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RESEARCH ARTICLE

Prenatal diagnosis and neonatal phenotype of a *de novo* microdeletion of 17p11.2p12 associated with Smith–Magenis syndrome and external genital defects

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Abstract. Smith–Magenis syndrome (SMS, OMIM: 182290) is a multiple congenital anomalies and intellectual disability syndrome due to a 3.45 Mb microdeletion involving 17p11.2 and is estimated to occur about one in 25,000 births. Up to now, the ultrasound findings of the foetus with SMS and their external genital defects in patients are rarely reported. This case indicates that foetus with SMS may present polyhydramnios and ventriculomegaly in the second trimester. The newborn male patient had an abnormal phenotype in which he has micropenis and his anus is close to the perineal body. The identification of this case may further expand the phenotypic spectrum of this genetic disorder.

Keywords. chr17p11.2p12 deletion; prenatal diagnosis; neonatal phenotype; Smith–Magenis syndrome; SNP array.

Introduction

The 17p11.2p12 locus is an unstable region that is predisposed to two known reciprocal copy number variant (CNV) syndromes named Smith–Magenis syndrome (SMS, OMIM: 182290) and Potocki–Lupski syndrome (PTLS, OMIM: 610883) (Shaw *et al.* 2002; Bi *et al.* 2003). SMS is caused in most cases by a 3.45-Mb microdeletion in chromosome 17p11.2. The size of the deletion is variable from 1.5 Mb to 9 Mb (Girirajan 2005). This disorder can also be caused by mutations in a dosage-sensitive gene named retinoic acid induced protein 1 (*RAI1*) (MIM: 607642), which are within the SMS chromosome region (Falco *et al.* 2017). The incidence of SMS is estimated to occur about one in 25,000 births (Girirajan 2005). This value may be consistently under reported due to misdiagnosis and missed diagnosis in prenatal diagnosis. Patients with SMS generally have flat, square, rather heavy facies. They are often obese and short with small hands and feet. They usually have a history of hypotonia in infancy, feeding difficulties, developmental delay and behaviour disturbance (especially self-injurious behaviours and sleep disturbances). They have mild to severe learning disabilities and may be prone to hearing loss caused by chronic ear infections. Their neurobehavioural abnormalities usually become more pronounced with age and adults with SMS were more dependent on caregivers than might be expected from their general level of intellectual function (Falco *et al.* 2017).

In this case, we demonstrated the application of 750K SNP array in prenatal diagnosis, which provided the rapid identification of a *de novo* microdeletion of 17p11.2p12 in a foetus with abnormal ultrasound findings. As far as we know, the reports about the ultrasound findings of the foetus with SMS syndrome and the external genital defects reported in SMS patients are very rare, and there are few descriptions about the phenotype of the newborn. Here, we present such a case.

Materials and methods

A 28-year-old woman, gravida 3 para 1, was referred to our centre (Department of Reproductive and Genetics, Hebei General Hospital, Shijiazhuang, China) at 27 weeks of

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gestation for genetic counselling. Both the parents were nonconsanguineous and healthy. Her family and medical histories were unremarkable. No drugs or infections and other adverse contact history were reported during the course of the pregnancy. Prenatal serologic screening was at low risk and glucose tolerance screening was normal in the second trimester.

As a routine practice, ultrasound was conducted to monitor the developmental status of the foetus. Genetic cordocentesis was performed under real time ultrasound guidance combined with free-hand technique for interventional prenatal diagnosis. Chromosome analysis was carried out on cultured cord blood lymphocytes by G-banding karyotype at ~320–400 band resolution, as described by Sun *et al.* (2020).

Microarray-based copy number analysis was performed using the Chromosome Analysis Suite software v. 4.0.0.385 (R28959) (Thermo Fisher Scientific) and the results were presented on the human genome assembly hg19. SNP array using Affymetrix CytoScan 750K SNP array (Affymetrix, Santa Clara, USA), was performed on DNA extracted from the uncultured cord blood.

Results

The ultrasound examination showed polyhydramnios and the foetus displayed left ventriculomegaly (figure 1). The cytogenetic analysis revealed an suspicious abnormal karyotyping of 46, XY, ? Del (17) (p12) (figure 2) with the limited banding resolution.

A 4.788-Mb deletion was detected by SNP array at chromosome 17p11.2-17p12 or arr[hg19] chr17p12p11.2 (15759453-20547625) x1 (figure 3). SNP array analyses of the parental blood were both normal and did not reveal any deletion at chromosome 17p.

