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Somatic Mosaicism for Partial Paternal Isodisomy in Wiedemann-Beckwith Syndrome: A Post-Fertilization Event

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Abstract

Genomic imprinting has been implicated in the aetiology of an overgrowth cancer-prone syndrome, the Wiedemann-Beckwith syndrome (WBS). We have demonstrated uniparental disomy (UPD) for paternal chromosome 11p markers in 5 out of 25 sporadic cases (20%). Delineation of the extent of the disomy region may help in understanding the mechanism and the stage, meiotic or mitotic, of disomy formation in this disease and in associated tumours. Our current studies in WBS patients with seventeen 11p and one 11q markers reveal paternal isodisomy, not heterodisomy, in the five cases. For one case we demonstrate unambiguously that partial isodisomy for 11p and somatic mosaicism for UPD resulted from a post-fertilization event. The restriction of isodisomy to part of 11p in another case, and somatic mosaicism for UPD in three other cases, suggest a mitotic recombinational event that must have occurred after fertilization. Mosaic phenotypes reflect the timing of their origin and the fate of the cells involved, as well as the cell-specific pattern of imprinting. Somatic mosaicism for UPD in four cases may thus explain the incomplete forms of WBS, the association of hemihypertrophy in sporadic WBS and even some cases of isolated hemihypertrophy. This is in agreement with a recent report of paternal isodisomy for 11p markers in a patient with hemihypertrophy, Wilms' tumour and adrenocortical carcinoma. Moreover, the risk of developing a tumour seems higher (50%) for patients with paternal 11p UPD than for WBS patients in general (7.5%).

Key Words
Imprinting
Chromosome 11
Isodisomy
Heterodisomy
Somatic mosaicism
Post-fertilization events
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Introduction

The Wiedemann-Beckwith syndrome (WBS) occurs with an incidence of 1 in 13,700 live births and is characterized by numerous growth abnormalities, including exomphalos, macroglossia, visceromegaly and gigantism [1, 2]. Other occasional abnormalities include adrenal cortical cytomegaly, neonatal hypoglycaemia, ear lobe creases and pits, hemihypertrophy and predisposition (7.5–10%) to several childhood malignancies [Wilms' tumour (or nephroblastoma), adrenocortical carcinoma, hepatoblastoma, rhabdomyosarcoma and, occasionally, pancreatic tumour and neuroblastoma] [3]. Interestingly, kidney, muscle and liver, the same organs that are involved in hemihypertrophy-associated neoplasia are also involved in the visceromegaly of WBS. The clinical findings in WBS patients are highly variable and tend to become less obvious with age.

Two lines of evidence suggest that genomic imprinting probably accounts for the unusual patterns of transmission of WBS and the parental bias in allele loss found in associated tumours [4]. First, rare WBS cases with different cytogenetic abnormalities involving region 11p15 show that duplications are always paternally inherited whereas balanced translocations are always maternally inherited [5–8]. Second, it is now widely accepted that familial forms of WBS (15%) are transmitted in an autosomal-dominant mode with reduced penetrance and variable expressivity [4]. Linkage analysis in three families revealed that the locus for the familial form mapped to region 11p15.5 [9, 10]. On the basis of the three-fold excess of female carriers, a sex-dependent mode of transmission has been demonstrated for familial cases [9, 11, 12].

Uniparental disomy (UPD), namely two paternal alleles without maternal contribution, was demonstrated in three out of eight informative sporadic cases of WBS [13]. Sporadic cases of WBS are significantly more frequently homozygous for 11p15 markers, including INS and IGF2, than normal controls [13]. Chromosome 11p uniparental paternal isodisomy was recently reported in a patient with no features of WBS, but with hemihypertrophy and two neoplasms, a congenital adrenal carcinoma and a Wilms' tumour, which are the hallmarks of WBS [14]. As previously suggested, the same underlying genetic abnormality, namely paternal UPD, in sporadic WBS and in hemihypertrophy, further supports the hypothesis that hemihypertrophy could represent incomplete expression of WBS [15].

