WT1 Mutation and 11P15 Loss of Heterozygosity Predict Relapse in Very Low-Risk Wilms Tumors Treated With Surgery Alone: A Children's Oncology Group Study

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ABSTRACT

Purpose

Children's Oncology Group defines very low-risk Wilms tumors (VLRWT) as stage I favorable histology Wilms tumors weighing less than 550 g in children younger than 24 months of age. VLRWTs may be treated with nephrectomy alone. However, 10% to 15% of VLRWTs relapse without chemotherapy. Previous studies suggest that VLRWTs with low *WT1* expression and/or 11p15 loss of heterozygosity (LOH) may have increased risk of relapse. The current study validates these findings within prospectively identified children with VLRWT who did not receive adjuvant chemotherapy.

Patients and Methods

Fifty-six VLRWTs (10 relapses) were analyzed for mutation of *WT1*, *CTNNB1*, and *WTX*; for 11p15 LOH using microsatellite analysis; and for *H19DMR* and *KvDMR1* methylation.

Results

11p15 LOH was identified in 19 (41%) of 46 evaluable VLRWTs and was significantly associated with relapse ($P < .001$); 16 of 19 were isodisomic for 11p15. *WT1* mutation was identified in nine (20%) of 45 evaluable VLRWTs and was significantly associated with relapse ($P = .004$); all nine cases also had 11p15 LOH. All evaluable tumors showing LOH by microsatellite analysis also showed LOH by methylation analysis. Retention of the normal imprinting pattern was identified in 24 of 42 evaluable tumors, and none relapsed. Loss of imprinting at 11p15 was identified in one of 42 tumors.

Conclusion

WT1 mutation and 11p15 LOH are associated with relapse in patients with VLRWTs who do not receive chemotherapy. These may provide meaningful biomarkers to stratify patients for reduced chemotherapy in the future. VLRWTs show a different incidence of *WT1* mutation and 11p15 imprinting patterns than has been reported in Wilms tumors of all ages.

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INTRODUCTION

Within the current Children's Oncology Group (COG) Favorable Histology Wilms Tumor (FHWT) protocols, a subset of patients has been defined as very low-risk Wilms tumor (VLRWT). This subset includes patients younger than 24 months of age with stage I FHWTs weighing less than 550 g. If eligible for this protocol, patients are treated with nephrectomy alone, without adjuvant chemotherapy. This approach is supported by the prospective evaluation of children with VLRWTs within the National Wilms Tumor Study-5 (NWT-5). From 1995 to 1998, 75 children with VLRWT were treated with nephrectomy alone; eight patients experienced recurrence, and three developed meta-

chronous contralateral tumors, resulting in a 2-year disease-free survival estimate of 86.5%.¹ As a result of pre-established stopping rules, this therapeutic arm was closed in 1998, and patients previously registered were recalled and given the option of receiving chemotherapy late in their course. From 1998 to 2002, 111 children with VLRWT continued to be registered on NWT-5 but were treated with vincristine and dactinomycin. The long-term follow-up of children with VLRWT registered on NWT-5 who did not receive adjuvant chemotherapy was recently compared with that of the patients who received adjuvant chemotherapy. Both groups showed an equivalent overall survival as a result of the higher than expected salvage rate in the chemotherapy-naïve patients.²

The previously mentioned studies indicate that approximately 10% to 15% of patients with VLRWT will experience relapse without chemotherapy. The ability to identify subsets of patients with VLRWTs who may have different risks of recurrence would enable further refinement of the criteria for selection of those children who may not benefit from chemotherapy. Genetic markers may allow for broadening of the somewhat arbitrary clinical criteria used to define candidates for nephrectomy only. Toward this end, patterns of global gene expression were recently reported for VLRWT.³ One subset was characterized by low expression of *WT1*, a gene located on 11p13 that is inactivated in up to 20% of Wilms tumors.⁴ This subset contained three of the six total relapses. Further, all evaluable patients with VLRWT who experienced relapse in this study had loss of heterozygosity (LOH) for 11p, a finding that is associated with *WT1* mutation.⁴⁻⁷ These data suggest that children with VLRWT whose tumors show *WT1* mutation and/or 11p LOH may have an increased risk of relapse. The goal of the current study is to validate these findings within a group of prospectively identified children with VLRWT who did not receive adjuvant chemotherapy.

