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A Third Wilms' Tumor Locus on Chromosome 16q[1]

Marion A. Maw, Paul E. Grundy, Lynn J. Millow, Michael R. Eccles, Roseanne S. Dunn, Peter J. Smith, Andrew P. Feinberg, Dave J. Law, Malcolm C. Paterson, Perry E. Telzerow, David F. Callen, Andrew D. Thompson, Robert I. Richards, and Anthony E. Reeve[2]

Molecular Carcinogenesis Laboratory, Department of Biochemistry, University of Otago, P. O. Box 56, Dunedin, New Zealand [M. A. M., L. J. M., M. R. E., A. E. R.]; Departments of Pediatrics and Medicine, Cross Cancer Institute and Faculty of Medicine, University of Alberta, Edmonton, Alberta, T6G 1Z2 Canada [P. E. G., M. C. P., P. E. T.]; Department of Pathology, University of Queensland, Herston, Queensland, Australia 4006 [R. S. D., P. J. S.]; Howard Hughes Medical Institute and Departments of Internal Medicine and Human Genetics, University of Michigan Medical School, Ann Arbor, Michigan 48109-0650 [A. P. F., D. J. L.]; and Department of Cytogenetics and Molecular Genetics, Adelaide Children's Hospital, North Adelaide, South Australia, 5006 [D. F. C., A. D. T., R. I. R.]

ABSTRACT

Loss of heterozygosity studies have been used to identify chromosomal regions which are frequently deleted and thus indicate areas which may harbor tumor suppressor genes. As a result, both the *WT1* gene located in chromosome 11p13 and an unidentified gene(s) within chromosome 11p15 have been implicated in Wilms' tumorigenesis. Cytogenetic and linkage studies suggest that additional non-chromosome 11 sites are involved in Wilms' tumor. Because these sites may also involve loss of heterozygosity, loci on 33 autosomal arms were screened for allele loss in a series of Wilms' tumors. We found that in addition to loss on chromosome 11p (11 of 25 informative tumors) there was significant loss on chromosome 16q (9 of 45 informative tumors), while the total frequency of allele loss excluding these loci was low (9 of 426 total informative loci). These data indicate that losses of both chromosome 11p and 16q alleles are nonrandom events and suggest that 16q is the location of a third tumor suppressor gene underlying Wilms' tumorigenesis. The parental origin of the lost chromosome 16q allele was determined in eight sporadic tumors. Alleles of paternal and of maternal origin were each lost in four sporadic tumors indicating that, unlike chromosome 11p, alleles of either parental origin are lost on 16q.

INTRODUCTION

Wilms' tumors are epidemiologically and histopathologically heterogeneous (1, 2). The molecular pathogenesis of Wilms' tumor is also complex and involves at least two genes on chromosome 11 (3–9). That chromosome 11 is not the only site involved in Wilms' tumor development has been suggested by cytogenetic analysis of tumor cells (10). Other loci are also implicated by the exclusion of linkage between familial Wilms' tumor and loci on 11p (11–13), the association of Wilms' tumor with neurofibromatosis type I which maps to chromosome 17q11.2 (14, 15) and the presence of putative precursor lesions of Wilms' tumor in the kidneys of patients with trisomy 13 and 18 (16, 17).

Mutational events sometimes involve extensive loss of one homologue of a chromosomal segment and DNA polymorphisms distributed along the chromosome can be used to determine the extent of the LOH[3] in individual tumors. The smallest region of LOH shared between different tumors can then be used to delineate the location of the relevant gene. In combination with cytogenetic data which direct the search towards potential areas of interest, LOH mapping has proven valuable in finding tumor suppressor genes (18, 19). Previous LOH analysis with polymorphisms mapping near genes known to act as suppressors in other cancers failed to detect the involvement of any of these sites in Wilms' tumor (20). In contrast, this study detected allele loss at loci on chromosome 16q which is a site implicated by cytogenetic analysis of Wilms' tumors. LOH mapping suggested that the region distal to chromosome 16q13 harbors a gene involved in the development of Wilms' tumor. It has been shown previously that the 11p alleles lost in Wilms' tumors and rhabdomyosarcomas are exclusively of maternal origin which suggests that a putative tumor-related gene in 11p15 may be imprinted (9, 21–24). We investigated the parental origin of the alleles lost on 16q and found that there was no bias for maternal allele loss which indicates that imprinting does not operate at this site.

