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Allele loss at 16q defines poorer prognosis Wilms tumour irrespective of treatment approach in the UKW1–3 clinical trials: A Children's Cancer and Leukaemia Group (CCLG) study

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ABSTRACT

Survival from Wilms tumour is excellent. Hence, better markers are required to restrict treatments causing late sequelae to those at highest risk of relapse.

We investigated the prognostic significance of loss of heterozygosity (LOH) on 1p and 16q in 426 favourable histology Wilms tumours treated with either immediate nephrectomy (63%) or preoperative chemotherapy (37%). Four years RFS and OS were 84.6% and 92.0%, respectively.

10.3% tumours had LOH 1p, 14.6% LOH 16q, with 2.6% at both loci. In multivariate analysis, LOH 16q was associated with an increased risk of relapse (hazard ratio (HR) 2.69, 95%CI: 1.47–4.92) and death (HR 2.67, 95%CI: 1.17–6.06). LOH 1p showed no significant associations. These results were not influenced by treatment approach.

LOH 16q is an adverse risk factor in favourable histology Wilms tumour, regardless of initial approach to therapy. Its relationship with histological risk groups defined after neoadjuvant chemotherapy requires analysis in a larger series, and is the subject of the current SIOP WT 2001 trial.

1. Introduction

Wilms tumour is highly curable since the 1970s as a consequence of consistent improvement in the treatment strategies using a combination of surgery, chemotherapy and radiotherapy. Treatment intensity has been adapted to the risk of relapse such that now the majority of low stage tumours receive a relatively minimal chemotherapy with vincristine and actinomycin D, whereas the therapies with greater potential for long-term side effects (anthracyclines,

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radiotherapy) are reserved for higher stage and high-risk histology tumours.[1] With this clinical approach to risk stratification, 15–20% of children will relapse, with the largest absolute numbers of relapses coming from the group that received a relatively mild first line therapy. There is therefore a need for additional prognostic factors that predict outcome.

The molecular biology of Wilms tumour has been studied well, and has served as a paradigm for the involvement of tumour suppressor genes in cancer development.[2] Use of loss of heterozygosity (LOH) assays to determine areas of allele loss has shown that the majority of Wilms tumours have few or no changes, and that these tend to be restricted to a few loci, principally at 11p, 1p, 16q, 11q and 22q.[3–7] The genetic changes involved at the two loci on the short arm of chromosome 11 have been identified, with mutation of the *WT1* gene at 11p13 occurring in up to 20% of Wilms tumours and epigenetic changes affecting the Beckwith Wiedemann syndrome locus at 11p15. However, allele loss of 11p is not associated with adverse outcome.[8] The genes affected by LOH at the other loci have not yet been elucidated; however LOH at 1p, 11q, 16q and 22q has been associated with adverse outcome.[3–6]

Historically, there are two main treatment approaches to Wilms tumour. The North American Children's Oncology Group (COG) recommends immediate nephrectomy, whereas the trials of the International Society of Paediatric Oncology (SIOP) group advocate preoperative chemotherapy with delayed nephrectomy. Both approaches use the tumour stage and the histological subtype determined at the time of nephrectomy for risk stratification. Based on its previous trial, the COG has now introduced the presence of combined LOH at 1p and 16q in otherwise favourable histology Wilms tumour to determine treatment intensity.[9] The SIOP group has revised its classification of histological response to preoperative chemotherapy to introduce a new high-risk category of 'blastemal type' Wilms tumour, but does not yet use molecular criteria for risk stratification.[10] The Childrens' Cancer and Leukaemia Group of the United Kingdom (formerly UKCCSG) recommended immediate nephrectomy in its first two trials (UKW1 and 2), and then performed a unique randomised clinical trial (UKW3) that compared the two treatment approaches.[11–14] The tumour stage and the histology-adapted use of chemotherapy and radiotherapy remained fundamentally unchanged during the 20-year period of these three trials (1980–2001), which registered >90% of children treated for Wilms tumour in Great Britain and Ireland. The UKW1–3 trials therefore provide the opportunity to assess the prognostic significance and the clinicopathological associations of allele loss at 1p and 16q in a population of Wilms tumours in which both these treatment approaches were applied.