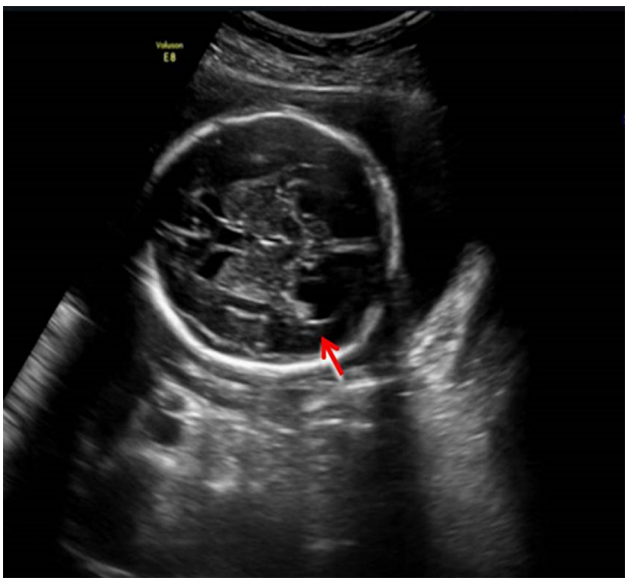

Figure 1. Ultrasound examination showing left ventriculomegaly (11.1 mm).

After genetic counselling and a fierce ideological struggle, the couples finally decided to continue the pregnancy, and made preparations to greet the angel with a broken wing. At 38 plus 3 weeks of pregnancy, a male neonate was born by cesarean section; his weight was 3750 grams, with a five-minute Apgar score of 10 points. The general condition of the newborn was good. He was feeding well, crying loud. He had normal bowel movements, normal body temperature, soft abdomen, dry umbilicus, and normal muscular tension of the limbs. His lung sounds clear without obvious rales. No other external or internal clinical symptoms and signs were observed except his anus is close to the perineal body, as well as micropenis, while the bilateral testes are located in the scrotum.

Discussion

Clinically, the positive findings at sonographic examination often indicated the chromosomal abnormality of foetus. However, the abnormal ultrasound findings of the foetus with a deletion of 17p11.2p12 associated with Smith–Magenis syndrome were rarely reported. In this case, polyhydramnios and left ventriculomegaly were detected at the 27th week of gestation. The foetus has 9.4 mm width at its right lateral ventricle and 11.1 mm width at the left lateral ventricle, respectively. Compared with the normal lateral ventricle width which is less than 10 mm, the left lateral ventricle is obviously abnormal, and that might be a soft marker of the foetus with Smith–Magenis syndrome.

In 1984, Patil and Bartleyre first reported a 4-year-old girl with an interstitial deletion of chromosome 17p11.2 who, except for moderate mental retardation, has no major malformation (Patil and Bartley 1984). Subsequently Smith *et al.* (1986) delineated in detail the phenotype associated with an interstitial deletion of 17p11.2 in nine unrelated patients. The clinical features consist of hypoplasia, prognathism, brachycephaly, midface, hypoplasia, speech delay, hoarse voice growth retardation and behavioural problems, with or without hearing loss. Since then, this kind of 17p11.2 deletion was known as Smith–Magenis syndrome. In the past 30 years, more than hundreds of patients with SMS have been reported (Moncla *et al.* 1991; Juyal *et al.* 1996; Andrieux *et al.* 2007) including several patients with heterozygous point mutations of the *RAI1* gene (Girirajan 2005; Bi *et al.* 2006; Vieira *et al.* 2012). However, the direct and first-hand descriptions on neonatal phenotypes of SMS were very rare, because the cases reported were mostly retrospective reports of patients aged 3–60 years who have been diagnosed later. To the best of our knowledge, there is only one case of SMS newborn that was born in spontaneous delivery with the neonatal phenotype of tachypnea, tracheomalacia, and mild hypotonia (Nijim *et al.* 2016). The newborn was confirmed to have an interstitial deletion of 46,

