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Iron Deficiency and Its Role in Sleep Disruption in Patients With Angelman Syndrome

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Abstract

Objective: To determine if Angelman syndrome patients with sleep complaints have an increased risk of iron deficiency, and if iron therapy improves their sleep difficulties. **Background:** About two-thirds of Angelman syndrome patients experience sleep difficulties, which are likely multifactorial. Because iron deficiency can contribute toward restlessness in sleep, we investigated whether it might be a contributing factor in this special population. **Methods:** This retrospective study involved medical record review of Angelman syndrome patients <18 years old who had attended our multidisciplinary Angelman syndrome clinic and had sleep complaints. Serum ferritin levels were compared to age- and sex-matched controls. Sleep history and nocturnal polysomnogram findings of the Angelman syndrome patients were also characterized. **Results:** Nineteen Angelman syndrome patients (9 female, mean age 6.2 ± 4.4 years) were identified. All 19 reported sleep difficulties. The mean serum ferritin level was 19.9 ± 8.5 μg/L, while that in controls was 27.8 ± 17.8 μg/L (*P* value .13). The odds ratio of iron deficiency in Angelman syndrome compared to controls was 4.17 (95% confidence interval 1.23-14.10), using normal serum ferritin level of 24 μg/L based on literature. Fifteen Angelman syndrome patients underwent nocturnal polysomnogram with 9/15 showing an elevated periodic limb movement index (overall mean 9.8 ± 10.4). Seventeen of 19 received iron therapy. Twelve had follow-up after iron therapy, with parents reporting improved sleep quality. Eight had serum ferritin levels rechecked after iron therapy, showing a mean increase of 24 ± 5.1 μg/L. **Conclusions:** Sleep difficulties in Angelman syndrome, though multifactorial, may in part be related to iron deficiency. Treatment with iron improved sleep to a modest degree in this population.

Keywords

ferritin, periodic limb movement, fragmentation of sleep, polysomnography, polysomnogram, UBE3A, HERC2

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Angelman syndrome is a neurodevelopmental disorder due to abnormal/reduced brain expression of the *UBE3A* gene (encoding ubiquitin protein ligase E3A) from the maternal allele within the 15q11-15q13 critical region, with maintained paternal methylation silencing.[1,2]

The clinical manifestations include intellectual disability, hypotonia, epilepsy, and prominent fragmentation of sleep.[2-5]

Up to 80% of Angelman syndrome patients have sleep initiation and maintenance difficulties.[3-6] Besides sleep-onset insomnia, it is not unusual for parents to report children having multiple night awakenings, with long periods of remaining awake that can be stressful to patients and caregivers alike.[3,5-7]

The pathophysiology of the sleep disruption is not well understood—it could be related to several factors, such as epilepsy, with or without nocturnal seizures, effects of antiseizure medications, obstructive sleep apnea, behavioral insomnia syndromes, environmental factors, and inadequate sleep hygiene.[8,9] Genetic factors may also play a role. For instance, absence of cortical expression of UBE3A, the ubiquitin protein ligase involved in Angelman syndrome, may disrupt sleep homeostasis.[10] This has been documented in the mouse model of Angelman syndrome, where maternal *UBE3A* deletion led to impairment of sleep homeostasis, although leaving intact circadian rhythmicity and the expression of UBE3A in the suprachiasmatic nucleus.[10]

Iron deficiency has been linked to sleep disturbances.[11,12] Our aim in this retrospective case series was to determine if the

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disrupted sleep in Angelman syndrome patients may be related to iron deficiency. We used reduced serum ferritin levels <24 μg/L as a marker for iron deficiency, and compared levels of serum ferritin in Angelman syndrome patients with those of age- and sex-matched controls. We also describe the response of Angelman syndrome patients to iron therapy from the standpoint of improving sleep quality.

