births, the syndrome is characterized by a range of well-described physical, developmental and neurobehavioral features that include a chronic circadian sleep–wake rhythm disorder associated with an abnormal inverted 24-h melatonin (MT) secretion rhythm (Smith et al. 2001). The inverted MT rhythm (daytime highs/nighttime lows), first recognized/reported by Potocki et al. 2000 and De Leersnyder, De Blois, Claustrat, et al. 2001, is felt to be pathognomonic in SMS, documented in the majority (> 90%) of studied cases (Potocki et al. 2000; De Leersnyder, De Blois, Claustrat, et al. 2001; Boudreau et al. 2009; Chik et al. 2010; Boone et al. 2011; Nováková et al. 2012; Spruyt et al. 2016).

Abbreviations: MT, melatonin; SMS, Smith Magenis syndrome.

A more detailed clinical summary on SMS can be found in GeneReviews available at https://www.ncbi.nlm.nih.gov/books/NBK1310/

1.1 | Endogenous MT Profiles in SMS

In typically developing individuals, MT levels are the lowest during daytime and highest during nighttime. Circadian rhythm of the MT synthesis in the pineal gland is synchronized to the light/dark cycle by information from photosensitive retinal ganglion cells, so that MT production is confined to the dark phase of the night. MT synthesis is blocked by light, preferably in the blue range (460–480 nm) (Cipolla-Neto and Amaral 2018).

In individuals with SMS, on the other hand, MT levels are high during the day and low at night. It is still not clear to what extent there is a completely inverted 24-h rhythm of MT, or whether there is an extremely advanced shifted MT rhythm. If there is an inverted 24-h MT rhythm based on an inverted circadian central clock, then other bodily functions that are controlled by the central clock should also have an inverted rhythm. However, 24-h patterns of body temperature are not inverted in SMS, although there is a slight advanced shift of 3 h, relative to matched controls (Smith et al. 2019). Additionally, there is no inverted pattern of cortisol, growth hormone or prolactin secretion (De Leersnyder, De Blois, Claustrat, et al. 2001).

A second notable difference is that light does not block MT synthesis in SMS, evidenced by the measurement of increased MT levels in daylight samples collected at task light levels (700–800 lx) (Chik et al. 2010). This could be caused by an abnormal functioning of the retinal–melanopsin system, as a result of which the pineal gland does not receive correct information about the light/dark cycle (Barboni et al. 2018).

Published data on MT levels in individuals with SMS indicate that some of them have high peak daytime MT levels and others have low MT levels (Potocki et al. 2000; Chik et al. 2010). Those that were on exogenous MT therapy tended to have a higher peak daytime MT level than those that did not use MT. After discontinuing MT therapy, average MT levels fell to values similar to those who did not use MT before the study (Chik et al. 2010).

In a study on 47 children with SMS from a Dutch outpatient clinic for individuals with an intellectual disability (Spruyt et al. 2016) mean salivary MT levels between 12 h and 14 h in those who were taking exogenous MT ($n = 15$) were 55.76 pg/mL. Mean salivary MT levels in those who were not taking exogenous MT ($n = 32$) were 10.71 pg/mL. Because MT is rapidly catabolized by the CYP1A2 enzyme, with a short half-life of approximately 40 min, it was concluded that the high daytime MT levels during exogenous MT use were probably caused by slow catabolism of MT, due to *CYP1A2* polymorphisms. In a study of 20 children with SMS (Comajuan et al. 2025), unexpectedly very high MT levels were found, even 48 h after taking the last MT intake. They also suspected that the high MT levels were caused by slow MT catabolism.

1.2 | Sleep in SMS

Sleep disturbance is virtually universal in SMS and remains a chronic, lifelong issue (Gropman et al. 2006; Smith et al. 2019). The inverted 24-h rhythm of MT in SMS leads to serious sleep problems, with daytime napping, difficulty staying asleep and frequent and early waking, which also seriously disrupts the parents' sleep. High daytime MT levels may also contribute to behavioral problems. A logical way to treat sleep problems in people with SMS is to restore a normal circadian MT rhythm. This can be done by creating normal nighttime MT levels with an evening dose exogenous MT, combined with a morning dose of a ß1-adrenergic antagonist for lowering elevated daytime MT levels (De Leersnyder, De Blois, Claustrat, et al. 2001). However, there still have been no well-controlled treatment trials to date for Smith-Magenis syndrome regarding dose and MT type (fast or sustained release), and when adding a beta blocker is indicated. Moreover, the results of MT treatment are often disappointing and guidelines are lacking. (Comajuan et al. 2025).

1.3 | Impact of Sleep Disturbance in SMS on Caregiver Experience and Well-Being

Raising a child with SMS is a major challenge and a heavy burden for parents/caregivers and the family. The chronic sleep disturbance not only impacts the child's daytime function (autism, communication problems, hyperactivity, aggression and tantrums) but takes a significant toll on the health and well-being of the entire family. Specifically, the nocturnal awakenings and early sleep offset with the child's strong motivation to interact with a parent/caregiver immediately can keep the entire family awake. Furthermore, it is common for a child with SMS to behave impeccably with people other than their parents, giving others the impression that the parents are greatly exaggerating the problems. It is extremely important that immediate and extended family and friends, as well as the treating physician, understand that the problems are often very serious and can provide appropriate help.

Past research highlights the challenge and heavy burden SMS takes on parents' and caregivers' mental health. In a survey using a self-report online questionnaire, 112 parents (97 mothers and 15 fathers) of individuals diagnosed with SMS described how their child's challenging needs impacted their own well-being (Foster et al. 2010). The most difficult aspects reported included chronic sleep disturbances, self-injurious behaviors, and explosive temper tantrums. Moreover, and disturbingly, a majority of parents reported suffering moderate to severe depressive symptoms (87.8% of mothers and 92.3% of fathers). Anxiety was prevalent, with 93.3% of mothers and 100% of fathers reporting moderate to high levels of anxiety. Although 40% of fathers and approximately 60% of mothers sought professional support, symptoms of anxiety and depression continued to persist despite professional intervention.