MATERIALS AND METHODS

Sample Collection. Samples of blood, normal kidney, and tumor from Wilms' tumor patients and of blood from their parents were collected from within New Zealand and Australia and through the Pediatric Oncology Group (St. Louis, MO), Study 9046. Histology and tumor stage were defined according to the National Wilms' Tumor Study nomenclature (25).

DNA Isolation, Digestion, Gel Electrophoresis, and Hybridization. Because the percentage of contaminating normal cells is less than 10% in most Wilms' tumors (Ref. 26; this laboratory), the tumor tissue was not histopathologically enriched prior to DNA isolation. DNA samples (3 μg) from paired Wilms' tumors and normal tissues (Epstein-Barr virus-transformed lymphoblastoid cell lines of patients, lymphocytes or kidney) were digested with appropriate restriction enzymes and electrophoresed in 0.8% agarose gels. The digested DNA was transferred onto nylon filters which were hybridized with [^{32}P]DNA probes in 0.5 M sodium phosphate (pH 7.2), 7% sodium dodecyl sulfate and 1 mM EDTA. Filters were washed with 0.1xSSC, 0.5% sodium dodecyl sulfate at 68°C and exposed to X-ray film. The DNA probes have been described (27, 28) and are listed in Table 1 together with the respective restriction enzymes used. The probes were supplied by the Howard Hughes Medical Institute, the Imperial Cancer Research Fund, ICI Diagnostics, or the American Type Culture Collection. Where more than one tumor occurred in a single patient, the tumors were scored separately for LOH at each polymorphic locus.

The probes used in the LOH analysis of chromosome 16 loci were: 3'HVR (D16S85), pEKMDA2.1 (D16S83), p16-1 (D6S23), p16-5(D16S27), pCETP.11A (CETP), ACHF3 (D16S10), hp2-α (HP), pEKXp3B (CTRB), and p79-2-23 (D16S7). 16AC6.21 (D16S301) is a multiallele $(AC)_n$ microsatellite (29) which was analyzed by polymerase chain reaction of genomic DNA and electrophoresis in 5% polyacrylamide urea gels. The regional localizations of the probes are those reported elsewhere (27–30).

Restriction fragment length polymorphism analyses with probes to the 11p13 loci *CAT* and *FSH* and to the 11p15 loci *CALCA, GLO, INS,* and *HRAS* were used to detect 11p allele loss. The chromosome 11p data on some of the tumors are already reported in more detail elsewhere (7).

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[2] To whom requests for reprints should be addressed.

[3] The abbreviation used is: LOH, loss of heterozygosity.