Methods

The electronic records system at Mayo Clinic–Rochester was used to identify Angelman syndrome patients under the age of 18 years who had established care through our Angelman syndrome clinic between January 1, 2013, through December 31, 2017. The diagnosis of Angelman syndrome was based on clinical and molecular genetic studies; demographic, clinical, sleep medicine, and polysomnographic data were reviewed. Inclusion criteria consisted of clinical and genetic confirmation of the diagnosis of Angelman syndrome, care established during the study dates, age <18 years old when care was initially established, formal sleep medicine consultation, and a serum ferritin level. Of note, because of the ubiquitous nature of iron deficiency in children with neurodevelopmental disorders, the measurement of serum ferritin levels is a routine practice in our sleep program. We reviewed parent reports of sleep quality, serum ferritin levels, and polysomnogram measures that had been obtained both before and after iron therapy. When indicated, treatment with iron was provided either by oral or intravenous route as part of routine clinical care. Polysomnogram measures included sleep efficiency, total time spent in N1, N2, N3, and rapid eye movement (REM) sleep, apnea-hypopnea index, arousal index, percentage of arousals from movement, percentage of arousals from breathing events, periodic limb movement index, and periodic limb movement arousal index.

Serum ferritin levels obtained at the time of the initial visit and before any iron therapy in Angelman syndrome patients were compared post hoc to age- and sex-matched controls in a 3:1 ratio (ie, 3 controls for each case) using basic descriptive statistics, Wilcoxon rank sum test, and odds ratio. Controls were retrospectively selected from a Mayo Clinic database, with chart review performed to ensure they did not have Angelman syndrome, had a serum ferritin level checked within 3 months of their age- and sex-matched comparable Angelman syndrome case, and did not have a medical condition that could have affected the serum ferritin level, such as a known or potential state of iron deficiency, use of iron supplementation, potential for restricted diets, and chronic systemic or inflammatory diseases.

Results

During the 5-year study period, there were 20 Angelman syndrome patients who met the study inclusion criteria. Of the excluded patients, only 2 did not meet inclusion criteria due to lack of sleep complaints. Of the patients meeting inclusion criteria, one patient had not authorized chart access for research, and was hence excluded from the study. The remaining 19 cases (9 female) constituted the study population. The mean age was 6.2 ± 4.4 years, range 0.7-16.7 years. Table 1 shows demographic information. Seventeen of 19 (89%) had epilepsy and were receiving anti-seizure medication (Table 2).

Table 1. Summary of Angelman Syndrome Patient Demographic and Medical Information.

Demographic information	
Total patients, n	19
Female, n (%)	9 (47)
Age, y, mean ± SD (range)	6.2 ± 4.4 (0.7-16.7)
Medical information, n (%)	
Genetic confirmation (deletion, UBE3A mutation, or abnormal methylation)	19 (100)
Comorbid epilepsy and use of antiseizure medications	17 (89)
Sleep diagnosis	
Disorder of initiation and maintenance of sleep	18 (95)
Periodic limb movement disorder	7 (37)
Obstructive sleep apnea	6 (32)
Circadian rhythm sleep disorder	3 (16)
REM-sleep behavior disorder (possible)	2 (11)
Catathrenia	1 (5)
Underwent polysomnography	15 (79)
Received iron therapy	17 (89)
Follow-up information available	12/17 (71)

At the time of the initial visit to the Angelman syndrome clinic, concerns about disrupted sleep had been raised by family members in all 19 patients. This had warranted referral to pediatric sleep medicine for further assessment (SK). Difficulty with sleep initiation and maintenance was present in 18/19 (95%) patients. Nocturnal polysomnogram was performed when indicated (n = 15 patients; Table 3). It showed reduced sleep efficiency of <90% in 13/15 (87%) patients and elevated periodic limb movement indices of >5 in 9/15 (60%) with mean periodic limb movement index of 9.8 ± 10.4 per hour. Four patients underwent a follow-up polysomnogram. One (Patient 12) showed resolution of elevated periodic limb movement index (8.7/hour on initial polysomnogram, then 0/hour on follow-up) 3 months after an intravenous iron infusion.