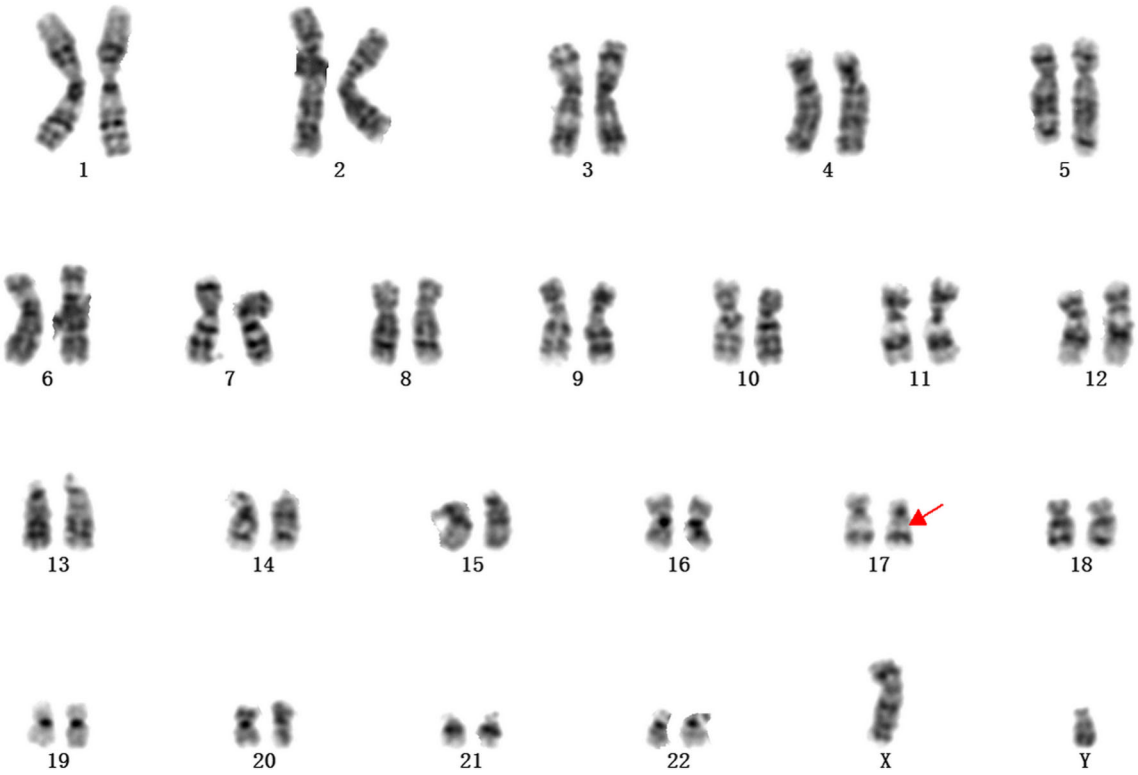

Figure 2. G-band karyotype obtained from the cord blood lymphocytes showing 46, XY, ? Del (17) (p12).

