HK J Paediatr (new series) 2006;**11**:310-316

Case Reports

Smith-Magenis Syndrome in Chinese with Previously Unreported Craniofacial Features and Association with Joubert Phenotype

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Abstract Smith-Magenis syndrome (SMS) is a clinically recognisable multiple congenital anomalies syndrome marked by distinctive craniofacial dysmorphism, behavioural and neurological abnormalities. Other additional abnormalities depend on the extent of contiguous genes involvement. We compared the clinical features of four SMS patients; and report new, consistent and previously unrecognised craniofacial features in the syndrome; and in addition, reporting the second case of SMS with Joubert phenotype in literature.

Key words Deletion 17p11.2; Joubert syndrome; *RAI1*; Smith-Magenis syndrome (SMS)

Introduction

Smith-Magenis syndrome (SMS) has long been regarded as a contiguous gene syndrome, caused by an interstitial deletion involving chromosome band 17p11.2. The size of deletions varies from 1.5 Mb to 9 Mb, with 4 Mb being the commonest, responsible for 75% of SMS cases.[1] Recent reports on retinoic acid induced 1 (*RAI1*) gene mutations have added new insights into pathological mechanism of SMS. Prevalence of this disorder is estimated to be around 1/25000 live births, and is generally believed to be under-estimated. The likely mechanism for the deletion cases has been attributed to non-allelic homologous recombination between flanking repeated gene cluster (SMS-REPs) in 17p11.2, namely the SMS-REPD (Distal), SMS-REPM (Middle), SMS-REPP (Proximal).

This syndrome has a distinct and clinically recognisable phenotype consisting of craniofacial dysmorphism, otolargngological, behavioural and neurological abnormalities. Typical craniofacial features include broad square-shaped faces with brachycephaly, prominent forehead, synophrys, up-slanting palpebral fissures, and deep set eyes. Other features include broad nasal bridge, mid-face hypoplasia, tented mouth, micrognathia in infancy, and relative prognathism as patient ages. Otolaryngological characteristics include bilateral sensori-neural deafness, hoarse voice, and high arched or clefted palate.

Behavioural and neurological abnormalities are highly distinctive and consistent in SMS patients. Sleep disturbance is present in all, with difficulty falling asleep, frequent prolonged awakenings and excessive daytime sleepiness. Stereotypic behaviours include self-hugging, teeth grinding, body rocking or spinning objects. Self-hitting or self-biting is common. Other self injurious behaviours, such as onychotillomania and polyemboilokomania, are less common but more specifically described in SMS. The self injurious behaviour may be aggravated in part by the relative pain insensitivity produced as part of contiguous gene involvement. Maladaptive behaviours include temper tantrum, attention seeking or simply disobedience or distractibility have been well described but highly variable in SMS patients.

Joubert syndrome (JS) is genetically heterogeneous,[2] and is a rare autosomal recessive condition that is characterised clinically by developmental delay, hypotonia and ataxia. Another major feature is agenesis or hypoplasia of cerebellar vermis with accompanying brainstem

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Received July 3, 2006

malformations that may present with either abnormal breathing pattern or abnormal eye movements.

A major locus for Joubert syndrome was identified at 9q34. A second locus at 17p11.2 was proposed after a large deletion at 17p11.2 was reported in a patient with SMS and JS phenotype.[3] In this paper, we report the second case of SMS with JS phenotype in literature.

Slager et al 2003 reported three patients with typical SMS features but in whom no deletion was detected by Fluorescence in situ hybridisation (FISH) analyses.[4] They sequenced the *RAI1* gene in telomeric end of 17p11.2, and found 3 deletion mutations causing frameshift in *RAI1* gene with resultant truncated *RAI1* protein; therefore resulting in haploinsufficiency for *RAI1*. This is a major advancement in diagnosing SMS patients. The *RAI1* gene is located at 17p11.2, and contains six exons coding for an ~8.5kb mRNA and a 1906 amino acid protein.[5]

This marked the beginning of new understanding of the underlying mechanism in which a single gene is implicated in most of SMS features while haploinsufficiency of neighbouring deleted genes may modify the overall SMS phenotype.[4]

Phenotypic variability is well described in deletion and non-deletion type of SMS.[6] It remains to be determined whether this variation is due to different levels of functional *RAI1*, allelic variations related *RAI1* expression, other functional modifiers or simply other genes in the SMS critical region.[6] More and more non-deletional cases are being reported, the previously FISH negative cases may rewrite the true incidence of the syndrome.