Loss of constitutional heterozygosity resulting in homozygosity or hemizygosity for region 11p15 has been observed in tumours of the same types as those observed in WBS. Furthermore, supporting the hypothesis of imprinting at a locus in the 11p15 region, these tumours, including Wilms' tumour, rhabdomyosarcoma, and adrenocortical carcinoma, showed a preferential loss of the maternal allele [for a review see ref. 16]. Loss of 11p15 maternal alleles was reported in the Wilms' tumour of one WBS case [17].

Reminiscent of WBS cases with 11p15 paternal disomy, chimeric mouse embryos with distal chromosome 7 di-paternal contribution were larger than normal embryos, while those with di-maternal contribution were smaller [18]. The distal region of mouse chromosome 7 is homologous to 11p15.5 and carries two genes, H19 and Igf2, imprinted in opposite directions. This led to the proposal that the different forms of WBS could result from an altered/increased expression of a growth promoter gene, perhaps IGF2, expressed from the paternal allele or from decreased expression of a tumour suppressor gene, perhaps H19, expressed from the maternal allele, or

from altered expression of both types of gene [19]. Nonetheless, whatever the function of the WBS gene and whatever the parental origin of the imprint, the different observations are apparently contradictory under a single-locus hypothesis. The involvement of either two genes with different roles or a single gene acting either as a growth promoter or suppressor, is more likely to account for the different observations [19].

The occurrence of UPD in sporadic WBS raised several interesting questions as to the mechanisms involved and their significance. (1) Are the patients isodisomic, heterodisomic or both? (2) Is the whole length of chromosome 11 involved or only parts of it? (3) Did these events occur during meiosis (I or II), or during mitosis at a stage after fertilization, thus leading to somatic mosaicism? (4) Are patients with UPD more prone to developing a tumour? To answer these questions we characterized the extent of disomy in five WBS patients with UPD. Somatic mosaicism and partial isodisomy suggest a post-fertilization event.

Patients and Methods

All twenty-five patients studied presented with typical sporadic WBS including exomphalos, macroglossia, gigantism, hypoglycaemia, and visceromegaly. Patients BW11P, BW15P, and BW21P have already been described [13]. In this report, we analysed 17 new sporadic cases (BW22P, BW23P, BW24P, BW25P, BW26P, BW27P, BW28P, BW29P, BW70P, BW71P, BW72P, BW73P, BW75P, BW76P, BW77P, BW78P, BW79P) and their parents. The constitutional karyotype analysis of WBS patients failed to reveal a structural or numerical chromosomal anomaly except for patient BW72P who carries a balanced translocation involving 11p. Patients BW11P, BW12P, and BW29P developed a Wilms' tumour, and patient BW22P developed a hepatoblastoma. Patients BW28P, BW15P and BW78P have not developed a tumour, at ages 4, 6 and 1, respectively.

Southern-Blot-Analysis

High-molecular-weight DNA was extracted from lymphocytes and lymphoblastoid cell lines. Aliquots of 5–10 µg of constitutional DNA were digested with restriction endonucleases according to the manufacturer's recommendations, resolved by electrophoresis through 0.8% agarose gels and transferred to nylon membranes (Hybond; Amersham). DNA probes were radiolabelled with $\alpha^{32}P$ using the random-priming method. Hybridization was carried out in 0.75 *M* NaCl, 50 µg/ml heparin, 1% SDS, 50 µg/ml salmon sperm DNA, 5% dextran sulphate at 65 °C overnight. The filters were washed and exposed to Hyperfilm-MP autoradiographic film (Amersham).

Southern blots were hybridized to the following probes that detect restriction-fragment-length polymorphisms (RFLPs) on chromosome 11 [20]: HRAS (c-Ha-ras1/BamHI), TH (tyrosine hydroxylase/TaqI), INS (insulin/HindIII, RsaI), IGF2 (insulin-like growth factor II/AvaII, ScaI), D11S774 (22.5.2/MspI), D11S12 (pADJ762/MspI), HBBP1 (β-globin pseudogene/HincII), HBB (β-globin/AvaII), CALCA (calcitonin 1/TaqI), PTH (parathyroid hormone/PstI), FSHB (follicle-stimulating hormone subunit β), D11S151 (p56H2.4/HindIII), D11S324 (p60H1.4/HindIII), D11S325 (p8B1.25/BamHI), CAT (catalase/AvaII), APOA1 (apolipoprotein A-I/XmnI).

