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Smith–Magenis syndrome: haploinsufficiency of *RAI1* results in altered gene regulation in neurological and metabolic pathways

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Smith–Magenis syndrome (SMS) is a complex neurobehavioural disorder characterised by intellectual disability, self-injurious behaviours, sleep disturbance, obesity, and craniofacial and skeletal anomalies. Diagnostic strategies are focused towards identification of a 17p11.2 microdeletion encompassing the gene *RAI1* (retinoic acid induced 1) or a mutation of *RAI1*. Molecular evidence shows that most SMS features are due to *RAI1* haploinsufficiency, whereas variability and severity are modified by other genes in the 17p11.2 region for 17p11.2 deletion cases. The functional role of RAI1 is not completely understood, but it is probably a transcription factor acting in several different biological pathways that are dysregulated in SMS. Functional studies based on the hypothesis that RAI1 acts through phenotype-specific pathways involving several downstream genes have shown that *RAI1* gene dosage is crucial for normal regulation of circadian rhythm, lipid metabolism and neurotransmitter function. Here, we review the clinical and molecular features of SMS and explore more recent studies supporting possible therapeutic strategies for behavioural management.

Duplications and deletions of the genome have long been thought to contribute to evolution of all species. Although there are rare cases for these copy number changes to confer an advantage to the said species, these types of changes are typically detrimental. Deletion and duplication syndromes are a major contributor to the overall number of recognised intellectual disability syndromes, which are being identified and characterised at a rapid pace with the

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advent of new technologies such as whole-genome array comparative genomic hybridisation (aCGH) (Ref. 1) and whole-genome sequencing technology, which has the ability to identify genomic variation between populations as well as disease regions (Ref. 2). Smith–Magenis syndrome [SMS; Online Mendelian Inheritance in Man (OMIM) accession 182290], Potocki–Lupski syndrome (PTLS; OMIM 610883), 1p36 deletion syndrome (OMIM 607827), DiGeorge syndrome (OMIM 188400), Wolf–Hirschhorn syndrome (OMIM 194190), Angelman syndrome (OMIM 105830) and Williams syndrome (OMIM 194050) all result from a duplication or deletion of specific regions of the genome and result in a constellation of features that include neurobehavioural problems, developmental delays and mild craniofacial dysmorphism.

SMS is a syndrome with multiple congenital abnormalities and intellectual disability, and can present with sleep disturbance, self-abusive and aggressive behaviour, craniofacial abnormalities, neurological abnormalities and obesity. SMS was initially thought to be a contiguous gene syndrome resulting from a deletion of the 17p11.2 region encompassing ~80 genes (Ref. 3); however, later molecular evidence showed that mutation or deletion of one gene – the *RAI1* (retinoic acid induced 1) gene – resulted in most of the phenotypes observed (Refs 4, 5). In summary, SMS is a sporadic, dominant syndrome defined by haploinsufficiency (where one copy of a gene is not sufficient for proper function) of *RAI1*, which has been identified by bioinformatic analysis and functional studies to encode a transcriptional regulator (Refs 5, 6, 7).

Typically, deletion syndromes are more common and phenotypically more severe than duplication syndromes. This is seen when comparing SMS (17p11.2 deletion) with PTLS, a disorder that arises from 17p11.2 duplication. In SMS, intellectual disability and craniofacial, sleep and metabolic abnormalities are typically much more challenging, and the number of subjects reported is >300; by contrast, ~75 cases have been reported with PTLS, a reportedly less severe disorder (Ref. 8). However, although there is some evidence showing that deletions are twice as likely to occur as duplications in the SMS region on 17p11.2 (Ref. 9), it is likely that the incidence of deletion and duplication events in the population is similar. The molecular events resulting in these two outcomes – duplication versus deletion – are similar and due to nonallelic homologous recombination (NAHR), mediated by low-copy repeats (LCRs) or segmental duplications in the 17p11.2 region (Ref. 10). Furthermore, in general, analysis of syndromes is more likely to find a deletion as the cause of the phenotype observed (Refs 8, 9); this is because haploinsufficiency of one or more core genes is the probable pathology behind the deletion syndromes, whereas duplication of genomic regions might or might not result in overexpression of the genes involved (depending on the regulatory regions affected) and the resulting phenotype can be more subtle, and thus more likely to be missed clinically and not evaluated at the molecular level (Ref. 11).

Clinical findings for SMS

SMS is a clinically recognisable syndrome characterised by a distinct set of physical, developmental, neurological and behavioural features. High-resolution karyotype analysis by G-banding and fluorescent in situ hybridisation are classical methods used to detect SMS deletions, whereas multiplex ligation-dependent probe amplification, aCGH and real-time quantitative PCR are the newer technologies used for diagnosis that might identify smaller deletions (Ref. 12). It is typically a de novo disorder, with an estimated prevalence of 1:15 000–25 000 live births (Ref. 12). However, there is one report of an SMS subject whose mother is mosaic for a del(17)(p11.2) (Ref. 13) and other clinically reported cases not published in the literature (S. Elsea, unpublished). Based on these cases, parental mosaicism for either a 17p11.2 deletion or an *RAI1* mutation is estimated at ~3–5%. SMS has many clinical features that overlap with other intellectual disability syndromes, such as Prader–Willi syndrome, Kleefstra syndrome, brachydactyly mental retardation syndrome and Williams syndrome. Interestingly, only ~20% of individuals referred for 17p11.2 deletion or *RAI1* molecular evaluation are positive for molecular changes leading to SMS (52/260, S. Elsea, unpublished; Ref. 14), suggesting that additional loci might cause similar phenotypes when disrupted.