We report four cases of SMS, all confirmed deletion in 17p11.2 by FISH; and compared their phenotypic variability with new unreported craniofacial features. We also report the second case of Smith-Magenis syndrome patient with Joubert phenotype in literature, and provided further evidence of a second locus for Joubert phenotype at 17p11.2.

Subject, Methods and Results

All four patients (Table 1) were ascertained through genetic counseling clinic at the Clinical Genetic Service (CGS). They were recognised by their craniofacial features, suspected on additional abnormal neurobehaviours, and diagnosed by karyotyping and FISH studies.

Table 1 Clinical features of four SMS patients

	Case 1	**Case 2**	**Case 3**	**Case 4**
Reason for referral	Dandy-walker malformation, dysmorphism	Suspected Prader Willi syndrome	Suspected Down syndrome	Global delay, hypotonia
Gender	Male	Male	Female	Male
Age at evaluation	5 months	15 months	21 months	29 months
Present age	9 years old	6.5 years old	6 years old	7.5 years old
Craniofacial/Skeletal				
Brachycephaly	+	+	+	+
Prominent forehead	+	+	+	+
Epicanthic fold	+	+	+	-
Broad nasal bridge	+	+	+	+
Midface hypoplasia	+	+	+	+
Prognathism (relative to age)	-	-	+	+
Tented upper lip broad, square face	+	+	+	+
Hypoplastic collumella	+	+	+	+
Thick earlobes	+	+	+	+
Brachydactyly	+	+	+	+
Short stature	<3%	3-10%	25%	10-25%
Small hands and feet	+	+	+	+

Table 1 Clinical features of four SMS patients (con't)

	Case 1	**Case 2**	**Case 3**	**Case 4**
Otolaryngological				
Chronic ear infections	-	-	+	-
Hoarse, deep voice	+	+	+	+
Neurological				
Pain insensitivity	+	+	+	+
Mental retardation	+	+	+	+
Speech delay	+No speech	+No speech	+	+
Motor delay	+Unable to walk, sit supported	+	+	+
Hypotonia	+	+	+	+
Sleep disturbance	+	+	+	+
CNS malformation	Dandy-walker malformation, cerebellar hypoplasia, hydrocephalus	Bilateral subependymal heterotopia	MRI brain: No abnormality detected	Asymmetry of hippocampal size Left cerebral atrophy
Behavioural				Body swinging
Aggressive	+	+	+	+
Self hugging	+	+	-	+
Onychotillomania	-	-	-	+ Onset 6 years old
Polyembolokoilomania	-	-	-	-
Head banging/face slapping	+	+	+	+
Hand biting	-	-	+	+ Onset 6 years old
Attention seeking	+	+	++	+
Hearing loss	Mod-Severe	Mod-Severe	Mild	Mild
Ocular abnormalities				
Myopia	+	+	+	+
Strabismus	+	+	+	-
Astigmatism	+	+	+	+
Synophrys	+	+	-	-
Scoliosis	+	+Cervical Hemivertebrae	-	-
Others				
Cardiovascular anomalies	ASD/VSD	VSD	-	-
Renal anomalies	-	-	VUR	-
Seizures	+	-	-	-
Cleft lip of palate	Submucous cleft	High arched palate	-	-
Small penis	+Right Cryptorchidism	+	-	Bilateral Cryptorchidism
Respiratory distress at birth	+	+	+	-
Karyotype	46,XY,del(17)(p11.2p13.1)	46,XY	46,XX	46,XY
De novo ish del(17)(p11.2p11.2) (SMS-)	+	+	+	+