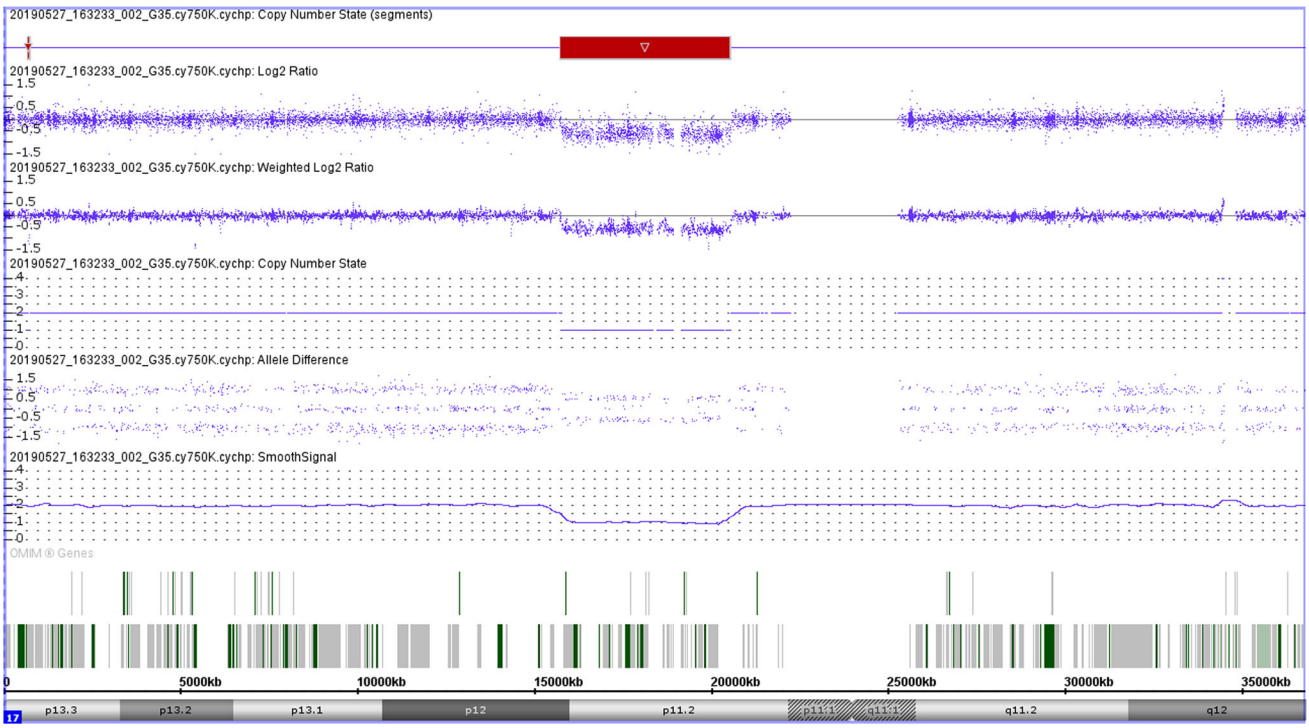

Figure 3. CMA plot showing the 4.788-Mb deletion of 17p12 p11.2 region (750K SNP array).

XY, del(17)(p11.2), detected by using conventional Giemsa-band karyotyping on metaphase cells and FISH testing. In our case, we describe a newborn male patient with the abnormal phenotype of having micropenis and anus closed to the perineal body. The features of SMS are likely to range in severity from person to person. The variable expressivity

Table 1. Summary of major and related abnormal phenotypes on the affected region of 17p11.2p12 deletion (data are based on DECIPHER, accessed 3 May 2020).

Patients with CNV matching 17p11.2-17p12 deletion or its genes		Patients with sequence variants matching 17p11.2-17p12 deletion or its genes		Present case 4.788-Mb deletion at 17p11.2-17p12
Abnormal phenotypes	Number of cases/total	Abnormal phenotypes	Number of cases/total	Abnormal phenotypes
Intellectual disability	87/252	Global developmental delay	7/32	Anus is close to the perineal body
Brachycephaly	39/252	Delayed speech and language development	6/32	Micropenis
Proportionate short stature	36/252	Congenital sensorineural hearing impairment	4/32	
Brachydactyly	35/252	Delayed gross motor development	4/32	
Midface retrusion	34/252	Intellectual disability	4/32	
Small hand	30/252	Coarse facial features	3/32	
Strabismus	30/252	Delayed fine motor development	3/32	
Aggressive behaviour	25/252	Depressed nasal bridge	3/32	
Wide nasal bridge	24/252	Developmental regression	3/32	
Muscular hypotonia	22/252	Downslanted palpebral fissures	3/32	
Microcephaly	20/252	Hypertelorism	3/32	
Abnormality of the pinna	19/252	Low-set ears	3/32	
Downturned corners of mouth	19/252	Mild short stature	3/32	
Two to three toe syndactyly	18/252	Moderate global developmental delay	3/32	
Broad palm	18/252	Preauricular pit	3/32	
Emotional lability	17/252	Tapered finger	3/32	
Frontal bossing	17/252	Urinary incontinence	3/32	
Micropenis	4/252	Abnormality of incisor morphology	2/32	
Abnormality of labia	3/252	Scrotal hypoplasia	2/32	

may relate to race and environment, and may be due to atypical deletions (larger or smaller).

In this case, G-band karyotype obtained from the cord blood lymphocytes showed a suspicious deletion at 17p12 with the limited banding resolution. In figure 2, one chromosome 17 is obviously smaller than the other and this can also occur when the staining was not done properly or the chromosome arm was not fully extended. The high resolution SNP array was subsequently performed on DNA extracted from uncultured cord blood to confirm the suspected cytogenetic finding, which revealed a result of arr [hg19] chr17p12p11.2 (15759453-20547625) x1 (figure 3). In the 4.788 Mb deleted region which covers the SMS region (16773072-20222149) completely, about 48 OMIM genes with already known or unknown functions are mapped. It contains 11 definite pathogenetic genes which have been implicated in a number of genomic disorders, including *TTC19* (MIM: 613814), *PIGL* (MIM: 605947), *UBB* (MIM: 191339), *TNFRSF13B* (MIM: 191339), *FLCN* (MIM: 607273), *G1D4* (MIM: 617699), *MYO15A* (MIM: 602666), *B9D1* (MIM: 614144), *SLC47A2* (MIM: 609833), *AKAP10* (MIM: 604694) and especially the known *RAI1* (MIM: 607642) which is believed to represent the critical gene involved in SMS. The haploinsufficiency of *RAI1* gene is responsible for most features of SMS, including behavioural, craniofacial, and neurologic signs and symptoms (Girirajan *et al*. 2006). Other related genes in the 4.788 Mb deleted section, encompassing *TRPV2* (MIM: 606676), *FAM83G* (MIM: 615886) and *SREBF1* (MIM: 184756) may also contribute to the variable features and overall severity of the syndrome.