Another international survey among parents of 40 children with SMS (UK, USA, Europe, and Australia) further highlights the impact of the sleep problems of children with SMS on their parents (Agar et al. 2022). Particularly striking was the high

rate of experiencing problems with early morning waking of toddlers (74%) and young children (84%) with SMS. Sleep problems decreased slightly with age, but 50% of parents still experienced sleep problems with night waking of their adult child with SMS. In this survey, parents reported the use of medication as the most effective strategy for treating sleep problems. Approximately 20 medications were mentioned, including a wide variety of psychotropic drugs in addition to melatonin and beta-blockers. Notable was the mention of omeprazole (a potent inducer of CYP1A2) and desmopressin (compensates for the inverted ADH rhythm), as well as the fact that only about one-third of the parents had received professional input to find a good strategy. Regarding the effect of melatonin, parents felt melatonin was very effective in a number of cases and that this effect remained good for years, while others indicated that melatonin was discontinued after some time due to a lack of effectiveness in their child.

1.4 | Aim of the Study

The primary aim of the study was to gain insight into the frequency of occurrence of significantly elevated MT levels after MT prescription and a possible relationship with the dose and type of MT. In addition, a secondary aim was to investigate whether data provided by parents about their child's sleep could provide insight into the effectiveness of MT treatment.

2 | Materials and Methods

At the request of the German Association of Parents with a Child with SMS (SIRIUS), head practitioner of the sleep center for persons with an intellectual disability's Heeren Loo in the Netherlands and researcher of the Gouverneur Kremers Centrum (WB) gave a lecture in 2018 about the experiences with the treatment of sleep problems in individuals with SMS (Spruyt et al. 2016). During this meeting, SIRIUS members were offered a measurement of midday MT levels in saliva and individualized guidance/treatment advice based on their child's test results. After this, guidance via email plus a second check of the MT level if necessary was possible. Written explanation of the method of saliva collection, aimed at people with intellectual disabilities, was provided. It was recommended to store saliva samples in the refrigerator until mailed. Parents also provided answers to questions about their child's sleep (bedtime, sleep latency, wake after sleep onset, number of night wakes, sleep offset time, first time at which the child falls asleep during the day) as well as information on health and co-medication. Parents of 94 children and adults with SMS have taken this offer. After individual guidance, data of each participant were stored anonymously in an Excel database. This is a retrospective study based on these data.

Statistical analysis: *t*-Tests for independent samples were conducted to test differences between different groups and *t*-tests for paired samples were conducted to test differences in MT levels in groups before and after MT start or stop.

A total of 137 saliva samples were submitted between 2019 and the end of 2024. Six samples were excluded due to an insufficient sample. So data of 131 saliva samples, obtained from 89 persons with SMS (Table 1) including 70 children (age 18y and under) and 19 adults were used. Subjects ranged in age from 1 to 39 years (mean age 11.8 years). Of these, 74 individuals (83%) had confirmed deletions and 15 had a pathogenic RAI1 variant (mutation). Time of saliva collection was chosen between 12:00 and 14:00, based on the data from Chik's study (2010).

Saliva samples received at the laboratory Chrono@work (Groningen, the Netherlands) were frozen and stored until analysis. MT concentrations were assessed using direct saliva MT radioimmunoassay test kits (RK-DSM; Bühlmann Laboratories, Alere Health, Tilburg, the Netherlands).

2.1 | MT and CYP1A2

MT is catabolized principally by CYP1A2, and to a lesser extent CYP1A1 and CYP2C19. MT half-life in the blood is about 40 min (Cipolla-Neto and Amaral 2018). This means that if there is a period of at least 16 h between evening MT intake and midday collection of saliva, MT should be catabolized fully, which means the measurement outcome is a reflection of the endogenous midday MT peak. In previous studies of people with SMS treated with MT, significantly higher MT levels were found than in those who did not use MT. This difference was thought to be due to the slow CYP1A2 metabolism (Spruyt et al. 2016; Comajuan et al. 2025).

To determine whether someone with SMS is a slow CYP1A2 metabolizer, *CYP1A2* genotyping can be done. In addition, results from two measurements, one without and one with exogenous MT use, can be compared (i.e., *CYP1A2* phenotyping), in which we used the following criteria:

1. There had to be a large change in absolute concentration (ΔC).
2. There had to be a large fold increase, with a ratio > 3.
3. Using a low MT dose was a strong supportive factor.

There remains a 'gray zone' in which the ratio is 2–2.5× and there was only a modest absolute ΔC.

A ratio ≈1, a ΔC within assay noise for salivary MT, and a relative high MT dose fits to a normal *CYP1A2* phenotype.

3 | Results

3.1 | MT Levels of MT Users Versus Non-MT Users

At time of their first saliva sampling, 43 participants were on exogenous MT, while 46 participants did not use MT (Table 1). Mean MT levels in participants on exogenous MT were significantly higher than in participants that did not use MT (mean 52.62 vs. 19.13 pg/mL, $p = < 0.0001$) (Figure 1a). This difference existed in children (mean 53.27 vs. 22.56 pg/mL, $p = < 0.0001$) as well as in adults (49.73 vs. 8.12 pg/mL, $p = 0.024$). This difference is also seen when the data from participants with a deletion

TABLE 1 | Demographics of SMS study group.

	# Subjects	Total samples[a]	S1[b]	S2	S3	S4
SMS study group	89 (47F/42M)	131	89	29	9	4
Genetic type						
Deletion	74 (42F/32M)	110	74	25	7	4
RAI1 Var (mutation)	15 (5F/10M)	21	15	4	2	
Age groups						
Adults (over 18 year)	19 (11F/8M)	32	19	9	3	1
Children (≤ 18 years)	70 (36F/34M)	99	70	20	6	3
Mean age (range)	11.78 years (1–39 years)					
Medication Use (at time of sampling)						
On BB	12 (6F/6M)	15	12	1	1	1
No BB	77 (41F/36M)	116	77	28	8	3
Exogenous MT	43 (23F/20M)	70	43	17	8	2
No MT	46 (24F/22M)	61	46	12	1	2
Behavior RXs Use	28 (11F/17M)	47	28	12	4	3
One behavior RX	22 (9F/13M)	37	22	9	3	3
Two behavior RX's	6 (2F/4M)	10	6	3	1	0
No behavior RXs	61 (36F/25M)	84	61	17	5	1
Anti-Epileptic RX use						
One or more AE (≥ 1)	9 (5F/3M)	12	9	3	0	0
No AE RX	80 (42F/39M)	119	80	26	9	4

Abbreviations: AE, Anti-Epileptic; BB, Betablockers; RX, medication; MT, melatonin.
[a]6 samples excluded due to insufficient volume for analysis.
[b]A breakdown of the number of samples is denoted by S1 (initial sample), with S2–S4 reflecting follow-up samples collected.