Table 1 *Loss of Heterozygosity at Autosomal Loci in Wilms' Tumor*

Chromosome	Locus	Probe	Enzyme	Allelic loss/informative cases		
				Current study	Mannens *et al.* (20)	Total
1p33-35	D1S7	LMS1	*Hinf*I	1/8		1/8
1q21-q23	MUC1	pMUC10	*Hinf*I	0/8		0/8
1q42-43	D1S8	λMS32	*Alu*I	0/8		0/8
2p	D2S44	pYNH24	*Taq*I	0/8		0/8
2q33-35	p5G1	CRYG1	*Taq*I	0/16		0/16
3p22-p21.33	THR	pBH302	*Hind*III		0/16	0/16
3q	D3S42	pEFD64.1	*Pvu*II	0/10		0/10
4p16.3	D4S125	pYNZ32	*Taq*I	1/13		1/13
4q11-qter	D4S24	CG16	*Pvu*II	0/9		0/9
5p13	D5S20	pJ071H-A	*Eco*RI	0/11		0/11
5q22-q31	D5S6	M4	*Bam*HI		1/17	1/17
5q35-qter	D5S43	λMS8	*Alu*I	0/5		0/5
6p	D6S44	pYNZ132	*Taq*I or *Pst*I	0/10		0/10
6q24-27	ESR	pOR3	*Pvu*II	0/13		0/13
7pter-p14	D7S135	TM102L	*Taq*I	0/11		0/11
7pter-q22	D7S21	λMS31	*Hinf*I	0/8		0/8
7q36-qter	D7S22	pλg3	*Hinf*I	0/7		0/7
8q	D8S39	pMCT128.2	*Pvu*II or *Pst*I	1/26		1/26
9p	NRASL1	pCN2	*Taq*I	0/9		0/9
9q34	D9S7	pEFD126.3	*Taq*I	0/9		0/9
10pter-p13	D10S28	cTBQ7	*Taq*I	0/17		0/17
10q21-26	D10S20	OS-3	*Taq*I	0/11		0/11
11p15.5	HRAS	pEJ 6.6	*Msp*I or *Taq*I	11/25	7/20	18/45
12p12.3-13.3	A2M	pHLA2M1	*Taq*I	0/8		0/8
12q24.3-qter	D12S11	λMS43	*Hinf*I	0/7		0/7
13q14	RB1	p68RS2.0	*Eco*RI		0/9	0/9
13q14	D13S22	pG14E3.8	*Hind*III		0/12	0/12
14q32.32	IGHJ	p3.4BHI	*Bam*HI	2/18		2/18
14q32.32–q32.33	D14S19	pHHH208	*Bam*HI	0/21		0/21
15pter-q13	D15S24	CMW1	*Taq*I	0/8		0/8
16p13.3	D16S83	pEKMDA2.1	*Taq*I	1/43		1/43
16q24	D16S7	p79-2-23	*Taq*I	9/45		9/45
17p13.3	D17S5	pYNZ22.1	*Bam*HI	1/31	1/27	2/58
17q23-q25.3	D17S4	pTHH59	*Pvu*II	1/22		1/22
18q21.3-qter	D18S5	OS-4	*Pst*I or *Taq*I	1/19		1/19
19p	D19S20	pJCZ3.1	*Pst*I or *Pvu*II	0/9		0/9
19q13.2	APOC2	pCII-711	*Taq*I	0/10		0/10
20p12	D20S5	pR12.21	*Pvu*II	0/7		0/7
20q	D20S19	pCMM6	*Taq*I	0/9		0/9
21pter-q21.1	D21S26	26C	*Pst*I	0/4		0/4
22q11	IGLV	pV3.3	*Taq*I		0/10	0/10

RESULTS AND DISCUSSION

DNA from paired Wilms' tumors and normal tissue samples was screened for allele loss with restriction fragment length polymorphisms mapping to loci from 33 chromosomal arms representing 20 autosomes. This analysis combined with data from a previous smaller survey (20) is shown in Table 1. Loss of heterozygosity was observed frequently on chromosome 11p (40%), as expected but was also moderately frequent on chromosome 16q (20%). If the loci on chromosomes 16 and 11 are excluded, then loss was observed at less than 5% of the total informative loci. Because the rate of background loss was low and the number of tumors informative for the locus D16S7 was large (45), it was evident that chromosome 16q represented a site of nonrandom LOH. The possibility that additional sites also lose heterozygosity at a moderate frequency was not investigated because there was insufficient DNA for a more extensive LOH analysis. It was, however, observed that the losses detected at 1p, 4p, 8q, 14q, 17p, 17q, and 18q occurred in separate tumors; *i.e.*, these losses did not result from analysis of a single massively rearranged tumor.

The losses detected on 11p are consistent with previous studies (5–9), while LOH on 16q suggests that this chromosomal region contains another tumor suppressor gene underlying Wilms' tumorigenesis. Although 11p LOH occurred at twice the frequency of 16q LOH, it is difficult to determine the relative importance of these allelic losses because 11p contains at least two loci involved in Wilms' tumor onset (3–9).

While the fraction of alleles lost in individual tumors may be associated with a more advanced disease (31), this may also reflect the number of specific events required to produce that cancer and/or the frequency of nonspecific loss. The frequencies of allele loss in this study are similar to those reported in another embryonal tumor, neuroblastoma (32), whereas the rates of both specific and general losses in some adult cancers are considerably higher (31, 33). Embryonal neoplasms such as Wilms' tumor and neuroblastoma may require fewer critical events compared with some adult cancers. This distinction might be due to differences in the cellular environment in which these tumors initiate (26). For example, Wilms' tumors initiate within the rapidly dividing nephrogenic mesenchyme (34) so the mutated cells can initially proliferate as part of the normal process of embryonic kidney development. By contrast, the expansion of a mutated clone in the adult may require multiple mutations to overcome growth restraints imposed by the mature surrounding tissues. A second explanation relates to the differing lengths of time during which embryonal and adult tumors arise. Wilms' tumors may have less time to develop and thus may be less likely than adult tumors to accumulate nonspecific chromosomal abnormalities.