Skeletal, developmental, growth and other features

Craniofacial anomalies in SMS include brachycephaly, frontal bossing, hypertelorism,

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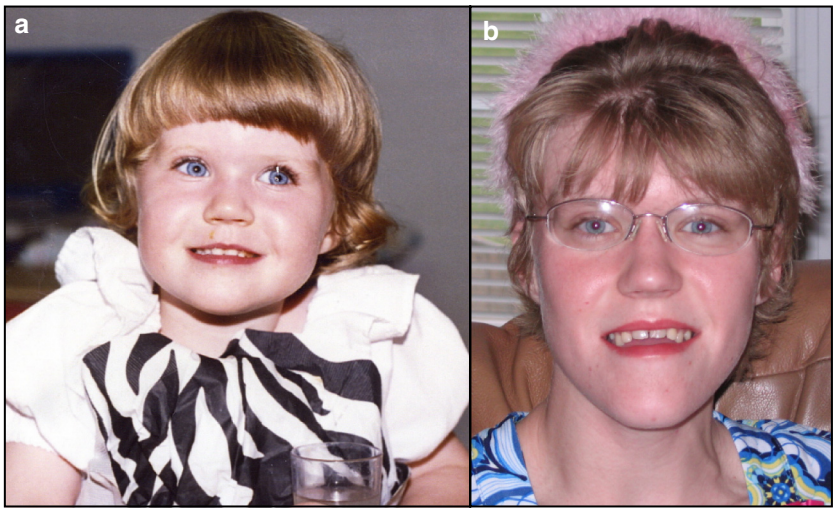

Craniofacial features in Smith–Magenis syndrome

Figure 1. Craniofacial features in Smith–Magenis syndrome. (a) Female child with common Smith–Magenis syndrome deletion, at age 2 years. Note broad, flat face and flat nasal bridge. (b) Same individual as in part a, at age 20 years. Note prognathism, midface hypoplasia and dental anomalies. Parental permission was obtained to use and publish these photos.

synophrys, upslanting palpebral fissures, midface hypoplasia, a broad square-shaped face, a flat nasal bridge and a tented upper lip (Ref. 15) (Fig. 1). Dental anomalies include tooth agenesis, especially of premolars, and taurodontism (Ref. 16). A cleft lip or palate is also reported at a higher rate than is seen in the normal population (Ref. 17) (Table 1).

Other skeletal and developmental abnormalities include short stature (<5th percentile in younger children), brachydactyly, scoliosis, fifth-finger clinodactyly, and forearm and elbow limitations. Adult height is typically less than expected based on parental heights, but will generally fall within the normal range, with an average between the 15th and 25th percentile. Early-onset obesity is observed, with >50% of individuals at or above the 85th percentile by age 9 years (Ref. 19) and truncal obesity most commonly noted. A wide range of cardiac defects are also present in ~30% of reported cases (Refs 17, 20).

Otolaryngological abnormalities such as hearing loss (Ref. 17), a hoarse deep voice, and vocal cord nodules and polyps are also common (Refs 21, 22, 23) (Table 1). Additionally, ophthalmological features are present in 64% of SMS patients, including myopia, iris anomalies [such as heterochromic irides or Wolfflin spots (iris hamartomas)], strabismus, microcornea and, rarely, retinal detachment (which can result from violent behaviours) (Refs 17, 24, 25).

Neurological findings and behaviour

The neurological and behavioural phenotypes seen in SMS consist of stereotypies/tics such as self-hugging and hand twirling, self-abusive behaviours including nail yanking and skin-picking, hyperactivity, and oral or motor dysfunction (Refs 4, 12, 17, 20, 22, 26, 27, 28, 29). Additionally, speech and motor delays are observed, with hypotonia typically present at birth in >90% of cases (Ref. 12). Lesser observed but significant phenotypic manifestations include seizures and polyembolokoilamania (Table 1).