Serum ferritin level was measured at the time of establishing care in all 19 patients. Iron deficiency (as defined by serum ferritin level <24 μg/L) was present in 15/19 (79%) patients. The mean ferritin level of Angelman syndrome patients was 19.9 ± 8.5 μg/L, which was lower than the mean ferritin level of controls, 27.8 ± 17.8 μg/L, but did not reach statistical significance, $P = .13$ (Wilcoxon rank-sum test). The odds ratio of iron deficiency in Angelman syndrome patients compared to the controls was, however, elevated at 4.17 (95% CI 1.23-14.10). The mean ferritin of Angelman syndrome patients with a deletion involving 15q13.1 was 22.4 ± 8.8 μg/L, which is not statistically different from Angelman syndrome patients without that deletion, 18.2 ± 8.3 μg/L.

Because of sleep disruption and associated iron deficiency, oral or intravenous iron was recommended to 17 Angelman syndrome patients (oral only in 9, intravenous only in 4, and oral plus intravenous in 4). The reason for use of oral versus intravenous forms was not specified in the medical record, but intravenous iron use is common at our institution. Follow-up data were available in 12 of 17 patients for whom iron therapy

Table 2. Demographic and Clinical Features of Angelman Syndrome Patients.

Patient	Age, y	Sex	Genetic findings	Sleep diagnoses reported when establishing care	Epilepsy	Medications	Initial Ferritin, μg/L	PSG
1	2.7	M	4.9-megabase deletion 15q11.2-15q13.1	DIMS, CRSD	Yes	Topiramate	39	No
2	1.1	M	6.1-megabase deletion 15q11.2-15q13.1	DIMS, PLMD	Yes	Levetiracetam	31	Yes
3	10.6	F	UBE3A mutation	CRSD, mild OSA, RBD, cataphrenia	Yes	Clobazam, zonisamide, trazodone	20	Yes
4	4.7	F	UBE3A mutation	DIMS	Yes	Levetiracetam, clonidine	19	No
5	2.5	F	5.1-megabase deletion 15q11.2-15q13.1	DIMS, OSA (s/p T&A)	Yes	Levetiracetam, melatonin	19	Yes
6	13.5	F	Mosaic abnormal methylation	DIMS, OSA (s/p T&A)	Yes	Valproic acid, ethosuximide, citalopram, melatonin, montelukast	9	Yes
7	6.7	F	UBE3A mutation	DIMS	Yes	Levetiracetam, methylphenidate, doxepin, clonidine, guanfacine	15	Yes
8	11.7	M	Deletion of 15q12	DIMS, OSA (s/p T&A), PLMD	Yes	Levetiracetam, topiramate, aripiprazole, melatonin	14	Yes
9	3.4	M	5.9 megabase deletion 15q11.2-15q13.1	DIMS, OSA, CRSD, PLMD,	Yes	Valproic acid, cyproheptadine	17	Yes
10	9.0	M	Mosaic abnormal methylation	DIMS, PLMD	Yes	Valproic acid, melatonin	37	Yes
11	3.5	F	UBE3A mutation	DIMS	No (but EEG showed IED)	None	5	Yes
12	9.2	F	Paternal uniparental disomy 15q11-13	DIMS, OSA (s/p T&A), PLMD, RBD	Yes	Topiramate, clonazepam, cannabis, guanfacine, melatonin, doxepin	19	Yes
13	16.7	F	Deletion 15q11-15q13	DIMS	Yes	Topiramate, clonazepam, melatonin	19	No
14	2.8	M	Deletion 15q11.2-15q13.1	DIMS	Yes	Levetiracetam, lacosamide, melatonin	11	Yes
15	2.9	M	Mosaic abnormal methylation	DIMS, PLMD	No	None	25	Yes
16	8.1	M	Deletion 15q11.2-15q13.1	DIMS	Yes	Levetiracetam, ethosuximide, clonazepam, clonidine, melatonin	20	Yes
17	0.7	M	Deletion 15q11.2-15q13.1	DIMS	Yes	Levetiracetam	23	Yes
18	5.4	M	UBE3A mutation	DIMS, PLMD	Yes	Levetiracetam	18	Yes
19	2.5	F	Abnormal methylation	DIMS	Yes	Levetiracetam, melatonin	19	No

