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Uniparental paternal disomy in a genetic cancer-predisposing syndrome

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THE 11p15.5 region of human chromosome 11 seems to contain a locus or loci involved in congenital overgrowth anomalies as well as in the genesis of many tumours associated with the Beckwith–Wiedemann syndrome (BWS)[1–6]. Given the unusual differential parental allele involvement in the different aetiological forms of BWS[5,7] and the loss of maternal alleles in associated tumours[8–10], we have now used 11p15.5 markers to determine the parental origin of chromosome 11 in eight sporadic cases of BWS. Probands in three informative families had uniparental paternal disomy for region 11p15.5. Further, an overall greatly increased frequency of homozygosity for several 11p15.5 markers in 21 sporadic BWS patients suggests that isodisomy probably accounts for an even higher proportion of BWS sporadic cases. This demonstrates that uniparental paternal disomy can be associated with a genetic cancer-predisposing syndrome.

All 21 sporadic BWS individuals studied had a typical phenotype of BWS, that is exomphalos, macroglossia, gigantism, hypoglycaemia and visceromegaly. Four had developed a Wilms' tumour (patients BW9P, BW11P, BW18P and BW21P). The constitutional karyotype analysis failed to reveal a chromosomal aberration. Restriction fragment length polymorphism (RFLP) analysis was done using nine RFLPs at six loci ranging from 11pter to 11p15.5 in the following order: HRAS1-(IGF2-INS-TH)-D11S12-HBB-HBBP. The genotypes of eight BWS patients and of 14 matched parents were determined (Table 1). In three BWS patients (BW11P, BW15P and BW21P), we have shown that there was an absence of maternal contribution for at least one marker mapping to 11p15.5 (Fig. 1*a*). Gene copy number determination revealed that all three patients had inherited two paternal copies (Fig. 1*b*, *c*, *d*) and thus displayed paternal disomy. Isodisomy rather than heterodisomy can be demonstrated only for BW21P (Fig. 1*a*). The region for which the patients are disomic includes INS-IGF2 and extends to HRAS1 (BW11P) and to HBBP (BW21P).

To test whether being uniparental for 11p15 disomy could be a common, yet unrecorded genetic event, we compared the genotypes of 18 unrelated control individuals with the genotypes of their parents. We did not detect uniparental disomy in this control population (data not shown). Therefore the occurrence of uniparental disomy in three out of eight sporadic BWS patients is probably not fortuitous (3/8 versus 0/18; P, 0.02, one-tailed test). A few cases with a duplication of 11p15.5 sequences have been reported[1,2]. All were almost exclusively of

TABLE 1 Genotypes of eight BWS patients and their parents for nine 11p15.5 RFLPs

	HRAS1 *Bam*HI	TH *Taq*I	INS *Rsa*I	INS *Hind*III	IGF2 *Sac*I	IGF2 *Ava*II	D11S12 *Msp*I	HBB *Ava*II	HBBP *Hinc*II
BW11P	1/1	2/2	1/1	1/1	2/2	1/1	2/2	2/2	1/1
BW11M	2/2	2/2	1/2	1/2	1/1	1/1	1/2	2/2	1/2
BW11F	1/1	2/2	1/1	1/1	2/2	1/1	1/2	2/2	1/2
BW12P	2/2	2/2	1/1	1/2	1/1	1/1	1/2	1/1	2/2
BW12M	1/2	2/2	1/1	1/2	1/1	1/2	1/1	1/2	2/3
BW13P	1/2	2/2	2/2	1/2	2/2	1/1	1/2	2/2	1/2
BW13M	1/2	2/2	2/2	1/2	2/2	1/1	1/2	2/2	2/3
BW13F	1/2	2/2	2/2	2/2	2/2	1/1	1/2	1/2	1/1
BW14P	1/2	2/2	1/1	2/2	1/2	1/1	1/2	2/2	1/1
BW14M	2/2	2/2	1/2	2/2	1/2	1/2	1/2	2/2	1/3
BW14F	1/2	2/2	1/2	1/2	2/2	1/1	1/2	2/2	1/1
BW15P	2/2	1/2	2/2	2/2	2/2	1/1	1/1	2/2	1/1
BW15M	2/2	2/2	1/1	1/2	2/2	1/1	1/2	1/2	1/1
BW15F	2/2	1/2	2/2	1/2	2/2	1/1	1/2	2/2	1/3
BW16P	1/2	2/2	2/2	1/2	1/2	1/2	2/2	1/2	1/3
BW16M	2/2	2/2	2/2	1/2	2/2	1/1	1/2	2/2	1/3
BW17P	2/2	2/2	1/2	1/2	2/2	1/2	2/2	2/2	1/3
BW17M	1/2	2/2	1/2	1/2	1/2	1/2	1/2	2/2	1/2
BW17F	2/2	2/2	1/1	1/2	1/2	1/1	2/2	2/2	1/3
BW21P	2/2	2/2	1/1	1/1	2/2	1/1	2/2	2/2	2/2
BW21M	1/2	2/2	2/2	2/2	1/2	1/2	1/2	1/2	1/3
BW21F	2/2	2/2	1/1	1/2	2/2	1/1	2/2	1/2	1/2

