Am. J. Hum. Genet. 44:711–719, 1989

Familial Wiedemann-Beckwith Syndrome and a Second Wilms Tumor Locus Both Map to 11p15.5

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Summary

Wilms tumor of the kidney occurs with increased frequency in association with two clinically and cytogenetically distinct congenital syndromes, the Wiedemann-Beckwith syndrome (WBS) and the triad of aniridia, genitourinary anomalies, and mental retardation (WAGR). Constitutional deletions in the latter situation and similar alterations in sporadic Wilms tumors have implicated the chromosomal 11p13 region in neoplastic development. In contrast, some sporadic cases of WBS have been reported to have a constitutional duplication of chromosome 11p15. In order to resolve this seeming paradox, we have analyzed a family segregating WBS for linkage to DNA markers mapped to chromosome 11p. Consonant with the cytogenetic alterations in sporadic WBS cases, we obtained evidence for tight linkage of the mutation causing the syndrome to markers located at 11p15.5. Also consistent with this localization, we identified a subset of Wilms tumors, not associated with WBS, which have attained somatic homozygosity through mitotic recombination, with the smallest shared region of overlap being distal to the beta-globin complex at 11p15.5. These data provide evidence that familial WBS likely results from a defect at the same genetic locus as does its sporadic counterpart. Further, the data suggest there is another locus, distinct from that involved in the WAGR syndrome, which plays a role in the association of Wilms tumor with WBS.

Introduction

Wiedemann-Beckwith syndrome (WBS) is characterized by macroglossia, gigantism, earlobe pits or creases, abdominal wall defects, and an increased risk for the development of tumors, especially Wilms tumor of the kidney, rhabdomyosarcoma, hepatoblastoma, and adrenal carcinoma (Wiedemann 1964; Beckwith 1969; Sotelo-Avila and Gooch 1976). Although most WBS cases are sporadic, families have been reported in which the disease segregates as an autosomal dominant trait with incomplete penetrance and variable expressivity (Kosseff et al. 1972; Best and Hoekstra 1981; Niikawa et al. 1986; Pettenati et al. 1986). It has been noted, however, that the incomplete penetrance follows a striking pattern (Kosseff et al. 1972, 1976; Lubinsky et al. 1974; Niikawa et al. 1986; Pettenati et al. 1986). In familial cases, sibs of affected individuals appear to have a 50% probability of being affected (full penetrance), whereas sibs of unaffected parents inferred to be carriers are themselves rarely affected (nonpenetrance). Although families have recently been reported that include examples of sibships that contain both unaffected carriers and affected individuals, the latter showed only minor manifestations (ear pits) or only part of the WBS syndrome (gigantism and/or macroglossia) (Niikawa et al. 1986; Pettenati et al. 1986). Furthermore, examples of a transmitting male, whether affected or an inferred carrier, with affected offspring are very rare. Proposed models to accommodate these facts have included autosomal dominant sex-dependent inheritance (Lubinsky et al. 1974), "delayed mutation" (Kosseff et al.

Received November 9, 1988; revision received January 6, 1989.

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0002-9297/89/4405-0012$02.00

1976), and extreme variation in expressivity (Niikawa et al. 1986).

In a few patients with sporadic WBS, constitutional duplications of chromosome 11p15 have been reported (Waziri et al. 1983; Turleau et al. 1984). However, confirmation by molecular dosage studies has not yet been reported (Saal et al. 1984; Jeanpierre et al. 1985). Further, other chromosomal alterations, including deletions of 11p11 and 11p13, have also been described in WBS patients (Haas et al. 1986; Schmutz 1986), and so it is not clear whether the same locus is involved in all cases.

