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CORRESPONDENCE

Hypomethylation of the *KCNQ1OT1* imprinting center of chromosome 11 associated to Sotos-like features

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In a screening for imprinting alterations in 268 patients with idiopathic intellectual disability, we have found a loss of methylation in *KCNQ1OT1* (MIM 604115) in one patient with clinical characteristics resembling Sotos syndrome. Sotos syndrome (MIM 117550) is mainly characterised by overgrowth (tall stature and macrocephaly), learning difficulties and facial gestalt (frontal bossing, high hairline, downslanting palpebral fissures, prognathism and pointed chin).[1] Hypomethylation of *KCNQ1OT1*, on the other hand, is the most common cause of Beckwith–Wiedemann syndrome (BWS, MIM 130650), but it previously was reported in two patients diagnosed with Sotos syndrome[2,3] (Table 1). *NSD1* gene deletions or point mutations account for 70–90% of cases of Sotos syndrome, whereas the disease-causing mechanism of other cases remains unknown.[4]

The patient is the third child of non-consanguineous healthy parents. He was born in the 36th week of gestation with a birth weight of 2940 g (75–50th percentile) and a length of 51 cm ($>90^{th}$ percentile), neonatal occipitofrontal circumference was unregistered. He could not walk until 16 months and now, at 12 years of age, he has poor coordination. He also had language delay and did not speak until 3 years.

Clinical examination at 12 years of age noted frontal bossing, sparse hair in frontoparietal area, macrocephaly and dolichocephaly. He also showed foetal finger pads, although not large hands (Figure 1). At that age, his height was 160 cm ($>97^{th}$ percentile). He is moderately mentally retarded and attends a special school. He presents sociability problems, ocular twitch and sometimes he talks alone. The absence of the typical facial gestalt (downslanting palpebral fissures and pointed chin), neonatal hypotonia, large hands or cardiac anomalies do not allow a clinical diagnosis of Sotos syndrome, in spite of the clear similarities.

Standard GTG-banding karyotype from peripheral blood cells was reported normal (46,XY). Subtelomeric rearrangements and classical microdeletion/microduplication syndromes (including Sotos syndrome) were discarded by multiplex ligation-dependent probe amplification (MLPA SALSA P036D, P064 and P245; MRC-Holland, Nederlands). Screening for dosage alterations was performed by a full coverage human-genome oligo-CGH-array from Agilent Technologies (44K oligo array G4410B; Palo Alto, CA, USA) with no relevant findings. All the laboratory analyses so far performed (routine biochemical and genetics analyses) were normal.

Therefore, the patient was included in a series of patients with idiopathic intellectual disability associated to congenital anomalies, in which we performed a screening for DNA methylation alterations in four differentially methylated regions (DMRs): *KCNQ1OT1* (11p15), *MEG3* (14q32), *H19* (11p15) and *SNRPN* (15q12) based on a methylation test described previously in Martínez *et al.*[5] The screening was performed by a multiplexed semi-quantitative PCR amplification, with and without digestion by methylation-sensitive enzyme HpaII, using fluorescently labelled primers and capillary electrophoresis. Data analysis was performed in Excel (manuscript in preparation).

The present case showed a hypomethylation of *KCNQ1OT1* (Figure 2a). His parents were also studied and no alteration of the methylation or the gene dosage in these regions was found. The results of the patient were confirmed by MLPA (SALSA MS030), with a demethylation of the KvDMR1 region of the *KCNQ1OT1* gene of the 80% (Figures 2b and c). Also, it should be noted that, to date, no false positives have been detected for this diagnostic procedure in 254 non-affected individuals. Three microsatellite markers in this region were studied in the patient and his parents (D11S1984, D11S2362 and D11S1999), showing no evidence of a paternal uniparental disomy (data not shown).

Only two patients with a hypomethylation of *KCNQ1OT1* associated to Sotos syndrome have been so far reported in a clinically-selected series, one of them due to a paternal isodysomy.[1,2] That study was based on the clinical similarities between BWS and Sotos syndromes, because both conditions share common clinical features such as macrosomia. We have found a third patient with this association in a screening of 268 patients with intellectual disability associated to highly variable clinical symptoms.[6,7] This finding further confirms the association between Sotos-like symptoms and epigenetic alterations in 11p15. Most importantly, as far as we know this is the first study that finds such association avoiding any clinical selection bias, which highlights its biological significance.