PATIENTS AND METHODS

Clinical Samples

Seventy-seven children younger than 24 months of age at diagnosis with stage I FHWT less than 550 g were initially treated with nephrectomy only on the NWTs-5 protocol. Of these, 53 children did not receive subsequent chemotherapy when the trial closed and therefore met our selection criteria. Also included were two patients with VLRWT who did not receive chemotherapy, but were not eligible because of late registration only. Lastly, NWTs-5 was searched for children who fully met the criteria for VLRWT but who received adjuvant chemotherapy and experienced relapse. All such patients were included because of the presumption that metastasis would have occurred had chemotherapy not been given. This results in a total of 56 patients within the current analysis. Nine tumors were also included in the prior gene expression analysis study.³ Institutional review board approval and informed consent for all patients were obtained. Specimens were obtained from the initial nephrectomy specimen, snap-frozen and stored at -80°C at the National Wilms Tumor Study Group Biology Reference Laboratory.⁸ A frozen section of each confirmed the presence of $\geq 80\%$ viable tumor. Diagnosis and local stage were determined by central pathology review.

Loss of Heterozygosity

Samples were analyzed prospectively from 1995 to 2002 using polymerase chain reaction (PCR) for loci on 11p15 as previously described.⁸ Polymorphic loci *INS*, *TH*, and *D11S1984* were evaluated initially. If these were noninformative, *D11S922* and *D11S2362* were also assessed. Loss of heterozygosity (LOH) was considered to be present if one of the two alleles in the constitutional DNA was absent or definitely reduced in the tumor DNA as determined by visual inspection of the ethidium stained gel by two individuals independently without knowledge of outcome. In cases in which only one locus was informative, the assay was repeated to ensure reproducibility.

11p15 Copy Number Analysis

Samples with LOH or presumed constitutional homozygosity for 11p15 were analyzed for 11p15 copy number using multiplex ligation-dependent probe amplification to assess for uniparental isodisomy (two identical copies of 11p15). Four probe sets were synthesized to loci on 11p15, and eight control probes were used for internal normalization. The probe sequences are provided in Appendix Table A1 (online only). Two normal control samples whose DNAs were prepared in the same manner as the test samples were likewise analyzed. After internal normalization, the test samples were normalized to the control samples to yield a ratio of relative copy number. Ratios less than 0.75 were interpreted as loss, and those greater than 1.35 were interpreted as gain.

Methylation Analysis at 11p15

Analysis of the extent of methylation of two differentially methylated regions (DMRs) on chromosome 11p15 was performed as previously described.⁹ The paternally methylated *H19* DMR (aberrantly methylated in many Wilms tumors) and the maternally methylated *KvDMR1* (whose methylation is independent of *H19*) both contain specific restriction sites recognized by methylation-sensitive enzyme *HpaII* and by *MspI*, which cuts irrespective of methylation. DNA was extracted using Qiagen DNeasy Blood and Tissue Kit (Qiagen, Santa Clarita, CA) following the manufacturer's protocol and was digested with either *RsaI*, *RsaI* plus *HpaII*, or *RsaI* plus *MspI* followed by quantitative PCR using primers described in Appendix Table A1 with the AB Power SYBR Green Kit (Applied Biosystems, Foster City, CA) following the manufacturer's protocol. Percent methylation was determined at each locus by comparing the quantity of amplified product after *RsaI* and *HpaII* digestion with the quantity of amplified product after cutting with *RsaI* alone. The *RsaI* and *MspI* digest was used to control for complete digestion. The cycle at which an arbitrary threshold level of fluorescence (the *Ct* value) was reached was determined. Retention of imprinting is demonstrated by 30% to 70% methylation of both *H19* and *KvDMR1*; loss of imprinting is demonstrated by 80% to 100% methylation of *H19* and 30% to 70% methylation of *KvDMR1*; loss of heterozygosity is demonstrated by 80% to 100% methylation of *H19* and 0% to 20% methylation of *KvDMR1*.

