

# *RAI1* gene mutations: mechanisms of Smith–Magenis syndrome

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**Abstract:** Smith–Magenis syndrome (SMS; OMIM #182290) is a complex genetic disorder characterized by distinctive physical features, developmental delay, cognitive impairment, and a typical behavioral phenotype. SMS is caused by interstitial 17p11.2 deletions, encompassing multiple genes and including the retinoic acid-induced 1 gene (*RAI1*), or by mutations in *RAI1* itself. About 10% of all the SMS patients, in fact, carry an *RAI1* mutation responsible for the phenotype. *RAI1* (OMIM \*607642) is a dosage-sensitive gene expressed in many tissues and highly conserved among species. Over the years, several studies have demonstrated that *RAI1* (or its homologs in animal models) acts as a transcriptional factor implicated in embryonic neurodevelopment, neuronal differentiation, cell growth and cell cycle regulation, bone and skeletal development, lipid and glucose metabolisms, behavioral functions, and circadian activity. Patients with *RAI1* pathogenic variants show some phenotypic differences when compared to those carrying the typical deletion. They usually have lower incidence of hypotonia and less cognitive impairment than those with 17p11.2 deletions but more frequently show the behavioral characteristics of the syndrome and overeating issues. These differences reflect the primary pathogenetic role of *RAI1* without the pathogenetic contribution of the other genes included in the typical 17p11.2 deletion. The better comprehension of physiological roles of *RAI1*, its molecular co-workers and interactors, and its contribution in determining the typical SMS phenotype will certainly open a new path for therapeutic interventions.

**Keywords:** 17p11.2, neurogenesis, sleep disorders, syndromic obesity, craniofacial abnormalities

## Introduction

### The Smith–Magenis syndrome

Smith–Magenis syndrome (SMS; OMIM #182290) is a complex genetic disorder firstly described by Smith in 1982.<sup>1</sup> Actual prevalence of SMS is one in 15,000, while birth incidence is estimated at one in 25,000, although this value may be consistently underreported, with no gender differences observed.<sup>2–4</sup>

The syndrome is due to interstitial 17p11.2 deletions, encompassing multiple genes and including the retinoic acid-induced 1 gene (*RAI1*), or to mutations in *RAI1* itself.<sup>5,6</sup> SMS is considered a sporadic condition. The vast majority of cases are due to de novo mutations, although familial cases have been described and others are anecdotally known.<sup>7–9</sup> A recurrent deletion of approximately 3.7 Mb due to nonallelic homologous recombination using flanking low copy repeats as substrates is observed in about 70–80% of all the deleted patients.<sup>3,10,11</sup> This deletion encompasses *RAI1*, whose haploinsufficiency is considered the primary cause for most of the SMS features.<sup>6,12,13</sup>

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About 10% of all the SMS patients, in fact, carry an *RAI1* mutation responsible for the phenotype (Figure 1).<sup>9</sup> Despite great overlap, some phenotypic differences have been reported between patients with 17p11.2 deletion and those with mutations in *RAI1*. Genotype–phenotype correlation studies have proposed that the other genes included in the commonly deleted region are responsible for the variability in phenotypic expression and severity seen between those two categories of SMS patients.<sup>14–17</sup>

SMS is, in fact, a multiple congenital anomalies and intellectual disability syndrome with a clinically recognizable phenotype mainly characterized by physical and neurobehavioral features that become more evident with age.<sup>3,9,14,18–24</sup>

### Physical and craniofacial features

Infants are usually born at term after a pregnancy remarkable for decreased fetal movement. Auxology at birth is normal; however, soon after birth, a deceleration of the growth curves is often observed, and several patients have head circumference below the third percentile for age. Feeding difficulties leading to failure to thrive are common, and hypotonia, accompanied by hyporeflexia, is reported in most infants.<sup>25</sup>

The facial appearance is characteristic and typically changes with age with features that become coarser. In the infancy/childhood age, a broad square-shaped face is common, with brachycephaly and prominent forehead, deep-set eyes with synophrys, and up-slanting palpebral fissures. The nasal bridge is broad. Midfacial hypoplasia with micrognathia and a fleshy everted vermilion of the upper lip is common. Over time, micrognathia changes into relative prognathism because of an excessive growth of the mandible with persisting midfacial retrusion, and eyebrows become thicker.

