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Report

Paradoxical NSD1 Mutations in Beckwith-Wiedemann Syndrome and 11p15 Anomalies in Sotos Syndrome

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Sotos syndrome is an overgrowth syndrome characterized by pre- and postnatal overgrowth, macrocephaly, advanced bone age, variable degrees of mental retardation, and typical facial features. Defects of the *NSD1* gene account for ⩾60% of cases of Sotos syndrome, whereas the disease-causing mechanism of other cases remains unknown. Beckwith-Wiedemann syndrome (BWS) is a distinct overgrowth condition characterized by macroglossia, abdominal-wall defects, visceromegaly, embryonic tumors, hemihyperplasia, ear anomalies, renal anomalies, and neonatal hypoglycemia. Deregulation of imprinted growth-regulatory genes within the 11p15 region is the major cause of BWS, whereas the molecular defect underlying a significant proportion of sporadic BWS cases remains unknown. Owing to clinical overlaps between the two syndromes, we investigated whether unexplained cases of Sotos syndrome could be related to 11p15 anomalies and, conversely, whether unexplained BWS cases could be related to *NSD1* deletions or mutations. Two 11p15 anomalies were identified in a series of 20 patients with Sotos syndrome, and two *NSD1* mutations were identified in a series of 52 patients with BWS. These results suggest that the two disorders may have more similarities than previously thought and that *NSD1* could be involved in imprinting of the chromosome 11p15 region.

Overgrowth syndromes are a heterogeneous group of disorders resulting from the dysfunction of various processes involving cell proliferation, cell growth, or apoptosis. Within this group, Sotos syndrome (MIM 117550) is characterized by the combination of overgrowth, specific facial features (prominent forehead with receding hairline, downslanting palpebral fissures, and pointed chin), large head circumference, and advanced bone age (Sotos 1964; Cole and Hughes 1990, 1994). Variable degrees of mental retardation are usually observed. Additional features include neonatal hypotonia, seizures, scoliosis, strabismus, congenital renal and heart defects, and tumor predisposition. Deletions and point mutations of the *NSD1* gene (MIM 606681) account for ⩾60% of cases of Sotos syndrome, but the disease-causing mechanism in other cases remains unknown (Kurotaki et al. 2002; Douglas et al. 2003; Rio et al. 2003).

On the other hand, Beckwith-Wiedemann syndrome (BWS [MIM 130850]) is a distinct overgrowth condition characterized by macroglossia, anterior abdominal wall defects, visceromegaly, and tumor predisposition (Elliot 1994). Additional features include earlobe creases, posterior helical ear pits, facial naevus flammeus, neonatal hypoglycemia, renal abnormalities, and hemihypertrophy. A cluster of genes on chromosome 11p15 is involved in the pathogenesis of BWS (Li et al. 1998; Maher and Reik 2000; Reik and Murrell 2000). Although the majority of cases are sporadic, a small number of familial forms are linked to chromosome 11p15. A minority of cases result from 11p15 chromosome duplications and translocations. Genetic causes (11p15 paternal uniparental disomy or a mutation in *CDKN1C*) account for 30% of cases, and most patients exhibit epigenetic defects, mostly demethylation of the *KvDMR1* region of the *KCNQ1OT* gene (MIM 604115) (60%) but also

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Table 1

Genotypes of Patient FD and His Parents at Polymorphic Loci on Chromosome 11p15

Locus[a]	Distance from Telomere[b] (Mb)	Genotype of Father	Patient	Mother
D11S4177	1.45	205	181/205	181/203
D11S4046	***1.92***	***121/123***	***121***	***113/123***
D11S1760	5.34	87/95	87	87
D11S1338	***5.94***	***261/263***	***261***	***255/263***
D11S1331	***7.25***	***192/196***	***192***	***194/196***
D11S909	8.81	116/118	118	114/118
D11S4188	***9.12***	***108/116***	***108***	***112***
D11S1346	11	268	268	268/280
D11S902	***17.52***	***157***	***157***	***159/161***
D11S904	***26.71***	***194/200***	***194***	***186/188***
D11S1751	33.79	204/214	204/212	212

NOTE.—Bold italic characters indicate genotypes that are consistent with a maternal deletion or paternal isodisomy.

[a] Loci are listed according to their relative chromosomal location from pter to centromere.