DNA was isolated from blood and/or lymphoblastoid cell lines. Southern blot analysis was performed as previously described[16]. Probes and restriction enzymes used for RFLP analysis were: HRAS1 (c-Ha-ras) with *Bam*HI, TH (Tyrosine Hydroxylase) with *Taq*I, INS (Insulin) with *Rsa*I and *Hind*III, IGF2 (Insulin-like Growth Factor II) with *Sac*I and *Ava*II, D11S12 (pADJ762) with *Msp*I, HBB (β-globin) with *Ava*II, and HBBP (β-globin, pseudogene) with *Hinc*II[17]. These markers were chosen because they were duplicated in two BWS patients with cytological duplications of 11p15.5 (ref. 3), and revealed loss of heterozygosity in several tumours[18–25]. RFLP alleles were named 1, 2 or 3 according to decreasing length. Parents were as follows: BW11M and BW11F are the mother and the father of patient BW11P. Genotypes in boxes: Loci for which the patients failed to inherit maternal alleles.

paternal origin. Further, in several families with the autosomal dominant syndrome, genetic linkage of the BWS locus to markers within 11p15.5 has been established[4,5]. In these families there is an excess of female carriers, suggesting a sex-dependent mode of transmission[5,7] partly due to a higher penetrance in individuals born to female carriers (C. Moutou, personal communication).

To determine whether isodisomy is a common event in sporadic cases of BWS, we compared the distribution of the different genotypes in the 21 unrelated BWS patients with that in 36 unrelated controls. There was a significant distortion of the genotype distribution in BWS individuals (Table 2). BWS patients were more frequently homozygous for four contiguous markers taken together, INS/*Rsa*I, INS/*Hind*III, IGF2/*Sac*I and IGF2/*Ava*II, than controls. The distortion suggests that uniparental isodisomy probably accounts for an even higher proportion of sporadic cases of BWS. Given the informativeness and the distance between them, the INS/IGF2 region can be considered as part of the region of disomy most probably common to the different patients.

The event triggering malignant proliferation in the same type of tumours as those observed in 10% of BWS patients, is a loss of 11p15.5 alleles. The alleles lost are always of maternal origin[8-10]. Of four BWS patients who developed a tumour, two, BW11P and BW21P, displayed paternal disomy. Paternal disomy with absence of maternal alleles is thus compatible with the development of Wilms' tumour. As patient BW15P, who is also disomic and has not developed a tumour at age five, this may suggest that the absence of maternal 11p15.5 alleles observed in many tumours is probably not sufficient for tumour development. One must await the cloning of the gene(s) to determine whether the remaining paternal allele(s) is functional or altered and the nature of the differential imprinting.