To estimate the proportion of disomic cells in patients with somatic mosaicism, the intensity of the hybridization signals for the different alleles was measured with a SEBIA densitometer. Values were estimated in three independent determinations. The ratio of the values of the allele inherited from the father versus the allele inherited from the mother (F/M) was calculated for each independent determination. This ratio was compared with that obtained for a heterozygous control individual (P/H) showing an equal contribution of maternal versus paternal alleles.

Results

Uniparental Paternal Disomy for Five WBS Patients

Using seventeen 11p and one 11q genes or anonymous DNA markers, we have genotyped by Southern-blot analysis a total of twenty-five sporadic WBS cases and their parents. These include a series of eight cases previously published but further analysed in this

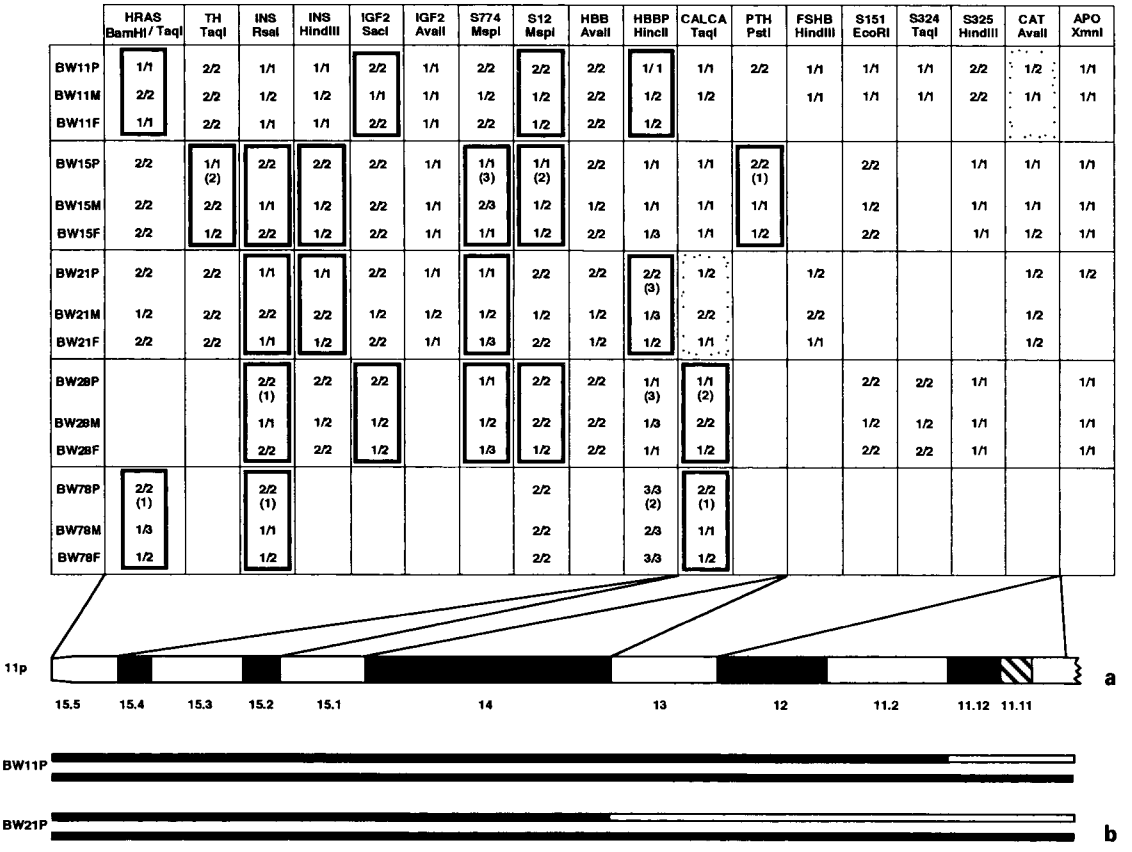

Fig. 1. Genotypes of five WBS patients and their parents for seventeen 11p and one 11q markers. **a** RFLP alleles were named 1, 2 or 3 according to decreasing length. Genotypes in boxes are those for which the patients showed paternal disomy with partial or complete absence of maternal contribution. Genotypes in dashed boxes are those which demonstrate the limit of isodisomy. For patients BW15P, BW21P, BW28P and BW78P the first line represents the main genotype, the second one, in parentheses, indicates the presence of one maternal allele underrepresented on some of the blots, demonstrating mosaicism. In the left hand column, P, M and F = patient, mother and father, respectively. S774 = D11S774; S12 = D11S12; HBBP = HBBP1; S151 = D11S151; S324 = D11S324; S325 = D11S325. **b** Schematic representation of the short arm of chromosome 11 with, below, the extent of isodisomy in patients BW11P and BW21P. Solid line = paternal origin; open line = maternal origin.