An inverted circadian rhythm, which results in nighttime awakenings and napping periods during the day and even during times of activity, complicates the neurological and behavioural phenotypes observed (Refs 30, 31). Sleep disturbance is reported in up to 100% of SMS cases and is a key diagnostic feature (Refs 4, 17,

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Table 1. Phenotypic features of subjects with deletion 17p11.2 and *RAI1* mutation

Phenotypic feature	Deletion 17p11.2 (%)[a]	*RAI1* mutation (%)[b]
Neurological/behavioural		
Intellectual disability	100	100
Speech delay	90	75
Motor delay	90	66
Hypotonia	90	63
Seizure	11–30	17
Sleep disturbance	70–100	100
Self-hugging or hand wringing	70–100	86
Attention seeking	80–100	100
Self-injurious behaviours	78–96	100
Onychotillomania	25–85	80
Polyembolokoilamania	25–85	90
Head banging or face slapping	71	60
Craniofacial/skeletal		
Brachycephaly	89	85
Midface hypoplasia	93	77
Prognathism	53	73
Tented upper lip	73	92
Broad square face	81	92
Synophrys	62	33
Cleft lip/palate	9	0
Brachydactyly (short fingers, toes, generalised)	85	83
Short stature	69	23
Scoliosis	49–67	46
Otolaryngological		
Chronic ear infections	85	55
Hearing loss	68	25
Hoarse, deep voice	80	100
Ocular		
Myopia	53	60
Strabismus	50	50

[a]Data compiled and modified from Ref. 4.
[b]Data compiled from Refs 4 and 18.

22, 26). Interestingly, infants usually experience hypersomnolence during the first year of life, with circadian changes beginning typically between 12 and 18 months of age (Ref. 28). The complicated sleep phenotype occurs in older children and includes reduced 24 h sleep time, reduced REM (rapid eye movement) sleep, and fragmented night sleep with daytime sleepiness (Refs 26, 30, 32). Until recently, these clinical phenotypes have been attributed to an inverted rhythm of melatonin secretion, but several instances of individuals with normal melatonin secretion and significant sleep disturbance have been reported (Refs 30, 33). These findings indicate a molecular role for RAI1 in proper sleep cycling, which should be further investigated.

Mechanisms of deletion of the 17p11.2 region

Chromosome 17p11.2 deletion or duplication results from NAHR between LCRs situated along the chromosome. Within the SMS region on chromosome 17p11.2 there are four distinct LCRs, termed LCR17pA, LCR17pB, LCR17pC and LCR17pD (Ref. 34). Additionally, three large highly homologous LCRs (SMS-REPs) are present in the region, and based on their chromosomal position are designated as

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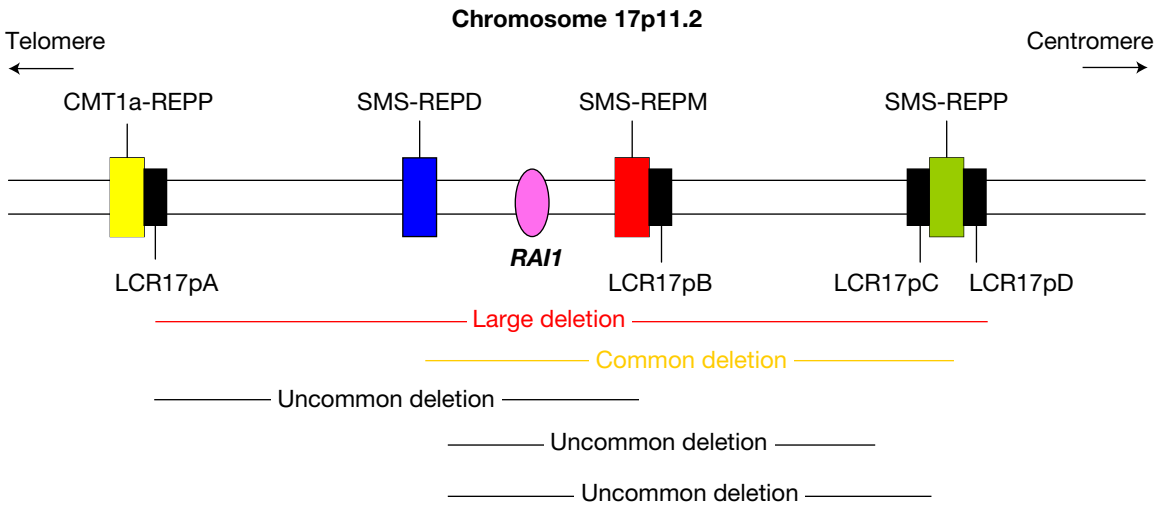

Genomic structure of chromosome 17p11.2 and mechanisms of deletion in this region

Figure 2. Genomic structure of chromosome 17p11.2 and mechanisms of deletion in this region. The *RAI1* (retinoic acid induced 1) gene is represented by the purple oval. SMS-REPs (highly homologous repetitive regions) are shown in blue (SMS-REPD), red (SMS-REPM) and green (SMS-REPP) (Ref. 36). Smaller and lesser utilised low-copy repeats are shown in black. The Charcot–Marie–Tooth disease type 1A (CMT1a) proximal REP is shown in yellow. Below the 17p11.2 schematic are examples of large (Ref. 4), common (Ref. 4) and uncommon deletions (Ref. 36). Additional examples of various deletions can be found in Refs 4 and 37. All deletions include the *RAI1* gene. Additional homologous repeat-rich regions are present but not shown, and details can be found in Refs 36 and 37. The figure is not drawn to scale. Abbreviation: SMS, Smith–Magenis syndrome.

