SMITH-MAGNIS SYNDROME AND ITS CIRCADIAN INFLUENCE ON DEVELOPMENT, BEHAVIOR, AND OBESITY

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Invitation Review

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SMITH-MAGENIS SYNDROME AND ITS CIRCADIAN INFLUENCE ON DEVELOPMENT, BEHAVIOR, AND OBESITY — OWN EXPERIENCE*

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Abstract

Smith-Magenis syndrome (SMS) is a complex genetic disorder characterized by sleep disturbance, multiple developmental anomalies, psychiatric behavior, and obesity. It is caused by a heterozygous 17p11.2 microdeletion containing the retinoic acid-induced 1 (RAI1) gene or mutation within RAI1. Sleep disorder is one of the most penetrant features of SMS. Molecular genetic studies indicate that RAI1 regulates circadian rhythm genes and when haploinsufficient, causes a distorted molecular circadian network that may be the cause of the sleep disturbance and the inverted rhythm of melatonin present in most individuals with SMS. RAI1 also regulates genes involved in development, neurobehavior, and lipid metabolism. Sleep debt, daytime melatonin secretion, and environmental stress often contribute to negative behavior in persons with SMS, and food entrained circadian rhythm also influences food intake behavior and humoral signals, which also affect development and neurobehavior. The cross-talk between circadian rhythm, development, metabolism and behaviors affect the multiple phenotypic outcomes in Smith-Magenis syndrome. These findings shed light on possible effective and personalized drug treatments for SMS patients in the future.

Key words: melatonin, RAI1, CLOCK, BDNF, intellectual disability, 17p11.2 deletion

1. PHENOTYPIC FEATURES AND MOLECULAR GENETICS OF SMS

Smith-Magenis syndrome (SMS) is a complex neurobehavioral disorder with an estimated prevalence of 1:15,000 to 1:25,000 live births [1]. Characteristic SMS features include sleep abnormalities (Figure 1A), craniofacial (Figure 1B) and skeletal anomalies, intellectual disability, self-injurious behaviors (Figure 1C), stereotypical behavior (Figure 1D), metabolic problems and obesity (Figure 1E) [2]. A more detailed clinical phenotypic spectrum of SMS catalog in sleeping disorder, developmental anomalies, neurological and behavior problems, and obesity is listed in Table I.

Molecular cytogenetic analyses of SMS patients show a common deletion in ~70% of individuals that spans ~3.8 Mb and contains 76 genes in chromosome band 17p11.2 [1, 3]. Within this region lies RAI1, the primary causative gene [4]. RAI1 spans ~130 kb and contains six exons, including 4 coding exons which encode a 1906 amino acid protein. Rai1 was first identified as a gene (designated Gt1) induced by retinoic acid in P19 mouse embryonic carcinoma cells [5]. It is localized in the nucleus and is expressed in migrating neural crest cells and the nervous system early in development, and also, at lower levels, in adult brain [6]. It functions as a transcriptional regulator with a PHD (plant homeodomain) motif [7] and acts as a “histone reader,” bridging specific histone modifications and other transcription factors [8].

About 90% of SMS patients carry a 17p11.2 deletion containing RAI1 [3], with the remaining 10% of individuals harboring mutations within the gene, including insertions or deletions within the coding region that result in frameshifts and truncated proteins, as well as missense and nonsense mutations [7, 9-11]. All reported mutations to date lie within the coding region of exon 3, which represents

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*This work was supported by Smith-Magenis Syndrome Research Foundation (SHE, SVM), National Science Foundation-China (NSFC) grant 31200937 (LC), and Shanghai Health and Family Planning Commission grant 2014Y0106 (LC).
Sleep disorder is one of the most penetrant features of SMS [18] and includes difficulties in falling asleep at night, reduced or absent rapid eye movement (REM) sleep, early waking, frequent night-time arousals, and daytime napping [18-21].