Additional findings might include skeletal abnormalities (brachydactyly, short stature, mild-to-moderate scoliosis, small arched feet conferring unusual gait), teeth anomalies (in particular malocclusion, taurodontism, and dental agenesis), and ocular and otolaryngological issues (strabismus, myopia, iris anomalies, and/or microcornea which may progress with age; recurrent otitis media, laryngeal anomalies, velopharyngeal insufficiency, oral sensorimotor dysfunction). Cardiac, urogenital, and thyroid abnormalities can occur; renal anomalies and cleft lip and/or palate occur in fewer than 25% of individuals.<sup>18,25–27</sup>

### Neurobehavioral features

SMS patients might show a wide range of variability in cognitive and adaptive functioning with the majority of them having mild-to-moderate intellectual disability. The

behavioral phenotype is typical and includes sleep disturbance, stereotypies, and maladaptive and self-injurious behaviors. Phenotypic food-related behaviors are also common. The neurobehavioral phenotype becomes recognizable, usually, from the second year of life and changes throughout the individual growth.<sup>19,20,28,29</sup>

In infancy, SMS patients present lethargy, hypotonia, and daytime sleepiness. Although some authors have documented fragmented sleep with shortened sleep cycles as early as 6 months of age, the typical sleep disturbance due to the inverted circadian rhythm of melatonin is not yet recognizable at this age.<sup>29</sup> Neurodevelopmental delay, for both gross and fine motor skills and speech delay, is common together with minimal maladaptive behaviors and normally developed social skills.<sup>30,31</sup> Global developmental delay, significant expressive language deficit, and emerging maladaptive behaviors are, usually, recognized by three years of age.<sup>29,30</sup> The final cognitive impairment, generally, falls into the mild-to-moderate range with relative weaknesses in sequential processing and short-term memory, and relative strengths in long-term memory.

Slowly, the typical behavior emerges and escalates with age. Head banging may begin as early as 18 months of age. Most patients exhibit inattention with or without hyperactivity and meet the clinical criteria for the pervasive developmental disorder. Maladaptive behaviors, which often escalate with onset of puberty, embrace outbursts and temper tantrums, attention seeking, impulsivity, aggression, and the typical self-injury behaviors including self-hitting, self-biting, skin picking, the distinctive spasmodic upper-body squeeze or “self-hug” behavior, the onychotillomania (nail yanking), and the polyembolokoilomania (insertion of foreign objects into body orifices).<sup>3,19,32–35</sup> A recent study pointed out that aggressive or destructive behaviors are not only self-inflicted but often directed toward others.<sup>36</sup>

Food-related behaviors, hyperphagia with deriving obesity, usually appear in adolescence and continue through the adult age and are the consequence of an impaired satiety signaling.<sup>37</sup> These disorders are similar, although less serious, to those observed in patients with Prader–Willi, the most frequent neurodevelopmental disorder with syndromic obesity due to hyperphagia caused by a severe impairment of satiety signals.<sup>36</sup>

While age and degree of developmental delay correlate with maladaptive behaviors, the degree of sleep disturbance remains a strong predictor of maladaptive behavior.<sup>33,34</sup> Individuals with SMS have difficulties in falling asleep, frequent awakenings during the nighttime sleep, and excessive

daytime sleepiness. This issue is chronic and ends affecting the caregivers' sleep attitude as well.<sup>38</sup> The sleep disturbances are in strict correlation with the inverted circadian rhythm of endogenous melatonin pattern, although some patients are described to have the typical sleep disturbances but a normal pattern of melatonin secretion suggesting the involvement of other factor.<sup>39–42</sup>

## The 17p11.2 deletion

The vast majority of SMS patients carry interstitial 17p11.2 deletions, encompassing multiple genes and including *RAII* (Figure 1).<sup>5</sup> A recurrent deletion of approximately 3.7 Mb is observed in about 70%–80% of all the deleted patients, with the smallest deletion associated with the SMS phenotype spanning at least 650 kb.<sup>43</sup> The common deletion includes the region from *TNFRSF13B* to *ULK2*, while the minimal critical region spans from *PEMT* to *MYO15A*.<sup>3,4,10,11,43–45</sup>

*RAII* haploinsufficiency is considered responsible for most of the SMS features, although some phenotypic differences between deleted patients and those with *RAII* mutations exist and probably reflect the role of the other genes included in the region.<sup>6,12–17</sup>

A genotype–phenotype correlation study, performed comparing patients with deletions of different sizes, suggested that the region between *SREBF1* and *SHMT1* could be implicated in the short stature, while the hearing loss could be influenced by haploinsufficiency of *LLGL1*, *FLCN*, and *MYO15A*.<sup>15</sup> In particular, mutations in *MYO15A*, which are responsible for the non-syndromic deafness (DFNB3; OMIM #600316), have been described in SMS patients with sensorineural hearing loss.<sup>46,47</sup>

Several other genes within the deletion interval have been implicated in other disorders, and the hemizygoty caused by the deletion may unmask the existence of autosomal recessive alleles leading to the possibility of two distinct genetic disorders coexisting in the same individual.