Although chromosomes 11p and 16q were the only common sites of LOH identified in this study, other chromosome arms may yet be shown to harbor tumor suppressor genes involved in Wilms' tumorigenesis. A more extensive analysis, with a large tumor series and using further probes for each chromosome arm, might detect a significant rate of LOH at other loci,

particularly on 1p, 4p, 8q, 14q, 17p, 17q, and 18q inasmuch as these arms lost alleles in the current study. In this study, as in most studies to date, only one DNA probe was used for the majority of chromosome arms (31–33). That genes may be overlooked was demonstrated in the case of colorectal cancers which lost chromosome 1p alleles at a high frequency (42%) with the probe D1S7, while previous workers detected only a low rate of loss (8%) with a different chromosome 1p probe (35). Another factor which could make tumor genes difficult to detect by LOH analysis is that LOH of a specific locus may occur in only a subset of tumors and this might not be readily apparent because of a small sample size. A further complication is that the significance of specific low frequency losses such as those observed in this study on 1p, 4p, 8q, 14q,17p, 17q, and 18q and on chromosomes 5q and 17p by Mannens *et al.* (20) is difficult to assess when the general frequency of LOH is low.

The chromosomal sites most commonly affected by cytogenetic alterations in Wilms' tumor have been studied by others (10). In view of the losses observed in this study, it is of interest that rearrangements resulting in partial monosomy of chromosomes 1p, 11p, 16q, and 17 have been reported (10). The allelic losses on 4p and 5q do not correspond to any known cytogenetic alterations, whereas the infrequent losses detected with the immunoglobulin gene probe at 14q32.3 may be important because translocations and deletions affecting chromosome 14q have been detected in Wilms' tumor patients (36–39). Frequent LOH of chromosome 14q has also been observed in neuroblastomas but the critical region has not been finely mapped (32). Chromosomes 8q and 18q are often trisomic in Wilms' tumors (10) whereas a low frequency of allele loss was observed for both regions in this study. The observation that the same site can be affected by allele loss or gain in different tumors resembles the situation observed with chromosome 11p15. LOH of this region is frequently observed in Wilms' tumors while triplication occurs in some patients with Beckwith-Wiedemann syndrome which predisposes to Wilms' tumor (5–9, 20, 40).

Chromosome 11p15 sequences lost in Wilms' tumors and rhabdomyosarcomas are of maternal origin (9, 21–24) which could indicate that a gene in this region is imprinted so that only one allele is capable of providing an activity required for either tumor suppression or transformation (22–23). Support for this view is provided by the observation that a gene located in chromosome 11p15 and expressed in many Wilms' tumors (41, 42), namely insulin-like growth factor II, is imprinted in the mouse (43). To determine whether chromosome 16q loss involves a similar mechanism, we examined a separate series of Wilms tumors. Of 63 tumors examined, 61 were constitutionally heterozygous for at least one locus on chromosome 16. In agreement with the previous analysis of the completely independent tumor series described above, 11 of 61 (18%) tumors showed loss of heterozygosity. Parental DNA was available on 8 of the 11 cases. Three of these eight cases also lost heterozygosity on chromosome 11p and in each case the losses were of maternal origin. The parental origin of the lost 16q alleles was determined for these eight tumors using D16S7 and some of the resulting autoradiographs are shown in Fig. 1. In four of the tumors the maternal allele was lost while in the other four cases the paternal allele was absent. Thus, in contrast to the extensive studies demonstrating preferential maternal loss on 11p, there is no evidence for an imprinting mechanism or for preferential mutation of either allele at the 16q locus (9, 21–24). This result supports the view that a characteristic of the chromosome 11p15 region dictates the parental bias in 11p allele loss and refutes the notion that a general mechanism operates which favors the loss of maternal alleles at all loci in this tumor.