The pineal gland in human brain suprachiasmatic nucleus (SCN) controls the central circadian rhythm and melatonin secretion through light stimulation in day-night cycles. Several studies have implicated an inverted rhythm of melatonin secretion in SMS patients as the underlying cause of the sleep disturbance [19, 22]. Individuals with SMS typically have elevated melatonin secretion from the pineal gland in the daytime in contrast to very low excretion at nighttime [21-23] (Figure 2A). Studies have shown that β1-adrenergic antagonist (acetbutolol) treatment during the day may alleviate daytime melatonin peaks and improve behavior, but melatonin levels at night are not improved with acetbutolol alone [24] (Figure 2B). However, the addition of a low dose of melatonin (<3 mg) before bedtime improves the sleep time duration of SMS patients [25] (Figure 2C). Combined treatment with β-blocker (acetbutolol) to block endogenous melatonin production during the day plus exogenous melatonin administration in the evening improved the overall sleep disturbance of SMS patients and resulted in fewer daytime naps and fewer awakenings throughout the night [26-28] (Figure 2D).

The inverted secretion of melatonin, while a common finding in SMS, is not present in 100% of patients. Recent studies reported two individuals with atypical 17p11.2 deletions having normal melatonin secretion but still have sleep disturbance, included a 5 year-old female carrying a ~5 Mb deletion that extended beyond the distal SMS-REP region and a 18 year-old female carrying a ~5.8 Mb deletion that extended beyond the proximal SMS-REP region detected by high resolution CGH and FISH [14, 19]. Given that melatonin is secreted during daylight hours, its secretion is not suppressed by light in most persons with SMS. However, a pulse of bright light temporarily inhibited melatonin secretion at night for the 18 year-old female SMS patient [14], suggesting that the sleep disturbances in SMS cannot be solely attributed to the abnormal diurnal melatonin secretion versus the normal nocturnal pattern. Additionally, while Rai1+/− mice do not have melatonin, they do have circadian abnormalities [29, 30], supporting a key role for Rai1 in circadian regulation without significant melatonin impact. These facts indicate that the dysregulation of sleep is not solely due to an altered circadian secretion of melatonin. Instead, the inverted melatonin maybe a secondary effect of a dysregulated molecular circadian network, thus, influencing sleeping patterns [22].

Circadian disturbances in SMS are likely due to abnormal functioning of RAI1, as SMS patients with point mutations in this gene have been reported with both sleep disturbance and altered melatonin rhythms [9]. Recent studies have shown that RAI1 is a critical player in maintaining the molecular clock system, both in the hypothalamus, where the suprachiasmatic nucleus (SCN) lies and is responsible for controlling the central circadian rhythm, and also within the peripheral circadian oscillators, including the liver, heart and kidney. Haploinsufficiency of RAI1 in SMS fibroblasts and Rai1+/− mice hypothalamus results in the dysregulation of critical genes involved in circadian biology, such as circadian locomotor output cycles kaput (CLOCK),
Table I. Clinical features of Smith-Magenis syndrome. Features are variable across the SMS population, with consistent neurobehavioral findings in all individuals.