Mutations in *TNFRSF13B* are responsible for the immunoglobulin A (IgA) deficiency-2 (OMIM #609529) and for the common variable immunodeficiency 2 (OMIM #240500) and could be involved in the IgA deficiency described in some SMS patients.<sup>3,5,15,18,48</sup>

*FLCN* is a tumor suppressor gene located in the commonly deleted region and associated with the Birt–Hogg–Dubé syndrome (OMIM #135150), a genodermatosis which predisposes to pulmonary cysts, spontaneous pneumothorax, and renal and skin tumors.<sup>49</sup> Intriguingly, recently a 58-year-old woman with SMS has been reported for having developed a bilateral renal tumor.<sup>50</sup>

Mutations in *ALDH3A2* gene cause the Sjogren–Larsson syndrome (OMIM #270200), an autosomal recessive neurocutaneous disease with ichthyosis, intellectual disabilities, spastic paraparesis, macular dystrophy, and leukoencephalopathy.<sup>51</sup> It has been observed that SMS patients with deletions present more frequently dry skin than those with mutations, suggesting a possible role of this gene in this feature.<sup>15</sup>

Many other genes are included in the region, but further studies are needed to determine whether these genes could contribute to specific features of the syndrome.

## RAII

*RAII* was firstly identified in 1995 as a main regulator of neuronal and glial differentiation in a mouse model of embryonal carcinoma cell line after treatment with high doses of retinoic acid.<sup>52</sup> Mutations causing haploinsufficiency of *RAII*, identified for the first time by Slager et al in 2003, are responsible for a phenotype similar, but not completely overlapping, to that described for the 17p11.2 deletion syndrome.<sup>6,12,13</sup>

*RAII* is a dosage-sensitive gene. The chromosomal segment that includes *RAII* is within the critical interval involved in both SMS and Potocki–Lupski syndrome (PTLS; OMIM #610883), with deletions and duplications, respectively. Murine models of SMS, deletions of the region containing *Rai1* or targeted gene inactivation, cause most of the clinical features observed in the SMS patients (obesity, craniofacial abnormalities, abnormal circadian rhythm, and seizures). On the other hand, murine models with duplication encompassing *Rai1* or with extra copies of *Rai1* showed diametrically opposing phenotypes in head circumference, body weight, percent of body fat, anxiety, preference for social novelty, dominant behavior, and activity levels.<sup>16,17,37,53,54</sup>

## Expression

*RAII* is widely expressed in many tissues with particularly high levels in brain, suggesting its crucial role in this tissue.<sup>55</sup> The subcellular distribution of human neuronal *RAII* indicated its presence in both cytoplasm and nucleus.<sup>56</sup> Expression studies performed in mice showed that *Rai1*, the mouse homolog of the human *RAII*, is primarily expressed in neurons, including pyramidal cells of the hippocampus, granule cells of the dentate gyrus, neurons in the neocortex, and Purkinje cells of the cerebellum.<sup>54</sup> Similarly, human *RAII* is highly expressed in hippocampal neurons, but not in glia, and in neurons of the occipital cortex. In the cerebellum, the second region of high expression, *RAII* is detected in Purkinje cells, but not granule cells. Northern blot analysis detected *RAII* mRNA in human frontal and temporal lobes.<sup>56,57</sup>

Aiming to investigate the possible role of *RAI1* as a contributor to the pathogenesis of neuropsychiatric disorders, a recent study identified two SNPs (rs9907986 and rs4925102) in the 5'-upstream region (5'-UTR) as putative regulatory elements. These two SNPs, that fall within the binding sites for the transcription factors *DEAF1* and for the retinoic acid RXR $\alpha$ -RAR $\alpha$ , account together for approximately 30–40% of the variance in *RAI1* mRNA expression in prefrontal cortex and temporal cortex.<sup>58–61</sup> Further supporting the role of *DEAF1* in regulating the *RAI1* expression is a recent study that identified, by exome sequencing, a mutation in *DEAF1* in a patient with clinical features resembling the typical SMS but negative for both the 17p11.2 deletion and *RAI1* mutations.<sup>62</sup>