Wilms tumor also occurs with high frequency in patients with the syndrome of aniridia, genitourinary anomalies, mental retardation (WAGR), and constitutional deletions including chromosome band 11p13, which is clinically distinct from WBS (Miller et al. 1964; Riccardi et al. 1978). Further support for the notion that this region contains a locus critical for the development of the tumor is that the majority of a series of independently ascertained sporadic Wilms tumors lost constitutional heterozygosity for 11p (Fearon et al. 1984; Koufos et al. 1984; Orkin et al. 1984; Reeve et al. 1984). These studies used DNA markers only from region 11p15. It has subsequently been shown that mitotic recombination occurs in Wilms tumors, with the breakpoint in each of seven cases being proximal to 11p13 (Dao et al. 1987). However, a mitotic recombinant distal to the PTH locus (11p15.1–15.4) has been reported in one Wilms tumor, although the data were misinterpreted since PTH was then thought to be at 11p11 (Raizis et al. 1985). As well, several sporadic rhabdomyosarcomas have been shown to lose heterozygosity for loci distal to the WAGR locus at 11p13 (Scrable et al. 1987). Finally, genetic linkage studies of two Wilms tumor families have excluded the tumor-predisposing locus from both the 11p13 region and the 11p15 region (Grundy et al. 1988; Huff et al. 1988).

In order to determine whether a genetic locus for familial WBS maps to 11p15, as suggested by some of the previous cytogenetic observations in sporadic occurrences, we analyzed an affected family for genetic linkage between WBS and several DNA markers for loci in the region from 11p13 to 11p15. Consistent with the cytogenetic data, the autosomal dominant mutation in this family mapped to 11p15.5. Furthermore, because of the discrepancy between this localization and the accepted location of the "Wilms tumor locus" we examined a series of Wilms tumors for their genotypes at loci on chromosome 11p. We found a subset of these tumors which had attained somatic homozygosity for portions of 11p; the smallest shared region of overlap for these mitotic recombination breakpoints was distal to the beta-globin complex in 11p15.5.

Material and Methods

DNA Analysis

High-molecular-weight DNA was isolated from normal tissue (peripheral leukocytes or normal kidney) and from primary or xenografted samples of histologically confirmed Wilms tumors passaged in nude mice. The DNA was digested to completion with the appropriate restriction endonuclease (Pharmacia), separated by agarose gel electrophoresis, and transferred to nylon membrane (Nytran®, Schleicher and Schuell). The membranes were hybridized with recombinant DNA probes radiolabeled by nick translation in the presence of [^{32}P]dCTP, washed, and autoradiographed according to a method described elsewhere (Koufos et al. 1985).

DNA probes and restriction enzymes were used to detect polymorphisms at the following loci, which are listed in chromosomal order from the 11p telomere (Grzeschik and Kazazian 1985): 11p15.5–HRAS1 (pTBB-2 [TaqI] [Goldfarb et al. 1982]); INS (pHins-310 [*Taq*I] [Bell et al. 1981]); D11S12 (pADJ762, [*Msp*I and *Bcl*I] [Barker et al. 1984*a*]); HBG2 (JW151 [*Hin*dIII] [Antonarakis et al. 1982]); 11p15.4–PTH (p20.36 [*Taq*I] [Schmidtke et al. 1984]); 11p15.1–15.4–CALCA (pHC9 [*Taq*I] [Hoppener et al. 1984]); 11p13–FSHB (pFSHO.5 [*Hin*dIII] [Watkins et al. 1987]); D11S16 (p32-1 [*Msp*I] [Feder et al. 1985]); and CAT (pCATint800 [*Taq*I] [Quan et al. 1985]).

Linkage Analysis

The pedigree of the individuals we investigated is shown in figure 1; the clinical details are reported elsewhere (Hadro et al. 1985; Aleck and Hadro, in press). DNA was extracted from peripheral blood leukocytes from the indicated individuals. Specimens were not available for I-1, or for the spouses of II-2, II-6, II-7, III-22, III-25, III-28, and III-30. Genotypes of the individuals are given in table 1. Genotypes for I-1 and for the spouse of II-7 could be inferred at some loci on the basis of segregation of the markers in their offspring. Individual I-1's genotypes for 11p15.5 loci were left unassigned for linkage analysis because there was a bias to the selection of his offspring who were sampled. Although monozygosity of individuals II-7 and II-8 was