Case 1 was a 9-year-old boy who was born to non-consanguineous parents at terms with birth weight 2.955 kg by caesarean section for antenatally detected hydrocephalus. Postnatally, it was complicated by respiratory distress. He was referred to CGS at aged 5 months (Figure 1) for dysmorphism, dandy-walker malformation, cerebellar hypoplasia, congenital heart defects (CHD) of atrial and ventricular septal defects (ASD/VSD), macrocephaly and generalised convulsions. He had persistent abnormal breathing pattern and sleeping difficulty. Diagnosis confirmed on karyotyping (Figure 2) and FISH (Figure 3). Parental testings were normal.

Case 2 (Figure 4) was a 6-year-old boy who was born to non-consanguineous parents at 42 weeks with birth weight 4.58 kg. He was admitted to neonatal intensive care unit on day 2 of life for recurrent choking, hypoglycaemia, heart failure with ventricular septal defect (VSD). He required assisted ventilation with tracheal intubation. Because of developmental delay, hypotonia, CHD and dysmorphism, he was referred to CGS at aged 15 months for suspected Prader Willi syndrome. There was no speech all along, and had persistent sleeping difficulty since birth. Clinical suspicion of SMS was confirmed on FISH testing. Parental testings were normal.

Case 3 (Figure 5) was a 6-year-old girl who was born to non-consanguineous parents at 39 weeks with birth weight

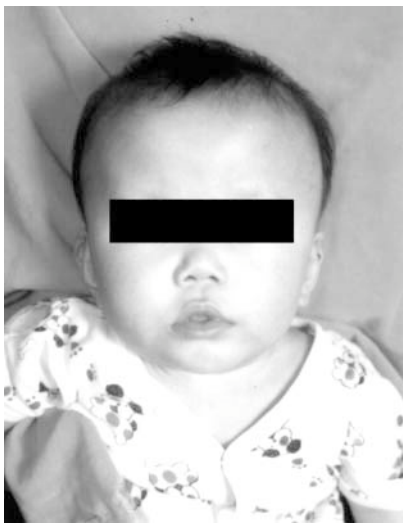

Figure 1 Case 1 with Smith-Magenis syndrome.

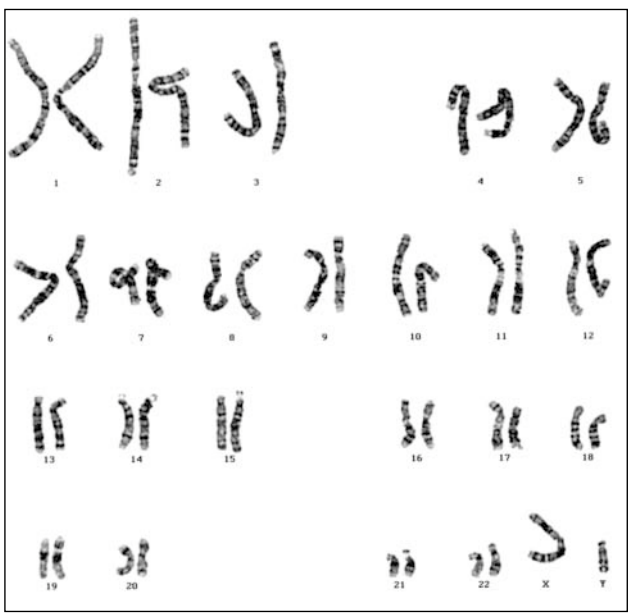

Figure 2 Case 1 karyotype showed 46,XY,del(17)(p11.2p13.1).