SMS-REPP (proximal), SMS-REPM (middle) and SMS-REPD (distal), each represented by a YAC clone (Ref. 35) (Fig. 2). These repeats are paralogous repeat gene clusters of ~200 kb. The distance between proximal and distal REPs was estimated to be ~4–5 Mb and is now calculated as 3.7 Mb (Refs 35, 37, 38). A common junction fragment was also identified in ~90% of SMS patient samples (Ref. 35).

Based on these data, it was predicted that SMS-REPP and SMS-REPD would mediate most of the recurrent rearrangements (duplications/deletions) associated with this region (Ref. 39) (Fig. 2). Genetic evidence of NAHR between the distal and proximal SMS-REPs was obtained by genotyping ~24 patients carrying 17p11.2 rearrangements (Refs 37, 40, 41). Data show that >75% of SMS deletions are due to recombination involving proximal and distal repeats, causing an ~3.7 Mb common deletion. Large deletions can be seen when recombination between LCR17pA and LCR17pD occurs, giving rise to an ~5 Mb deletion in ~4% of SMS cases (Ref. 37) (Fig. 2). Additionally, uncommon deletions occur in ~16% of cases when alternate LCRs are paired in recombination (Ref. 37). Rearrangements mediated by nonhomologous mechanisms involving repetitive elements such as Alu–Alu recombination have also been reported (Ref. 38).

Mutation of *RAI1*

De novo mutations in the *RAI1* gene that result in SMS are observed in ~10% of cases (Refs 4, 5, 12, 17, 18, 42, 43, 44) (Fig. 3). *RAI1* contains six exons, four of which are protein coding (Fig. 3). Mutations include small deletions and insertions within the *RAI1* coding region, which cause frameshifts and premature stop codons; also observed are nonsense mutations, which again produce a

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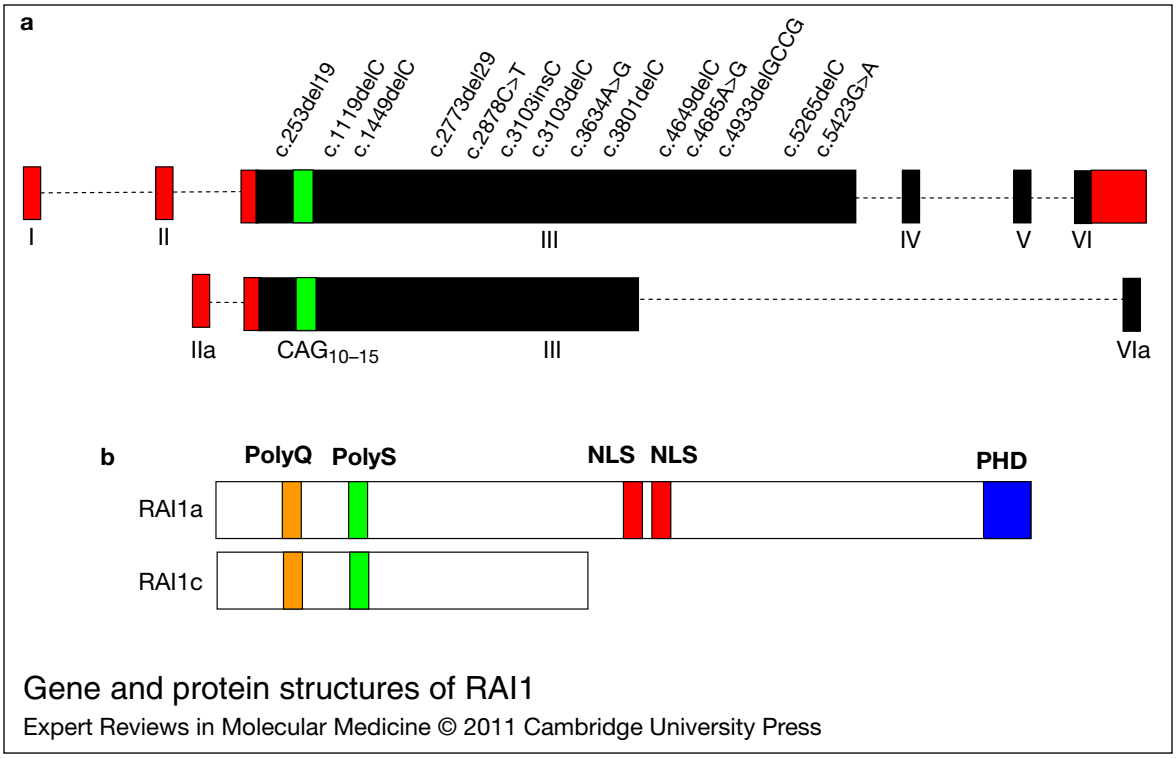