<table>
<thead>
<tr>
<th>Smith-Magenis Syndrome clinical findings</th>
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<tbody>
<tr>
<td>Sleep disturbance</td>
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<tr>
<td>Reduced total sleep, difficulty falling asleep, diminished rapid eye movement</td>
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<tr>
<td>sleep, fragmented and shortened sleep cycles, early-morning awakenings</td>
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<tr>
<td>Developmental anomalies</td>
</tr>
<tr>
<td>Craniofacial anomalies</td>
</tr>
<tr>
<td>Mid-face hypoplasia, brachycephaly, tented upper lip, micrognathia and</td>
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<tr>
<td>prognathism</td>
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<tr>
<td>Skeletal anomalies</td>
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<tr>
<td>Brachydactyly, short stature, scoliosis, vertebral anomalies</td>
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<tr>
<td>Otolaryngological anomalies</td>
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<tr>
<td>Hearing loss, chronic ear infections, deep hoarse voice</td>
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<tr>
<td>Ophthalmologic anomalies</td>
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<tr>
<td>Myopia, strabismus, microcornea, Wolfflin-Kruckman spots</td>
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<tr>
<td>Other anomalies</td>
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<tr>
<td>Cardiovascular defects (VSD, ASD, tetralogy of Fallot), enlarged and ectopic</td>
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<tr>
<td>kidneys, immunological abnormalities, failure to thrive</td>
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<tr>
<td>Neurological and behavioral features</td>
</tr>
<tr>
<td>Motor aspect</td>
</tr>
<tr>
<td>Infantile hypotonia, hyporeflexia, delayed fine motor skills, sensory</td>
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<tr>
<td>integration problems</td>
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<tr>
<td>Cognitive aspect</td>
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<tr>
<td>Mild to moderate intellectual disability, delayed speech, short-term memory</td>
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<tr>
<td>impairment</td>
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<tr>
<td>Gait aspect</td>
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<tr>
<td>Seizures, an abnormal gait, toe walking, balance problems</td>
</tr>
<tr>
<td>Central nervous system defects</td>
</tr>
<tr>
<td>Decreased gray matter in the insula and lenticular nucleolus, underdeveloped</td>
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<tr>
<td>cerebellar vermis, malformed brain stem, enlarged ventricles</td>
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<tr>
<td>Self-injurious behaviors</td>
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<tr>
<td>Skin picking, wrist biting, head banging, pulling out of nails, and</td>
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<tr>
<td>insertion of objects into bodily orifices, insensitivity to pain</td>
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<tr>
<td>Maladaptive behaviors</td>
</tr>
<tr>
<td>Frequent outbursts, temper tantrums, attention seeking, impulsivity,</td>
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<tr>
<td>aggression, disobedience, hyperactivity, attention deficits</td>
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<tr>
<td>Stereotypical behaviors</td>
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<tr>
<td>Spasmodic upper body squeeze, self-hugging with excitement, autistic-like</td>
</tr>
<tr>
<td>behaviors, mouthing of objects, bruxism, spinning or twirling objects</td>
</tr>
<tr>
<td>Metabolic problems</td>
</tr>
<tr>
<td>Feeding difficulties, hypercholesterolemia, early on-set obesity, impaired</td>
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<td>satiety</td>
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brain and muscle Arnt-like protein-1 (BMAL1), period circadian protein homolog genes (PER1, PER2, PER3), cryptochrome gene (CRY1, CRY2), nuclear receptor subfamily 1 group D (NR1D1, NR1D2), and RAR-related orphan receptor A genes (RORA, RORC). Functional studies have shown that RAI1 siRNA knockdown in a transgenic cell line that carries the firefly luciferase gene under the control of the BMAL1 gene promoter results in a shortened period and reduced amplitude of BMAL1 expression [32]. Furthermore, ChiP-Chip and luciferase data showed that RAI1 also binds to the first intron of CLOCK and positively regulate its transcriptional activity in vitro, suggesting that RAI1 acts as an enhancer to bind, directly or in a complex, to the CLOCK gene, and plays an important role in the circadian loop of transcription [31].
usually includes questions about sleep and napping [43].

3. SMS CIRCADIAN DEFECT INFLUENCES DEVELOPMENT, BEHAVIOR, AND METABOLISM

The outputs of endogenous circadian oscillator and melatonin secretion rhythm influence a series of physiology and development event, neurological behavior and metabolism, in response to environmental changes and physiological homeostasis [32]. Besides synthesis and release of melatonin by the pineal gland abnormally, the circadian defect in SMS might also affect the entrainment pathway (retinohypothalamic tract) and pacemaker functions (suprachiasmatic nucleus), thus further contributing the physiological processes and metabolic disturbances observed in SMS patients [28].