Mutation Analysis

Tumor DNAs were assessed for *WT1* point mutations and small insertions/deletions by sequence analysis of PCR products from all 10 exons, including flanking intronic sequence. Quantitative real-time PCR was performed to identify deletions encompassing one or more exons. Eight amplicons were used for this analysis: promoter/exon 1, exon 2/3, exon 4, exon 5, exon 6, exon 7, exon 8/9, and exon 10 (Appendix Table A1). Tumor DNA was amplified in duplicate using SYBR Green PCR Master Mix (Applied Biosystems) with amplicons from *WT1* as well as two reference amplicons¹⁰; the intron 13–exon 14 region of *NDST1* (*N*-deacetylase/*N*-sulfotransferase, located at 5q33.1) and the intron 4–exon 5 region of *FAH* (fumarylacetoacetate hydrolase, located at 15q25.1). These reference amplicons were used because chromosomes 5 and 15 are rarely amplified or deleted in Wilms tumors^{11,12} and both are unique, single-copy genes. Tumor DNAs were analyzed for point mutations in *CTNNB1* as previously described.¹³ The *WTX* locus was assessed for deletions by quantitative real-time PCR as described.¹⁰ Additionally, regions of *WTX* where functional mutations were previously observed were sequenced.¹⁰

RESULTS

Among the 56 patients assessed, 10 patients experienced relapse. This included seven patients with pulmonary metastasis detected 96, 140, 154, 164, 182, 202, and 303 days after diagnosis. Three patients developed local recurrence within the operative bed at 94, 133, and 383 days after diagnosis. The average follow-up for those patients who did not experience relapse was 2,733 days (range, 676 to 3,976 days).

LOH Analysis

11p15 LOH analysis was performed on 49 tumors using microsatellite analysis, and the data are provided in Tables 1 and 2. Of note, three tumors (patients 1 through 3) demonstrated constitutional homozygosity for all five loci sampled. Although these are technically noninformative using this method, this is most consistent with uniparental disomy, a recognized event in patients with Wilms tumor. Excluding the patients with constitutional homozygosity, the overall rate of LOH in VLRWTs was 16 (35%) of 46, compared with an overall published prevalence within FHWT of 33%.⁸ LOH for 11p was significantly associated with relapse in VLRWTs ($P = .001$, sensitivity

Table 1. Mutation and LOH Analysis

Relapse	11p15 LOH Microsatellite				WT1 Mutation			11p15 LOH Methylation			
	LOH	H	ROH	ND	Mutation	No Mutation	ND	LOH	LOI	ROI	ND
Relapse, n = 10	6	2	1	1	5	3	2	8	0	0	2
Lung, n = 7	4	2	1	0	4	2	1	6	0	0	1
OR bed, n = 3	2	0	0	1	1	1	1	2	0	0	1
No relapse, n = 46	10	1	29	6	4	33	9	9	1	24	11
Total, n = 56	16	3	30	7	9	36	11	17	1	24	13

Abbreviations: LOH, loss of heterozygosity; H, constitutional homozygosity for all five loci; ROH, retention of heterozygosity; LOI, loss of imprinting; ROI, retention of imprinting; ND, not done; OR, operative.

85.7%, specificity 74.4%, positive predictive value 37.5%, negative predictive value 96.7%).

WT1 Analysis

WT1 analysis was performed on 45 tumors for which sufficient frozen tumor sample was available, and the data are provided in Tables 1 and 2. Overall, WT1 abnormalities were identified in nine (20%) of 45 tumors. Homozygous WT1 mutations or deletions were identified in the tumors of five patients (Table 2). Mutations identified included truncating mutations in exon 1 (patients 1 and 5) and exon 7 (patient 6), deletion of exons 4 to 10 (patient 4), and a 3 base pair insertion 8nt 5' of exon 6, the functional significance of which is not clear (patient 2, who did not experience relapse). Heterozygous deletion of the entire WT1 locus was identified in four patients (patients 16 through 19). Six of nine tumors with WT1 abnormalities were associated with intralobar nephrogenic rests, many multiple (Table 2). Of the nine tumors with WT1 abnormalities, all demonstrated either LOH or constitutional homozygosity at 11p15 (Table 2). WT1 abnormalities were significantly associated with relapse in VLRWTs ($P = .004$, sensitivity 62.5%, specificity 89.2%, positive predictive value 55.5%, negative predictive value 91.7%). Of note, elimination of tumor 2 from the analysis would increase the significance of the association.

