

Smith-Magenis syndrome in monozygotic twin fetuses presenting with discordant phenotypes and uteroplacental insufficiency

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Abstract. Smith-Magenis syndrome (SMS) is a rare condition with multiple congenital malformations caused by the haploinsufficiency of RAI1 (deletion or mutation of RAI1). However, the correlation between genotype and phenotype is not well understood. The present study describes the prenatal diagnosis of monozygotic twins with a 17p11.2 deletion, which is indicative of SMS, who presented with discordant phenotypes and uteroplacental insufficiency. A high-resolution genome-wide single nucleotide polymorphism array revealed a 3.7-Mb deletion in the 17p11.2 chromosome region. Accurate breakpoints of the deletion in these patients were used to identify correlations between SMS and the concomitant phenotypes, particularly uteroplacental insufficiency, which has rarely been investigated in SMS. In addition, no exonic mutations were identified in or affected known disease-associated loci that could explain the congenital anomalies, according to a model that accounts for the possibility of incomplete penetrance. Furthermore, a novel benign copy number variation (a duplication of 195 kb at 13q12.13) was identified but was unlikely to be clinically significant in the discordant phenotypes of the twins. The present study showed that multiple interacting genetic and environmental factors are involved in determining the variance of the SMS phenotype.

Introduction

Smith-Magenis Syndrome (SMS) is a rare neurodevelopmental disorder caused by a microdeletion on chromosome 17p11.2 and has an incidence of ~1 in 25,000 live births (1).

Individuals with SMS exhibit wide phenotypic variability. Certain patients show mild to moderate mental retardation, while others exhibit atypical facial features, and cardiac, renal and otolaryngologic abnormalities (1-3). However, the correlation between genotype and phenotype is not well understood in SMS (3-5).

For a number of decades, monozygotic (MZ) twin comparisons have been used to identify the contributions of nature (heredity) and nurture (environment) (6). Recently, several studies have suggested that genetic and epigenetic factors exhibit a role in phenotypic variance (2,3,7). Phenotypic variability is often observed between unrelated or related individuals or twins with the same microdeletion syndrome, such as 22q11.2 (3,8-10). Observation of the genetic history of a syndrome in MZs often leads to a greater understanding of the phenotypic variability (11). Recently, a high resolution single nucleotide polymorphism (SNP)-array allowed for the detection of copy number variants (CNVs) as well as SNPs. Halder *et al* (12) reported a case in which twins carrying the 22q11.2DS microdeletion had discordant phenotypes with a different sized genetic deletion. Another technique, exome sequencing, offers an efficient and affordable method to investigate the genetic factors involved in human diseases (13-16). B.D. Solomon performed exome sequencing on MZs discordant for VACTERL (vertebral anomalies, anal atresia, cardiac malformations, tracheo-esophageal fistula, renal anomalies and limb abnormalities) association-type congenital malformations (17). It was hypothesized that this method may reveal discordant variants that are able to explain the cause(s) of disease (17). Hicks *et al* (11) reported a case of MZs with SMS with different phenotypes; however, further molecular investigation of the discordant phenotypes was not conducted. The standard study design in these cases is to use current molecular techniques, such as an SNP array and exome sequencing, in order to identify correlations between SMS and the concomitant phenotypes, in addition to investigating the factor that may contribute to the phenotypic variability in MZs with SMS. In the present study, current molecular techniques, including as an SNP array and exome sequencing, were used in order to identify correlations between SMS and the concomitant phenotypes, in addition to investigating the factors, which may contribute to the phenotypic variability in MZs with SMS

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Patients and methods

Clinical description. A 24-year-old woman, gravida 3, para 1, with a monochorionic diamniotic twin pregnancy was referred to the prenatal department of the Department of Obstetrics and Gynaecology, The First Affiliated Hospital of Sun Yat-Sen University (Guangzhou, China) at 28 weeks and 4 days gestation. The patient presented with the presence of a ventricular septal defect and stenosis of the main pulmonary artery in one twin; the other twin was normal. Her two previous pregnancies were uncomplicated, and the third pregnancy was conceived spontaneously with a 25-year-old man. Ultrasound examination (GE Voluson 730 Expert; GE Healthcare Life Sciences, Vienna, Austria) at the cessation of menstruation, at 9 weeks, and at 16 weeks revealed that the twins were developmentally delayed by 2 weeks. These ultrasound findings and her delayed menstrual cycle postponed the expected delivery date by 2 weeks. A thin dividing membrane between the fetuses was visualized on ultrasound. The twins (Fig. 1A) were diagnosed with fetal growth retardation (FGR) at 28 weeks and 4 days gestation due to the 2-week developmental delay. A sonographic examination suggested measurable growth discordance. In addition, one twin (twin 1) was observed to have a ventricular septal defect (3 mm) and stenosis of the main pulmonary artery (Fig. 1C), while the other twin (twin 2), with the exception of FGR showed no abnormalities (Fig. 1B). The amniotic fluid volume (AFV) of twin 1 and twin 2 was 48 mm³ and 31 mm³, respectively. Following genetic counseling, an ultrasound-guided amniocentesis of the fetuses was performed for a cytogenetic analysis and chromosomal microarray analysis (CMA) at 29 weeks and 4 days gestation. The ultrasound examination of the AFV of twin 1 and 2 prior to cordocentesis were 13 and 21 mm³, respectively.