Many psychiatric disorders are known to involve sleep disturbance, such as autism spectrum disorder (ASD) [33], brachydactyly mental retardation syndrome (BDMR) [34], PTLS [35], Rett syndrome (RTT) [36], 2q23.1 deletion syndrome [37], fragile X syndrome [38], and Prader-Willi syndrome [39]. Several studies have shown that the severity of sleep disturbances and degree of developmental delay are proportionate to the behavior and learning problems [40–42]. Evaluation of a child with developmental delays or cognitive disability usually includes questions about sleep and napping [43]. And adverse sleep patterns often correlated to higher levels of depression and anxiety [44]. For SMS children, sleep debt, daytime melatonin secretion, and expectations from school, society and family often make them even more irritable. Stereotypical behaviors including body squeeze, self-hugging with excitement, autistic-like behaviors, and maladaptive behaviors including temper tantrums, attention seeking, aggression, and self-injurious behaviors are unique in SMS patients. Response to anxiety, in addition to insensitivity to pain, which is a consistent finding in persons with SMS, are thought to be the major contributors to the observed self-injurious behaviors [45]. Improvement of sleep quality and quantity have a direct positive effect on behavioral adherences in persons with SMS [45]. Resetting the molecular circadian network by a combined treatment with β-blocker (acetybutolol) to block endogenous melatonin production during the day plus exogenous melatonin administration in the evening, resulting suppressed melatonin level in the daytime and increased melatonin level at night.

Food entrained circadian rhythm also reinforces the humoral signals, such as hormones and blood glucose, and forms a feedback loop between circadian, development, metabolism, and behaviors [47]. Brain-derived neurotrophic factor (BDNF) is a growth
factor crucial for the growth of striatal neurons and is involved in several neuropsychiatric disorders like depression, schizophrenia, and obsessive-compulsive disorder. BDNF is also known to be involved in energy metabolism pathways and satiety signals [48] and is reported to be downregulated in the hypothalamus of Rai1+/− mice, which are hyperphagic, have an impaired satiety response, develop adult onset obesity, and consume more food during light phase. Rai1+/− mice also have altered fat distribution, with increased abdominal fat deposition and a reduced proportion of subcutaneous fat in females [49]. Luciferase reporter studies also showed that RA11 regulates BDNF expression, via intrinsic enhancer elements. In vitro, RAII isoform 1 (RAI1a, long form, localized to nucleus) increased BDNF expression ~2-fold, while RAII isoform 4 (RAI1c, not localized to nucleus) does not enhance transcription [49]. Also, a study has shown that SMS mice fed a high carbohydrate or a high fat diet gained weight at a significantly faster rate than wild type mice and exhibited an altered fat distribution pattern. This finding suggested that mice that are haploinsufficient for RAI1 are more susceptible to diet induced obesity, and that a high fat or high carbohydrate diet may exacerbate early onset obesity outcomes in SMS patients [50]. Individuals with RAII mutations are more likely to exhibit obesity and somatic overgrowth compared to those with 17p11.2 deletions [51]. These data provide evidence that RAII is likely involved in the regulation of brain development and probably contributes to behavior, growth, and developmental problems in SMS patients.

Complicated cross-talk and feedback loops exists across circadian, behavioral, developmental and metabolic processes. For example, Rai1+/− mice exhibit altered circadian rhythm, including a shorter period and disrupted circadian rhythm, and abnormal neurological responses, such as pain insensitivity, gating problems, muscle weakness, and seizures. Df11(17)/+ mice, with a SMS-equivalent deletion that includes Rai1, exhibit a shorter period and a dysregulated rhythm in the dark phase, similar to what was found in Rai1+/− mice [52]. Reduced expression of BDNF has been associated with obesity, hyperphagia, and behavioral abnormalities in mice and human [53, 54], similar to the phenotypes of Rai1+/− mice. Furthermore, Clock mutant mice also develop obesity [55], indicating there might be a complex feedback loop within RAII, CLOCK and BDNF, and that they may share common regulatory and downstream pathways. Among SMS patients, obesity is prevalent, starting in early adolescence and throughout adulthood; this may be due to a combination of the disrupted function of RAII and its impact on both CLOCK and BDNF.