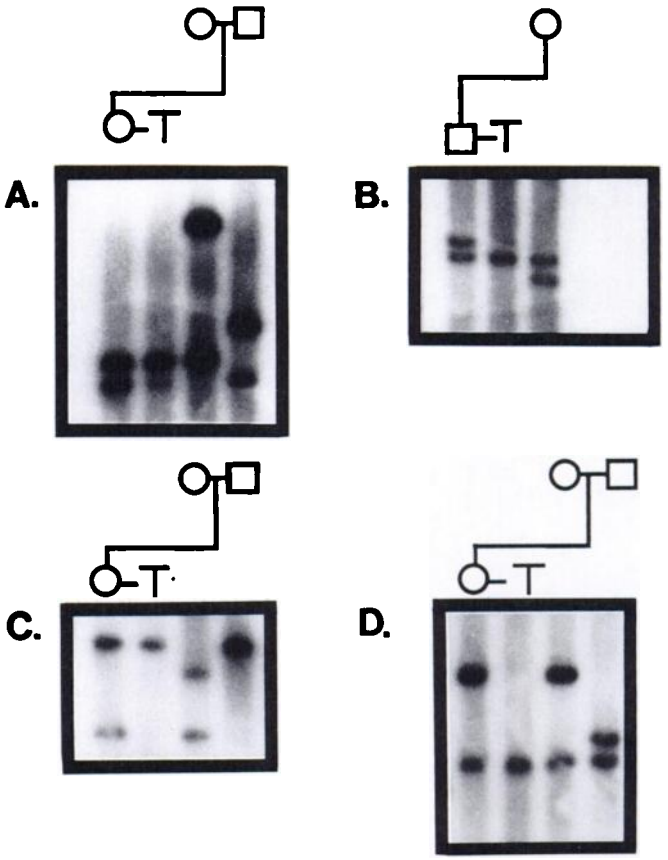

Fig. 1. Parental derivation of allelic loss on 16q. Autoradiograms of Southern blots of DNA from four Wilms' tumor patients, their tumors (*T*) and parents probed with p79–2–23 (D16S7). The parental symbols above each blot are directly aligned with the respective DNA samples. *A*, Case 41; *B*, Case 42; *C*, Case 53; *D*, Case 71.

The region of chromosome 16 LOH in each of the tumors always involved the long arm (data not shown). The smallest region of overlap for 16q allele loss was determined using polymorphic DNA probes (Fig. 2). Eight tumors retained heterozygosity for at least one locus on 16q and were therefore useful for determining the critical chromosomal region. Tumors 1–7 showed allelic loss which overlapped between 16q21 (D16S10) and 16q24.1 (CTRB), while tumor 8 had interstitial LOH only at 16q13–16q21 (CETP). The relative locations of CETP, D16S10, and D16S301 in Fig. 2 were based upon linkage and physical mapping data (28–30). However, one explanation for the nonoverlap of LOH between tumors 7 and 8 is that the marker D16S10 maps either proximal to CETP or distal to D16S301. The nonoverlap of these deletions would also be explained if a complex chromosomal rearrangement had occurred in tumor 8 resulting in multiple deletions. Alternatively, it is possible that there are two loci on chromosome 16q analogous to the two Wilms' tumor loci on chromosome 11 (3–9).

Translocations resulting in deletions of 16q have been reported not only in Wilms' tumors (10, 44) but also in several adult and embryonal carcinomas (45, 46). Furthermore breast, prostate, and hepatocellular carcinomas have recently been shown to involve 16q LOH at high frequencies (33, 47, 48).