One week following surgery, the AFV of the two fetuses recovered to normal with volumes of 37 and 28 mm³, in twins 1 and 2, respectively, but the ventricular septal defect was enlarged to 3.5 mm in twin 1. Additionally, twin 1 developed a more severe form of FGR and absent end-diastolic flow of the umbilical artery. The ultrasound testing performed 2 weeks after amniocentesis revealed oligohydramnion of both fetuses, with AFVs of 9.7 mm³ (twin 1) and 12 mm³ (twin 2). Doppler investigations (GE Voluson 730 Expert; GE Healthcare Life Sciences) of the umbilical arteries and ductus venosus revealed no abnormal findings. Analysis of the amniocentesis sample by CMA revealed a 17p11.2 deletion, which was characteristic of SMS. The patient was called back for additional genetic counseling to discuss the results and prognosis of both fetuses. The patient decided to continue the pregnancy after multiple discussions.

Ultrasound examination at 34 weeks and 3 days gestation revealed the intrauterine fetal death of both twins and the absence of amniotic fluid. The two fetuses were delivered by stillbirth at the Obstetric Department of The First Affiliated Hospital of Sun Yat-Sen University). Both fetuses were visually nearly normal. However, organ malformations could not be determined as the parents did not consent to a fetal autopsy.

Ethical approval was obtained for this study from the Ethics Committee of the First Affiliated Hospital of Sun Yat-Sen University. All data were collected with the informed consent of the patient.

Cytogenetic analysis. Routine cytogenetic analysis using G-banding techniques at a resolution of 550 bands was performed. Briefly, a 10 ml sample of amniotic fluid was collected and subjected to amniocyte culture according to the standard cytogenetic protocol (18). A 5 ml sample of parental blood was collected from each parent and subjected to lymphocyte culture according to the standard blood cytogenetic protocol (18).

Whole-genome high-resolution SNP array. CMA on the uncultured amniotic fluid was performed using an Affymetrix cyto HD Array (Affymetrix, High Wycombe, UK). DNA was amplified, labeled, and hybridized to a CytoScan HD array platform, according to the manufacturer's protocol. The array was designed specifically for cytogenetics research and offers more than two million markers across the genome, including SNP probes and probes to detect copy number variations (Cyto-arrays). CEL files, obtained by scanning the CytoScan arrays, were analyzed with Chromosome Analysis Suite software (Affymetrix) using the annotations of genome version GRCH37 (hg19). Only those achieving the manufacturer's quality cut-off measures (MAPD \leq 0.25; SNP QC \geq 15.0; waviness standard deviation \leq 0.12) were included in the analysis. Gains and losses that affected a minimum of 50 markers over a 100-kbp length were initially considered. Changes in copy number were compared with the CNVs catalogued in the Database of Genomic Variants (DGV; <http://dgv.tcag.ca/dgv/app/home>) and the University of Santa Clara in California (UCSC; <http://genome.ucsc.edu/>) genome browser. The gene content of the CNVs of interest was determined using the UCSC browser, based on the Genome Reference Consortium Human Genome (GRCH; build 37; <http://genome.ucsc.edu/>).