The molecular basis for this association is unknown. It might well be speculated that some unknown gene, implicated in maintaining the methylation of DMRs, can also cause Sotos or Sotos-like syndrome. Up to date, only three genes are known to cause imprinting disorders by *trans*-acting loss of imprinting: *ZFP57*,[8] *NALP2*[9] and *NALP7*.[10] Mutation screening by direct sequencing of these genes in the patient did not allow us to find any pathogenic mutation. On the other hand, as the three presently known patients are sporadic, it cannot be rejected to be consequence of a new kind of epimutation without genetic cause. In any case, the methylation test of

Table 1 Comparison of phenotype of our patient and the patients described by Baujat *et al.*[2,3] with the main features of the Sotos Syndrome and the BWS

		Cases described by Baujat et al.[2,3]			
	Sotos syndrome	*FD*	*RD*	*Present case*	*Beckwith–Wiedemann syndrome*
Clinical features					
Prenatal overgrowth	+	+	+	+	+
Gestational age		34 weeks	40 weeks	36 weeks	
Birth weight (g)		2140 (75–50th percentile)	3380 (75–50th percentile)	2940 (75–50th percentile)	
Birth length (cm)		44 (50–25th percentile)	49 (50th percentile)	51 (>90th percentile)	
Postnatal Overgrowth	+	+	+	+	+
Age		11 years	10 years	12 years	
Height (cm)		156 (97th percentile)	146.5 (97th percentile)	160 (>97th percentile)	
Sotos clinical features					
Macrocephaly	+	+	+	+	
Dolichocephaly	+		+	+	
Frontal bossing	+	+	+	+	
High hairline	+	+	+	+	
Downslanting palpebral fissures	+	+	+		
Prognathism	+				
Pointed chin	+	+			
Large feet and hands	+	NR	NR		
Advanced bone age	+	+	+	NR	
Heart defect	+	+	+		
Psycomotor delay	+	+	+	+	
Speech delay	+	NR	NR	+	
Intellectual disability	+	+	+	+	
Behavioral problems	+	NR	NR	+	
Seizures	+	+	+		
BWS clinical features					
Macroglossia					+
Earlobe creases					+
Abdominal wall defect					+
Neonatal hypogycemia					+
Gene/*Locus*	*NSD1* (5q35)	11p15 paternal isodisomy	KvDMR1 (11p15) demethylation	KvDMR1 (11p15) demethylation	KvDMR1 (11p15)
Phenotype		Sotos syndrome	Sotos syndrome	Sotos-like	

Abbreviations: BWS, Beckwith–Wiedemann syndrome; NR, not reported.
FD and RD the code used to identify the patients in Baujat *et al.*[3]

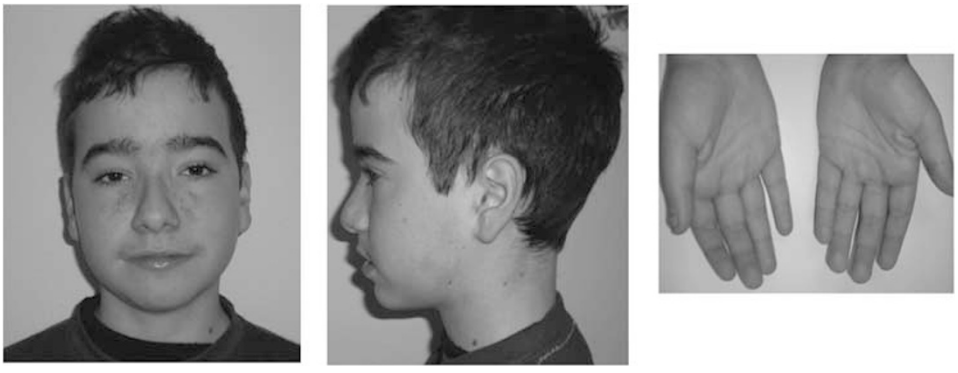

Figure 1 Hands, frontal and lateral facial views. All the images were taken when the patient was 12 years old with parental consent.