CTNNB1 and WTX Mutation

All tumors were also evaluated for CTNNB1 and WTX mutations. Of 45 tumors analyzed, five from male patients were deleted for WTX (11%). None of these also showed mutation of WT1, and no tumors with WTX deletion relapsed (Table 2). Of 45 tumors analyzed for CTNNB1 mutation, five sustained missense mutations (11%) and two of five relapsed. CTNNB1 mutations identified included Ser45Tyr (patients 1, 4, and 13), Ser45Pro (patient 2), and Ser45Phe (patient 6). Four of these also carried WT1 mutations, including both patients with CTNNB1 mutations who experienced relapse (Table 2). The presence of CTNNB1 mutation was associated with skeletal muscle differentiation within the Wilms tumor, as previously described.^{14,15} Neither WTX nor CTNNB1 mutation was significantly associated with relapse.

11p15 Copy Number Analysis

All samples with LOH or presumed constitutional homozygosity for 11p15 were analyzed for 11p15 copy number to assess for uniparental isodisomy (copy number neutral 11p15 LOH; Table 2). Of these, all but three patients with 11p15 LOH contained at least two

copies of 11p15, consistent with isodisomy. One tumor showed gain consistent with three copies of 11p15 (patient 8).

Of the four patients with heterozygous deletion of WT1, three also showed both copy number loss and LOH of 11p15, most consistent with loss of a chromosomal segment including both 11p13 and 11p15.

11p15 Methylation Analysis

To further confirm isodisomy and to investigate the potential role of 11p15 imprinting abnormalities in those tumors that lacked LOH, the 42 tumors with remaining available DNA underwent H19 DMR and KvDMR1 methylation analysis (Table 2). All 17 tested tumors showing LOH by microsatellite analysis displayed only a paternal methylation pattern, consistent with paternal isodisomy. Of the remaining 25 patients who lacked LOH, all but one demonstrated retention of imprinting by 11p15 methylation analysis, and none of these experienced relapse. A single tumor retained heterozygosity at the 11p15 microsatellite loci, but showed loss of imprinting at 11p15. LOH as determined by 11p15 methylation analysis was significantly associated with relapse in VLRWTs ($P < .0001$, sensitivity 100%, specificity 73.5%, positive predictive value 47%, negative predictive value 100%).

DISCUSSION

Treatment with vincristine and dactinomycin constitutes the chemotherapeutic backbone for patients with FHWT. However, this therapy may cause serious myelosuppression and hepatic toxicity, particularly in very young children. The evidence collected thus far suggests that children with VLRWT have an excellent prognosis when treated with nephrectomy only.^{1,16-18} However, the definition of VLRWT is in many ways arbitrary. The criteria include weight of the tumor (< 550 g), age of the patient (< 24 months), and stage of the tumor (stage I). Although the application of such criteria is straightforward, it is philosophically and intellectually challenging, particularly for those tumors that weigh 551 g or in patients 25 months of age, for example. This has led to efforts to define different subtypes within VLRWT on the basis of inherent tumor characteristics, with the hope of finding differences in outcome within these subtypes.

To accomplish this, a recent gene expression analysis of 39 VLRWTs was performed that identified three subsets with different risks of relapse (subset 1, zero of nine relapses; subset 2, three [23%] of