We have shown that BWS can occur following erroneous events during meiosis or after fertilization leading to complete or partial uniparental isodisomy (or heterodisomy), even in the absence of obvious clinical signs in the parents. There are two possible explanations to account for the occurrence of BWS in children with paternal disomy. First, BWS in the three disomic cases could be due to inheritance of a recessive paternal mutation as reported in two patients with cystic fibrosis (CF) and short stature[11,12]. Second, the BWS locus (or loci) more probably undergoes differential genomic imprinting[13]. Although the number of cases reported here is insufficient to demonstrate that uniparental disomy is always of paternal origin in BWS, our findings are similar to data in two phenotypically different

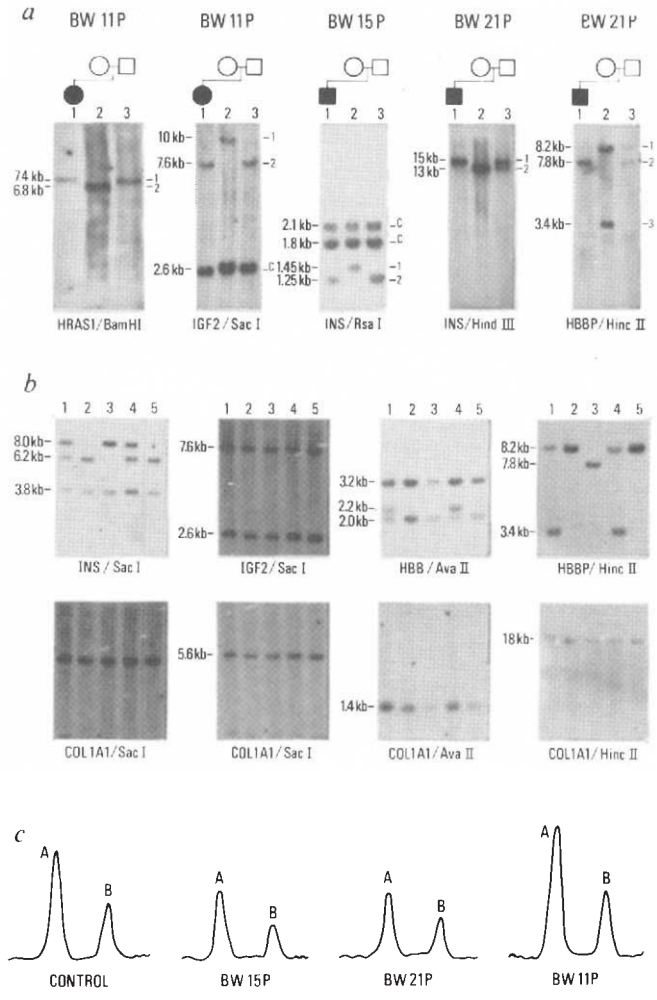

FIG. 1 Paternal uniparental disomy in three BWS patients with cytogenetically normal chromosome 11. *a*, Absence of maternal alleles for 3 BWS patients: Southern blot experiments. BW11P, Paternal origin of HRAS1/*Bam*HI and IGF2/*Sac*I in patient BW11P. The 7.4-kilobase (kb) HRAS1 allele and the 7.6 kb IGF2 allele is shared by the patient BW11P (lane 1) and her father BW11F (lane 3), whereas the patient inherited neither the 6.8-kb HRAS1 allele nor the 10-kb IGF2 allele from her mother BW11M (lane 2). The 2.6-kb IGF2 band is a constant band. BW15P, Paternal origin of INS/*Rsa*I in patient BW15P. The same 1.45-kb allele is shared by the patient BW15P (lane 1) and his father BW15F (lane 3), whereas the patient (BW15P) did not inherit a 1.25-kb allele from his mother BW15M (lane 2). The 2.1 and 1.8 kb bands are constant bands. BW21P, Paternal origin of INS/*Hind*III and HBBP/*Hinc*II in patient BW21P. The patient BW21P (lane 1) only displays the 15-kb INS alleles from his heterozygous father (lane 3, 15-kb and 13-kb alleles). This patient did not inherit a 12-kb INS allele from his mother BW21M, homozygous for this allele (lane 2). Similarly the patient displays only one 7.8-kb HBBP allele from his heterozygous father and did not inherit the 8.2-kb or 3.4-kb allele from his mother. Isodisomy rather than heterodisomy can account for this pattern. Using non-11p highly polymorphic markers, maternity and paternity testing confirmed that in all three cases the alleles present were indeed of paternal origin (data not shown). *b*, Gene copy number determination for patients BW11P, BW15P, BW21P and control individuals: Southern blot experiments. Top, 11p15 probes, INS, IGF2, HBB, and HBBP; bottom, the same filter was hybridized with a non-11p probe, COL1A1, used as the internal control. Lanes 1 and 4, control individuals; lane 2, BW15P; lane 3, BW21P and lane 5, BW11P. All three patients displayed two copies for each of the markers tested. *c*, Densitometry data. Densitometer scanning profiles for IGF2 (A) and COL1A1 (B). *d*, Densitometry values obtained by scanning of the blots shown in *b*. *R*, Ratio obtained by dividing the intensity for the 11p15 marker by the intensity of the internal control (COL1A1); *P*/*C*, ratio obtained by dividing the preceding ratio for the patient by that of the control individuals; *C*, mean value for control individuals in lane 1 and 4.
METHODS. Methods and probes were as described in Table 1. The same filters were successively hybridized with probes for which the patients were disomic and with a non-chromosome 11 probe (collagen type I alpha 1, COL1A1, on chromosome 17). The intensity of the hybridization signals was measured with a SEBIA densitometer. The values for normal control subjects and for patients were estimated through three independent determinations. The value of the 11p15.5 probe versus non-11 internal control probe ratio was calculated for each independent determination. The patient versus control ratio (*P*/*C*) is indicated for each patient showing paternal disomy. For each patient, the *P*/*C* ratio was not very different from 1 (which means that there are two copies of the markers in the DNA of the control and in the DNA of the patient).