(mean 59.93 vs. 20.29 pg/mL, $p = < 0.0001$) or with a mutation (mean 31.31 vs. 10.69 pg/mL, $p = 0.058$) are assessed separately (Figure 1b).

3.2 | MT Levels During and After Discontinuation of MT Use

In 9 participants who used exogenous MT and had very high MT levels, we measured MT levels again after 2 or more weeks of discontinuation of exogenous MT use, in order to be able to determine the endogenous MT level (Figure 2a). Mean MT levels during MT use were significantly higher than their MT level after discontinuation of MT (65.7 vs. 13.8 pg/mL, $p = 0.0090$). However, in one of them MT level still was > 50 pg/mL, so we suspected that even after 2 weeks of discontinuation the MT accumulation still was not washed out. It is noteworthy that in one other participant the MT level 2 weeks after stopping treatment with MT had increased from 17.2 to 23.7 pg/mL. This was most likely caused by the simultaneous stopping of treatment with the beta blocker propranolol, which inhibits MT synthesis (De Leersnyder, de Blois, Vekemans, et al. 2001). Details of these 9 participants are listed in Table 2.

Because previous research already suggested that high MT levels during MT use could be caused by a polymorphism of *CYP1A2*, *CYP1A2* genotyping was performed in five participants with high MT levels during MT use. Although clinically suspected to be a poor CYP1A2 metabolizer, all five turned out to have haplotype *CYP1A2*1F*. This haplotype is generally associated with increased enzyme activity (see discussion 4.4 MT catabolism).

3.3 | MT Levels Before and After Starting MT Therapy

In 12 cases in which the first measurement was made without the use of exogenous MT, a second measurement was taken as a control after the start of treatment with MT (Figure 2b). On average, MT levels during this check were significantly higher ($p = 0.0281$) than baseline measurement before treatment with MT (47.22 vs. 12.33 pg/mL). Details of these 12 participants are listed in Table 3.

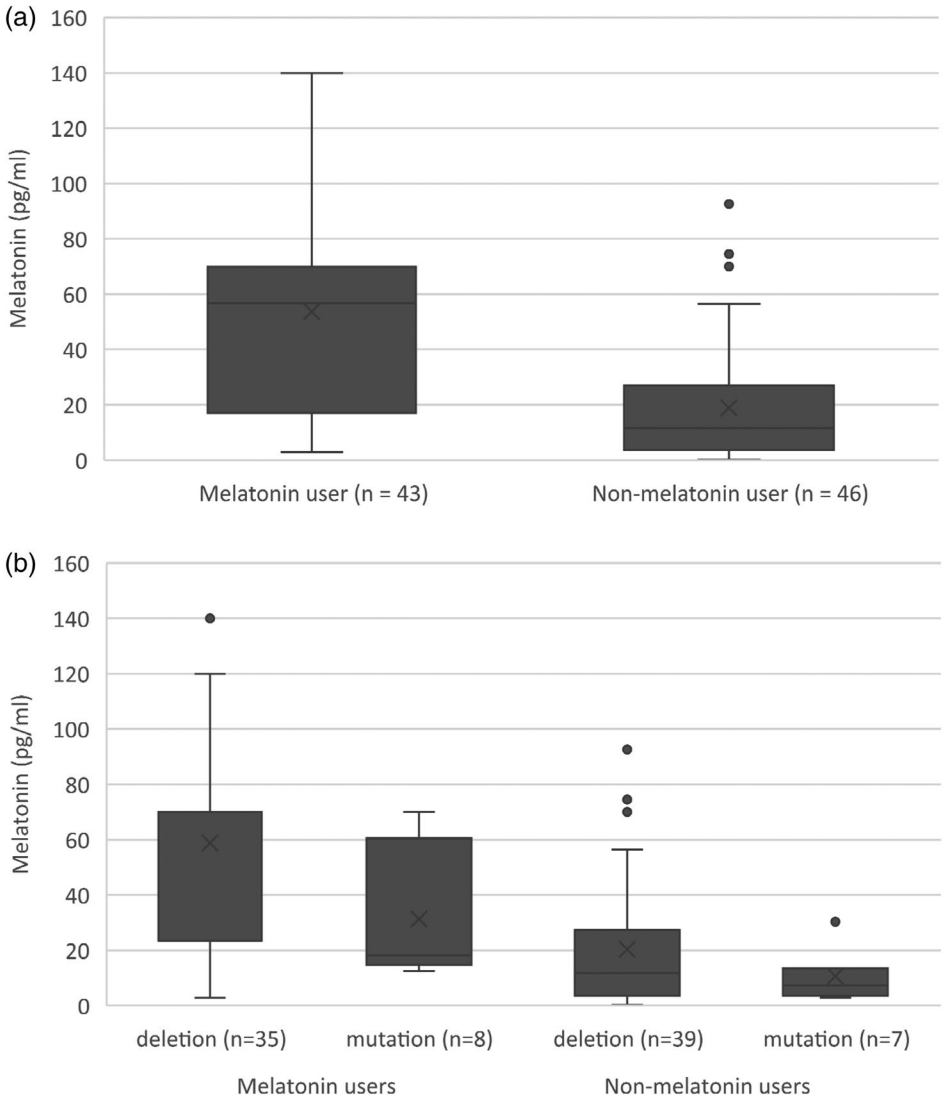

FIGURE 1 | (a) Mid-daytime salivary MT levels in patients with SMS according to MT use at first visit. (b) Mid-daytime salivary MT levels in patients with SMS according to MT use and SMS type (deletion or mutation) at first visit.

3.4 | Endogenous MT Levels and Age

To explore the relationship between endogenous MT levels and age, data of 46 participants that did not use exogenous MT at first measurement (S1) were combined with those of 13 participants that had stopped exogenous MT for at least 2 weeks (Figure 3a). The endogenous MT level was high in the first 5 years of life and showed a normal slow decline, as in the general population. Endogenous MT levels in SMS mutation type do not show the usual decline pattern with age as MT levels in SMS deletion type (Figure 3b).

Endogenous MT levels in SMS mutation type do not show the usual decline pattern with age as MT levels in SMS deletion type (Figure 3b). and were lower than in deletion type (mean 9.67 vs. 18.77 pg/mL). This difference did not reach statistical significance ($p = 0.29$), reflecting limited power due to the small number of mutation cases not taking MT ($n = 8$). Nevertheless, the observed effect size and consistent direction suggest a biologically relevant reduction in MT levels in mutation carriers.