Relapse Prediction in Very Low-Risk Wilms Tumors

Table 2. Clinical, Pathologic, and Genetic Data

Patient	Relapse	11P LOH Summary	11p15 Copy No.	11p15 Methylation	Mutation Summary	Interpretation	Histology	% Muscle	Nephrogenic Rests
1	Lung	H	2 copies	LOH	<i>WT1, Bcat</i>	Constitutional 11p15 isodisomy; homozygous <i>WT1</i> mutation	Mixed	5	Multiple ILNR
2	None	H	2 copies	LOH	<i>WT1, Bcat</i>	Constitutional 11p15 isodisomy; homozygous <i>WT1</i> mutation	Mixed	70	Multiple ILNR
3	Lung	H	2 copies	LOH	<i>None</i>	Constitutional 11p15 isodisomy	Mixed	1	ILNR
4	Lung	LOH	2 copies	LOH	<i>WT1, Bcat</i>	Isodisomy for 11p15; homozygous <i>WT1</i> deletion	Mixed	60	Multiple ILNR
5	Lung	LOH	2 copies	LOH	<i>WT1</i>	Isodisomy for 11p15; homozygous <i>WT1</i> mutation	Mixed	0	Multiple ILNR
6	None	LOH	2 copies	ND	<i>WT1, Bcat</i>	Isodisomy for 11p15; homozygous <i>WT1</i> mutation	Mixed	50	Multiple ILNR
7	OR bed	LOH	2 copies	LOH	<i>None</i>	Isodisomy for 11p15 in tumor	Mixed	0	Multiple ILNR
8	Lung	LOH	3 copies	LOH*	<i>None</i>	Three copies of 11p15, two isodisomic	Mixed	0	Multiple ILNR
9	None	LOH	2 copies	LOH	<i>None</i>	Isodisomy for 11p15 in tumor	Epithelial	0	Multiple PLNR
10	None	LOH	2 copies	LOH	<i>None</i>	Isodisomy for 11p15 in tumor	Blastemal	0	Multiple PLNR
11	None	LOH	2 copies	LOH	<i>None</i>	Isodisomy for 11p15 in tumor	Blastemal	0	Multiple PLNR
12	None	LOH	2 copies	LOH	<i>WTX</i>	Isodisomy for 11p15 in tumor	Mixed	0	ILNR plus PLNR
13	None	LOH	2 copies	LOH	<i>Bcat</i>	Isodisomy for 11p15 in tumor	Mixed	1	None
14	None	LOH	2 copies	ND	<i>WTX</i>	Isodisomy for 11p15 in tumor	Mixed	0	One ILNR
15	None	LOH	2 copies	LOH	<i>WTX</i>	Isodisomy for 11p15 in tumor	Mixed	0	ILNR plus PLNR
16	Lung	LOH	1 copy	LOH	<i>WT1</i> het del	Loss of 1 copy of 11p13-15	Mixed	0	Inconclusive
17	OR bed	LOH	2 copies	LOH	<i>WT1</i> het del	Isodisomy for 11p15; heterozygous <i>WT1</i> deletion	Mixed	0	None
18	None	LOH	1 copy	LOH	<i>WT1</i> het del	Loss of 1 copy of 11p13-15	Blastemal	0	Inconclusive
19	None	LOH	1 copy	LOH	<i>WT1</i> het del	Loss of 1 copy of 11p13-15	Mixed	0	Multiple ILNR
20	None	No LOH	2 copies	ROI	<i>WTX</i>	Two copies of 11p13 and 11p15	Mixed	0	Multiple ILNR