report with additional markers [13], and seventeen new patients, all of whom, except one (BW72P), had a normal karyotype. The absence of maternal contribution for some of these markers has already been described for three patients out of the first series of eight, namely BW11P, BW15P and BW21P (fig. 1) [13]. In the present report, two additional cases (BW28P, BW78P), with partial absence of maternal 11p alleles have been characterized. There was evidence for paternal UPD at two loci in 11p, INS/RsaI (fig. 2) and

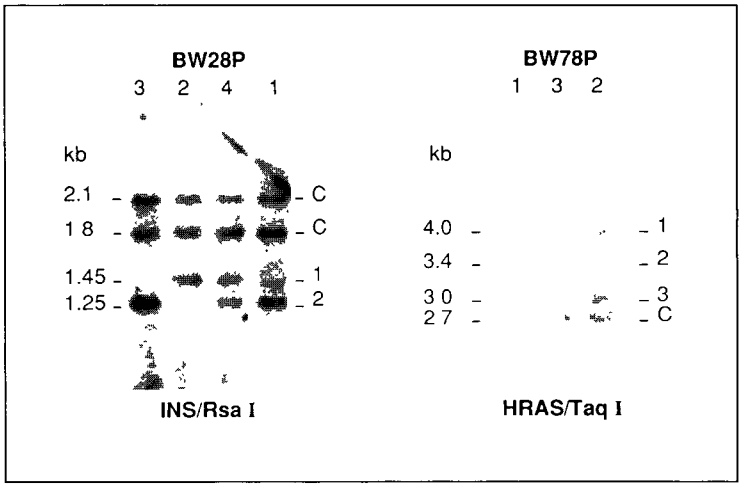

Fig. 2. Paternal UPD in two WBS patients with cytogenetically normal chromosomes 11. Examples of Southern blots showing the partial absence of the maternal allele. In patient BW28P, for INS/RsaI the mother (lane 2) is homozygous for allele 1, the father (lane 3) is homozygous for allele 2. The signal obtained for patient BW28P (lane 1) for allele 2 inherited from the father is significantly higher than the signal for allele 1 inherited from the mother (see table 1). Lane 4 corresponds to the unaffected brother of patient BW28P. In patient BW78P, for HRAS/TaqI the mother (lane 2) is heterozygous for alleles 1 and 3, the father (lane 3) is heterozygous for alleles 1 and 2. The signal obtained for patient BW78P (lane 1) for allele 2 inherited from the father is significantly higher than the signal for allele 1 inherited from the mother (see table 1). C = Constant band.

CALCA/TaqI for patient BW28P, and at three loci in 11p, HRAS/TaqI (fig. 2), INS/RsaI and CALCA/TaqI (fig. 3) for patient BW78P (fig. 1). Densitometer scanning of the blots using a non-11p marker as an internal control revealed the presence of two paternal copies (data not shown). Genotyping of the fifteen remaining cases of WBS and their parents failed to disclose evidence for such a mechanism (data not shown).