3.5 | Salivary MT Levels and Sleep

Sleep data from three participants was unavailable. We were therefore able to evaluate the sleep data reported by the parents of 86 participants.

Day time napping was reported for 79 of 86 (92%), and in half of them it started before noon (Figure S1). In SMS deletion type, daytime napping was as frequent as in mutation type. However, in mutation type, it seemed to occur a bit later in the afternoon (Figure S2). Earlier daytime napping was associated with higher daytime MT levels (Figure S3). Those who did not show daytime napping had the lowest MT levels.

The first time of night waking after sleep onset (WASO) appeared to occur earlier in young children than in older children and adults (Figure S4). However, if we take age into account, it turned out that almost half of the group that first woke up before midnight consisted of children under 10 years old (Figure S5). First wake up time was earlier in MT users than in those who do not use MT (Figure S6).

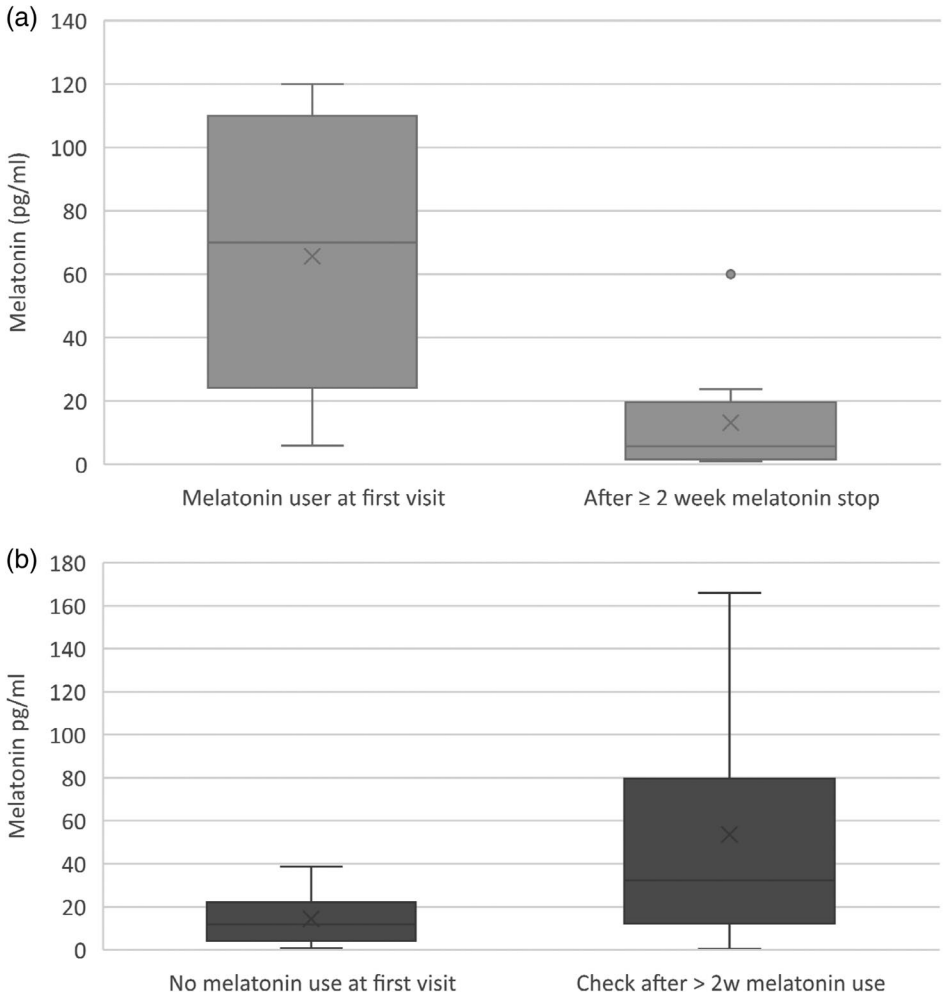

FIGURE 2 | (a) Mid-daytime salivary MT levels at first visit in 9 patients who used MT and in whom we measured MT levels again two or more weeks after stopping MT treatment. (b) Mid-daytime MT levels in 12 cases before and during use of exogenous MT.

TABLE 2 | Comparison of MT measurement at first visit and after stopping MT treatment in 9 SMS subjects.

				Exogenous melatonin		Melatonin 12 h–14 h		
Code	**Gender**	**Age (Year)**	**Genetics**	**Imm/Pro**	**Dose in mg**	**Mel use**	**Mel stop**	**Slow MT catabolism?**
S25	M	14	del	Pro	4	31.1	1.5	yes
S68	F	21	mut	Imm	3	70	1.5	yes
S97	F	25	del	Pro	2	120	3.6	yes
S51	F	3	del	Pro	7	100	5.8	yes
S34	M	8	del	Imm	0.3	56.9	6.2	yes
S30	F	17	del	Pro	6	120	15.4	yes
S57	M	24	del	Imm	6	5.9	0.9	gray zone
S37	F	9	del	Pro	4	17.1	23.7	no
S90	F	4	del	Imm	0.5	70	60	no

Abbreviations: Exogenous MT: Imm = immediate release, Pro = prolonged release; Gender: F = female, M = male; Genetics: del = deletion, mut = mutation; gray zone: change in MT levels did not meet criteria for slow CYP1A2 metabolism; MT 12–14 h: MT level at baseline and during MT use.

Sleep off-time was earlier in younger compared to older children (Figure S7). There appeared to be no clear correlation between the MT levels measured during the day and sleep offset time.

Total sleep time was estimated based on data on bedtime and sleep off time, since no data on sleep latency were available. Estimated sleep time in MT users was 8.46 h and in non-MT users 8.50 h.

TABLE 3 | Comparison of MT measurement at baseline and after start of MT treatment in 12 SMS subjects.