## Structure

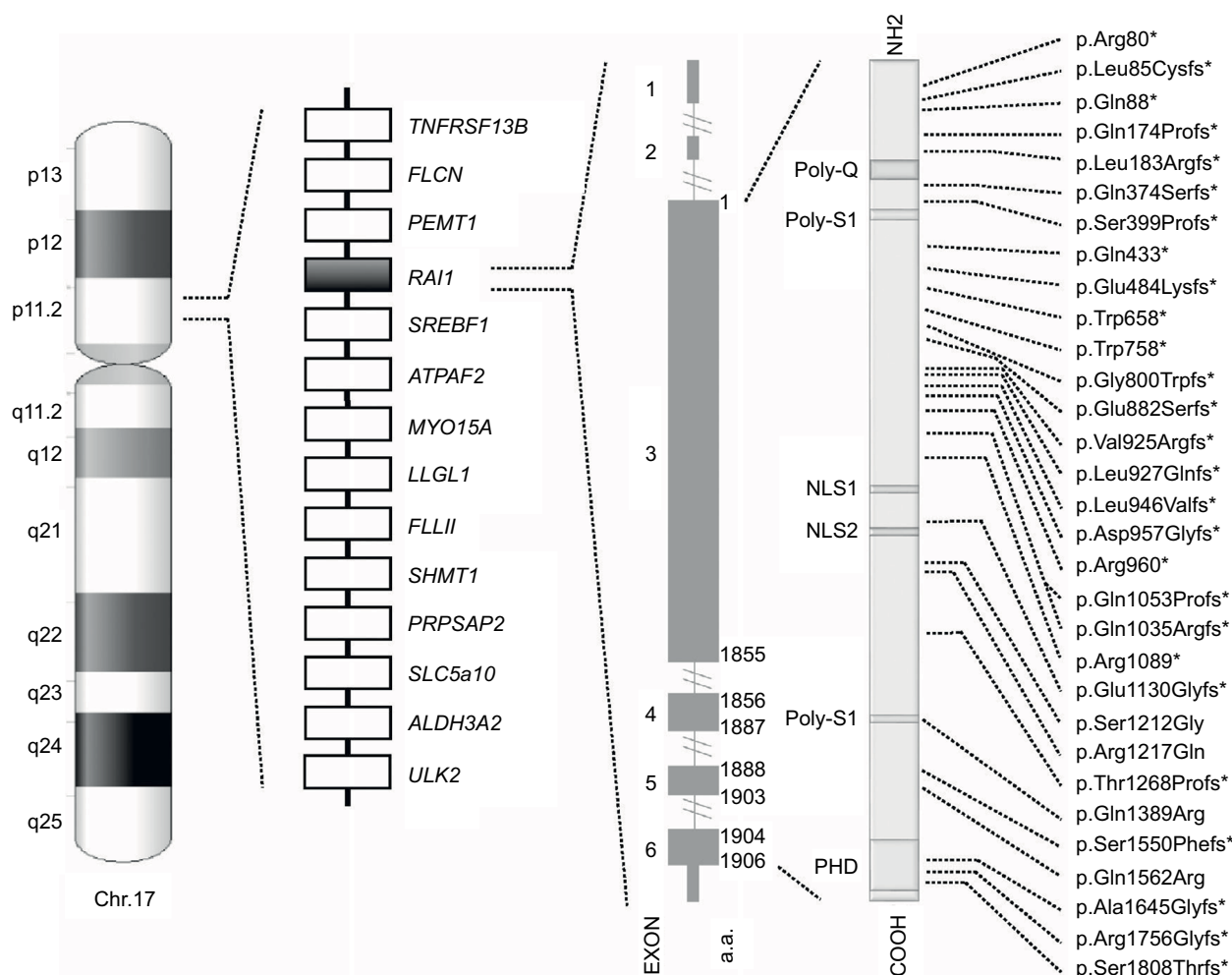
*RAI1* is highly conserved among species. Human *RAI1*, which shows high levels of homology to the mouse *Rai1*, contains

six exons and encodes for a 1906-amino-acid protein localized in the cellular nucleus (Figure 1).<sup>55,57,63</sup>

*RAI1* is a nuclear chromatin-binding protein with several functional domains (Figure. 1). Moving forward from N-terminus, the following have been identified: a polyglutamine-rich domain (Poly-Q), a first polyserine-rich domain (Poly-S1), bipartite nuclear localization signals (NLS1 and NLS2), a second polyserine-rich domain (Poly-S2), and a plant homeodomain (PHD).<sup>63,64</sup>