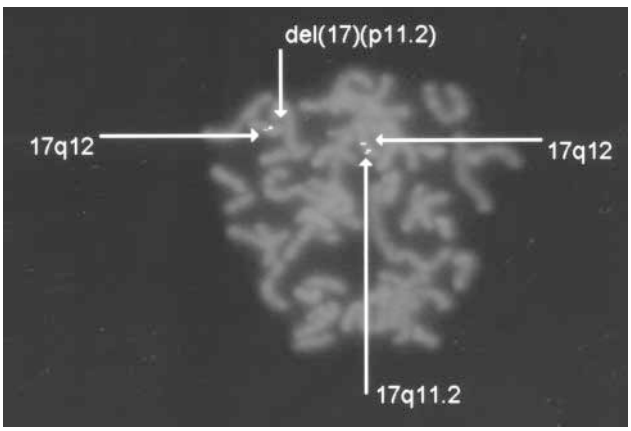

Figure 3 Case 1 FISH showed del (17)(p11.2p11.2)(SMS-).

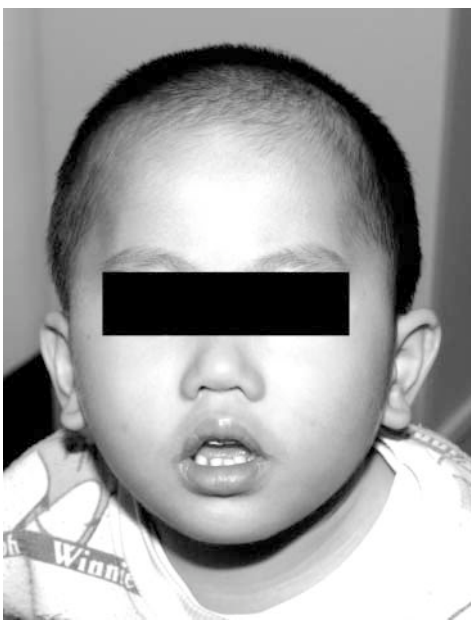

Figure 4 Case 2 with Smith-Magenis syndrome.

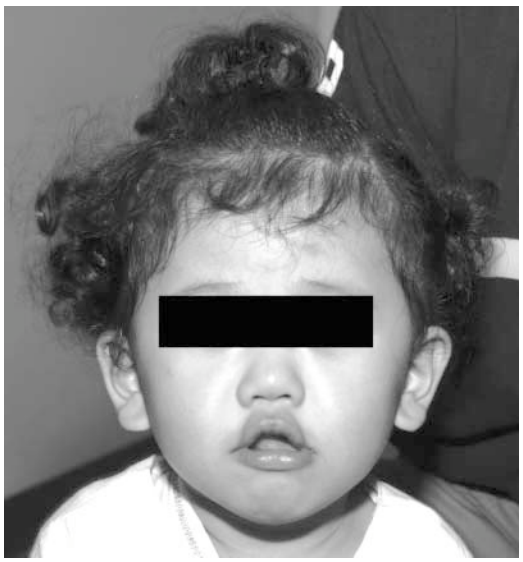

Figure 5 Case 3 with Smith-Magenis syndrome.

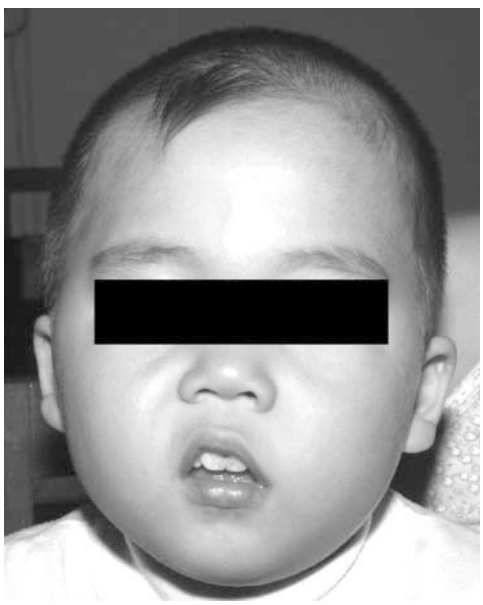

Figure 6 Case 4 with Smith-Magenis syndrome.