				Exogenous melatonin		Melatonin 12–14 h		
Code	**Gender**	**Age (Year)**	**Genetics**	**Imm/Pro**	**Dose in mg**	**Baseline**	**Mel use**	**Slow MT catabolism?**
S11	F	2.5	del	Imm	0.5	0.7	70	yes
S74	F	8	del	Imm	0.3	1.3	120	yes
S64	F	10	del	Pro	4	18	70	yes
S82	F	6	del	Imm	0.3	10.6	166	yes
S04	M	2	del	Imm	0.3	26	89.4	yes
S03	F	23	del	Imm	0.6	2.2	5.5	gray zone
S23	F	39	del	Pro	2	12.5	28.1	gray zone
S28	M	8	del	Pro	2	11.7	22.2	gray zone
S94	F	12	del	Pro	5	29.9	32.4	no
S66	M	7	del	Pro	5	17.9	18.9	no
S77	M	5	mut	Pro	2	11.3	3.3	no
S69	M	30	mut	Pro	0.2	5.9	0.4	no

Abbreviations: Exogenous MT: Imm = immediate release, Pro = prolonged release; Gender: F = female, M = male; Genetics: del = deletion, mut = mutation; gray zone: change in MT levels did not meet criteria for slow CYP1A2 metabolism; MT 12 h–14 h: MT level at baseline and during MT use.

3.6 | Comedication

At first visit 28 participants (31.5%) used psychotropic drugs: methylphenidate ($n = 7$), neuroleptics ($n = 20$, of whom 6 used 2 different neuroleptics) and one participant used two antidepressants. More participants with a *RAI1* mutation used psychotropic drugs (40%) than with a deletion (29.6%). Males were also prescribed psychotropic drugs more often than women (40.5% vs. 23.4%). Children used relatively less psychotropic drugs than adults (27.5% vs. 45%).

SMS participants that do not use psychotropic drugs have slightly higher MT levels (39.6 pg/mL) than those that use psychotropic drugs at first visit (27.3 pg/mL) (Figure S8). This difference is not statistically significant and is biased by a higher percentage MT users among psychotropic drug users.

Use of psychotropic drugs was slightly higher among MT users (32.6%) than those who did not use MT (26.5%) (Table S1). This difference was greater in participants with a mutation (22.2% vs. 66.7%) than with a deletion (35.3% vs. 24.3%).

At first visit 12 participants were using a betablocker to inhibit the endogenous production of MT. Four betablockers were used: propranolol (6), metoprolol (3), acebutolol (2), and atenolol (1). 10 also were prescribed MT. Mean MT levels in these 10 MT users were lower than in MT users that did not use a betablocker (37.98 vs. 58.43 pg/mL).

Nine participants were on anti-epileptic drugs (7 monotherapy): carbamazepine (2), lacosamide (1), lamotrigine (4), levetiracetam (1), and valproate (3). MT levels in 5, who also were exogenous MT users, were much higher than in 4 who did not use exogenous MT (70.22 vs. 4.85, $p = 0.0704$) (Figure S9).

4 | Discussion

This study presents data of daytime salivary MT and sleep from a retrospective study in the largest sample so far of individuals with SMS, a rare genetic disorder characterized, among other things, by a disturbance in the circadian sleep–wake rhythm. A unique aspect of this study is that multiple measurements were taken in a number of participants before starting and during MT use, or during MT use and after stopping treatment, as this provides a clear insight into the change in MT levels that can occur during MT treatment.

The primary aim of the study was to gain insight into the variability of endogenous MT levels and the frequency of occurrence of significantly elevated MT levels after MT prescription and a possible relationship with the dose and type of MT. We also wanted to gain insight into the variability of endogenous MT levels and the chance of developing high MT levels while using exogenous MT, as it was our experience that the chance of this increased as the endogenous MT level was lower.

In addition, a secondary aim was to investigate whether data provided by parents about their child's sleep could provide insight into the effectiveness of MT treatment, as it was our experience that the effect of MT could become worse as daytime MT levels had become very high.

4.1 | Endogenous MT in SMS

Mean endogenous MT levels in individuals with SMS were significantly lower than in participants that took exogenous MT. This difference confirms the previously found indications in smaller groups (Potocki et al. 2000; Chik et al. 2010).