Figure 3. Gene and protein structures of RAI1. (a) *RAI1* (retinoic acid induced 1) gene structure, including noncoding exons (red) and coding regions (black). Represented are those cDNAs cloned from biological samples and subsequently evaluated, encoding isoform a (top: NP109590, EAW55692.1) and isoform c (bottom: AAH21209.1, BC021209). A CAG repeat region is shown in green. Other isoforms are predicted to exist but have not been confirmed. Shown above the gene structure for isoform a are all reported mutations in the *RAI1* gene. (b) RAI1 protein isoforms a and c, including polyglutamine tract (polyQ, orange), polyserine tract (polyS, green), bipartite nuclear localisation signals (NLS, red) and plant homeodomain (PHD) (blue).

truncated protein product, and missense mutations, which lead to nonfunctional or reduced-function proteins. To date, all mutations observed have been in coding exon 3 of the *RAI1* gene, and recently a 'hotspot' was identified in this region for frameshift of *RAI1* (c.3103delC and c.3103insC) (Ref. 18). Thus far, the mutations reported include ten frameshift, one nonsense and three missense changes in the *RAI1* gene (Refs 4, 5, 42, 43, 44) (Fig. 3). These molecular data combined with clinical information support haploinsufficiency of *RAI1* as the primary gene defect responsible for most of the phenotypic load in SMS, accounting for mild intellectual disability, attention-seeking behaviour, self-injurious behaviour, circadian defects, craniofacial features and other minor anomalies (Table 1). To date, major organ anomalies have not been associated with mutation of *RAI1*, and so other genes are probably contributing to these developmental defects.

Potocki–Lupski syndrome

As described above, *RAI1* is a dosage-sensitive gene, and duplication of the 17p11.2 region results in 'duplication 17p11.2 syndrome', also known as PTLS. PTLS results in mild to severe intellectual disability, infantile hypotonia, failure to thrive, cardiovascular anomalies, sleep-disordered breathing, developmental delay, hyperactivity and autism (Refs 45, 46, 47). Craniofacial features include a triangular face, microcephaly, micrognathia, a broad nasal bridge, a highly arched palate, hypertelorism, and dental anomalies in the form of malocclusion of the teeth (Ref. 39). Limb abnormalities, including flexion deformity of the fingers and club foot, have also been described,

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as well as pharyngeal dysphagia and electroencephalogram abnormalities (Refs 45, 46). Less commonly reported features are hearing impairment, structural otolaryngological defects and ophthalmic abnormalities, such as myopia and iris hamartoma. Other rarely reported features are genitourinary or renal anomalies, scoliosis and hypercholesterolaemia (Ref. 46).

As discussed in the introduction, although the predicted incidence of 17p11.2 duplication syndrome is the same as that of 17p11.2 deletion, it is probably underdiagnosed because the phenotype can be much more subtle than that of SMS (Ref. 8). Reported duplications of this 17p11.2 region range between 0.4 and 13.3 Mb; 3.7 Mb is the most common duplication (between distal and proximal repeats flanking *RAI1*) and 5 Mb is the least common recurrent duplication (located between LCR17pA and LCR17pD), having been recently identified in only two individuals (Ref. 8).

The clinical and molecular findings for deletion and duplication syndromes such as SMS and PTLS that involve the same genomic region, with evidence that the same gene is contributing to the phenotypes in both disorders, illustrate the dosage sensitivity of the key gene in that genomic region (Refs 5, 8). SMS and PTLS data, in addition to the mouse models described below, indicate the need for tight regulation of expression of *RAI1*.

Mouse models for SMS and PTLS

Mouse models that mimic human conditions are invaluable tools to help understand the molecular, developmental and behavioural role of a gene or genes. The sequencing of the mouse genome has allowed for regions of synteny to the human genomic sequence to be mapped and utilised for research (Ref. 48).

Murine chromosome 11qB1.3–B2 is syntenic to human chromosome 17p11.2. An ~34 cM region surrounding human *RAI1* and murine *Rai1* is conserved between human and mouse genomic structure, making this region fit for creation of knockout and transgenic mice modelling human conditions arising from changes in gene dosage. Mice lacking [*Df(11)17*/+] or duplicated [*Dp(11)17*/+] for a 2 Mb region containing seven genes syntenic to human chromosome 17p11.2, including *Rai1*, were originally created by Walz et al. (Ref. 49), using chromosome engineering technology, to mimic the common deletion or duplication seen in SMS or PTLS, respectively. These mice recapitulate many of the core features observed in PTLS or SMS, including lean body weight, hyperactivity and altered sociability in *Dp(11)17*/+ mice, and obesity, abnormal behaviour and craniofacial features in *Df(11)17*/+ mice. Such features are also seen in later gene-targeted models of altered *Rai1* expression (Refs 6, 19, 50, 51, 52) (Table 2).

Recently, Girirajan and colleagues (Refs 50, 55) generated and performed an in-depth behavioural analysis of bacterial artificial chromosome (BAC) transgenic *Rai1*-overexpression mice (*Rai1-Tg*) harbouring two additional copies of the *Rai1* gene. These mice showed a variety of defects, including neurological deficits and growth retardation, which resolved by 20 weeks of age (Ref. 55). Abnormal maternal behaviour was observed wherein *Rai1-Tg* mothers could not properly take care of their pups (1/5 survival average for wild-type or transgenic pups); however, when pups were placed with a wild-type foster mother, all (5/5, average) survived (Ref. 50). Additionally, *Rai1*-overexpressing mice show a reduction in the levels of the serotonin metabolite 5-HIAA, suggesting a defect in the regulation or expression of monoamine oxidase, which converts serotonin to 5-HIAA. Further, these mice have altered social behaviour, with impaired nesting behaviour, social dominance, aggression, reduced social memory, hyperactivity, anxiety-related behaviour and altered sociability – all 5-HIAA-mediated behaviours (Refs 50, 55, 56).