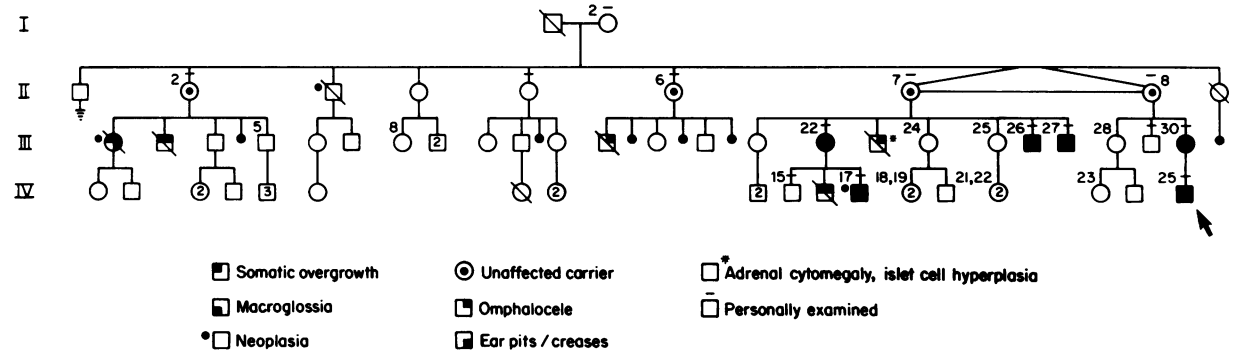


Figure 1 Abridged pedigree of family segregating WBS. DNA samples were available from all individuals labeled with a pedigree number, except for I-1. This figure was redrawn from Aleck and Hadro (in press) by kind permission.

Table 1

Genotypes[a] of Family Members, at 11p13 and 11p15.5 Loci

	Locus						
	11p13			11p15.5			
Pedigree No.	D11S16 *Msp*I	FSHB *Hin*dIII	p13 hap[b]	D11S12 *Msp*I	D11S12 *Bcl*I	INS *Taq*I	HRAS1 *Taq*I
I-1	...	(1,2)	...	...	...	...	...
I-2	1,3	1,1	A,B	2,2	1,2	3,3	3,3
II-2	3,3	1,2	B,C	2,2	1,2	3,3	3,4
II-6	1,3	1,1	A,B	2,2	2,2	3,3	3,4
II-7	1,3	1,2	A,C	2,2	2,2	3,3	3,4
Spouse	...	...	...	(1,2)	(1,1)	(1,3)	...
II-8	1,3	1,2	A,C	2,2	2,2	3,3	3,4
Spouse	2,2	1,2	D,E	1,2	1,1	1,3	3,3
III-5	3,3	1,2	B,C	2,2	1,2	3,3	3,3
III-8	1,1	1,1	A,A	1,2	1,1	1,3	3,4
III-22	1,3	1,2	A,C	1,2	1,2	1,3	3,4
III-24	1,3	1,2	A,C	2,2	1,2	3,3	3,4
Spouse	1,2	1,2	A,E	2,2	2,2	1,3	3,4
III-25	1,1	1,1	A,A	2,2	1,2	1,3	3,3
III-26	1,1	1,1	A,A	1,2	1,2	1,3	3,4
III-27	1,1	1,1	A,A	1,2	1,2	1,3	3,4
III-28	2,3	2,2	C,E	1,2	1,2	1,3	3,3
III-30	2,3	1,2	C,D	2,2	1,2	3,3	3,4
IV-15	1,3	1,2	A,C	1,1	1,1	1,1	3,3
IV-17	1,3	1,2	A,C	1,2	1,2	1,3	3,4
IV-18	1,3	1,2	A,C	2,2	1,2	3,3	3,4
IV-19	2,3	2,2	C,E	2,2	1,2	1,3	4,4
IV-21	1,1	1,1	A,A	2,2	1,2	...	1,3
IV-22	1,1	1,1	A,A	2,2	2,2	3,3	1,3
IV-23	2,3	1,2	...	2,2	2,2	3,3	3,3
IV-25	1,2	1,2	D,F	2,2	2,2	3,3	2,4

[a] Numbers 1–4 represent the alleles observed at each locus in order of decreasing size. The actual sizes, in kilobases, were: D11S16–11, (7.6, 6,4), and (6,4,3); FSHB—15, 12; D11S12—11.6 and 4.3 (for *Msp*I) and 2.1 and 1.7 (for *Bcl*I); INS—6.1 and 4.6; HRAS1—4.4, 3.1, 2.9, and 2.7. Genotypes in parentheses were inferred.

[b] Letters represent the haplotypes of D11S16 and FSHB genotypes: A = 1,1; B = 1,3; C = 2,3; D = 1,2; E = 2,2; F = 2,1.

not rigorously established, linkage analysis was performed under the conservative assumption that they were identical (they were considered as one individual with two spouses).