## Function

Despite great advancement in our knowledge about *RAI1* functions, much is yet to be clarified. In brief, *RAI1* is a transcriptional factor implicated in cell growth and cell cycle regulation, bone and skeletal development, lipid and glucose metabolisms, embryonic neurodevelopment and neuronal differentiation, behavioral functions, and circadian activity.<sup>64–66</sup>



**Figure 1** Schematic representation of chromosome 17, common 17p11.2 deletion, and *RAI1*. From left to right, the following are shown: the ideogram of G-band pattern of human chromosome 17; a schematic representation of the Smith-Magenis syndrome region with some representative genes; the *RAI1* genomic and protein structure – glutamine-rich domain (Poly-Q), bipartite nuclear localization signals (NLS1 and NLS2), two serine-rich domains (Poly-S1 and Poly-S2), and C-terminal plant homeodomain (PHD); and the *RAI1* mutations indexed in the HGMD™ Professional (version December 2015.4) known to date. \*Indicates either frameshift or nonsense mutations.

A recent study in genetically engineered mice shows that *Rai1* interacts with chromatin, occupies DNA regions near active promoters (CpG islands, 5'-UTRs, and promoters, but not intergenic or repetitive regions), and enhances the expression of genes involved in circuit assembly and neuronal communication. In this study, the authors also show that pan-neuronal loss of *Rai1* causes a severe SMS-like phenotype. They also point out that the haploinsufficiency of *RAI1* in specific cellular types causes deficits in motor function, learning, and food intake. These data underlie the relevant role of the subcortical excitatory neurons in the pathogenesis of the SMS.<sup>64</sup>

Bioinformatic analyses and gene expression assays strongly suggest that human *RAI1* directly or indirectly influences transcription. First, the presence of a nuclear localization signal is consistent with the role in the regulation of gene expression. Second, the Poly-Q tracts are common in regulatory proteins, such as transcription factors, and contribute to transcriptional activation in mammalian cells. Furthermore, the PHD motif at the C-terminal is homologous to the PHD motif of the transcriptional co-activator *TCF20* and is presumably important, in both proteins, to carry out their role as “histone readers.” *RAI1*, thus, is able to “read” the specific posttranslational modifications of histones and to recruit proteins that regulate, among other metabolic functions, the transcription of specific DNA regions.<sup>12,37,63,67–69</sup>

### RAI1 mutations

To date, more than 30 variations in *RAI1* have been associated with SMS (HGMD Professional, version December 2015.4), all clustered in exon 3, which encodes more than 95% of the protein (Figure 1). Most are frameshift, predicted to inactivate the gene product, whereas few are missense mutations occurring within highly conserved sequences. All of them, hampering with the gene expression and/or the normal protein structure/function, and thus, result in haploinsufficiency and lead to the SMS phenotype.<sup>6,13,70</sup>

### Animal models

To investigate in vivo the SMS disease mechanisms and the *RAI1* functions, several animal models, in particular mouse and *Xenopus* models, have been created and extensively used.

In 2003, Walz et al generated the first SMS mouse model bearing the 17p11.2 deletion in the mouse chromosome region syntenic to the SMS critical interval. The deleted mice showed craniofacial abnormalities, seizures, and weight abnormality as many SMS patients do.<sup>53</sup>

Following the publication of reports on SMS patients carrying mutations in *RAI1* in place of the 17p11.2 deletion,

engineered mouse models were generated to investigate *RAI1* expression patterns and to explore its functions. In 2005, Bi et al generated mice with an *Rai1* null allele (*Rai1*<sup>+/−</sup>) through the insertion of a lacZ reporter gene encoding β-galactosidase. These mice allowed to define the endogenous expression pattern of *Rai1* and to prove that some SMS phenotypes, already reported in mice carrying the 17p11.2 deletion, were also observed in a mouse model of *Rai1* haploinsufficiency.<sup>71</sup> In addition, it was observed that homozygous null mice (*Rai1*<sup>−/−</sup>) displayed embryonic lethality and almost all died in utero; the few mice that survived exhibited severe postnatal growth retardation, craniofacial and skeletal abnormalities, motor dysfunction, and fear-learning deficits.<sup>54</sup>