Later, Burns et al. (Ref. 19) showed that $Rai1^{+/-}$ mice are obese and hyperphagic but lack metabolic disease, such as signs of insulin resistance commonly associated with high fat stores (Ref. 19). Female $Rai1^{+/-}$ mice also have altered fat deposition, with increased deposition of truncal fat when compared with wild-type female mice. However, these mice have increased leptin levels, which might account for their increased food intake after fasting. These data suggest a role for Rai1 in the satiation response or metabolism/synthesis of adipose tissue.

Taken together, *Df(11)17*/+, $Rai1^{+/-}$, *Dp(11)17*/+ and *Rai1-Tg* mice have proved to be good models for their respective human syndromes. $Rai1^{+/-}$ mice have craniofacial, behavioural and developmental abnormalities consistent with those of SMS.

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Table 2. Phenotypes of *Rai1*-engineered mice

	Mouse strain[a]				
	Df(11)17/+	*Rai1*$^{+/-}$	*Rai1*$^{-/-}$	*Dp(11)17/+*	*Rai1-Tg*
Feature[b]					
Craniofacial defect	+	+	+	–	–
Developmental defect	+	+	+	+	+
Overweight	+	+	–	–	–
Underweight	–	–	+	+	+/–
Circadian rhythm abnormality	+	N/A	N/A	–	–
Impaired conditioned fear	+	–	+	+	N/A
Hyperactivity	–	–	–	+	+
Seizure	+	+	+	–	–
Abnormal EEG	+	+	+	–	N/A
Abnormal maternal behaviour	N/A	N/A	N/A	N/A	+
Altered mendelian transmission	+	+	N/A	+	+

[a]*Df(11)17/+*: SMS common deletion model; 2 Mb deletion includes seven genes syntenic to human chromosome 17p11.2 (Refs 49, 53).
Rai1$^{+/-}$: heterozygous gene-targeted disruption of *Rai1* (Refs 6, 52).
Rai1$^{-/-}$: homozygous gene-targeted disruption of *Rai1* (Ref. 52).
Dp(11)17/+ : PTLS common duplication model; includes 2 Mb duplication of seven genes syntenic to human chromosome 17p11.2 (Refs 49, 53, 54).
Rai1-Tg: Rai1 BAC transgenic overexpression model (Ref. 50).
[b]'+' indicates presence of feature; ' – ' indicates absence of feature; 'N/A' indicates feature not assessed (Refs 6, 49, 50, 52, 53, 54).
Abbreviations: BAC, bacterial artificial chromosome; EEG, electroencephalogram; PTLS, Potocki–Lupski syndrome; Rai1, retinoic acid induced 1; SMS, Smith–Magenis syndrome.

Additionally, *Dp(11)17/+* duplication and *Rai1-Tg* mice have behavioural, neurological and developmental abnormalities consistent with those of PTLS. These data reinforce the dosage sensitivity of *RAI1*. A summary of the phenotypes seen in these mice is provided in Table 2.

Molecular function of RAI1

Although data show that haploinsufficiency of the *RAI1* gene results in SMS, little is known about the molecular function of RAI1. Given the phenotypic consequences of *RAI1* mutation or deletion, RAI1 must be involved in pathways associated with development, behaviour, neurological function and circadian rhythm.

Mutations in transcription factors have long been associated with human disease, including ATRX syndrome (X-linked α-thalassaemia/mental retardation syndrome), myeloid leukaemia and autoimmune dysfunction (Ref. 57). Because transcription factors can regulate many genes, deciphering their global function can be difficult. With regard to RAI1, there is an additional layer of complexity because, other than insights from bioinformatic analysis, very little is known about the true molecular function of RAI1.

Rai1, originally called *Gt1*, was identified in mouse P19 embryonal carcinoma cells, which can be differentiated into neurons and glial cells when treated with retinoic acid. It was found to be expressed in all tested tissues and highly expressed in neurons (Refs 5, 58). At least three human isoforms are predicted by bioinformatic

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analysis. RAI1 contains a plant homeodomain (PHD) and a bipartite nuclear localisation signal, which are commonly seen in transcription factors. PHD domains typically bind zinc and are involved in chromatin remodelling, whereas nuclear localisation signals have the ability to recruit chaperone proteins, thus facilitating transport into the nucleus (Ref. 59) (Fig. 3). Indeed, RAI1/Rai1 protein localises to the nucleus and has transactivational activity in a luciferase reporter system (Refs 6, 7, 19). Complicating protein analysis, the three predicted isoforms of RAI1 might serve different functions dependent on spatial and temporal expression.