Exome sequencing. The zygoty of the twins was confirmed using small tandem repeat markers. Polymorphic DNA marker analysis was performed on the parental and fetal DNAs using an ABI Prism 3500 (Applied Biosystems, Foster City, CA, USA). Solution hybridization exome capture was performed using the SureSelect Human All Exon v5 systems (Agilent Technologies, Santa Clara, CA, USA) using biotinylated RNA baits to hybridize sequences that corresponded with exons (19). The manufacturer's software version 1.5, which is compatible with Illumina paired-end sequencing software (Illumina, San Diego, CA, USA), was used. The manufacturer's specifications for the v5 kit report that the capture regions total ~50 Mb, which corresponded with the Consensus Conserved Domain Sequences database (<http://www.sanger.ac.uk/resources/databases/encode/>) that contains >1,000 non-coding RNAs as well as Gencode Project defined exons. Targeted regions included the exons of 18,113 genes of the Consensus Conserved Domain Sequences database, exons of additional genes, miRNAs, and non-coding RNA genes, totaling 30,241 genomic features and 51,646,629 targeted bases. Flowcell preparation and sequencing were performed according to the protocol for the HiSeq 2000 sequencer (Illumina) using 100-bp paired-end reads to generate sufficient data such that \geq 85% of the targeted bases were accurately genotyped, with an average coverage of 150x. Image analysis and base calling were performed on all data lanes using the Illumina HiSeq Control Software (HCS) v2.0.5 and RTA software v1.17.20 (Illumina) with default parameters.