(*d*)

	INS	COL1A1	$R=\frac{\text{INS}}{\text{COL1A1}}$	$\frac{P}{C}$	IGF2	COL1A1	$R=\frac{\text{IGF2}}{\text{COL1A1}}$	$\frac{P}{C}$	HBS	COL1A1	$R=\frac{\text{HBB}}{\text{COL1A1}}$	$\frac{P}{C}$	HBBP	COL1A1	$R=\frac{\text{HBBP}}{\text{COL1A1}}$	$\frac{P}{C}$
C	57	235	0.24	—	497	234	2.12	—	367	217	1.69	—	369	43	8.58	—
BW15P	28	131	0.21	0.87	294	130	2.26	1.06	326	190	1.71	1.01	461	64	7.20	0.83
BW21P	35	122	0.28	1.16	252	122	2.06	0.97	167	81	2.06	1.21	169	20	8.45	0.98
BW11P	65	258	0.25	1.04	569	238	2.39	1.12	223	115	1.93	1.14	454	45	10.08	1.17

TABLE 2 Genotypes of 21 sporadic BWS patients for nine 11p15.5 RFLPs

	HRAS1 *Bam*HI	TH *Taq*I	INS *Rsa*I	INS *Hind*III	IGF2 *Sac*I	IGF2 *Ava*II	D11S12 *Msp*I	HBB *Ava*II	HBBP *Hin*cII
BW1P	1/1	2/2	2/2	1/1	1/2	1/2	2/2	1/2	2/3
BW2P	1/1	2/2	2/2	1/2	1/2	1/2	2/2	1/2	2/3
BW3P	1/2	2/2	2/2	2/2	2/2	1/1	1/2	2/2	1/1
BW4P	1/2	2/2	2/2	2/2	2/2	1/1	2/2	2/2	1/1
BW5P	1/1	2/2	1/2	2/2	2/2	1/1	2/2	2/2	1/1
BW6P	1/2	2/2	2/2	2/2	2/2	1/1	1/2	1/2	1/1
BW7P	1/2	2/2	2/2	1/1	2/2	1/1	2/2	2/2	1/1
BW8P	2/2	2/2	1/1	1/1	1/2	1/2	1/2	2/2	1/2
BW9P	1/2	1/2	2/2	2/2	2/2	1/1	1/2	1/2	1/1
BW10P	1/2	2/2	1/1	1/1	1/2	1/1	2/2	1/2	1/1
BW11P	2/2	2/2	1/1	1/1	2/2	1/1	2/2	2/2	1/1
BW12P	2/2	2/2	1/1	1/2	1/1	2/2	1/2	1/1	2/2
BW13P	1/2	2/2	1/1	1/2	2/2	1/1	1/2	2/2	1/2
BW14P	1/2	2/2	1/1	2/2	1/2	1/1	1/2	2/2	1/1
BW15P	2/2	1/2	2/2	2/2	2/2	1/1	1/1	2/2	1/1
BW16P	1/2	2/2	2/2	1/2	1/2	1/2	2/2	1/2	1/3
BW17P	2/2	2/2	1/2	1/2	2/2	2/2	2/2	2/2	1/3
BW18P	2/2	2/2	1/2	2/2	2/2	1/1	2/2	2/2	1/1
BW19P	2/2	1/2	2/2	2/2	2/2	1/1	1/2	2/2	1/3
BW20P	1/2	1/2	2/2	2/2	2/2	1/1	1/2	2/2	1/2
BW21P	2/2	2/2	1/1	1/1	2/2	1/1	2/2	2/2	2/2
F_{BWS}	0.52	0.80	0.85	0.76	0.71	0.80	0.57	0.66	0.57
F_C	—	0.78 (28/36)	0.52 (19/36)	0.51 (18/35)	0.51 (18/35)	0.58 (21/36)	—	—	—
$F_{C/HGM10}$	0.52	0.73	0.52	0.45	0.55	0.62	0.73	0.68	0.51