In a functional network module of SMS, small interfering RNA technology was used to achieve in vitro haploinsufficiency (50% knockdown) of *RAI1* (Ref. 60). Differentially expressed transcripts identified in this study included genes important in (but not limited to) metabolism, the cell cycle, insulin signalling, lipid biosynthesis and cholesterol homeostasis. Additionally, knockdown of *RAI1* resulted in reduced expression of *NR1D2* (nuclear receptor subfamily 1, group D, member 2), which is an essential component of the circadian clock. It has been well established that disruption of the circadian clock can lead to obesity: the $Clock^{\Delta 19}$ mutant mouse model presents with obesity, hyperphagia and metabolic disease, including hyperleptinaemia, hyperlipidaemia, hepatic steatosis, hyperglycaemia and hypoinsulinaemia (Ref. 61). CLOCK is a transcriptional regulator of the circadian clock, including NR1D2 as well as PER1/2 (period homologue 2) and CRY1/2 (cryptochrome 1/2). Taken together, these data and the well-established sleep disturbance in SMS suggest a possible role for RAI1 in the circadian loop.

Recent studies in mice showed that haploinsufficiency of *Rai1* results in reduced expression of brain-derived neurotrophic factor (*Bdnf*) (Ref. 19). BDNF promotes striatal neurons, and reduced expression has been associated with schizophrenia, depression and obsessive compulsive disorder (Refs 62, 63, 64). Additionally, tissue-specific knockout of *Bdnf* in mouse hypothalamus results in obesity and hyperphagia, much like that seen in $Rai1^{+/-}$ mice. Furthermore, a luciferase reporter system showed that *RAI1* overexpression induces *BDNF* expression (Ref. 19), lending support to the idea that these two genes function in a common pathway with RAI1 acting as an enhancer of *BDNF*. These facts present an interesting opportunity to exploit the RAI1–BDNF relationship by targeting *Bdnf* expression through ampakine treatment in mice; ampakine compounds have been shown to enhance *Bdnf* expression in several animal model studies, suggesting a possible pharmacological target for treatment in humans (Refs 65, 66, 67, 68). Additionally, given the fact that RAI1 has been shown to upregulate the transcription of the *BDNF* gene in vitro and that *Bdnf* expression is reduced in $Rai1^{+/-}$ mice, it is likely that RAI1 serves as an enhancer that is integral to modulating genes involved in metabolism, and these pathways should be explored in detail in future studies.

RAI1 has also been implicated in metabolism and neurobehaviour in a recent publication that shows that *RAI1* expression levels are reduced in subjects with brachydactyly mental retardation syndrome (BDMR) (Ref. 69). This syndrome results from a deletion of chromosome 2q37 or a mutation of the histone deacetylase 4 gene (*HDAC4*) (Ref. 69). Individuals with BDMR have a strikingly similar phenotype to individuals with SMS (including obesity, sleep disturbance and self-abusive behaviour) and have been previously misdiagnosed with SMS (Ref. 14). All BDMR patient lymphocytes tested for expression of *RAI1* had reduced levels, indicating that *RAI1* is probably downstream of HDAC4. One major difference between SMS and BDMR is the distinctive brachydactyly type E that BDMR subjects often present with, which is not seen in SMS subjects. These phenotypic differences should be taken into account when considering the differential diagnosis.

Clinical implications/applications

The study of SMS, and RAI1 in particular, provides an interesting opportunity to analyse the molecular aspects of a single-gene disorder involving intellectual disabilities, neurobehavioural problems, sleep disturbance and obesity. Because of the phenotypic overlap with many other syndromes in the diagnostic spectrum, it will be important to analyse the 'finer details' of these molecularly distinct syndromes in the clinic. Supporting this, a recent study showed that up to 50% of subjects referred for molecular evaluation of SMS did not

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have SMS, indicating the need for close evaluation of behaviour and attention to the physical phenotype, including craniofacial and skeletal clues (Ref. 14).

Proper diagnosis is key to all downstream treatments. Because of the strong overlap of SMS with so many other syndromes, this is a challenge every day for clinical geneticists. It would be appropriate to devise a detailed panel of phenotypes to aid in distinguishing the clinical differences between SMS and overlapping syndromes, taking into account the following: neurobehavioural features including, but not limited to, stereotypies, self-injurious and aggressive behaviours, and sleep disturbance; craniofacial features, including a broad face, a flat nasal bridge and brachycephaly; and skeletal features including short stature, brachydactyly and displaced toes 3–5. This is a short list, but if specific combinations of features are matched with most likely syndromes, the diagnostic process might be simplified. Development of a tiered molecular testing panel to facilitate correct diagnosis would further support this process and should include aCGH (to detect 17p11.2 and 2q37.3 deletions as well as other phenotypically overlapping syndromes), *RAI1* mutation analysis and *HDAC4* mutation analysis. Further studies of gene expression changes in *RAI1* (and *HDAC4*) could support diagnosis in complex cases not identified by the above methodologies.