To determine whether specific symptoms/phenotypes observed in the affected patients were the results of *Rai1* requirement in specific cell types, in 2016 Huang et al generated *Rai1* knock-in and *Rai1* conditional knock-out mouse models.<sup>64</sup> They engineered *Rai1* alleles in order to characterize *Rai1* expression patterns (knock-in mice with tandem FLAG and myc peptides fused to the carboxyl terminus of endogenous *Rai1*). Also, they explored the neural functions of *Rai1*, and how the loss of *Rai1* affects the transcriptome, by conditionally deleting *Rai1* in the nervous system using a pan-neural Nestin<sup>Cre</sup> line and producing Nestin<sup>Cre</sup>; *Rai1*<sup>CKO</sup> mice.<sup>64</sup> Furthermore, they explored if the phenotypic consequences of *RAI1* haploinsufficiency were the result of *RAI1* loss in specific neuronal subtypes. To do that, they knocked out *Rai1* in different neuronal cell types using Gad2<sup>Cre</sup> that targets most GABAergic inhibitory neurons, Emx1<sup>Cre</sup> that targets cortical and hippocampal excitatory neurons and glia, mGfap<sup>Cre</sup> that targets astrocytes and subsets of adult neural progenitors, and Vglut2<sup>Cre</sup> that targets subcortical excitatory neurons.<sup>64</sup>

On the other hand, these studies also pointed out that *Rai1*<sup>+/−</sup> mice have a significantly reduced fecundity and an altered transmission pattern of the mutant *Rai1* allele. These observations limited the use of these models for large, extended studies. In 2014, Alaimo et al created an *Rai1*<sup>+/−</sup> mouse model by breeding C57Bl/6J *Rai1*<sup>+/−</sup> mice with FVB/NJ mice to create offspring in a mixed genetic background, which ameliorated both fecundity and *Rai1* allele transmission throughout generations and provided a more robust platform for larger phenotypic studies.<sup>72</sup>

Finally, in 2014, Tahir et al used the *Xenopus* as a tool to uncover the developmental mechanisms regulated by *Rai1*. They took advantages by the fact that, unlike the mouse, *Xenopus* embryo develops externally. Both *Xenopus tropicalis* and *Xenopus laevis* share 44% and 42% overall identity,



respectively, with the human *RAI1* protein with even higher shared identity in specific functional domains, such as the PHD domain. A targeted knock-down approach using antisense oligos stabilized with morpholino was used to generate this animal model.<sup>73</sup>

## ***RAI1* functions and mechanisms for the SMS phenotype**

### ***RAI1* and neurogenesis**

*RAI1* influences memory, behavior, and motor function through the retinoic acid metabolism. Expression studies demonstrate, overall, that human *RAI1* is a highly expressed neuronal protein whose distribution matches with its role in cognitive and motor skills.<sup>56</sup>

Recent studies in mouse models show that *Rai1* is highly expressed during the early stage of neurodevelopment. Specifically, *Rai1* is expressed in the post-mitotic neurons of the cortical plate but not in progenitor neurons actively proliferating. This suggests its role in maintaining the functions or differentiation of correct mature neurons.<sup>64</sup>

Transgenic *Rai1*<sup>+/-</sup> mice show abnormal electroencephalogram (EEG) with overt seizure and neurobehavioral abnormalities, learning impairment, and motor dysfunction. Moreover, *Rai1* null mice die during embryogenesis in almost all cases. Surviving *Rai1*<sup>-/-</sup> mice exhibit craniofacial and skeletal abnormalities, more severe neurobehavioral issues with less learning ability, overt seizures at younger age, motor dysfunction, and fear-learning deficits delineating a more severe clinical phenotype. These observations confirm a dosage-sensitive effect of *Rai1* in neurodevelopment.<sup>54</sup>

Mutations in *RAI1* are, thus, considered to have a causal, leading, role in the pathogenesis of most of the neurobehavioral features typically observed in the SMS patients, including autistic trait, intellectual disability, self-injurious behavior, EEG abnormalities, and sleep disturbances.

Moreover, further supporting its role in the neurodevelopment, *RAI1* is associated with non-syndromic autism disorder, spinocerebellar ataxia 2 (SCA2; OMIM #183090), and neuroleptic response in patients with schizophrenia.<sup>54</sup>

### ***RAI1* and sleep disorders**

Several studies, both in vitro and in vivo, demonstrated the crucial role of *RAI1* as a circadian regulator. *RAI1* positively regulates the transcription of Circadian Locomotor Output Cycles Kaput (*CLOCK*), a key component of the mammalian circadian oscillator that, in turn, transcriptionally regulates many critical circadian genes such as *PER2*, *PER3*, *CRY1*, and *BMAL1*. Haploinsufficiency of *RAI1*, thus, results in the

disruption of the circadian rhythm with consequence on the sleep–wake cycle.<sup>66</sup> Sleep disturbances, including difficulty in falling asleep, abnormality in rapid eye movement phase of sleep, and daily sleepiness represent the typical features in SMS, present in almost all cases.<sup>74</sup>