Smallest Region of Proven Uniparental Paternal Disomy

As shown in figure 1, in the five patients with UPD, the 'smallest region of proven disomy' is bracketted by two informative markers for which the parents are homozygous for a different allele: HRAS/BamHI and IGF2/SacI for BW11P; TH/TaqI and PTH/PstI for BW15P; INS/RsaI and HBBP1/HincII for BW21P; INS/RsaI and CALCA/TaqI for BW28P, and HRAS/TaqI and CALCA/TaqI for BW78P. In all five cases this region includes INS and IGF2 which are possible candidates for WBS.

Somatic Mosaicism for Paternal UPD

Depending on the stage at which a postzygotic recombinational event occurred, this may result in somatic mosaicism. In four patients, either direct examination (BW28P and BW78P), or overexposure and/or over-

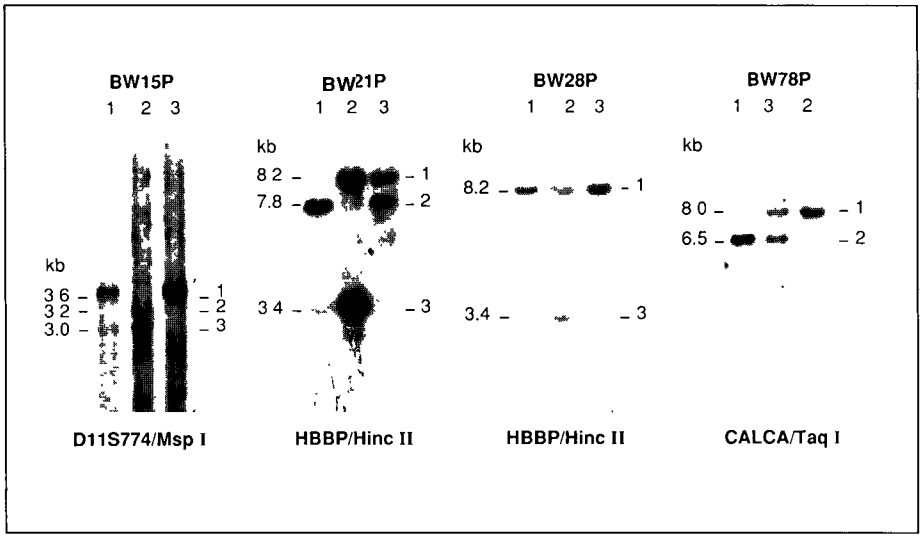

Fig. 3. Evidence for mosaicism in four WBS patients presenting with paternal disomy. Southern-blot experiments showing a faint signal, with the markers used, for the allele inherited from the mother within the region of paternal disomy. Patient BW15P's father (lane 3) is homozygous for allele 1 (D11S774/MspI), the mother (lane 2) is heterozygous 2/3 for the same marker. Both alleles 1 and 3 are detectable in patient BW15P (lane 1). However allele 3 inherited from the mother is underrepresented. Patient BW21P's father (lane 3) is heterozygous 1/2 (HBBP1/HincII), the mother (lane 2) is heterozygous 1/3 for the same marker. Both alleles 2 and 3 are detectable in patient BW21P (lane 1). However, allele 3 inherited from the mother is underrepresented. Patient BW28P's father (lane 3) is homozygous for allele 1 (HBBP1/HincII), the mother (lane 2) is heterozygous 1/3 for the same marker. Both alleles 1 and 3 are detectable in patient BW28P (lane 1). However, allele 3 inherited from the mother is underrepresented. Patient BW78P's father (lane 3) is heterozygous 1/2 (CALCA/TaqI), the mother (lane 2) is homozygous for allele 1 for the same marker. Both alleles 1 and 2 are detectable in patient BW78P (lane 1). However, allele 1 inherited from the mother is underrepresented.