A recent study also demonstrated that RAI1, as a histone reader, recognizes a set of histone modification marks and binds histones and the nucleosome core through C-terminus PHD domain in vitro [8]. Acting as a chromatin remodeling factor, RAII may mediate interactions between chromatin, chromatin modulators, and transcriptional regulators, and regulate its downstream genes epigenetically [56]. Furthermore, histone deacetylase 4 (HDAC4) and methyl-CpG binding domain protein 5 (MBD5) are reported to indirectly regulate RAI1 expression [57]. HDAC4, a histone deacetylase, acts as an eraser in histone modification, while MBD5 is a methyl-CpG binding protein and acts as a reader in DNA methylation [58]. RAI1 expression is reduced in cells from individuals with HDAC4 deletion or mutation [34]. Haploinsufficiency of MBD5 in both 2q23.1 deletion patient cell lines and SH-SY5Y cells causes a decrease in RAI1 and alters circadian gene expression, including CLOCK, PER1, PER2, PER3, NR1D2, CRY1, CRY2, RORB [37]. These data suggested RAI1 may have direct or indirect effects on these pathways or have multiple targets in these pathways, and likely further modulates the phenotypic spectrum of SMS through multiple genetic networks.

Gene expression microarray and pathway studies also showed that RAI1 acts as a transcription factor to regulate its downstream genes in several phenotypespecific biological pathways that are dysregulated in SMS. RAI1 gene dosage is crucial not only for normal regulation of circadian rhythm but also for neurotransmitter function and lipid metabolism, as well. Haploinsufficiency of RAI1 expression results in dysregulation of its downstream genes and pathways, including growth signaling and insulin sensitivity, neuronal differentiation, lipid biosynthesis and fat mobilization, circadian activity, behavior, renal, cardiovascular and skeletal development, gene expression, and cell-cycle regulation and recombination, all reflecting the spectrum of clinical features observed in SMS [59]. These dysregulated genes have been confirmed in the SMS mouse models and/or SMS patient cell lines and are potential drug targets in SMS treatments.

Since RAI1 is a dosage sensitive gene, and thought to function as a transcription factor and histone reader, these data imply that RAI1 serves as a master switch for multiple genes involved in development, neurobehavior and metabolic regulation, explaining the diverse range of symptoms seen in SMS. (Figure 3).

4. FUTURE RESEARCH AND TREATMENT OF SMS

Given the broad phenotypic spectrum of SMS, future research may identify additional genetic and environmental modifiers. Molecular cytogenetic analysis suggests that other genes in the SMS common deletion region need further investigation and may play a role in modifying circadian rhythm, cognitive development, neurobehavior, and obesity [12, 13]. As a possible contributor to neuropsychiatric disorders, RAI1 function in specific brain regions or different developing stages of the brain needs to be investigated. The predictor region of RAI1 and its regulatory sequences are not well defined; thus, elucidating its transcriptional regulators and regulatory mechanisms will help to screen drug targets for SMS. Restoring expression of both RAI1 and its downstream genes could rescue some of SMS phenotypes, such as sleep disturbance, cognitive function, behavioral problems, and obesity.
Early accurate diagnosis is essential for patients, families, and the society as a whole. Some SMS phenotypic features are subtle in infancy and early childhood and overlap with many other neurodevelopmental disorders like Down syndrome, Prader-Willi syndrome, Williams syndrome, Sotos syndrome, 9q34 deletion syndrome, 2q37 deletion syndrome, and Bardet-Biedl syndromes [60]. Some of the core and early onset SMS features such as craniofacial anomalies, sleep disturbance, and self-injury may help to diagnose SMS distinct from other disease. Early intervention to address developmental delays, such as speech, motor function, and behavior and sleep management, will improve quality of life for the patient and family. Further, evaluations to assess other medical complications often associated with SMS can be targeted and addressed early before negative consequences occur.

So far, most pharmacological interventions used to alleviate the multisystem impact of SMS are not consistently effective, or many are effective only for a limited period. Elucidating pathophysiological mechanisms of this complex genetic disorder will help to provide evidence for molecular targets for pharmaceutical intervention and personalized therapeutic approaches for SMS patients.

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Conflicts of interest/Konflikt interesu
The Authors declare no conflict of interest. Autorzy pracy nie zgłaszają konfliktu interesów.

Received/Nadesłano: 02.03.2015 r.
Accepted/Zaakceptowano: 10.03.2015 r.

Published online/Dostępne online

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