The four observed alleles at HRAS1 were coded as a three-allele system with the two most common alleles (2.9 and 2.7 kb) both assigned frequencies of .4 and with the remaining six alleles pooled, with a frequency of .2. For D11S12, haplotypes were constructed from the observed genotypes of two polymorphisms identified by separate enzymes. Three of the four possible haplotypes were observed (1–1, 2–1, and 2–2, after *MspI* and *BclI* digests, respectively), and D11S12 was coded as a three-allele locus with frequencies of .11, .57, and .32, respectively, obtained by normalizing the reported frequencies to the sum of the three (Barker et al. 1984*a*). Allele frequencies assigned for the remaining loci were as follows: CAT—.5 and .5; D11S16—.5, .18, and .32; FSHB—.5 and .5; HBG2—.5 and .5; and INS—.3 and .7 (Human Gene Mapping 9 1987; authors unpublished results).

Linkage analysis was carried out with the computer program LINKAGE (V4.7) (Lathrop et al. 1984). On the basis of (*a*) an estimate of the incidence of WBS of 1:13,500 (Thorburn et al. 1970), (*b*) the apparent .15 proportion of all cases which are familial, and (*c*) given the incomplete penetrance (Pettenati et al. 1986), we chose .0001 as a conservative estimate of the population disease-gene frequency. The spontaneous mutation rate at the WBS locus is not known; we assumed that it was 10^{-5} in all reported analyses. Further, it was confirmed that variation of these parameters through a broad range caused no significant change in the results. Individuals were assigned to one of two liability classes: class 1 consisted of family members of generations I and II and all in-marrying spouses, while class 2 consisted of family members who were descendants of couple I-1/I-2 in generations III and IV. Penetrances for the first liability class were .05 and 1.0 for the disease gene heterozygote and homozygote, respectively, and for liability class 2 the corresponding values were 1.0 and 1.0. Further, to take into account the possibility that a case of WBS was sporadic because of a mechanism other than germinal mutation, such as somatic mutation or an environmentally induced phenocopy, the penetrance for the homozygote normal was assumed to be .0001 in both liability classes. The order, HRAS1–INS–D11S12, has been established elsewhere (Kazazian and Junien 1987). The recombination rates between HRAS1 and INS (.03) and between INS and D11S12 (.06) were derived as the average of the male and female rates as reported elsewhere (Leppert et al. 1987).

Densitometric Analysis

Southern blots of *Taq*I-digested normal and tumor DNA from the Wilms tumor patients were hybridized simultaneously to the radioactively labeled probes pTBB-2 (chromosome 11) and DOSLC-4, homologous to a locus on chromosome 17 (Barker et al. 1984*b*). The resultant autoradiograms were scanned with an LKB Ultroscan XL laser densitometer with an internal integrator. The area under the curve representing the signal density from the chromosome 11 probe was divided by that from the chromosome 17 probe, for both normal and tumor DNAs. The number of copies of the chromosome 11 locus in the tumor tissue was obtained by dividing the chromosome 11:17 ratio in the tumor by that in the normal DNA.

Results

A detailed pedigree of the WBS family we analyzed is shown in figure 1, and the genotypes for the 11p13 loci (D11S16 and FSHB) and the 11p15.5 loci (D11S12, INS, and HRAS1) are given in table 1. Linkage analysis was performed assuming an autosomal dominant mode of inheritance with two liability classes of penetrance. This was done to accommodate the previously noted fact that sibs of unaffected carriers are rarely affected (low penetrance), while half the sibs of affected individuals are themselves affected (full penetrance). As well, the possibility of sporadic disease was allowed by assigning a low penetrance value to the homozygous normal individual. Thus, in liability class 1, the probabilities for expression of the disease phenotype were .0001, .05, and 1.00 for individuals homozygous for the normal gene, heterozygous for the disease gene, and homozygous for the disease gene, respectively (low penetrance). Included in this class were sibships containing unaffected carriers, i.e., generations I and II, and all in-marrying spouses. In liability class 2 (full penetrance), the corresponding probabilities were .0001, 1.0, and 1.0 and included all descendants of couple I-1/I-2 in generations III and IV.