21	None	No LOH	2 copies	ROI	<i>WTX</i>	Two copies of 11p13 and 11p15	Mixed	0	ILNR plus PLNR
22	Lung	No LOH	ND	ND	ND	ND	Mixed	0	None
23	OR bed	ND	ND	ND	ND	ND	Blastemal	0	Inconclusive
24	None	No LOH	ND	LOI	Neg	Two copies of 11p13 and 11p15	Mixed	3	ILNR
25	None	No LOH	ND	ROI	Neg	Two copies of 11p13 and 11p15	EDT	0	None
26	None	No LOH	ND	ROI	Neg	Two copies of 11p13 and 11p15	EDT	0	None
27	None	No LOH	ND	ND	Neg	Two copies of 11p13 and 11p15	EDT	0	Inconclusive
32	None	No LOH	ND	ROI	Neg	Two copies of 11p13 and 11p15	EDT	0	None
29	None	No LOH	ND	ROI	Neg	Two copies of 11p13 and 11p15	EDT	0	None
36	None	No LOH	ND	ROI	Neg	Two copies of 11p13 and 11p15	EDT	0	None
33	None	No LOH	ND	ROI	Neg	Two copies of 11p13 and 11p15	EDT	0	Inconclusive
40	None	No LOH	ND	ROI	Neg	Two copies of 11p13 and 11p15	EDT	0	ILNR
41	None	No LOH	ND	ROI	Neg	Two copies of 11p13 and 11p15	EDT	0	None
44	None	No LOH	ND	ROI	Neg	Two copies of 11p13 and 11p15	EDT	0	None
54	None	ND	ND	ROI	Neg	Two copies of 11p13 and 11p15	EDT	0	None
35	None	No LOH	ND	ROI	Neg	Two copies of 11p13 and 11p15	Epithelial	0	None
38	None	No LOH	ND	ROI	Neg	Two copies of 11p13 and 11p15	Epithelial	0	None
45	None	No LOH	ND	ROI	Neg	Two copies of 11p13 and 11p15	Epithelial	0	Inconclusive
28	None	No LOH	ND	ROI	Neg	Two copies of 11p13 and 11p15	Blastemal	0	None
30	None	No LOH	ND	ROI	Neg	Two copies of 11p13 and 11p15	Mixed	0	ILNR
31	None	No LOH	ND	ROI	Neg	Two copies of 11p13 and 11p15	Mixed	0	None
34	None	No LOH	ND	ROI	Neg	Two copies of 11p13 and 11p15	Mixed	0	Multiple ILNR
37	None	No LOH	ND	ROI	Neg	Two copies of 11p13 and 11p15	Mixed	0	None
39	None	No LOH	ND	ROI	Neg	Two copies of 11p13 and 11p15	Mixed	0	Inconclusive
42	None	No LOH	ND	ROI	Neg	Two copies of 11p13 and 11p15	Mixed	0	None
43	None	No LOH	ND	ROI	Neg	Two copies of 11p13 and 11p15	Mixed	0	None
46	None	No LOH	ND	ROI	Neg	Two copies of 11p13 and 11p15	Mixed	0	None
50	None	No LOH	ND	ND	ND	ND	Epithelial	0	Inconclusive
48	None	No LOH	ND	ND	ND	ND	Epithelial	0	Multiple PLNR
47	None	No LOH	ND	ND	ND	ND	Mixed	0	Multiple ILNR plus PLNR
49	None	No LOH	ND	ND	ND	ND	Mixed	0	None
51	None	ND	ND	ND	ND	ND	Mixed	40	ILNR
52	None	ND	ND	ND	ND	ND	Mixed	0	Inconclusive
56	None	ND	ND	ND	ND	ND	Mixed	0	None
55	None	ND	ND	ND	ND	ND	EDT	0	None
53	None	ND	ND	ND	ND	ND	Blastemal	0	ILNR