[b] Allele sizes are given in base pairs.

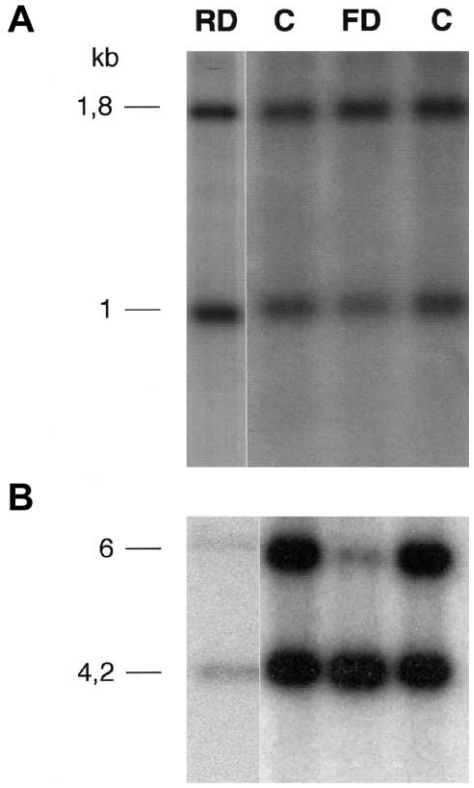

Figure 1 Methylation analysis of *H19* and *KCNQ1OT* genes in patients (RD and FD) and normal subjects (C). *A, H19* methylation was assessed by digestion with *Pst*1 and *Sma*I. The 1.8-kb *Pst*1 fragment is cut with *Sma*I, resulting in a 1-kb fragment. The *H19* methylation index was determined by scanning autoradiographs and calculating the ratio (1.8-kb fragment)/(1.8-kb fragment + 1-kb fragment) × 100. *B, KCNQ1OT* methylation was assessed by digestion with *Bam*HI and *Not*I. The 6-kb *Bam*HI fragment is cut with *Not*I, resulting in a 4.2-kb fragment. The *KCNQ1OT* methylation index was determined by scanning autoradiographs and calculating the ratio (4.2-kb fragment)/(4.2-kb fragment + 6-kb fragment) × 100.

hypermethylation of the *H19* gene (MIM 103280) (10%). Altogether, these genetic and epigenetic abnormalities of the 11p15 region account for ⩾80% of all BWS cases (Li et al. 1998).

Although Sotos syndrome and BWS are believed to be clinically distinct conditions, they share common clinical features—namely, macrosomia, neonatal hypoglycemia, and cardiac anomalies. On the basis of this phenotypic overlap, we hypothesized that some forms of unexplained Sotos syndrome could be related to 11p15 anomalies, whereas unexplained BWS cases could be related to *NSD1* deletions or mutations.

We first analyzed genomic DNA from 52 patients with BWS for deletion of the *NSD1* gene. Inclusion criteria were the presence of at least two of the four major signs—namely, (i) macrosomia, (ii) macroglossia, (iii) abdominal-wall defect, and (iv) organomegaly—and two minor signs. In all cases, routine G-banding and R-banding chromosome analyses showed a normal karyotype with no evidence of deletions or duplications. Molecular analyses of the 11p15 region ruled out chromosomal rearrangements as well as abnormal methylation patterns and mutations in the *CDKN1C* gene. Patients were then genotyped (according to the method of Rio et al. 2003) using four polymorphic microsatellite markers (two intragenic and two adjacent to the *NSD1* gene) (UCSC). In all cases, a microdeletion encompassing *NSD1* was excluded by the detection of two distinct alleles for at least one of the intragenic markers. The 12/52 patients with some degree of mental retardation were then selected for *NSD1* sequencing. It is interesting that two patients with BWS were found to have *NSD1* mutations—namely, a 1-bp insertion in exon 14 (patient PB) and a 4-bp deletion in exon 23 (patient BA). Both mutations were found to have occurred de novo.

Conversely, 20 patients with Sotos syndrome were investigated for chromosomal and methylation anomalies of the 11p15.5 region. They all presented with typical facial gestalt, macrocephaly (head circumference >+2 SD), overgrowth, and developmental delay. In all cases, routine G-banding and R-banding chromosome analyses showed a normal karyotype, and molecular analyses ruled out fragile X syndrome and *NSD1* deletion or point mutation.