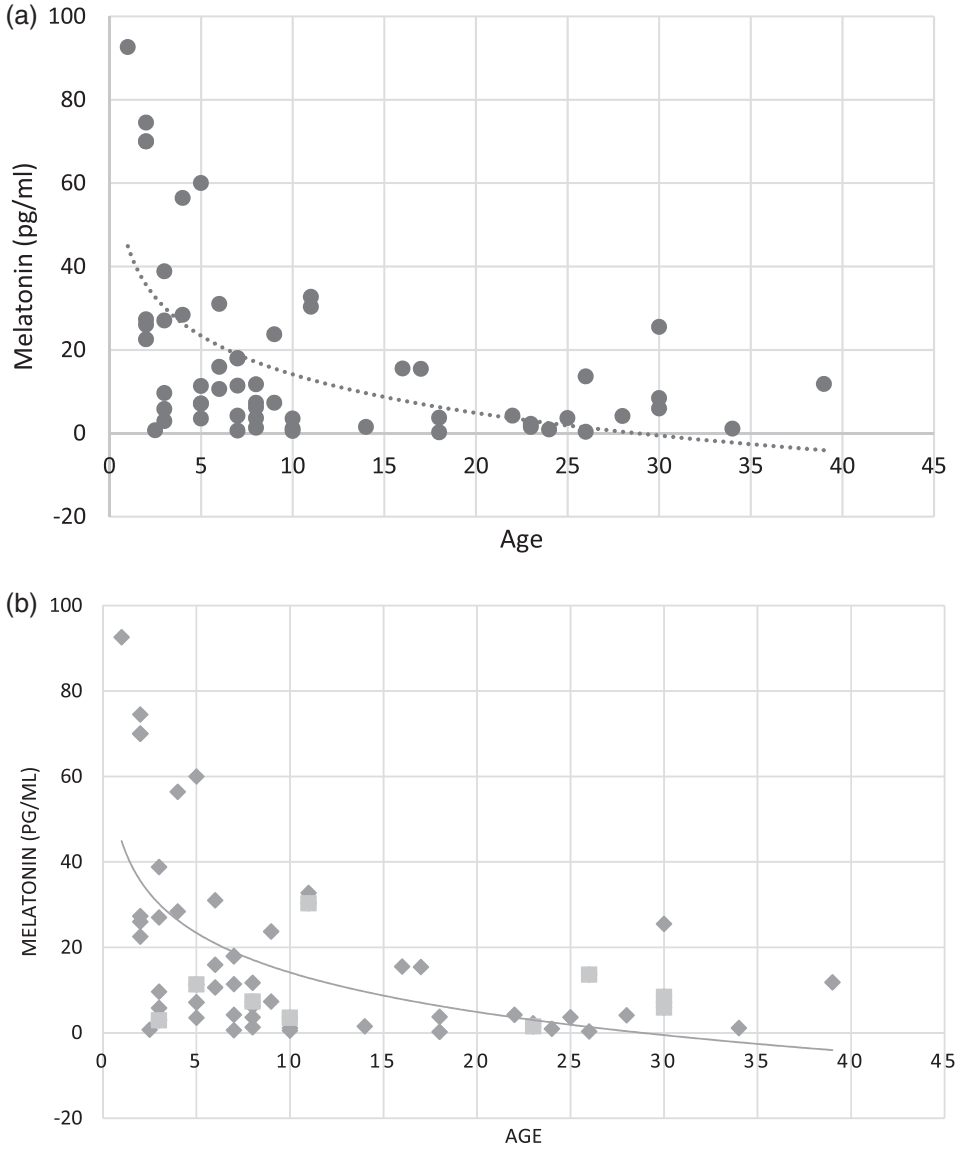

FIGURE 3 | (a) Mid-daytime endogenous MT levels in relation to age. (b) Mid-daytime endogenous MT levels in relation to age in deletion (filled ◊) and mutation type (filled □).

There was a clear relationship between age and endogenous MT levels, with high levels before the age of 5 and a subsequent decline with age, as found in the general population (Figure 2a). This seems to contradict Chik's study (Chik et al. 2010), which found no relationship between endogenous MT levels and age. However, they included some patients who used exogenous MT but had temporarily discontinued MT use for at least 36 h. This period was clearly too short to catabolize any excess of MT. If these patients were excluded, there would also have been a normal decline with age in this study.

4.2 | Endogenous MT in Deletion Type Versus Mutation Type

Endogenous MT levels in individuals with SMS caused by a mutation are lower than in individuals with SMS deletion type. However, this difference was not significant due to the small number of participants with a mutation. This finding is consistent with Chik et al. 2010, who reported lower daytime MT levels in two *RAI1* mutation cases compared to the larger deletion cohort. Endogenous MT levels in individuals with SMS caused by a mutation also do not show the normal decline pattern with age. Both findings have not been published before. The addition of MT levels on 15 mutation cases expands data threefold, adding to the four previously published *RAI1* cases (Chik et al. 2010; Boone et al. 2011).

4.3 | MT Levels During Exogenous MT Therapy

Mean MT levels in participants on exogenous MT were significantly higher than in participants that did not use MT. Since exogenous MT is identical to endogenous MT, the measured MT level in exogenous MT users consists of the sum of endogenous MT and any non-catabolized exogenous MT taken the evening before. This can complicate the interpretation of a measurement in someone already using exogenous MT.

4.4 | MT Catabolism

MT is catabolized principally by CYP1A2, and to a lesser extent CYP1A1 and CYP2C19. MT half-life in the blood is about 40 min (Cipolla-Neto and Amaral 2018). This means that if there is a period of at least 16 h between evening MT intake and midday collection of saliva, the significantly higher MT levels found in our study are likely caused by non-catabolized exogenous MT due to slow CYP1A2 metabolism. Even a washout period of 2 weeks may still be too short to determine endogenous MT levels, as was the case in 2 of the 8 participants in whom we still measured very high MT levels after 2 weeks of washout. This raises the question of whether it is possible that in case of very high serum levels MT, a fat-soluble substance, may be stored in adipose tissue. However, current scientific literature does not provide evidence supporting this phenomenon.

Higher MT levels were also found after starting exogenous MT in 12 cases (Figure 2b). Because a measurement without exogenous MT had previously been carried out in these 12 individuals, both results could be compared (Table 3). On average, MT levels during this check were significantly higher than before treatment with MT. In one SMS subject (S11) there was a more than 90-fold increase in MT levels during use of a very low dose of MT (0.3 mg). In another subject (S66) MT level did not rise after starting MT use. However, almost simultaneously methylphenidate was also initiated. Methylphenidate may inhibit MT synthesis (Cubero-Millán et al. 2014), which may compensate for any increase in MT levels due to MT therapy.

In subject S64 prolonged release MT 4 mg was replaced by fast release MT 3 mg. On a fixed day of the week no MT was given. After day 6 the MT level was 47.2 and after the stop day 25.9 pg/mL. A schedule with a fixed stop day every 1 or 2 weeks can be used when the MT level is still too high during MT use despite giving a lower dose of short-acting MT.

There are indications that MT inhibits the activity of CYP1A2 at high blood levels (Chang et al. 2010). It is very possible that, in some cases, the activity of CYP1A2 is so low that the resulting increase in MT levels becomes so great that this in itself, as in a vicious circle, inhibits the activity of CYP1A2 even further.

It is remarkable that in most individuals with SMS deletion type MT levels increased significantly after starting exogenous MT, while this was not the case with SMS mutation type. It is also notable that in individuals with SMS deletion type, the MT level during MT use showed a clearly stronger increase, as the endogenous MT level was low for age (Table 3). This also applied to a lesser extent to the decrease in MT levels after stopping MT treatment (Table 2). This could mean that as the endogenous MT level of someone with SMS is low for age, the greater the chance that this person is a poor CYP1A2 metabolizer. This also suggests that in SMS there may be a link between CYP1A2 activity and endogenous MT synthesis, and that as CYP1A2 activity is lower, endogenous MT levels are also lower. If this is correct, *CYP1A2* could be involved in the epigenetic regulation of MT synthesis in the pineal gland.

Support for this hypothesis is provided by the result of a study by Ursing et al. (2002) who studied caffeine clearance and nocturnal serum MT levels in 12 healthy subjects. Their hypothesis was that subjects with high caffeine clearance (high CYP1A2 activity) should have lower endogenous MT levels in serum than individuals with low caffeine clearance. However, the expected inverse correlation between MT levels and caffeine clearance was not found. They suggested that an increased MT secretion probably counterbalances an increased hepatic MT catabolism. In the case of SMS this would mean that a low endogenous MT level is expected to go with a low CYP1A2 activity and a high MT level with a normal/fast CYP1A2 activity. Therefore, based on our data (Figure 3a) we recommend using the following estimated cut-off values as a rough indication beyond distinguishing between poor and extensive CYP1A2 metabolizers: < 5 years 15 pg/mL, 5–15 years 10 pg/mL and > 15y 7 pg/mL. These are not absolute values, but global values below which the probability of slow MT catabolism increases and below which this probability decreases.