Abbreviations: CRSD, circadian rhythm sleep disorder; DIMS, disorder of initiation and maintenance of sleep; IED, interictal epileptogenic discharges; OSA, obstructive sleep apnea; PLMD, periodic limb movement disorder; PSG, polysomnogram; RBD, rapid eye movement sleep behavior disorder; T&A, adenotonsillectomy.

had been recommended. Parents reported subjective improvements in sleep quality in all 12 patients with follow-up information available, which consisted of either a clinic visit or a telephone call performed a few weeks through several months later. Eight patients had a repeat ferritin level checked within months after iron therapy; all 8 had increased levels, with a mean rise in serum ferritin of 23.8 ± 5.1 μg/L (see Table 4 for details). Of the 5 without follow-up data, 4 had oral iron supplements recommended and 1 patient received an intravenous iron infusion and had oral iron recommended.

Discussion

All 19 Angelman syndrome patients in our study had disrupted sleep, likely on a multifactorial basis. However, iron deficiency, as measured by reduced serum ferritin level (<24 μg/L) was present in 15/19 (79%) patients. As compared to controls, the Angelman syndrome study population with sleep complaints was at increased risk for iron deficiency (OR 4.17, 95% CI 1.23-14.10). It is possible that the association between Angelman syndrome and iron deficiency might be

Table 3. Polysomnography Results per Patient.[a]

Patient (initial and repeat studies)	% sleep efficiency (>90%)	% N1 (10%-15%)	% N2 (50%-55%)	% N3 (20%-25%)	% REM (18%-20%)	Apnea-hypopnea index (<1/h)	Arousal index (<8/h)	% breathing arousals	% PLM arousals	PLM index (≤5/h)	PLM arousal index
2	62.3	3	49.9	29.2	17.8	6	12.8	20.9	0	21.2	0
3, first	84.6	0.1	45.9	34.6	19.4	3	2.3	43.8	0	0	0
3, second	92.5	1.9	84.0	0.1	13.9	4	8.5	29.0	0	0	0
5, first	32.8	2.7	49.9	43.6	3.8	6	3.6	18.2	0	0	0
5, second	49.8	0.7	43.0	52.2	4.1	4	3.7	0	0	0	0
6	83.2	3	50.8	19.4	26.9	1	7.3	4.1	0	0.4	0
7	98.8	0	70.4	29.3	0.3	3	17.7	4.3	12.3	6.1	2.2
8	79.9	10.5	68.1	17.2	4.2	16	16.5	21.4	42	25.7	6.9
9	82.1	2.5	63.5	33.8	0.1	4	21.9	7.9	58.3	32	12.8
10	80.3	9.3	42.6	37.2	10.9	1	10.6	0	0	16.6	0
11	69.6	5.3	40.5	37.3	16.9	0	5.4	0	24.1	7.1	1.3
12, first	82.2	6.4	72.5	21.1	0	2	7.5	0	0	8.7	0
12, second	69.5	0.6	31.4	57.6	10.4	4	16.4	14.6	0	0	0
14	41.5	8.9	83.9	7.1	0	3	13.3	2.4	0	0	0
15, first	94.5	10.7	46.3	23.6	19.4	4	19.9	10.3	43.6	17.2	8.7
15, second	83.7	10.3	39.1	32.5	18.1	8	19.4	24.6	26.3	10.9	5.1
15, third	93.5	5.8	54.1	28.7	11.4	2	11.3	3.4	31.8	9.5	3.6
16	57.1	4.5	58.1	26.3	11.2	1	7	2.9	0	0	0
17	62.7	8.7	24	44.9	22.4	5	10.1	13.2	23.7	3.5	2.4
18	74.4	5.6	31.8	41.7	20.9	2	14.4	7.8	31.1	8	4.5
Summary of the 15 initial studies: mean ± SD (range)											
15 total	72.4±18.4% (32.8%-98.8%)	5.4±3.6% (0%-10.7%)	53.2±16.2% (24.0%-83.9%)	29.8±10.8% (7.1%-44.9%)	11.6±9.5% (0%-26.9%)	3.8 ±3.8 (0-16)	11.4 ±6.0 (2.3-21.9)	10.5±11.8% (0%-43.8%)	15.6±20.0% (0%-58.3%)	9.8 ±10.4 (0-32)	2.6 ±4.0 (1.3-12.8)

Abbreviations: PLM, periodic limb movement; REM, rapid eye movement.