To screen for deletions or uniparental disomies of the 11p15.5 region, three polymorphic microsatellite markers (D11S4046, D11S1338, and D11S1346) (UCSC) were tested. Analysis of one patient with Sotos syndrome (patient FD) revealed the unbalanced segregation of two

Table 2

Clinical Manifestations in Children with *NSD1* Mutations and 11p15 Anomalies

Clinical Features or Diagnosis	Manifestations in Patient			
	PB	BA	FD	RD
Clinical Features Common for Sotos and BWS:				
Birth history:				
Duration of pregnancy	40 wk	38 wk	34 wk	40 wk
Birth weight (g)	3,680	2,700	2,140	3,380
Birth length (cm)	54.5	47.5	44	49
Birth head circumference (cm)	39	36	32	35
Complications of prematurity	No	No	No	No
Neonatal hypoglycemia	No	Yes, with hyperinsulinism, persistent to date	No	No
Postnatal overgrowth:				
Age	5 years 6 mo	5 years	11 years	10 years
Height (cm)	122.5 (97th percentile)	130 (>97th percentile)	156 (97th percentile)	146.5 (97th percentile)
Weight (kg)	26.5 (97th percentile)	29 (97th percentile)	50 (>97th percentile)	35 (50th percentile)
Head circumference (cm)	57 (>97th percentile)	57 (>97th percentile)	59 (>97th percentile)	58.3 (>97th percentile)
Heart defect	No	No	Patent ductus arteriosus and ventricular sepral defect	Septal hypertrophy
Genito-urinary abnormalities	2 Renal cysts	Persistent vesicoureteral reflux	No	No
Advanced bone age	No	No	Yes	Yes
Malignant or benign tumors	No	No	No	No
Sotos clinical features:				
Sotos facial features:	No	No	High hairline, frontal bossing, antimongoloid slant of palpebral fissures, pointed chin	Dolichocephaly frontal bossing, antimongoloid slant of palpebral fissures
Development delay:	Yes (moderate)	Yes (mild)	Yes (moderate)	Yes (severe)
Age able to walk unaided	2 years	2 years	3 years	4 years
Special education	Yes	Normal education, orthophonist	Yes	Yes
Hands	Deep creases	Deep and brittle nails	Deep nails	Arachnodactyly
Cerebral malformation	No	No	Ventriculomegaly	No
Seizures	No	No	Yes	No
Skeletal abnormalities	Craniostenosis	Scoliosis	no	Severe scoliosis at age 1 year
BWS clinical features:				
Craniofacial features:				
Macroglossia	No	No	No	No
Earlobe creases	No	No	No	No
Posterior helical ear pits	No	No	No	No
Frontal angioma	No	No	No	No
Abdominal-wall defect	Large umbilical hernia	Abdominal-wall hypotonia	No	No
Visceromegaly	No	No	No	No
Hemihypertrophy	Yes, left side	No	No	No
Unclassified features:				
Craniofacial morphology (cf. fig. 3*A*–3*D*)	Scaphocephaly ptosis, epicanthus, large ears	Plagiocephaly, low hairline (diazoxyde), horizontal palpebral fissures		
Initial diagnosis	BWS	BWS	Sotos syndrome	Sotos syndrome
Molecular diagnosis	NSD1: exon 14: 4976 ins 5(G)	NSD1: exon 23: 7968 del (GACA)	11p15: paternal isodisomy	11p15: demethylation of *KCNQ1OT*