Extreme high MT levels found at midday in individuals who use exogenous MT have been found in previous studies in individuals with developmental disabilities (Braam et al. 2010). This usually involved individuals whose sleep problems initially responded favorably to MT, but for whom the effect had diminished over time. Often the dose was subsequently increased one or more times with temporary favorable results. This decrease in effect was supposed to be caused by high MT levels, as a result of slow MT catabolism, probably due to *CYP1A2* polymorphisms (Braam et al. 2013).

Common *CYP1A2* haplotypes include *CYP1A2*1A*, *CYP1A2*1C*, *CYP1A2*1F*, and *CYP1A2*1 K*. *CYP1A2*1A* is considered the reference haplotype. Compared with *CYP1A2*1A*, *CYP1A2*1F* is generally associated with increased activity, while *CYP1A2*1C* and *CYP1A2*1 K* are associated with decreased CYP1A2 activity in vivo (Tian et al. 2019). All 5 participants in our study with high MT levels during exogenous MT use, in whom *CYP1A2* genotyping was performed, however, turned out to have haplotype *CYP1A2*1F*. This haplotype is generally associated with increased activity, so genotyping of *CYP1A2* did not explain slow MT catabolism. Moreover, this result only caused confusion for the practitioner and parents.

Our finding of a higher incidence of poor CYP1A2 metabolism in SMS, not related to a causal *CYP1A2* polymorphism, has not been published yet. We are not aware of any study to directly explain this substantial higher incidence of poor CYP1A2 metabolism in SMS. *CYP1A2* is a clock-controlled gene. Its expression follows a circadian rhythm (more active at daytime than at nighttime). The clock gene that regulates *CYP1A2* is primarily *BMAL1* (Chen et al. 2020). *RAI1* is a transcriptional regulator that helps maintain normal circadian function by, among other things, regulating *BMAL1* expression, which can influence the expression of *CYP1A2* (Williams et al. 2012). This leads to the hypothesis that *RAI1* haploinsufficiency can directly lead to lower *CYP1A2* activity, and therefore in those cases indirectly to a lower MT production in the pineal gland. Another hypothetical explanation of the co-occurrence of low AANAT activity and low CYP1A2 activity could be explained by shared circadian clock regulation, as both genes are directly transcribed by the same CLOCK/BMAL1 and NPAS2/BMAL1 heterodimers via E-box elements in their promoters

(Takahashi 2017). Any disruption of this clock machinery simultaneously reduces pineal melatonin synthesis and hepatic CYP1A2 expression, whereby CYP1A2 can be functionally silenced.

4.5 | Assessing *CYP1A2* Activity by Genotyping or Phenotyping?

The activity of metabolizing enzymes can be assessed by genotyping and phenotyping. Genotyping is sufficient in case the variability of an enzyme's activity is mainly determined by the presence of known polymorphisms. However, genotyping will not be able to provide an accurate estimate of an enzyme's activity if other important factors are involved in the activity of an enzyme, such as enzyme induction and inhibition. This applies to a large extent to the activity of CYP1A2, which makes it difficult to determine the likelihood of slow CYP1A2 metabolism in an individual by genotyping. Therefore, phenotyping is necessary to determine the activity of the CYP1A2 enzyme (Faber et al. 2005; Fekete et al. 2022). This can be done by analyzing *CYP1A2* mRNA expression in leukocytes. However, this is not available as a routine laboratory test. For an accurate prediction of the CYP1A2-metabolizing capacity, a function test with a probe drug is therefore an alternative. Several probe drugs have been proposed to estimate the activity of the CYP12 enzyme, including caffeine and MT (Faber et al. 2005).

In healthy individuals, a MT clearance test is usually done during the day, in which the endogenous MT level is usually <2 pg/mL. Any use of MT should be discontinued for at least 3 weeks. The test requires one zero-line measurement before and several measurements after the intake of the MT probe dose. However, for individuals with SMS, such a clearance test is not possible because a zero-line measurement is only possible at night. This is not feasible in practice in the home situation. In our opinion, measuring the MT level before and during use of MT, equivalent to a MT clearance test, is a way to estimate the capacity of CYP1A2.

Therefore, we suggest the following protocol for individuals with SMS when MT is not used:

1. Saliva sampling 12:00–14:00 h.
2. At bedtime, a probe dose MT (dose depending age).
3. Next day saliva sampling at the same time as first sampling was done.

In a normal CYP1A2 metabolizer, the result in both samples will be more or less the same. In a slow CYP1A2 metabolizer, the result in the second sample will be significantly higher than in the first sample.

In case exogenous MT is used, the result of one saliva sample taken between 12 and 14 h can be suggestive for slow CYP1A2 metabolism in case the MT level is >50 pg/mL (>50 in very young children). Therefore, to estimate the capacity of CYP1A2 in MT users, exogenous MT has to be stopped for at least 3 weeks.

An alternative option, if parents do not dare to stop taking MT for 3 weeks:

1. Day 1. Saliva sampling 12:00–14:00 h.
2. Day 1 and Day 2 no MT use.
3. Day 3. Saliva sampling same time as first sampling.

Because it is possible that both results are higher than 50 pg/mL (and presented as >50), it must be agreed in advance with the laboratory that both saliva samples are diluted 1 in 4.

4.6 | MT and Sleep

Daytime napping was reported for 92% of participants. Napping in participants who do not use MT appeared to occur slightly later or not at all, compared to those who use MT. Higher MT levels were associated with more frequent and earlier occurrence of daytime napping. The very frequent occurrence of daytime napping, as well as its relation to elevated MT levels, is mentioned in several other studies (Gropman et al. 2006; Smith et al. 2019; Comajuan et al. 2025). This means that an increase in daytime napping can be seen as an indication that the MT dose is probably too high.

Higher MT levels during the day were also associated with an earlier onset of waking at night after sleep onset (WASO) and poorer sleep maintenance. This was especially true for people who use MT. The higher their MT levels during the day, the earlier they woke up at night. This difference was also found in a recent study (Comajuan et al. 2025), where in children with SMS who used MT, WASO was clearly shorter than without MT use (80 versus 60 min).

The relationship between high MT levels and poor sleep maintenance has previously been described in people with intellectual disabilities with or without coexisting autism (Braam et al. 2010, 2013). The findings in this study confirm that worsening sleep maintenance can be a clear indication that the prescribed dose of MT has become too high. High MT levels can lead to an increase in sleep problems due to MT receptors becoming less sensitive to MT (Braam et al. 2010, 2013). This means that a decrease in sleep maintenance can also be seen as an indication that the MT dose is probably too high.