2.7 kg. Antenatally, amniocentesis was performed for previous child with trisomy 18, and the result was normal. She was referred for suspected Down syndrome at aged 21 months. She had the characteristic facial features of SMS, and it was well recognised that it may look similar to Down syndrome. She was the mildest of our four affected patients with typical craniofacial features and abnormal neurobehaviours; however, no associated internal organ anomalies. Her diagnosis was confirmed on FISH study. Parental testings were normal.

Case 4 (Figure 6) was a 7-year-old boy who was born to non-consanguineous parents at terms with birth weight 4.11 kg. He was referred at aged 29 months for global delay and hypotonia. He was also mildly affected as compared to case 1 & 2, and had characteristic abnormal neuro-behaviours and no major internal organ anomalies. He was last seen studying at a normal school but poor performance. His diagnosis was confirmed on FISH study. Parental testings were normal.

Cytogenetic Studies

Standard and high resolution G banding chromosomal analyses were performed on metaphases spread obtained from probands and their parents' peripheral blood lymphocytes.

FISH was performed using LSI SMS Smith-Magenis probe from VYSIS: LSI SMS probe (17p11.2) for the target and LSI RARA control probe (17q21.1) for chromosome 17. Patient's peripheral blood lymphocyte cultures were used for metaphase spreads. The probes were denatured and hybridised according to the manufacturer's protocols. Following the post-hybridisation wash, chromosomes were counterstained with DAPI. Preparations were then examined using a fluorescence microscope with appropriate filters.

Parental consents for publication that included photos and radiographs were kindly given and signed in all four cases.

Discussion

Smith-Magenis syndrome has long been regarded, since its first description by Ann Smith in 1982, as a multiple congenital anomalies syndrome with manifestation; as in any other contiguous gene syndrome, involving multiple organs. Variable clinical phenotypes were attributed to variable deletion sizes, with minimal overlapping deletion region refined to 650 kb.[7] All four cases reported here demonstrated this quite well ranging from case 1 with multiple congenital anomalies, the only cytogenetically visible deletion in our series, still unable to speak and walk at age 8; to case 4 with normal karyotype, no multiple congenital anomalies, borderline to mildly delayed with abnormal behaviour; walked and ran well, communicate well in conversations, and enjoyed playing computer games at age 7.

Case 2 although was not as severe as in case 1, but was second in terms of severity in the series with no speech,

cardiac and vertebral anomalies; and moderate to severe hearing loss. Case 1 and 2 had congenital cardiac defects (CHD) of ASD/VSD and VSD respectively, and these were reported to be common CHD in SMS.[8] Case 3 and 4 were relatively mild in presentation, with only basic features of SMS, and no internal organ anomalies. From Table 1, the most consistent features of SMS amongst these four patients were the craniofacial dysmorphism, hoarse deep voice, pain insensitivity, mental retardation, hearing and ocular problems, behavioural and sleep abnormalities. In fact, all these features are present in either "deletion" or "nondeletion" SMS patients.

Haploinsufficiency of *Rail* has been shown to cause obesity and craniofacial abnormalities in SMS mouse models.[9,10] Sleep disturbances of SMS was thought to be due to inverted circadian rhythm caused by involvement of neighbouring gene in 17p11.2.[11] Since then, many therapeutic measures that include melatonin and growth hormone had been tried with variable success.[12-14] However, in the recent reports of "non-deletion" SMS patients, sleep disturbance was also a major consistent feature. The mechanism of how *RAI1* causes inverted circadian rhythm/ sleep disturbances is intriguing and unknown; and the answers of which await future research. Similarly for pain insensitivity which was thought to be a "contiguous gene" feature, but now also observed in "non-deletion" patients.

The overall understanding of the syndrome was further complicated by the fact that *RAI1* contained a polymorphic CAG repeat that coded for a polyglutamine tract in the amino terminal domain of the protein *RAI1*.[15] CAG repeat length in *RAI1* had been implicated as a possible contributor to SCA2 neurodegeneration[16] or was thought to be involved/ or as a modifier in the etiology of Schizophrenia.[17] Further studies would be needed to delineate the role of *RAI1* played in causing the abnormal neurobehaviour, and is it responsible for progressively worsening of neurobehaviour with age that had been observed in SMS; and whether expansion of *RAI1* polyglutamine tract has any role in this disease phenotype.