### ***RAI1* and obesity**

Obesity and overeating issues are more frequently observed in individuals with *RAI1* mutations compared to those carrying the 17p11.2 deletions.<sup>14</sup> Food intake, moreover, can alter the function of brain regions responsible for the regulation of the circadian rhythm, reinforcing humoral signals and connecting metabolism, circadian rhythm, behaviors, and development.<sup>75</sup>

Several evidences show how *RAI1* contributes to the hyperphagia and obesity seen in SMS. The brain-derived neurotrophic factor (*BDNF*) is a growth factor involved in development, differentiation, and survival of neurons and in the energy homeostasis. It is expressed both in the central nervous system and in peripheral tissues, such as liver, muscle, and adipose tissue.<sup>76</sup> Low serum levels of *BDNF* have been associated with obesity, hyperphagia, and behavioral abnormalities even in humans.<sup>77</sup> Studies in mice demonstrate that *Rai1* haploinsufficiency downregulates *Bdnf* and contributes to the obesity with hyperphagia and abnormal fat distribution. *Rai1* regulates, through its PHD domain, the transcription of *Bdnf* via intronic enhancer element. *Rai1* haploinsufficiency is also responsible for altered hypothalamic expression of other genes. It downregulates proopiomelanocortin (*Pomc*), a precursor of melanocortins involved in energy homeostasis, whose mutations have been associated with obesity in mice and human.<sup>37</sup>

### ***RAI1* and coarse face**

Craniofacial abnormalities have been observed in both 17p11.2-deleted and *RAI1*-mutated patients suggesting that *RAI1* might also play a crucial rule in the onset of facial dysmorphism in SMS. Similar midface abnormalities have also been reported in mouse models and in *rail* *Xenopus* morphants.<sup>17,73</sup>

Yan et al observed, in fact, that, in mouse models, deletion size and genetic background influenced the penetrance of the craniofacial phenotype suggesting that *Rai1* was playing a major role in determining this phenotype but also that *Rai1*-surrounding regions might contain regulatory elements which could modify the phenotype penetrance.<sup>17</sup>

More recently, Tahir et al showed, in vertebrate models *X. laevis* and *X. tropicalis*, that *rail*, the frog homologous of *RAI1*, is expressed in the developing craniofacial tissues and that its haploinsufficiency results in defects in the developing brain

and face which exhibits midface hypoplasia and malformed mouth shape. They also offer insight about the mechanism. The formation of the face depends largely on neural crest development. During the embryogenesis, the neural crest was not migrating properly. The authors, in fact, observed fewer neural crest cells, and the migration itself seemed delayed and with migratory streams less defined. This defective neural crest development affected the formation of its derivative cartilage (ethmoid plate, infraorbital, Meckel's, ceratohyal and gill cartilages) which appeared reduced in size. The whole process was considered responsible for the observed malformation.<sup>73</sup>

## RAI1 role in other disorders

*RAI1* is not only associated with SMS. Duplications of the chromosomal segment that includes *RAI1* are responsible for the PTLs. Furthermore, *RAI1* mutations have, also, been linked to other neurodegenerative and neuropsychiatric disorders. The length of the polyglutamine rich domain (Poly-Q) at the N-terminus of the protein is associated with the response to neuroleptics in schizophrenia and with the age of onset of SCA2. Additionally, *RAI1* has been identified as a candidate gene for the susceptibility to non-syndromic autism spectrum disorder.<sup>78–83</sup>

A recent study by Thaker et al identified a de novo *RAI1* mutation in a child with morbid obesity and a clinical diagnosis of the Rapid-onset Obesity with Hypothalamic dysfunction, Hypoventilation and Autonomic Dysregulation (ROHHAD) syndrome. The authors suggest to consider *RAI1* as a candidate gene in children with morbid obesity, especially if presenting a phenotype consistent with the SMS or the ROHHAD syndrome.<sup>84</sup>

Finally, whole-exome sequencing analyses performed on 123 patients affected by non-syndromic hearing loss and negative for mutations in *GJB2* revealed in several affected members of two unrelated families a homozygous missense mutation of *RAI1*. Despite the genotype–phenotype correlation studies indicating that the hearing issues in the SMS patients appear to be more frequently associated with the deletions, and thus, with the influence of genes other than *RAI1*, the authors of this study speculate about the possible role of *RAI1*, and of the retinoic acid signaling, in the mammalian organ of Corti development and, thus, in the pathogenesis of the hearing loss.<sup>14,15,85</sup>

## Discussion

Accurate phenotype description and molecular mechanism underlying a phenotype are two aspects strictly entwined in medical genetics. The better a phenotype is described, the

more successful the diagnostic path will be. Besides, a well-described phenotype can be further analyzed in its different traits and, as a consequence, each trait can be investigated under a pathogenetic point of view. On the other hand, experimental models of diseases allow to investigate, and understand, the biological pathways underlying the onset of a specific phenotype, or a trait of a phenotype, giving eventually insight for specific medical interventions.