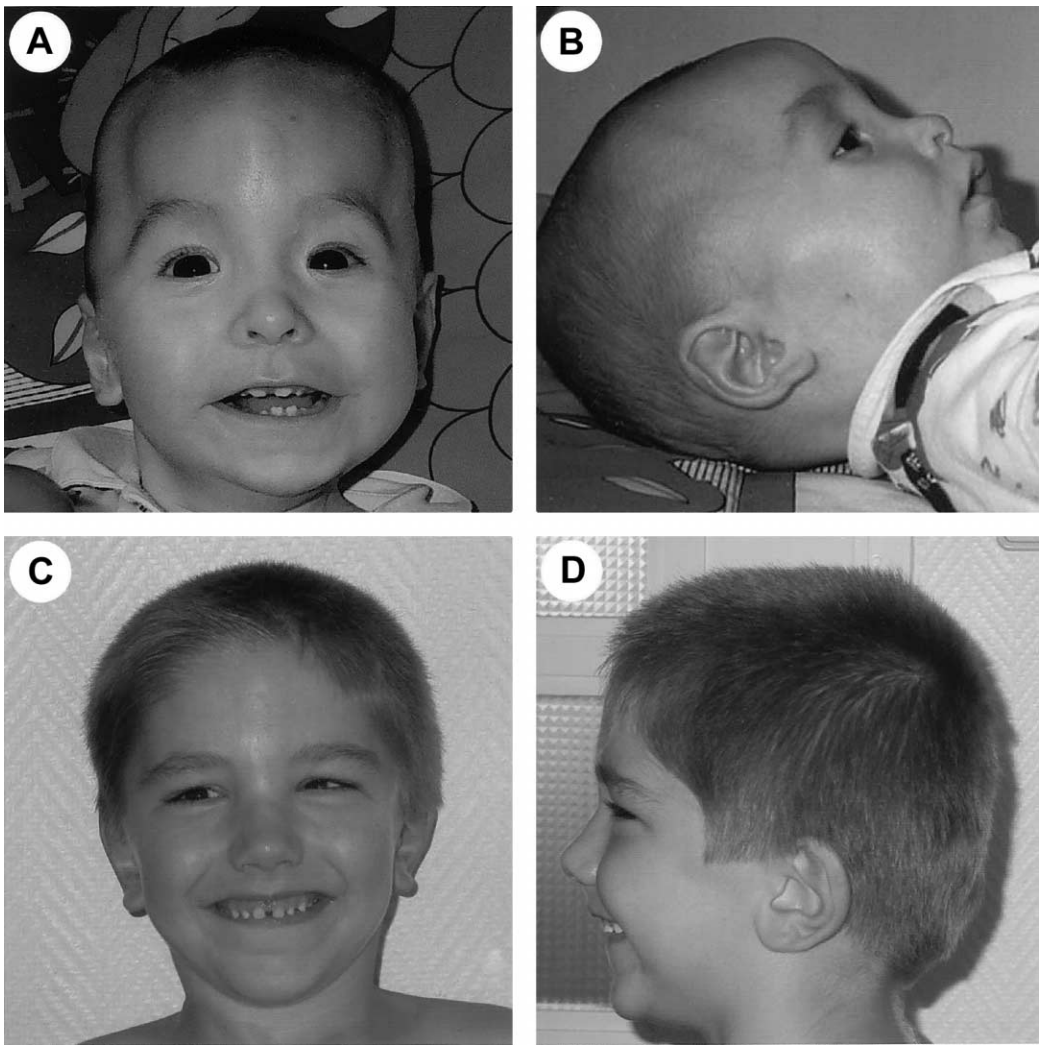

Figure 2 Facial features of patients with BWS who have *NSD1* mutations. *A* and *B*, Patient PB at age 1 year. Note that the craniostenosis may hide the classic facial gestalt of Sotos syndrome. Frontal bossing and antimongoloid slant of the palpebral fissure are not typical. *C* and *D*, Patient BA at age 4 years. Note the absence of typical facial characteristics of Sotos syndrome: normo-versed nares and absence of prominent forehead, dolichocephaly, and prominent chin.

microsatellite DNA markers, with no maternal contribution, at loci D11S4046 and D11S1338 (table 1). Genotyping eight additional polymorphic markers confirmed the lack of maternal contribution at loci D11S1331, D11S4188, D11S902, and D11S904 and a balanced contribution at loci D11S4177 and D11S1751. Therefore, the disease region encompassed 25 Mb between D11S4046 and D11S904. FISH analyses performed using YAC 896B12 and PAC RPCI-5 908H22, located on distal chromosome 11p, detected two spots for each probe, ruling out a maternal deletion and supporting paternal isodisomy.