loading (BW15P, BW21P) of the Southern blots disclosed the presence, for some markers, of one of the two maternal alleles in the region of UPD. As shown in figures 1 and 3, the DNA from patient BW15P contains trace amounts of his mother's allele 3 for S774/MspI, the DNA from patient BW21P contains trace amounts of allele 3 for HBBP1/HincII from his mother, the DNA from patient BW28P contains trace amounts of allele 3 for HBBP1/HincII as well as allele 1 for INS/RsaI (fig. 2) inherited from his mother, and the DNA from patient BW78P contains trace amounts of allele 1 for HRAS/TaqI (fig. 2) and allele 1 for CALCA/TaqI from his mother (fig. 3). This cannot be due to contamination by maternal DNA which would result in the simultaneous presence of the other maternal allele for BW15P (S774/MspI), BW21P (HBBP1/HincII) and BW78P /HRAS/TaqI (fig. 1, 3). Densitometer-scanning determination of the respective signal intensities indicated the presence of two types of cells: one with a normal biparental contribution and

one with only paternal contribution. The percentage of disomic cells was estimated in blood DNA at 50%, 53%, 51% and 17% for patients BW15P, BW21P, BW28P, and BW78P, respectively (table 1).

Isodisomy or Heterodisomy?

To determine whether isodisomy, heterodisomy or both could account for paternal UPD we used two approaches: first, isodisomy was unambiguously proven when the patient was homozygous for at least one marker within the 'smallest region of proven disomy' while his father was heterozygous, and, second, the 'extent of contiguous homozygosity' was determined by counting the number of contiguous 11p markers for which the patient was homozygous, hence his probability of having inherited two identical alleles from his father.

Isodisomy was unambiguously proven in BW15P for TH/TaqI, INS/HindIII, S12/MspI, HBBP1/HincII and PTH/PstI; in BW21P for INS/HindIII, S774/MspI, HBB/AvaII and HBBP1/HincII; in BW28P for IGF2/SacI, S774/MspI, S12/MspI and CALCA/TaqI, and in BW78P for HRAS/TaqI, INS/RsaI and CALCA/TaqI. Moreover, the 'extent of contiguous homozygosity' was significantly increased with sixteen markers for BW15P, ten markers for BW21P and twelve markers for BW28P. In patient BW11P, none of the markers within the smallest region of proven disomy was informative. However, in this case, homozygosity for sixteen contiguous markers of the region extending from HRAS to S325 strongly suggested isodisomy. Furthermore, the father was heterozygous for two markers (S12 and HBBP1) while the child was homozygous, suggesting isodisomy. The marker CAT for which the patient is heterozygous possibly represents the limit of isodisomy.

Table 1. Densitometric determination of the percentage of disomic UPD cells versus normal cells in patients presenting with mosaicism

	F/M	P/M	DC, %
BW15P (P)	2.82	3.00	50%
BW15M (H)	0.94		
BW21P (P)	4.34	3.31	53%
BW21M (H)	1.31		
BW28P (P)	3.75	3.07	51%
BW28M (H)	1.22		
BW78P (P)	1.88	1.42	17%
BW78M (H)	1.32		

P = Patient; H = heterozygous individual (mother); F = allele inherited from the patient's father; M = allele inherited from the patient's mother; DC = uniparental disomic cells. In the context of a post-fertilization event leading to UPD, the percentage of disomic cells was obtained using the proportionality of the signal. The number of disomic cells = number of normal cells $\times$ [(P/H) – 1/2.

Extent of Isodisomy

The involvement of a limited portion of chromosome 11 versus that of the entire length of the chromosome can be unambiguously discriminated with a marker for which a maternal contribution is fully restored. This is the case only for patient BW21P at the CALCA/TaqI locus where the child has inherited allele 2 in normal amounts from his homozygous mother (fig. 1, 4). Only a postzygotic mitotic recombination can account for these findings. When no such informative marker is available, the limit of the homozygosity can be considered as the limit of isodisomy. The first proximal marker for which BW11P is heterozygous is CAT in 11p13. For patients BW15P, BW28P and BW78P the limit of the extent of isodisomy could not be determined.