In 2003, Slager et al first reported mutations in *RAI1* associated with non-deletional type of Smith-Magenis syndrome.[4] Since then numerous authors reported similar findings that the distinct clinical phenotype of Smith-Magenis syndrome namely, craniofacial dysmorphism, otolaryngological and neurobehavioural abnormalities, were attributable to *RAI1* gene mutations. All our "deletion" cases shared all these characteristics as in reported "non-deletion" cases, with a slightly Down syndrome like craniofacial features. When young, they had a pugilistic look; and when they got older, additional feature of prognathism became prominent. We also noted previously unreported consistent craniofacial features of hypoplastic columella; which was marked when young, and became less prominent as patient aged, and fleshy budda-like earlobes. All our patients remained on the low side in height but progressively gained height as they got older.

Boddaert et al (2004) reported anatomical and functional evidence of bilateral insulo-lenticular anomalies in all their five Smith-Magenis syndrome patients, and that this may have a causal relationship with the neurobehavioural feature of Smith-Magenis syndrome.[18] It is interesting to note that all; except case 3, of our cases had brain imaging abnormalities. With *RAI1* generally believed to be a transcriptional regulator involved in neuronal development;[5,9] the structural abnormalities may be related to the clinically observed hallmark of the syndrome that included the distinctive neurobehavioural and sleep abnormalities.

Natacci et al (2000) reported a Smith-Magenis syndrome patient with Joubert Syndrome phenotype.[3] The authors here report the second case of Joubert phenotype in a Smith-Magenis syndrome patient.

The major diagnostic criteria of JS was published by Saraiva and Baraitser in 1992 that included developmental delay, hypotonia, cerebellar vermis hypoplasia; together with either abnormal breathing or abnormal eye movements.[19] Case 1 is now 9 years old, still unable to speak or stand, with history of abnormal breathing requiring home oxygen and assisted ventilation in the form of CPAP. Brain imaging 9 years ago revealed Dandy-walker malformation, cerebellar vermis hypoplasia, and hydrocephalus with dilated ventricles managed by shunts. Although the specific feature of JS, "Molar tooth" sign was not mentioned in the radiology report; the overall picture was compatible with Joubert phenotype in this SMS patient. In addition to being the most severely presented out of the four; case 1 was the only case with cytogenetically visible deletion 17p11.2. This is in keeping with the larger the deletion size, the more complex is the clinical presentation due to contiguous genes involvement.

Girirajan et al (2005) suggested a logarithm for investigating suspected Smith-Magenis syndrome patient.[5] After initial karyotyping, this is then followed by FISH (Fluorescence in situ hybridisation) with an *RAI1* containing probe. "Non-deletion" cases would then be followed by *RAI1* sequencing analysis. Positive cases would then be confirmed de novo by parental testing. It has been several years since the first report of the new underlying molecular pathology in which a single gene *RAI1*, is implicated in

essential features of SMS. Since commercially available Smith-Magenis syndrome FISH probe does not include *RAI1* gene. The authors emphasised the importance of commercial FISH manufacturers to base their FISH probes on *RAI1*,[20] which is currently not the case in commercially available FISH probes, so that small deletion cases are not missed, thus allowing estimation of true prevalence of SMS.