We subsequently investigated the methylation status of the 11p15 region in our 20 patients with Sotos syndrome. That region contains two distinct domains that are regulated by two independent imprinting centers separated by a nonimprinted region. The telomeric domain 1 contains the maternally expressed *H19* gene and the paternally expressed *IGF2* gene. The centromeric domain 2 contains six known imprinted genes, including the paternally expressed *KCNQ1OT* gene (LIT1) and the maternally expressed *CDKN1C* gene (p57KIP2). The methylation status of the *KCNQ1OT* and *H19* genes was assessed as described elsewhere (Brannan et al. 1990; Gaston et al. 2001). As expected, patient FD, who had a paternal isodisomy, exhibited a strong demethylation pattern of the *KCNQ1OT* gene (methylation index [MI] 9.2%; normal mean ± 1 SD = 51.6 ± 2.5%) (fig. 1*B*). It is surprising, however, that we found an almost-normal methylation pattern of the *H19* gene (MI 61%) (fig.

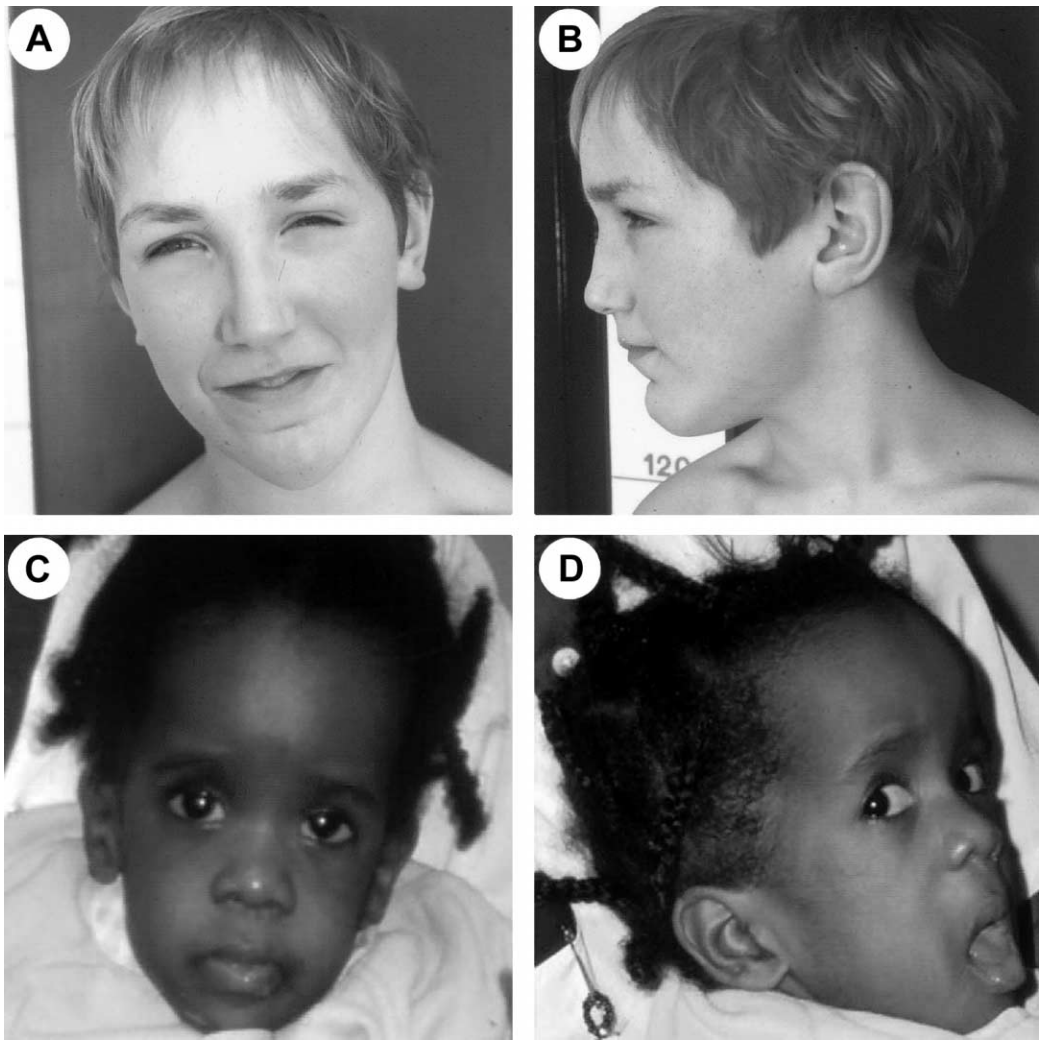

Figure 3 Facial features in patients with Sotos who have 11p15 anomalies. *A* and *B,* Patient FD at age 11 years. Note the downslanting palpebral fissures and pointed chin. *C* and *D,* Patient RD at age 15 mo. Note the dolichocephaly, ocular hypertelorism, frontal bossing, sparseness of hair in frontoparietal region, and antimongoloid slant of the palpebral fissures.