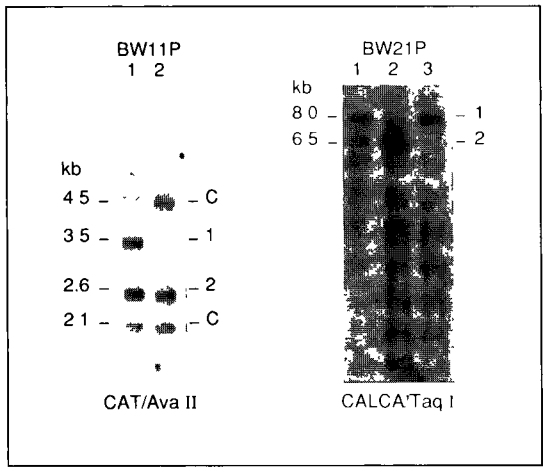

Fig. 4. Extent of isodisomy for two patients. Patient BW11P (lane 1) is heterozygous for CAT/AvaII, which is the only heterozygous marker disrupting the probable paternal isodisomy (homozygosity for sixteen contiguous markers). Lane 2 = mother; C = constant band. Patient BW21P (lane 1) is heterozygous for CALCA/TaqI which is the only heterozygous marker disrupting the proven paternal isodisomy. Lane 2 = mother; Lane 3 = father. The isodisomy thus extends from pter to CAT excluded for patient BW11P, and from pter to CALCA excluded for patient BW21P.

Discussion

UPD for the entire length of the chromosome could result from non-disjunction during meiosis I or II and lead to heterodisomy or isodisomy [21]. Alternatively, postzygotic recombination events would only lead to isodisomy and might be associated with somatic mosaicism. Partial or total isodisomy could result from mitotic recombination or from non-disjunction followed by reduplication of the remaining chromosome, respectively. However, the proportion of disomic cells in lymphocytes may not reflect the situation in the rest of the body tissues. Detection of somatic mosaicism for UPD will depend on the stage and on the cells in which this event occurred. Moreover, there may be selective growth effects associated with paternal duplication/maternal deficiency of this chromosomal region. Conversely, somatic mosaicism for UPD precludes a meiotic event and is not compatible with heterodisomy [22].

We have demonstrated total or partial absence of 11p maternal contribution in five out of twenty-five informative sporadic WBS cases (20% of the cases). In one patient, BW21P, partial isodisomy and somatic mosaicism allowed us to demonstrate unambiguously that the mitotic recombination event responsible for UPD occurred at a post-fertilization stage. UPD was limited to CALCA in patient BW21P. For patients BW15P, BW28P and BW78P, isodisomy could be due to either a meiotic or a mitotic event. Nevertheless, somatic mosaicism implies the occurrence of a post-fertilization event in these patients. In patient BW11P, without detectable mosaicism, we could not determine whether isodisomy (limited to CAT) was due to a mitotic or to a meiotic event. Independently, chromosome 11p paternal uniparental isodisomy was also recently demonstrated in a patient with hemihypertrophy and embryonal neoplasms, Wilms' tumour and adrenocortical carcinoma [14]. Thus all six patients reported so far are isodisomic, not heterodisomic, with paternal UPD for 11p markers. This is in contrast with the Prader-Willi and Angelman syndromes where meiotic events probably account for chromosome 15 uniparental isodisomy and heterodisomy [21, 23–26]. This is not surprising, since trisomy 15, which can lead to UPD by loss of one chromosome 15, represents 1% of neonatal chromosomal abnormalities. However, mechanisms that may explain aneuploidy for the X, the Y, and chromosomes 15, 16, 21 and 22 do not necessarily apply to other chromosomes such as chromosome 11. Mosaicism for trisomy 11 was not observed in the karyotype of our patients.

It can also be postulated that normal development in humans is not compatible with meiotic UPD leading to complete 11p paternal UPD and absence of maternal alleles in all tissues. This happens to be the case for mouse embryos disomic for the distal region of chromosome 7 which show early or late lethality depending on whether they are di-paternal or di-maternal [18]. Thus only WBS cases due to a postzygotic UPD would be seen. In line with this thinking is the finding that in nine cases of monozygotic twins with WBS, all females, each twin pair was discordant for the expression of WBS [27]. In contrast, five cases of monozygous twins all concordant for Prader-Willi syndrome have been reported [28]. This stresses again the possible difference between these syndromes: a postzygotic event in WBS and a meiotic event in Prader-Willi syndrome.