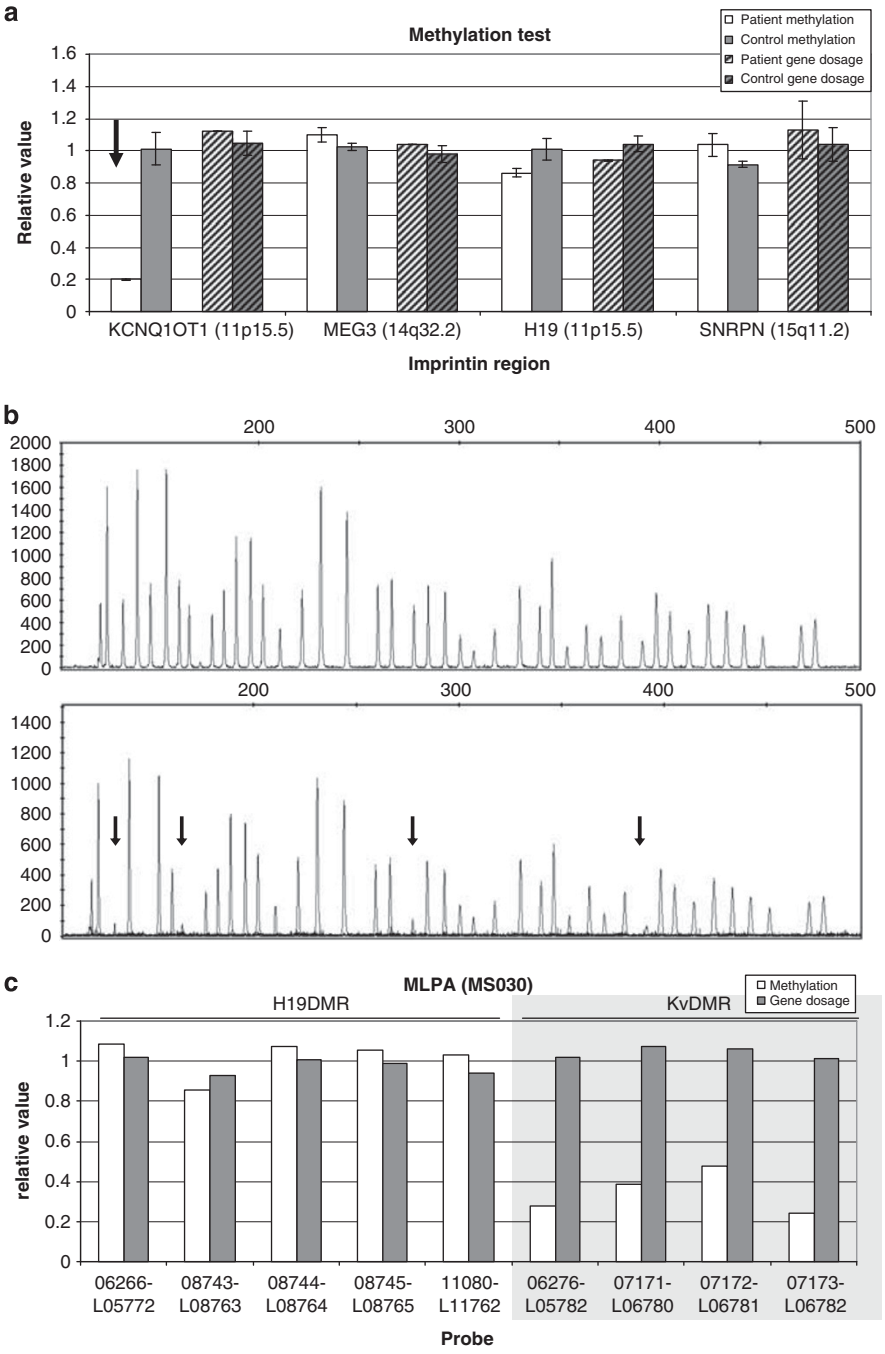

Figure 2 (**a**) Methylation studies. The dosage of four imprinting regions (*KCNQ1OT1*, *MEG3*, *H19* and *SNRPN*) was measured in the patient and control samples simultaneously. It is considered as normal a relative value within 1 ± 0.2. The experiment was carried out twice by independent replicates. The hypomethylation of *KCNQ1OT1* is indicated with a black arrow. (**b** and **c**) MLPA studies. MLPA SALSA MS030 patient results for the probes located in the two imprinting regions, H19DMR (*H19*) and KvDMR (*KCNQ1OT1*). Also, the normal relative values lie between 0.8 and 1.2. (**b**) Methylation electropherogram from control sample (above) and from the patient sample (below). The KvDMR probes are indicated with black arrows. (**c**) Analyzed result of the MLPA. Raw data were normalized against five healthy controls. All the four CpG sites tested showed a loss of methylation in a similar degree to that seen in the semi-quantitative PCR amplification.

KCNQ1OT1 may well be used as a new diagnostic marker among Sotos-like patients of unknown cause. This marker would allow to establish a new (epi)genetically homogeneous entity and, in turn, it can facilitate the identification of the causing gene and/or a more accurate genetic counselling.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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