Medical management, behavioural modifications and other pharmacological treatments are probably appropriate for people with SMS, but any treatment should be on a case-by-case basis in order to improve daily life. No single medication works for all people with SMS (Ref. 70); thus, additional research into the affected molecular and metabolic pathways is required in order to target appropriate pharmaceuticals to this population. A promising avenue to explore in the future is sleep rhythms and their impact on behaviour and metabolism. Circadian rhythms are present in living organisms ranging from bacteria to mammals. Disrupted circadian rhythms are associated with obesity and increased risk for high blood pressure and heart attack in shift workers (Ref. 71). Because of the strong circadian phenotype seen in SMS, directly addressing this basic biological problem is key for improved behaviour overall. Very basic treatments, such as light therapy and strictly enforced feeding schedules, might significantly improve the behaviour and metabolic outcomes in this population. Studies have shown that robust and appropriate circadian rhythms of transcription can be restored in mice lacking circadian rhythm ($Cry1^{-/-}/Cry2^{-/-}$) (Ref. 72) by enforcing a restricted feeding schedule, which holds promise that patterned feeding schedules during the day for people with SMS might provide an avenue for more consistent sleep patterns at night. Restoration of a consistent sleep pattern would probably have a positive impact on the behavioural and metabolic aspects of SMS (Ref. 31).

Outstanding research questions

There are many aspects of research that are yet to be uncovered in the field of intellectual disabilities syndromes, especially SMS. Further molecular analysis of the *RAI1* gene, its transcriptional targets and the genes regulating RAI1 is important if targeted pharmacological treatments are to be pursued in a meaningful way.

Any proteins that RAI1 might bind in order to exert its gene-regulatory activity, and whether it functions only as an enhancer or can act as a repressor too, are unknown. Molecules that modulate transcription rarely work alone. If binding partners are identified, it will be easier to identify specific points at which RAI1 functions in metabolic, neurological and circadian pathways. Additionally, the exact targets of RAI1 are still undefined. This can be analysed by tissue-specific temporal chromatin immunoprecipitation with sequencing (ChIP-seq) analysis, which will give an insight into the genes that RAI1 regulates during and after development.

To that end, it would be especially useful to create an inducible *Rai1*-knockout mouse in order to separate the developmental impact of loss of Rai1 from the postnatal influence of Rai1 on metabolism and neurological development and behaviour. This approach would also provide data to indicate whether RAI1 would be a good target for pharmacological intervention in SMS. If the primary impact of *Rai1* haploinsufficiency is on the developing fetus, then it is unlikely that simply increasing the expression level of *RAI1* would make an effective treatment strategy. However, it is more likely that haploinsufficiency of *RAI1* has a

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major role throughout life and that targeting this gene pharmacologically could be explored using conditional mouse models with the ability to focus on specific affected pathways, such as satiety, control of behaviours and circadian rhythm.

At the very basic level, the specific structure of the RAI1 protein, the composition and function of the various isoforms, and any tissue specificity are not understood. These details are especially of interest to identify the specific role that RAI1 has in critical molecular and metabolic pathways.

Given that proper dosage of *RAI1* is crucial to normal development, investigation into the regulation of *RAI1* expression is important, and understanding the players involved might lead to the identification of other genes that, when insufficient, might result in phenotypes with significant overlap to SMS. In this same line, we question whether *RAI1* might be targeted by microRNAs. Is there an antisense transcript that might be targeting *RAI1* in a temporal pattern? These details hold a wealth of information for future work, and if there are microRNAs targeting *RAI1*, this might be a crucial step in the creation of therapeutics in the future. A screen of known microRNAs and subsequent evaluation of *RAI1* expression would provide the first indication of whether this is a possibility. Bioinformatic analysis should also be assessed when seeking these answers.

In summary, RAI1 has a major role in pathways that impact metabolism, neurology, development and circadian rhythm. Although the specific role of RAI1 in these pathways is unknown, the SMS (and PTLS) phenotype illustrates the importance of this gene and proper gene dosage. Additionally, because of the phenotypic overlap and emerging molecular role for RAI1 in other syndromes with intellectual disability and congenital abnormalities, it is essential that RAI1 not be overlooked. A combined, collaborative and focused clinical and research strategy is of the utmost importance and will aid progress in the future.

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Further reading, resources and contacts

The NCBI Bookshelf entry for SMS provides a comprehensive review, including medical management recommendations:
http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=gene&part=sms

The SMS support group PRISMS (Parents and Researchers Interested in Smith–Magenis Syndrome) provides excellent information for families, educators and clinicians:
http://www.prisms.org/start.htm

An online community of families with children with SMS can be found at:
http://smithmagenissyndrome.ning.com/

Features associated with this article

Figures
Figure 1. Craniofacial features in Smith–Magenis syndrome.
Figure 2. Genomic structure of chromosome 17p11.2 and mechanisms of deletion in this region.
Figure 3. Gene and protein structures of RAI1.

Tables
Table 1. Phenotypic features of subjects with deletion 17p11.2 and *RAI1* mutation.
Table 2. Phenotypes of *Rai1*-engineered mice.

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