4.7 | Use of Comedication

Behavioral problems in SMS (such as aggression and temper tantrums) are related to poor sleep (sleep deprivation), daytime somnolence and high daytime MT levels. The use of psychotropic drugs in SMS (28 of 89 participants, 31.5%) may be seen as a reflection of the occurrence of behavioral problems. Children with an *RAI1* mutation are supposed to have more internalizing behavioral problems than deletion type (Linders et al. 2023). We found more participants with a *RAI1* mutation used psychotropic drugs than with a deletion (Table S1).

Moreover, psychotropic drug use was less among children compared to adults (27.5% vs. 45%). The fact that adults with SMS

use psychotropic drugs relatively often may be partly due to the often older age at which they were diagnosed with SMS. The relatively large number of participants who used psychotropic drugs without also using MT is remarkable. Since behavioral problems in SMS are more often related to sleep deprivation, when treating behavioral problems, one should first consider treating the sleep problems.

There was a striking difference in MT levels in 5 participants who used anti-epileptic drugs, as well as exogenous MT, versus 4 using anti-epileptics but not MT. It is obvious to think of an interaction between anti-epileptics and MT catabolism by CYP1A2. Only carbamazepine is a strong inducer of metabolic activity of CYP1A2 (Klomp et al. 2020). But the 5 who used MT used the same anti-epileptics (carbamazepine, lamotrigine, and valproate) as the 4 who did not use MT. We cannot therefore provide an explanation for the difference in MT levels in participants using anti-epileptic drugs with or without concomitant use of exogenous MT.

When prescribing MT, one should be aware of several potential interactions with other medications (Papagiannidou et al. 2014). As MT is primarily metabolized by CYP1A2, strong CYP1A2 inhibitors (e.g., fluvoxamine) may substantially increase MT levels, whereas inducers (e.g., carbamazepine) may reduce circulating concentrations. Furthermore, it is important to realize that because of the large number of people with SMS that are poor CYP1A2 metabolizer, they should be prescribed lower doses of medications that are also primarily metabolized by CYP1A2 (Zhou et al. 2009). Clinically relevant CYP1A2 substrates encountered in SMS practice include clozapine and olanzapine (antipsychotics), duloxetine (SNRI) and tizanidine (α2-agonist).

4.8 | Prescribing MT

In many countries, MT is considered a 'dietary supplement'. Various MT-containing products are available over the counter and their MT content may differ from what is stated on the packaging. A recent study (Nogrady 2025) pointed to the high degree of variability of MT content in popular sleep supplements. Eight unregistered brands of MT gummies were tested by the Australian drug regulator TGA. They were advertised as containing 1–10 mg of MT but had anywhere from 112% to 417% of the stated dose. Three other brands were found to contain less MT than advertised, including one brand in which no MT could be found at all.

In some countries MT cannot be prescribed because it is not approved as a medicine there, as it is considered a nutritional supplement. This is especially true in the USA, where MT is not an FDA-approved drug. However, compounding pharmacies can prepare MT capsules, suspensions, or lozenges in precise doses if a doctor writes a prescription for a compounded medication (See Text S1). Prolonged release MT (Circadin and Slenyto) is also not approved by the FDA and not commercially marketed in the US However, importing it for individual use is sometimes allowed under the FDA's personal importation policy.

In all cases we recommend starting with a low dose of immediate release MT (see Table 4).

TABLE 4 | Recommended starting dose of MT by age and metabolizer status.

Age	Normal MT metabolizer	Slow MT metabolizer
< 5 years	0.5–1 mg	0.1–0.2 mg
5–15 years	2 mg	0.3 mg
> 15 years	3 mg	0.5 mg

A decrease in effect of MT is a strong indication that the dose is too high and that MT levels are too high. Monitoring MT levels is recommended. Pending the results, the dose should be reduced. In addition to lowering the dose, taking a MT-free day during the week is also an option.

If lowering the dose of MT does not improve sleep problems, omeprazole could be prescribed as an off-label medication, as it is a potent inducer of the gene expression of *CYP1A2* in vitro and in vivo (Klomp et al. 2020).

4.9 | Strengths and Limitations

This paper represents the largest study on salivary MT levels and sleep in SMS. The number of participants with a RAI1 mutation ($n = 15$) in this study forms the largest group described so far. The availability of data on MT levels before starting and during MT use, or during MT use and after stopping treatment, is unique and reinforces the conclusions of this study.

However, a limitation of this study is that the parents of most participants may have requested a measurement of MT levels because of their child's sleep problems. The fact that we do not have data on children with SMS without sleep problems constitutes a bias. Nevertheless, the results of this study point to an underestimated problem that the treatment of sleep problems with MT can entail, namely MT accumulation due to slow catabolism of MT by CYP1A2.

4.10 | Conclusions

Circadian sleep problems are one of the main issues in SMS. When prescribing MT, it is strongly recommended to measure a MT level prior to treatment in order to determine the dose to be given. Checks of the MT level during treatment are necessary due to the frequent occurrence of poor CYP1A2 metabolism. Since *CYP1A2* genotyping does not provide adequate information about the level of CYP1A2 enzyme activity, we propose a simple way to determine *CYP1A2* phenotype.

Author Contributions

Wiebe Braam: design and conceptualization, data curation, formal analysis and interpretation of the data, including tables and figures, writing original draft, critical review, revisions and finalizing the manuscript for submission. **Ann C. M. Smith:** formal analysis and interpretation of the data, including tables and figures, writing original draft,

critical review, revisions and finalizing the manuscript. Both authors have read and agreed to the published version of the manuscript.

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The authors have nothing to report.

Ethics Statement

All procedures were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Time of onset of daytime napping. **Figure S2:** Time of onset of first nap in SMS deletion type compared to mutation type. **Figure S3:** Relationship between daytime melatonin levels and first time of daytime napping. **Figure S4:** Time of first wake after sleep onset (WASO). **Figure S5:** Time of first wake after sleep onset (WASO) in relation to age. **Figure S6:** Time of first wake after sleep onset (WASO) in relation to MT level. **Figure S7:** Sleep offset time in relation to MT levels. **Figure S8:** Relationship between daytime melatonin level and use of psychotropic drugs. **Figure S9:** Relationship between daytime melatonin levels during anti-epileptic use and whether or not exogenous melatonin is used at the same time. **Text S1:** Availability of melatonin. **Table S1:** Use of melatonin combined with psychotropics, or single use.