References

1. Vlangos CN, Yim DK, Elsea SH. Refinement of the Smith-Magenis syndrome critical region to approximately 950kb and assessment of 17p11.2 deletions. Are all deletions created equally? Mol Genet Metab 2003;79:134-41.
2. Blair IP, Gibson RR, Bennett CL, Chance PF. Search for genes involved in Joubert syndrome: evidence that one or more major loci are yet to be identified and exclusion of candidate genes EN1, EN2, FGF8, and BARHL1. Am J Med Genet 2002;107: 190-6.
3. Natacci F, Corrado L, Pierri M, et al. Patient with large 17p11.2 deletion presenting with Smith-Magenis syndrome and Joubert syndrome phenotype. Am J Med Genet 2000;95:467-72.
4. Slager RE, Newton TL, Vlangos CN, Finucane B, Elsea SH. Mutations in RAI1 associated with Smith-Magenis syndrome. Nat Genet 2003;33:466-8.
5. Girirajan S, Elsas LJ 2nd, Devriendt K, Elsea SH. RAI1 variations in Smith-Magenis syndrome patients without 17p11.2 deletions. J Med Genet 2005;42:820-8.
6. Bi W, Saifi GM, Shaw CJ, et al. Mutations of RAI1, a PHD-containing protein, in nondeletion patients with Smith-Magenis syndrome. Hum Genet 2004;115:515-24.
7. Schoumans J, Staaf J, Jonsson G, et al. Detection and delineation of an unusual 17p11.2 deletion by array-CGH and refinement of the Smith-Magenis syndrome minimum deletion to approximately 650 kb. Eur J Med Genet 2005;48:290-300.
8. Myers SM, Challman TD. Congenital heart defects associated with Smith-Magenis syndrome: two cases of total anomalous pulmonary venous return. Am J Med Genet A 2004;131:99-100.
9. Bi W, Ohyama T, Nakamura H, et al. Inactivation of Rai1 in mice recapitulates phenotypes observed in chromosome engineered mouse models for Smith-Magenis syndrome. Hum Mol Genet 2005;14:983-95.
10. Yan J, Keener VW, Bi W, et al. Reduced penetrance of craniofacial anomalies as a function of deletion size and genetic background in a chromosome engineered partial mouse model for Smith-Magenis syndrome. Hum Mol Genet 2004;13:2613-24.
11. De Leersnyder H, De Blois MC, Claustrat B, et al. Inversion of the circadian rhythm of melatonin in the Smith-Magenis syndrome. J Pediatr 2001;139:111-6.
12. De Leersnyder H, Bresson JL, de Blois MC, et al. Beta 1-adrenergic antagonists and melatonin reset the clock and restore sleep in a circadian disorder, Smith-Magenis syndrome. J Med Genet 2003;40:74-8.
13. Itoh M, Hayashi M, Hasegawa T, Shimohira M, Kohyama J. Systemic growth hormone corrects sleep disturbance in Smith-Magenis syndrome. Brain Dev 2004;26:484-6.
14. McBride G: Melatonin disrupts sleep in Smith-Magenis syndrome. THE LANCET 1999;354(Nov):1618.
15. Seranski P, Hoff C, Radelof U, et al. RAI1 is a novel polyglutamine encoding gene that is deleted in Smith-Magenis syndrome patients. Gene 2001;270(1-2):69-76.
16. Hayes S, Turecki G, Brisebois K, et al. CAG repeat length in RAI1 is associated with age at onset variability in spinocerebellar ataxia type 2 (SCA2). Hum Mol Genet 2000;9:1753-8.
17. Joober R, Benkelfat C, Toulouse A, et al. Analysis of 14 CAG repeat-containing genes in schizophrenia. Am J Med Genet 1999; 88:694-9.
18. Boddaert N, De Leersnyder H, Bourgeois M, Munnich A, Brunelle F, Zilbovicius M. Anatomical and functional brain imaging evidence of lenticulo-insular anomalies in Smith Magenis syndrome. Neuroimage 2004;21:1021-5.
19. Saraiva JM, Baraitser M. Joubert syndrome: a review. Am J Med Genet 1992;43:726-31.
20. Vlangos CN, Wilson M, Blancato J, Smith AC, Elsea SH. Diagnostic FISH probes for del(17)(p11.2p11.2) associated with Smith-Magenis syndrome should contain the RAI1 gene. Am J Med Genet A 2005;132:278-82.