The results of a multipoint linkage analysis of WBS to the three 11p15.5 marker loci (HRAS1, INS, and D11S12) are given in table 2. There was evidence for tight linkage, with the maximum LOD score $Z = 3.47$ at $\theta = .0$ with the one LOD down interval extending to $\theta = .15$. Since there were no recombinants between the disease locus and any of the 11p15.5 marker loci,

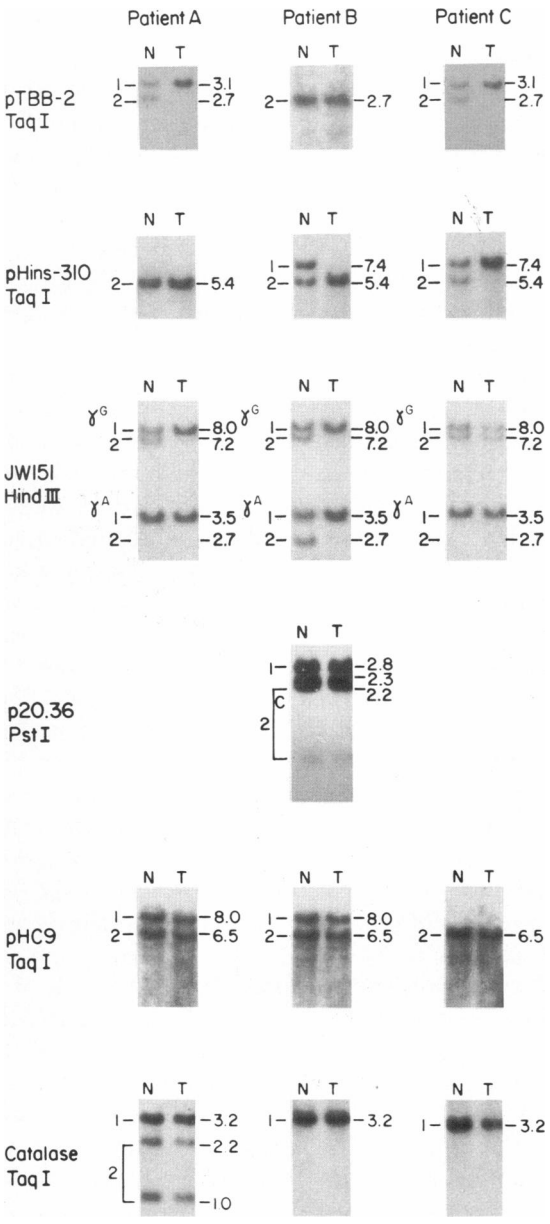
Table 2

Lod Scores for Linkage between WBS and Loci on 11p

Location, Locus	Lod Score at θ of				
	.00	.10	.20	.30	.40
11p13, Haplotype[a]	−7.14	−2.65	−1.27	−.57	−.18
11p15.5, HRAS1-INS-D11S12[b]	3.47	2.84	2.15	1.40	0.60

[a] Haplotype of D11S16, FSHB genotypes, as shown in table 1.

[b] The recombination fraction between HRAS1 and INS was fixed at .03 and that between INS and D11S12 was fixed at .06; the order was established elsewhere (Kazazian and Junien 1987).



the WBS locus cannot be ordered among these markers. Increasing the estimated disease frequency to as high as .001 did not affect the significance of the results, nor did varying the marker-allele frequencies within a broad range.

The possibility of linkage to the 11p13 loci was also examined. Since no recombinants between D11S16 and FSHB were observed and since in physical mapping studies these two loci appear to be very close (Glaser et al. 1987), haplotypes were constructed as shown in table 1. Affected individual III-30 (haplotype C/D) does not share either haplotype with affected individuals III-26 and III-27 (A/A). Further, she did not transmit haplotype C, inherited from her carrier mother, to her affected son, IV-25. Tight linkage to D11S16 and FSHB was also excluded by LOD scores of less than −2.00 at θ = .0 to .1, which remained negative to θ = .4 (table 2). Since FSHB flanks the WAGR locus at 11p13, at a distance estimated to be no greater than 6 cM (Glaser et al. 1986), these results effectively exclude close linkage of the WBS and WAGR loci.