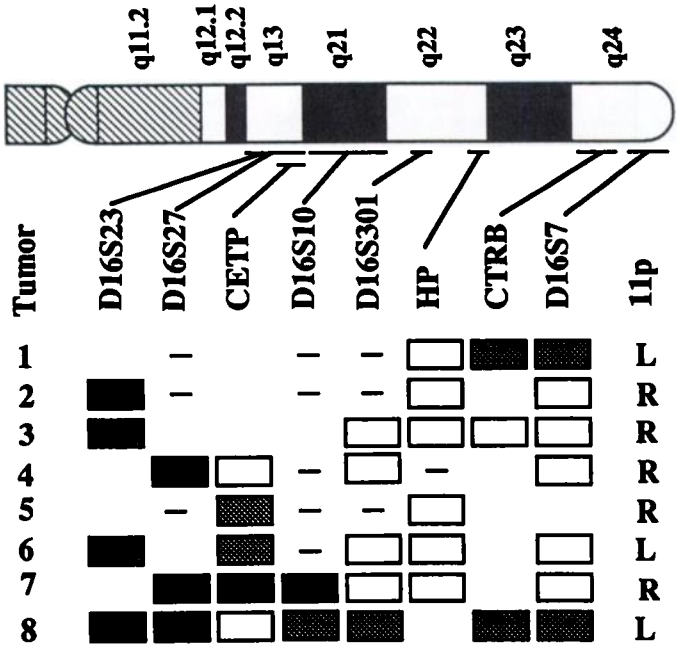

Fig. 2. Mapping of chromosome 16q allele loss in Wilms' tumor. Retention of both alleles (■), loss of one allele (□), uninformative locus (*blank space*), and sample not tested (—). The regional localization of each probe is indicated in the ideogram (27–30). Loss of one allele at one or more informative chromosome 11p loci (*L*), retention of both alleles at all informative loci (*R*).

LOH mapping of prostate and hepatocellular carcinomas has implicated a gene(s) located in distal 16q22.1-qter in these cancers (48, 49). This chromosomal region overlaps with the area lost in Wilms' tumors and suggests that a single gene could be involved in all of these cancers.

A potential candidate for the critical Wilms' tumor gene on chromosome 16q is uvomorulin. This gene maps in 16q22.2 between D16S301 and HP (28–30) and thus is within the region of loss in Wilms' tumors but is located proximal to the losses in prostate and hepatocellular carcinomas. Uvomorulin encodes a cell adhesion molecule. This is of interest because cell adhesion molecules may act as tumor suppressors (50). For example, the *DCC* gene has sequence similarity with neural cell adhesion molecules and is mutated in colorectal carcinomas (19). Uvomorulin may play a role in preventing tumor invasion in some cancers (51–55) and it has been shown that invasion of collagen gels by undifferentiated breast carcinoma cells could be prevented by transfection with E-cadherin complementary DNA but was restored when treated with anti-cadherin antibodies (53). It is conceivable that this protein could play a role in the development of some Wilms' tumors, as uvomorulin is expressed in the epithelial cells of the developing chicken kidney (56) and in the distal tubules and collecting ducts of the adult human kidney (52). However, in a preliminary Southern blot analysis with 37 tumors using a partial uvomorulin complementary DNA clone no rearrangements were found (data not shown).

Previous work has suggested that chromosomes 11p13, 11p15, and at least one non-chromosome 11 locus are involved in predisposition to Wilms' tumor (3–9, 11–13). This study has demonstrated that sporadic Wilms' tumors involve the nonrandom loss of chromosome 16q alleles. Whether this chromosome 16q locus represents the familial Wilms' tumor-predisposing locus in families which do not show linkage to chromosome 11p (11–13) is currently under investigation. Alternatively, this region may harbor a fourth gene involved in Wilms' tumorigenesis. A comparison of tumors with and without LOH for 16q did not reveal any differences with regard to stage, histological features or age at diagnosis (data not shown). It is possible that a more direct assay of the activity of the critical locus will be required to determine the role of this region in the disease.

Allele losses at chromosomes 11p and 16q were observed in individual tumors at neither, one, or both sites. These data are insufficient to determine which combinations of 11p and 16q mutations are necessary and/or sufficient for the initiation or progression of Wilms' tumor. Previous studies using LOH and cytogenetic analysis have shown that Wilms' tumors involve multiple genetic alterations (10, 11, 57–61) yet epidemiological data has suggested that only two rate-limiting events are involved in Wilms' tumorigenesis (62). Collectively, these results suggest that additional non-rate-limiting steps may also play an important role in Wilms' tumorigenesis. Further investigation of the Wilms' tumor locus on chromosome 16q should therefore increase our understanding of the genetic complexity of this pediatric tumor.

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