Abbreviations: LOH, loss of heterozygosity; H, constitutional homozygosity for all 5 loci; OR, operative; ILNR, intralobular nephrogenic rests; ND, not done; LOI, loss of imprinting; ROI, retention of imprinting; PLNR, perilobular nephrogenic rests.

*Seventy-seven percent *H19DMR* methylation, 35% *KvDMR* methylation, consistent with LOH in two of three copies.

13 relapses; subset 3, three [17%] of 17 relapses).³ Subset 2 was characterized in part by low *WT1* expression. These data suggest that inactivation of *WT1* may be of prognostic significance in VLRWT patients undergoing nephrectomy only. Of note, patients with constitutional *WT1* abnormalities who develop FHWT do not show differences in overall outcome when treated with standard chemotherapeutic protocols.¹⁹ The ability to evaluate outcome was limited in this previous study because it included both children who did and did not receive adjuvant chemotherapy.

In addition to *WT1* mutation, 11p15 LOH was also highly associated with relapse in children with VLRWT in the previous study. 11p15 LOH has been consistently recognized to be important in the development of Wilms tumor. 11p15 LOH may result from 11p15 deletion, chromosome loss, or somatic recombination.²⁰ Chromosome loss often results in duplication of the remaining chromosome 11, resulting in copy number neutral LOH. Additionally, loss of imprinting (LOI) for 11p15 occurs in 30% to 50% of sporadic Wilms tumors, with aberrant methylation of the maternal allele of H19 and biallelic expression of insulin-like growth factor 2.^{21,22} Both copy number neutral 11p15 LOH and 11p15 LOI are predicted to result in overexpression of insulin-like growth factor 2. During NWT-5, 11p15 LOH was assessed for all tumors registered. 11p15 LOH has been shown to occur in 33% of all patients with FHWT and has not been associated with differences in outcome for patients treated with standard chemotherapy.²³

The current study validates the significant association of both *WT1* mutation and 11p15 LOH with relapse within a group of prospectively identified children with VLRWT who did not receive adjuvant chemotherapy. It further demonstrates that all patients with *WT1* mutation or deletion also had 11p15 LOH and that 16 of 19 tumors with 11p15 LOH were isodisomic for 11p15 (copy number neutral LOH), either confined to the tumor (13 of 16 cases) or constitutional (three of three cases). Lastly, it demonstrates a low incidence of LOI of 11p15 in this group of young patients, present in only one of 43 analyzed tumors compared with the reported overall incidence in sporadic Wilms tumors of 30% to 50%.²¹ No tumor that retained the normal 11p15 imprinting pattern relapsed in this study. It should be noted that all epithelial tubular differentiated VLRWTs from the previous gene expression analysis and in the current study retained the normal 11p15 imprinting pattern and did not relapse. These findings suggest that Wilms tumors in patients younger than 2 years display an increased frequency of *WT1* mutations, an increase in 11p15 uniparental isodisomy, and a rather dramatic decrease in frequency of LOI. These features are associated with differences in relapse-free survival.

Although *WT1* mutation and 11p15 LOH and LOI are considered to represent two independent pathogenetic mechanisms for Wilms tumors, a large proportion of patients with *WT1* mutation also show 11p15 LOH. Therefore, in such tumors, mutation of one *WT1* allele is followed by loss of the second (wild-type) allele due to chromosomal mechanisms such as whole chromosome loss, deletion, or somatic recombination, which also result in 11p15 LOH. Therefore, in the current study, *WT1* mutation and 11p15 LOH are likely not independent variables, and the underlying evaluation of each locus independently may be difficult. The observations that (1) three of 10 relapses occurred in patients who lacked *WT1* mutation but demonstrated 11p15 LOH with uniparental isodisomy and (2) all patients with *WT1* abnormalities demonstrated 11p15 LOH suggests that anal-

ysis of 11p15 LOH may provide a more meaningful clinical test if the goal is to maximize sensitivity.

The ability to identify patients with VLRWT who have an increased risk of relapse in the absence of chemotherapy offers the hope of broadening the currently arbitrary definition of children who may not benefit from chemotherapy by either expanding the age or tumor weight criterion, or both. It is not possible to test this directly using patients previously treated within NWT-5 because all such patients received chemotherapy and few experienced relapse. However, the United Kingdom Childhood Cancer Study Group investigated 242 patients with stage I FHWT (not restricted by age or tumor weight) treated with vincristine alone.²⁴ Excluding the development of a contralateral tumor, this group of patients had a risk of relapse (31 of 242; 13%) that was similar to that experienced by children with VLRWT in NWT-5 who received no adjuvant chemotherapy (eight of 75; 11%). The United Kingdom Childhood Cancer Study Group study demonstrated no significant difference in relapse between patients younger than 2 years (93%) and those 2 to 4 years of age (87%). However, patients more than 4 years of age at diagnosis had a significantly worse outcome (71%; $P < .001$). Importantly, tumor weight in this study was not an independent predictor of relapse. These findings suggest that the criteria for VLRWT may be broadened to include a larger range of patient age (up to 4 years) and any tumor weight.

In summary, the current study shows an association between *WT1* mutation and 11p15 LOH with relapse in a group of prospectively identified children with VLRWT who did not receive chemotherapy. This association may in part be due to inherent differences in pathogenesis of VLRWT. These findings must be validated in an independent group of prospectively identified patients with VLRWT before using any of these biomarkers to stratify patients for different therapeutic approaches.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

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