To date, there is no specific treatment for the SMS, and all the available therapeutic approaches are symptomatic treatments.<sup>40,92–94</sup> As for most genetic conditions, the only way to find a cure is to better understand the biological role of the gene causing disease. This is true for the monogenic conditions but also for the contiguous gene deletion/duplication syndromes in which the definite contribution of one (or more) gene has been established. That is why, in our opinion, studies aiming to further elucidate the biological role of *RAI1* are essential to identify putative biological targets for better medical strategies. Interest toward the clinical research and the translational medicine in order to improve the living conditions of the patients is noteworthy, and necessary. Nevertheless, we should give proper credit to the amount of knowledge acquired after years of basic-research studies in cellular and animal models. The studies, reviewed herein, have contributed in elucidating structure, expression pattern, and biological function of *RAI1* among different tissues. All these studies offer hypothesis on how *RAI1* can be responsible, when disrupted, for the onset of the SMS, a complex syndrome with different clinical manifestations.

Moreover, other recent publications identified a cohort of genes which might interact, directly or indirectly, with *RAI1* and its network.<sup>62,95</sup> These works provide a good example of how entwined “good characterization of a phenotype”, on one side, and “molecular basis of a disease”, on the other, are. In fact, in those two studies starting from a phenotype strongly resembling the SMS (which was excluded by the molecular testing) and applying new testing technologies (the whole-exome sequencing), the authors identified a cohort of putative candidate genes causing disease and proposed a common disease network. All these studies and hypotheses are essential to further elucidate the mechanism of the SMS pathogenesis and to develop new targeted therapies.

## Conclusion

About 10% of all the SMS patients carry an *RAI1* mutation which is responsible for the phenotype.<sup>9</sup> Despite great overlap, these patients show several phenotypic differences when compared to those carrying the typical deletion. They

usually have less cognitive impairment and lower incidence of hypotonia, short stature, hearing loss, and cardiac and renal defects than those with 17p11.2 deletions suggesting a minor role for *RAI1* in these clinical features and, most likely, reflecting the haploinsufficiency of other genes as for typical contiguous gene deletion syndromes.<sup>14,15</sup> On the other hand, they are more likely to exhibit overgrowth phenotypes, and the behavioral characteristics of the syndrome – including polyembolokoilamania, skin picking, and self-hugging – and overeating issues with obesity tendency.<sup>4</sup>

*RAI1* is a dosage-sensitive gene, highly expressed in neurons during the early stage of neurodevelopment, and its protein distribution matches with its role in cognitive and motor skills neurodevelopment.<sup>54,56</sup> *RAI1* carries out its role as a circadian regulator influencing the transcription of *CLOCK*. Therefore, *RAI1* haploinsufficiency results in the disruption of the circadian rhythm with a consequence on the sleep–wake cycle.<sup>66</sup> Likewise, *RAI1* haploinsufficiency downregulates *Bdnf* and *Pomc* contributing to obesity with hyperphagia and abnormal fat distribution.<sup>37</sup> *Rai1* also affects the neural crest migration and the development of the derivative cartilage elements. This partially explains its role in the pathogenesis of the craniofacial abnormalities observed in the SMS patients.<sup>73</sup> As observed by Tahir et al, in fact, craniofacial abnormalities have already been linked to anomalies in the neural crest development also in different conditions, such as the Williams–Beuren syndrome (OMIM #194050), the DiGeorge syndrome (OMIM #188400), the fragile-X syndrome (OMIM #300624), the Prader–Willi syndrome (OMIM #176270), and the Down syndrome (OMIM #190685), sharing common features with the SMS.<sup>86–91</sup>

Finally, it is worth mentioning that, besides the SMS, *RAI1* is associated with non-syndromic autism disorder, SCA2, and neuroleptic response in patients with schizophrenia.<sup>54</sup>

## Disclosure

The authors report no conflicts of interest in this work.

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