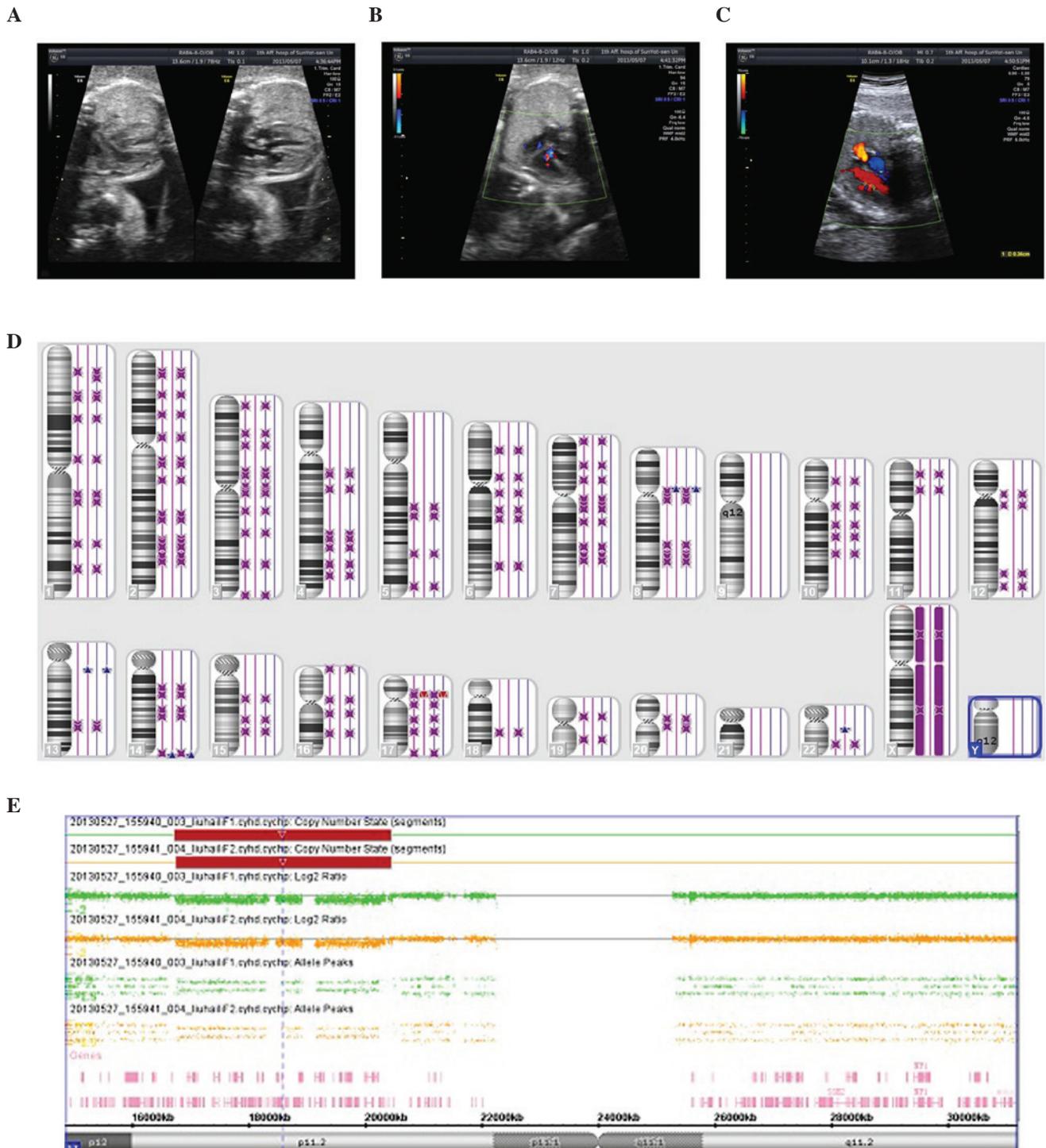


Figure 1. (A) Sonographic examination of both twins. (B) Sonographic examination of twin 1. (C) Sonographic examination of twin 2. (D) Long-contiguous stretch of homozygosity patterns observed in the twins (twin 1 and twin 2, respectively) shown in the purple star. CNV patterns are shown in the red (loss) and blue (gain) boxes. (E) Microdeletion at 17p11.2. The microarray profile of the 17p11.2 microdeletion is represented by the bold red line. The schematic diagram is shown below, and the deleted region is shown by a red box (twin 1 and twin 2, respectively).

Results

Chromosome analysis. The chromosome analysis revealed a normal male karyotype in both fetuses. The parents also had a normal karyotype. However, the CMA indicated that the fetuses had a deletion at chromosome 17 as arr 17p11.2 (16, 761, 814-20, 433, 502)x1 in one fetus, and, arr 17p11.2 (16, 761, 814-20, 433, 502)x1 in the other fetus. Both harbored the RAI1

gene, which is a critical gene involved in SMS (Fig. 1E). The parents had a normal CMA result, indicating a *de novo* deletion in both fetuses.

CNV identification. With the exception of the 17p11.2 deletion, the CMA identified a total of 9 CNVs. The identified CNVs were classified as being absent in the DG) or present in the DGV (Table I). The sizes of these CNVs vary from

Table I. Identity of copy number variants in the family members.

Family member	CNVs	Size (kb)	Gene	Type	Inherited
Father	13q12.13 (26, 803, 491-26, 918, 775)x3	115	CDK8	Novel	
	14q32.33 (106, 072, 250-106, 728, 149)x3	656	KIAA0125, ADAM6	PD	
	16p11.2 (33, 596, 761-33, 774, 726)x1	178		PD	
	19p12 (20, 588, 836-20, 716, 153)x1	127	ZNF826P	PD	
	22q11.22 (23, 063, 020-23, 258, 369)x3	195	MIR650, IGLL5	PD	
Mother	1p11.2 (121, 225, 582-121, 339, 317)x3	114	EMBP1	PD	
	12p13.31 (8, 006, 510-8, 124, 048)x3	118	SLC2A14, SLC2A3	PD	
	19p12 (20, 588, 836-20, 720, 705)x1	132	ZNF826P	PD	
	14q32.33 (106, 251, 147-106, 728, 149)x3	477	KIAA0125, ADAM6	PD	
Twin 1	13q12.13 (26, 803, 491-26, 918, 933)x3	115	CDK8	Novel	pat
	8p11.22 (39, 247, 097-39, 386, 952)x3	140	ADAM5P, ADAM3A	PD	dn
	14q32.33 (106, 072, 264-106, 728, 149)x3	656	KIAA0125, ADAM6	PD	pat
Twin 2	13q12.13 (26, 803, 491-26, 927, 389)x3	124	CDK8	Novel	pat
	8p11.22 (39, 254, 032-39, 384, 337)x3	130	ADAM5P, ADAM3A	PD	dn
	14q32.33 (106, 072, 264-106, 728, 149)x3	656	KIAA0125, ADAM6	PD	pat
	22q11.22 (23, 063, 020-23, 258, 369)x3	195	MIR650, IGLL5	PD	pat

CNVs, copy number variants; PD, present in the database of genomic variants; novel, absent in the database of genomic variants; pat, paternal; dn, *de novo*.

114 to 656 kb. In the fetuses, the duplication at 13q12.31 was inherited from the father, and the other CNV at 8p11.22 was a *de novo* event. Exome sequencing revealed the same disorder in the twins. A polymorphic DNA marker showed that the twins were a perfect match monozygosity (data not provided) and confirmed the twin pairs by high-density SNP microarray analysis (Fig. 1D). In brief, neither microarray analysis nor exome sequencing revealed an obvious discordant genetic anomaly (CNV or exome mutation) that would readily explain the presence of the congenital anomalies in one of the twins.

Discussion

The present study reports a pair of MZ twins, with a 3.7 Mb microdeletion at 17p11.2. This type of deletion is 'common' as 70% of patients with SMS have an ~3.7 Mb microdeletion in the 17p11.2 region (<https://decipher.sanger.ac.uk/syndrome/8#overview>). However, the twin pair presented with discordant phenotypes: One with a ventricular septal defect and stenosis of the main pulmonary artery, and the other as nearly normal.