F_{BWS}, Frequency of homozygous individuals in the BWS population; F_C, frequency of homozygous individuals in the control population. The numbers in brackets indicate the number of homozygous individuals versus the total number of individuals tested. $F_{C/HGM10}$, Frequency of homozygotes as from HGM 10 (ref. 16); *P*, probability that the random distribution of the alleles is responsible for the higher homozygosity in patients than in controls. For the four contiguous loci (INS/*Rsa*I, INS/*Hind*III, IGF2/*Sac*I, and IGF2/*Ava*II) taken together, the frequency of homozygotes was 0.48 (10/21) for BWS patients and 0.18 (6/34) for controls. Methods and probes were as described in Table 1. The test used is a simple one-tailed X^2 homogeneity test (valid since all the expected values are greater than 6); $\chi^2 = 5.65$, $P < 0.01$. The one-tailed test was used because in the hypothesis of isodisomy the frequency of homozygotes is expected to be higher than in controls.

syndromes: the Prader-Willi syndrome with a lack of 15q11-q13 paternal alleles[14], and the Angelman syndrome with a lack of 15q11-q13 maternal alleles[15]. Taken together the lack of maternal alleles in the three sporadic BWS cases reported here, paternal duplication in trisomic BWS patients, retention of paternal alleles in tumours, and higher penetrance in individuals born to female carriers in familial BWS corroborate the involvement of genomic imprinting. Thus we conclude that an unbalanced dosage of maternal and paternal alleles is a common feature for the different aetiological forms of BWS and associated tumours. □

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Embryological and molecular investigations of parental imprinting on mouse chromosome 7

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MOUSE embryos with duplications of whole maternal (parthenogenetic and gynogenetic) or paternal (androgenetic) genomes show reciprocal phenotypes and do not develop to term[1,2]. Genetic complementation has identified the distal region of chromosome 7 (Chr 7) as one of the regions for which both a maternal and paternal chromosome copy are essential for normal development, presumably because of the presence of imprinted genes whose expression is dependent on their parental origin[3,4]. Embryos with the maternal duplication and paternal deficiency of distal Chr 7 are growth retarded and die around day 16 of gestation; the reciprocal paternal duplication embryos die at an unidentified earlier stage[4]. We report here the incorporation of cells with the paternal duplication into chimaeras, resulting in a striking growth enhancement of the embryos. One gene located on mouse distal Chr 7 (ref. 5) is the insulin-like growth factor 2 (*Igf2*) gene, an embryonic mitogen[6]. In embryos with the maternal duplication of distal Chr 7, the two maternal alleles of the *Igf2* gene are repressed. The presence of two paternal alleles of this gene in many cells is probably responsible for the growth enhancement observed in chimaeras. We propose that there are other imprinted genes in