Since WBS showed clear evidence of linkage to 11p15.5—as opposed to linkage to 11p13—a series of Wilms tumors were examined to determine whether any showed evidence for mitotic recombination in the distal region. Figure 2 shows, for three such patients, the constitutional and tumor genotypes at loci on chromosome 11p. Patient A developed a unilateral Wilms tumor at age 5 mo, without signs of associated malformations. The patient's mother and two uncles had had

Figure 2 Mitotic recombination in Wilms tumors, resulting in shared homozygosity for chromosome 11p15.5. Autoradiograms of Southern blots of normal and tumor DNA, from three patients with Wilms tumor, hybridized to the indicated radiolabeled probes. Patient A = CHOP S87–463, patient B = CHMC 433256; patient C = CHMC 320478.

Wilms tumors, and the maternal grandmother had hemihypertrophy (Meadows et al. 1974; Grundy et al. 1988). The tumor was associated with multifocal, bilateral perilobar nephroblastomatosis. Comparison of his normal and tumor DNA revealed maintenance of constitutional heterozygosity at CAT (llp13) and CALCA (11p15.1-15.4). The constitutional DNA was also heterozygous and therefore informative at HBG2 and HRAS1 (11p15.5). Examination of the tumor DNA revealed loss of the 7.2-kb allele at HBG2 and of the 2.7-kb allele at HRAS1. This tumor therefore lost heterozygosity for all loci examined distal to CALCA.

Patient B, also without malformations on examination, was diagnosed at age 18 mo. with a unilateral Wilms tumor associated with perilobar nephroblastomatosis. There was no family history of Wilms tumor or malformations. Patient B's tumor DNA maintained constitutional heterozygosity at both CALCA and PTH (11p15.4) but had lost alleles at the HBG2 and INS (11p15.5) loci. The most distal locus, HRAS1, was uninformative in this patient. The third patient, C, presented at age 29 mo with a unilateral anaplastic histology tumor without evidence of nephbroblastomatosis. There were no malformations or family history of associated conditions. Patient C's constitutional DNA was not informative at the more proximal loci but was heterozygous at HBG2, INS, and HRAS1 (p15.5). The tumor DNA, although still heterozygous at HBG2, retained only the 7.4-kb and the 3.1-kb alleles at the INS and HRAS loci, respectively. The smallest overlapping region that attained somatic homozygosity in these tumors was the region distal to the beta-globin locus at 11p15.5.

Densitometric comparisons of the hybridization signals from tumor and normal DNA were performed to quantify the number of copies of chromosome 11 loci that were within the region which had become homozygous. The ratio of chromosome 11 signal intensity to chromosome 17 signal intensity was similar between normal and tumor DNA in each case (data not shown), consistent with two copies of the HRAS1 locus being present in each tumor. Thus, the mechanism underlying the loss of heterozygosity for chromosome 11p sequences was either a mitotic recombination event or partial loss with reduplication of the remaining homologue.

Discussion

The chromosomal location of a mutation responsible for WBS was suggested by cytogenetic observation of duplications of 11p15 in some patients (Waziri et al. 1983; Turleau et al. 1984). Whether all cases of WBS were due to alterations of the same locus was not known, however, since these observations were confined to presumed sporadic cases of the syndrome and since reports of normal karyotypes have been more common (Saal et al. 1984; Niikawa et al. 1986; Pettenati et al. 1986). Furthermore, several other chromosomal abnormalities have been described (Haas et al. 1986).

The results of our linkage analysis of a family segregating WBS strongly support 11p15.5 as the location of the disease locus. Since this is the same region most frequently reported to be abnormal in sporadic cases, it is likely that WBS is homogeneous with respect to the location of the underlying genetic defect, although the size and nature of such a defect may vary between unrelated individuals. The linkage analysis was performed assuming autosomal dominant inheritance with incomplete penetrance, an assumption that is supported by many reported pedigrees (Niikawa et al. 1986; Pettenati et al. 1986). For the purposes of the analysis only, we chose a value of 1.0 for the penetrance in the disease gene homozygote, although the phenotype—or even the viability—of an individual with such a genotype is not known. In any event, this genotype would be a rare occurrence and thus does not significantly affect the linkage analysis. We modeled the incomplete penetrance as two liability classes, consistent with the pattern in reported families, even though the underlying mechanism is not known. As of 1976, there were no reported cases of affected sibs of unaffected carriers (Kosseff et al. 1976). A few such families have now been reported, although the affected individuals had only minor signs or partial forms of the syndrome and variable expressivity was suggested as the explanation for this pattern (Niikawa et al. 1986; Pettenati et al. 1986). Nevertheless, two distinct classes of sibship have been apparent, one with full penetrance and usually high expressivity and one with very low penetrance or expressivity. The pedigree of the kindred we investigated fits this pattern, and a model invoking an average penetrance of approximately .5 for all sibships (Pettenati et al. 1986) does not seem as appropriate for linkage analysis.