The majority of SMS features are due to an RAI1 haploinsufficiency (20), while the variability and severity of the disorder are modified by other genes in the 17p11.2 region. The functional role of RAI1 is not completely understood, but based on homology and preliminary studies, it is likely to be involved in transcription (21,22). A phenotypic comparison between patients with deletions and patients with RAI1 mutations shows that 21 out of 30 SMS features are the result of an RAI1 haploinsufficiency, whereas cardiac anomalies, speech and motor delay, hypotonia, short stature and hearing loss are associated with 17p11.2 deletions rather than RAI1 mutations (7). In addition to RAI1, the 3.7 Mb region deleted in the present case

contains at least 50 genes, including mitogen-activated protein kinase 7 (MAPK7) activated by an upstream cascade of kinases in response to a wide variety of extracellular stimuli. Specific PRKM kinases (MAPKKs or PRKMKs) have been shown to phosphorylate and activate specific PRKMs in a given signaling pathway. Hayashi *et al.* (23) concluded that the MAPK7 pathway is critical for endothelial function and the maintenance of blood vessel integrity. In addition, a common cause of intrauterine growth restriction in humans is uteroplacental vascular insufficiency, which increases the incidence of perinatal asphyxia and neurodevelopmental disorders (24). A recent study showed that the cardiac dimensions are spared and may be used for gestational age estimation in growth-restricted fetuses resulting from uteroplacental insufficiency (25). A case report of MZ twins with SMS also shows both fetuses presenting with FGR (11), which is concordant with the present study. However, whether a fetus with SMS is more prone to FGR and whether haploinsufficiency of the PRKM7 gene is connected with uteroplacental insufficiency requires further investigation.

The twins also had a duplication at 13q12.31 which was inherited from the father. It was absent in the DGV and there was no reported CNV in this region. The CNV only included one protein-encoding gene CDK8 (cyclin-dependent kinases 8,*603184), which encodes a member of the mediator complex, located at 13q12.13, a region of recurrent copy number gain in a substantial fraction of colon cancers (26). Spore investigation confirmed the importance of Cdk8 at multiple stages of Dictyostelium development, although the severity of the defect in spore production depends on the genetic background (27). Although, it is important to consider the possibility of incomplete penetrance, the family did not know of any history of cancer. CDK8 is also a haploinsufficiency

gene in DECIPHER (28). While, deletions in the region have clinical relevance, duplication of the same interval may be benign (29). The present study also used phenotype databases, such as OMIM or gene/mutation-specific databases included in the specific CNV, and, considering the normal phenotype of the father, the duplication at 13q12.31 appears to be a benign polymorphism in the family and did not affect the twins SMS-associated features.

The zygosity of the twins, first confirmed by a polymorphic DNA marker and genomic DNA analysis on a high-resolution SNP array, showed the twins were a perfect match, except for the size of the CNV. However, except for experimental error and its proximal endpoint located in a low copy number, repeat or segmental duplication region, as evidenced on the UCSC genome browser (hg19), no CNV or exonic mutations were identified or affected known disease-associated loci that would explain the congenital anomalies for the possibility of incomplete penetrance. In this study, a high-resolution SNP array and exome sequencing of the MZ but phenotypically discordant twins did not explain why only one member of the twin pair was affected with features associated with SMS. There are multiple possible explanations: The presence of a regulatory factor that affects gene expression or a coding-region CNV, the presence of mutations only in the affected individual, or the mutations occurred in a region that may not be revealed by current methods of CNV analysis. Eventually, developments in genome testing may be able to evaluate these hypotheses, and there may be a discordant mutation not detected by the applied CNV. Microarray studies were conducted on DNA extracted from amniotic fluid, and similar testing based on other tissue types may yield greater success, as has recently been demonstrated in Proteus syndrome (30). The occurrence of a twin pregnancy may act on susceptible alleles to result in congenital malformations. Although parental studies were performed in this twin pair, molecular studies indicated that there were variants present in the parents but not the 17p11.2 deletion. Additionally, other testing modalities, such as methylation analysis, in conjunction with CNV, may shed more light on disease pathogenesis. Finally, it is possible that the causes of these congenital malformations may not be directly gene-related and may involve a primary, currently unidentified, environmental factor. Finally, on a level more specific to SMS, it is likely that multiple interacting genetic and environmental factors are involved in determining phenotype.

Further studies are ongoing related to several genetic variants of high interest (not located in the genes previously shown to be associated with human disease) that were found in the two twins and that may act in concert as susceptibility factors.

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