[a]Normal values are presented within parentheses in the column heads. Apnea-hypopnea, arousal, PLM, and PLM arousal indices are reported in events per hour.

Table 4. Results of Iron Therapy.

Patient	Route of administration	Subjective sleep quality at follow-up	Other confounding therapies	Ferritin change at follow-up	Change in PLM Index on PSG
1	None	–	–	–	–
2	Oral	Improved	Melatonin	+21	–
3	Oral and IV	Improved	CPAP (for coexisting OSA)	+21	No change (0 to 0)
4	Oral	Improved	–	–	–
5	Oral	Improved	Adenotonsillectomy (for OSA)	+24	No change (0 to 0)
6	Oral	Improved	–	+33	–
7	Oral, then IV	Improved	–	+24	–
8	IV (twice)	Improved	–	–	–
9	IV (twice)	Improved	Adenotonsillectomy (for OSA)	+30	–
10	Oral and IV	Improved	–	+21	–
11	IV	Improved	–	–	–
12	IV	Improved	–	–	From 8.7 to 0
13	None	–	–	–	
14	Oral	–	–	–	
15	Oral (stopped early because taste and GI upset)	N/A	–	–	8.7, 5.1, 3.6
16	Oral	–	–	–	–
17	Oral	Improved	–	+16	–
18	Oral and IV	–	–	–	–
19	Oral	–	–	–	–
Summary[a]	8/17 receiving iron had at least 1 IV infusion	12/12 with follow-up reported improvement		Mean change was increase of 23.75 ± 5.1 μg/L	The one with elevated PLM index showed resolution on repeat PSG

Abbreviations: CPAP, continuous positive airway pressure; GI, gastrointestinal; OSA, obstructive sleep apnea; PLM, periodic limb movement; PSG, polysomnography.

[a]In summary, 17 patients had iron therapy recommended. All (12/12) with follow up available reported subjective improvements. All (8/8) with repeat ferritin check showed improvement (mean increase of 24 μg/L). One patient with elevated PLM index had a repeat PSG, which showed resolution of the PLM.

even more robust because the control group was clinic-based rather than being a community sample.

Defining iron deficiency on the basis of reduced serum ferritin has not been clear until recently. In a study of 1257 children aged 12-36 months, the mean serum ferritin level was 23.7 μg/L.[13] Our serum ferritin threshold of <24 μg/L for defining iron deficiency is therefore consistent with the literature. In the largest study of children with restless legs syndrome and periodic limb movement disorder (n=105, mean age 10.2 years),[14] the baseline mean of serum ferritin level was 27 μg/L. It is important to distinguish that although iron deficiency is defined as ferritin level <24 μg/L, clinical improvement in restless legs syndrome or periodic limb movement disorder becomes apparent when serum ferritin rises >50 μg/L.[14,15]

The association of iron deficiency with Angelman syndrome may be related to *UBE3A* dysfunction. UBE3A has interactions with another ubiquitin ligase, *HERC2*, which is colocalized to 15q13.1 and involved in regulating iron metabolism.[16-20] Moroshi et al[18] have demonstrated that HERC2 regulates the basal turnover of FBXL5 (F-box and leucine rich protein 5). Based on their work, one can postulate that there might be a loss of HERC2 function in Angelman syndrome, leading to upregulation of FBXL5 activity, which in turn decreases bioavailable iron. This might be the basis for iron deficiency in Angelman syndrome. We did not, however, observe lower ferritin levels in the Angelman syndrome patients with deletion of 15q13.1 as compared to those without a deletion. The mechanism for iron deficiency in Angelman syndrome is therefore likely complex.