A sex-dependent autosomal dominant mode of inheritance of WBS has been hypothesized because examples of affected children born to transmitting males, whether affected or assumed carrier, have been rare (Lubinsky et al. 1974). In this pedigree, it is interesting that the 11p15 haplotype segregating with WBS was transmitted by the unaffected male I-1 who had four unaffected but obligate-carrier daughters. These four

females in turn had affected children, both males and females. A model involving sex-specific genomic imprinting (Sapienza et al. 1987), as has been proposed for Wilms tumor (Wilkins 1988), could account for these patterns. If the paternally transmitted allele at the WBS locus is always functionally inactivated, then offspring who inherit the mutation from their father, even if he himself is affected, would not be expected to manifest the syndrome; rather, they would be unaffected carriers. Offspring of females carrying the mutation would be expected to be affected in a proportion consistent with a fully penetrant dominant condition. It is possible that decreased fertility in the affected male could account for the paucity of examples of affected-male-to-affected-offspring transmission, but the possibility of altered fertility in the unaffected male carrier is not supported by numerous examples in published pedigrees (Niikawa et al. 1986). Further follow-up and molecular studies of families such as these will allow testing of this hypothesis.

The present mitotic recombination mapping results in Wilms tumors provide evidence for a locus at 11p15.5. Although we cannot rule out a localized genetic defect at more proximal loci, the smallest shared chromosomal region lost from the tumors is distal to the beta-globin complex. This region is clearly distinct from the WAGR locus at 11p13 and provides an explanation for the independent association of Wilms tumor with the clinically disparate syndromes WBS and WAGR. These observations further support the previous suggestion that Wilms tumor is genetically heterogeneous (Grundy et al. 1988; Huff et al. 1988). It is noteworthy, however, that all Wilms tumors previously reported to have lost heterozygosity for 11p markers appear to have lost a copy of both the 11p13 and 11p15 loci. It thus remains a possibility that alterations at both loci are necessary for tumorigenesis. Alternatively, loss of the distal p15 locus in these latter cases may simply be an epiphenomenon of the mechanisms underlying loss of heterozygosity for the 11p13 locus, e.g., mitotic recombination, whole chromosome loss, or partial chromosome loss (Dao et al. 1987).

WBS patients have an increased susceptibility to other pediatric embryonal neoplasms in addition to Wilms tumors (Sotelo-Avila and Gooch 1976). These neoplasms include rhabdomyosarcoma, hepatoblastoma, and adrenal tumors. Sporadic rhabdomyosarcomas and hepatoblastomas have been shown to lose heterozygosity for 11p (Koufos et al. 1985), and a locus for rhabdomyosarcoma has recently been mapped to 11p15.5 by mitotic recombination mapping (Scrable et al. 1987). Also, two tumors—an adrenal adenoma (Hayward et al. 1988) and a hepatoblastoma (Little et al. 1988)—from patients with WBS have been reported to have lost heterozygosity for 11p15.5 loci, although the more proximal loci examined were noninformative. It is possible that a pleiotropic mutation at 11p15.5 underlies the etiology of the congenital syndrome as well as these related tumors. Alternatively, these diseases may be due to defects at closely linked but separate loci.

Acknowledgments

We would like to thank Drs. M. Wigler, J. Hoppener, H. H. Kazazian, H. Mayer, R. L. White, G. I. Bell, R. A. Gravel, and P. C. Watkins for kindly providing us with the DNA probes used in this study. We also thank Dr. A. T. Meadows for providing the samples from patient A, Drs. K. Bove, J. Chatten, J. P. de Chaderavian, and J. B. Beckwith for reviewing the histology of the Wilms tumors, and Drs. Tim Bishop and Jurg Ott for helpful discussions. K. M. was supported in part by NSERC grant A3354 and Programme d'Actions structurantes of the Québec Ministère de l'Enseignement Supérieur et de la Science.

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