1*A*). It is interesting that a second patient with Sotos syndrome (patient RD) also displayed an abnormal methylation status, as shown by the demethylation pattern of the *KCNQ1OT* gene (MI 33.8%) (fig. 1*B*). The methylation status of the *H19* gene was normal (MI 49.5%) (fig. 1*A*). The other patients with Sotos syndrome had a normal methylation pattern at the two loci.

Table 2 shows the clinical findings of the two patients with BWS who had *NSD1* mutations and the two children with Sotos syndrome with anomalies of the 11p15 region. In all cases, careful analysis supported the initial clinical diagnosis. Indeed, patient PB, who had a 1-bp *NSD1* deletion, first received a diagnosis of BWS based on pre- and postnatal overgrowth, umbilical hernia, and hemihyperplasia. He also had mild developmental delay, craniostenosis, and multiple renal cysts. Moreover, the patient did not have the typical facial gestalt observed in patients with *NSD1* anomalies (fig. 2*A* and 2*B*). Similarly, patient BA, who had a 4-bp *NSD1* deletion, first received a diagnosis of BWS based on postnatal overgrowth, hypoglycemia with hyperinsulinism, a significant umbilical hernia, and persistent vesicoureteral reflux. This patient did not have the typical Sotos facies either (fig. 2*C* and 2*D*).

Conversely, patient RD was believed to have Sotos syndrome on the basis of the association of postnatal overgrowth, characteristic facial appearance, and developmental delay (fig. 3*A* and 3*B*). The patient had a partial isolated demethylation of *KCNQ1OT*, a feature that is already reported in patients with BWS and is probably due to mosaicism (Gaston et al. 2001). Similarly, facial gestalt, advanced age bone, macrosomia and

overgrowth, ventriculomegaly, and ventricular septal defect were consistent with the diagnosis of Sotos syndrome in patient FD (fig. 3*C* and 3*D*). The patient had a paternal isodisomy, including both *KCNQ1OT* and *H19* loci, and should therefore have an altered *H19* methylation. His unusual methylation profile may therefore be the cause of the atypical overgrowth phenotype observed in him.

In conclusion, this study suggests that 11p15 anomalies could account for ⩾10% of unexplained cases of Sotos syndrome and, conversely, that *NSD1* mutations could account for ⩾5% of the unexplained cases of BWS. These findings illustrate the difficulty in clinically recognizing the various overgrowth syndromes, owing to their phenotypic overlap. These results suggest giving consideration to testing the *NSD1* gene and 11p15 region, respectively, in individuals with atypical features of BWS (i.e., unexplained developmental delay) and Sotos syndrome (i.e., hypoglycemia secondary to hyperinsulinism). Finally, these results are also important for genetic counseling and definition of recurrence risk in affected families.

The mechanism by which mutations in the *NSD1* gene and deregulation of 11p15 genes result in similar phenotypes remains unknown. *NSD1* was isolated in a screen to isolate C-regulators for retinoic acid receptors (Huang et al. 1998). It encodes a nuclear protein containing an SET (su(var)3-9, enhancer-of-zeste, trithorax) domain and multiple PHD (plant homeodomain protein finger) domains. Recent analyses of the mouse protein have shown that the nsd1 SET domain has an intrinsic histone methyltransferase with a unique substrate specificity for both Lys36 of histone H3 and Lys20 of histone H4 (Rayasam et al. 2003), suggesting an essential function in the transcriptional gene silencing by histone methylation. On the basis of these findings, it is tempting to speculate that the NSD1 protein may also be involved in the establishment and/or maintenance of the imprinting of the chromosome 11p15 region. We hope that further molecular and genetic studies will elucidate the exact link between NSD1 and imprinting.

Acknowledgments

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Electronic-Database Information

The URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for Sotos syndrome, BWS, *NSD1, KCNQ1OT,* and *H19*)

University of California–Santa Cruz (UCSC) Genome Bioinformatics, http://genome.cse.ucsc.edu/ (for chromosome 11 physical map [updated July 2003])

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