Features of restless legs syndrome (such as restlessness, limb jerks, hyperkinesia) are present at an increased rate in Angelman syndrome patients compared with control groups, either with reports of nocturnal hyperkinesia, 68% in Angelman syndrome compared with 29% for controls,[6] or on measuring periodic limb movement index on polysomnogram.[21] Increased periodic limb movement index, a general marker of iron deficiency, was seen in 9/15 (60%) patients on polysomnogram in our study. It has also been shown that a recently proposed diagnostic entity termed restless sleep disorder, which is characterized by large-amplitude movements in sleep and normal periodic limb movement index, is also associated with iron deficiency.[22] It is unclear whether any of our patients had restless sleep disorder. In our study, iron therapy was

recommended for 17/19 Angelman syndrome patients, including all 9 with increased periodic limb movement index. For the patients with increased periodic limb movement index, 7/9 received an intravenous iron infusion. Of those 7, 6 with follow up data available reported improvements in sleep quality, and the one patient with a repeat polysomnogram showed resolution of the periodic limb movement (periodic limb movement index decreased from 8.7/hour to 0/hour). For the 2 patients with elevated periodic limb movement index and oral iron therapy, 1 was lost to follow-up and 1 stopped it because of bad taste and gastrointestinal upset. Overall, it appears that iron therapy is helpful in treating elevated periodic limb movement in Angelman syndrome patients, particularly intravenous iron infusion, which was effective and well tolerated. For those Angelman syndrome patients without elevated periodic limb movement index, who also underwent a trial of iron therapy, parents reported improvements in sleep quality in 5/5 patients. This suggests that iron is helpful for improving sleep quality in Angelman syndrome patients without increased periodic limb movement.

Our polysomnography data correlate well with previously published polysomnography data in Angelman syndrome patients.[7,21,23] The Miano et al[21] study of 10 Angelman syndrome children showed reduced sleep efficiency and percentage of REM sleep, along with increased periodic limb movement index—this resembles our findings. What we are also able to add to the existing literature is the association of markers of disrupted sleep in Angelman syndrome with iron deficiency, and a commensurate improvement in sleep following supplementation with iron. The low REM sleep percentage in Angelman syndrome may be related to the characteristic EEG finding of slow wave activity.[23] The EEG in Angelman syndrome characteristically shows high-amplitude 2-3 Hz notched delta activity with spike and slow-wave discharges, including sleep-activated generalized epileptiform discharges.[24] These findings may alter the relative percentages of time spent in N3 and REM sleep. In addition to nocturnal seizures, antiseizure medication may also contribute to impairment in sleep quality.[25]

The limitations of our study include the open-label, retrospective design without placebo control, selection bias from only including subjects with sleep complaints, lack of consideration of the role of epilepsy or its treatment may have played in sleep disruption, lack of standardized questionnaire to assess sleep prior to and after intervention, and incomplete follow-up. Also, the control patients used for comparing serum ferritin level were not truly healthy controls because the serum ferritin level was checked for a clinical reason. Nevertheless, given the ubiquitous nature and complexity of the sleep disturbance in Angelman syndrome and the lack of satisfactory treatment, our finding of iron deficiency and improved sleep following iron supplementation warrants further study. We also propose a novel genetic mechanism for iron deficiency in Angelman syndrome.

Conclusion

There is increased prevalence of sleep-wake disruption in children with Angelman syndrome. In part, this is related to iron deficiency, with or without the associated polysomnographic marker of increased periodic limb movement in sleep. The treatment of Angelman syndrome–related sleep fragmentation with oral or intravenous iron may be helpful in improving subjective sleep quality.

Authors' Contributions

CR, SK, WE, JM, RG, LWK.

CR and SK conceived the research project; CR and SK organized it; and CR, SK, RG, and LWK were responsible for its execution. SK, JM, and WE designed the statistical analysis; JM, CR, and WE were responsible for its execution; and SK, RG, and LWK reviewed and critiqued it. CR wrote the first draft of the manuscript and SK, WE, JM, RG, and LWK reviewed and critiqued the final version for publication.

Declaration of Conflicting Interests

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Ethical Approval

This study was approved by the Institutional Review Board (Mayo Clinic IRB# 19-004312).

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