These are, to our knowledge, the first examples of somatic mosaicism for UPD in humans. As reviewed by Hall [29], chromosomal mosaicism has long been recognized in cultured lymphocytes: the presence of a normal set of chromosomes in lymphocytes, with mosaicism for a chromosomal abnormality in fibroblast cells, has been described for an increasing number of phenotypically abnormal individuals. Either somatic or germline mosaicism for point mutations has also been reported for several human diseases.

According to Engel's [30] theory on UPD, homozygosity is expected to result in the phenotypic expression of recessive disorders. But there is now striking evidence from observations in humans and mice that parent-of-origin differences can also account for phenotypic differences in the association with UPD. Furthermore, the different forms of WBS are characterized by a sex-dependent mode of transmission which strongly suggests that imprinting is implicated in WBS. Accordingly, including the recently published case of UPD in a patient with hemihypertrophy [14], all six cases reported so far show partial or complete absence of maternal, not paternal, contribution. This probably endorses differential imprinting rather than the inheritance of two recessive mutant WBS alleles from either the father or the mother.

Three different loci are involved in the predisposition to Wilms' tumour: WT1 in 11p13, WT2 (identical to WBS) in 11p15 and WT3, as yet unmapped [31]. In Wilms' tumours, specific losses of alleles (LOH) for 11p markers have been found in about one-third of the cases. A significant proportion of Wilms' tumours with LOH for 11p markers showed that the loss of alleles was limited to 11p15 [16]. Whenever identifiable, the 11p alleles lost in Wilms' tumour (29/30), in rhabdomyosarcoma (7/7) and in adrenocortical carcinoma (2/2) were of maternal origin and the vast majority of the cases (50%, 42% and 47%, respectively) concerned region 11p15, not 11p13, thus strongly suggesting imprinting of the WT2/WBS locus [16, 17, 32–35]. When the overall frequency of tumours in WBS (7.5%) [3] is compared with the figure in WBS cases with 11p paternal UPD (2/5 cases = 40%) [13], and in WBS cases with dup11p15 (1/15 case = 7.5%) [5, 33], there seems to be an increased risk of tumours in cases with UPD. Moreover, this figure increases to 50% if all cases of UPD, including the case reported by Grundy et al. [14], are taken into account. Hemihypertrophy, either partial or complete, was noted in 12.5% of all WBS patients, and in more than 40% of the WBS children with neoplasms [3]. Thus the congenital absence of the maternal allele would confer a higher risk than the duplication of the paternal allele in trisomic patients. Since not all cases with paternal 11p UPD and/or hemihypertrophy develop a tumour, this may suggest that a second event is still required. Imprinting would be the equivalent

of germline mutation in hereditary cases [35]. The risk of developing a tumour may be directly related to the proportion of cells with UPD in a given tissue. This proportion of cells may remain unchanged, or these cells may possess a growth advantage in certain tissues where 11p genes are imprinted and may override the growth of normal cells. In the context of a post-fertilization event leading to UPD, since the proportion of disomic cells in lymphocytes of patients BW15P, BW21P, BW28P and BW78P was 50%, 53%, 51% and 17%, respectively, this would reflect an early event. Although UPD cases are still too rare to confirm this hypothesis, this would have important consequences in terms of surveillance of such children and genetic counselling. Obviously, more WBS patients and especially those with hemihypertrophy have to be analysed to test whether the same mechanism holds for all patients.

Note Added in Proof

Two WBS patients showing a loss of heterozygosity in the DNA of a nephroblastoma, of the healthy kidney, leucocytes and fragments of tongue have been reported by Schneid H, Vazquet MP, Sevrin D, le Bouc: Loss of heterozygosity in non-tumoral tissue in two children with Wiedemann-Beckwith Syndrome. Growth Regul 1991;1:168–170.

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