There are 252 patients with CNVs and 32 patients with sequence variants matching the variant or genes of the proband have been reported by decipher database (https://decipher.sanger.ac.uk/syndrome/8#genotype/cnv/15/patient-overlap/cnvs). Sixty-eight per cent of the 252 patients with CNVs are *de novo* constitutive. It indicated that 17p11.2p12 is an unstable region and prone to variation. The total of 284 patients (including 252 CNVs and 32 sequence variants) contributed more than 160 various clinical phenotypes in

decipher database. The major abnormal phenotypes differences between the CNVs group and the sequence variants group are compared in table 1.

However, no cases sharing the same abnormal phenotype of anus is closed to the perineal body is as our case. Interestingly, four male patients were reported to have the phenotype of micropenis which is the same as our case and three female patients were reported to have the abnormality of labia in decipher database (table 1). And they all shared the same 9.12 Mb deleted segment of chr17p12p11.2 (16259275-25375873) which comprised the affected region of our case (4.788-Mb deletion at 17p11.2p12) entirely. These findings suggest that the deletion of 17p11.2p12 may contribute to external genital development. Although external genital defects are among the most common congenital anomalies in humans (Perriton *et al.* 2002), the molecular mechanism that underlies the morphogenesis of external genitalia in humans or other mammals have not been intensively investigated. Traditionally, the genital tubercle (GT) constitutes the anlage of external genitalia for penis and clitoris (Dolle *et al.* 1991; Kondo *et al.* 1997). Now, it is believed that the molecular regulation of genital development is a complex and continuous process (Cunha and Baskin 2019). The initiation of paired genital swellings generative process formation is a prerequisite for GT formation. It is a *Sonic hedgehog* (*Shh*) independent process mediated by fibroblast growth factor (*Fgf8*), but the exogenous growth and differentiation of GT depend on the ability of *Shh* to directly or indirectly program a large number of genes, including its receptor *Ptch1*, endoderm derived *Fgf8* or *Fgf10*, *Bmp2*, *Bmp4*, *Wnt5A*, *Msx1* and *Hoxd13*. *Shh* signal transduction obstruction can lead to downregulation of these genes, so *Shh* expressed in urethral epithelial cells plays a decisive role in the early development and differentiation of GT (Haraguchi *et al.* 2001; Haller and Ma 2019; Kajioka *et al.* 2020). After the formation of GT, testicular mesenchymal cells convert cholesterol to testosterone through enzymes such as P450 side chain cleavage (P450scc), 3-beta-hydroxysteroid-dehydrogenase type II (3-beta-HSDII), 17alpha-hydroxylase/17, 20-lyase (P450c17 or CYP17), and 17beta-hydroxysteroid-dehydrogenase type III (17beta-HSDIII). Testosterone is subsequently converted to dihydrotestosterone (DHT) by 5-alpha-reductase. Finally, DHT is mediated by androgen receptors acts on GT to form penis (Klonisch *et al.* 2004; Cunha and Baskin 2019). Therefore, androgens are crucial for the differentiation of GT into male genitalia, and any problem in the biosynthesis of androgens will lead to malformation of male genitalia, such as hypospadias (Hyuga *et al.* 2019; Mitsui *et al.* 2020). Sterol regulatory element-binding protein-1 (*SREBF1*) (MIM 184756) within the deleted region of chromosome 17p11.2 that is a structurally related protein which control cholesterol homeostasis by stimulating transcription of sterol-regulated genes. We hypothesized that *SREBF1* might contribute to external genital defects via regulating cholesterol metabolism in the proband. Interestingly, there are two patients with *MYO15A* mutation were reported to have scrotal hypoplasia (table 1) (https://decipher.sanger.ac.uk/syndrome/8#genotype/cnv/15/patient-overlap/snvs). This indicated that the *MYO15A* gene may also contribute to external genital defects, but the mechanism is still unknown.

In conclusion, the phenotypes of SMS patients previously reported are mostly focussed on intellectual disability, global developmental delay and sleep disturbance. The ultrasound findings of the foetus with SMS and the external genital defects in SMS patients are rarely reported. The identification of this case may further expand the phenotypic spectrum of SMS. Up to now, there is still no specific treatment for the SMS, and all the available therapeutic approaches are confined to symptomatic treatments. The better understanding of the biological role of these genes will be the only way to find out a proper cure for this syndrome.

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