2. Patients and methods

Snap frozen and paraffin-embedded tumour and matching normal tissue samples were collected from eight CCLG centres, which had registered 58% of the total of 1472 patients with Wilms tumour in the UKW1–3 trials. All participants had given informed consent for inclusion in the relevant clinical trial, each of which had institutional ethical approval. This specific biological study had a national ethical approval that included access to research use of archival tissues without further consent, although the more recent patients had given such consent. All patients were treated on the UKW1, 2 or 3 trials. UKW1 (1980–1986) included a non-randomised trial of single agent vincristine for children with stage I FH Wilms tumour.[11] UKW2 (1986–1991) confirmed the excellent outcome for stage I tumours treated with only 10 weeks of vincristine.[12,13] UKW3 (1991–2001) randomised patients with localised Wilms tumour between the two established surgical approaches (immediate nephrectomy versus preoperative chemotherapy and delayed surgery), and showed that the preoperative chemotherapy gave a more advantageous stage distribution, and hence lowered overall burden of therapy without adversely affecting the outcome.[14] In all the three trials, use of doxorubicin and radiotherapy was restricted to patients with stage III or IV tumours or anaplastic histology. Clinical data were obtained from case report forms that were held at the CCLG. 68% of tumours had been reviewed by the relevant trial pathology panel. Only anaplasia was recognised as a high-risk histological feature and, for the purposes of this analysis, the term favourable histology (FH) is used to describe all tumours without evidence of anaplasia, regardless of pre-treatment with chemotherapy.

2.1. *LOH analysis*

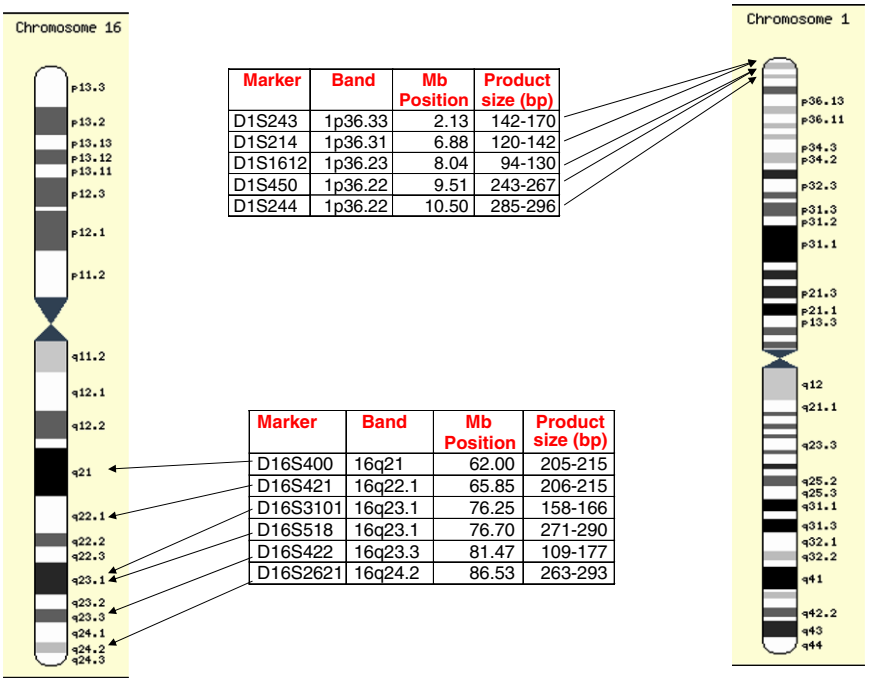
Tumour cell content was assessed on an H&E section cut prior to DNA extraction, and only those with at least 85% tumour were deemed suitable for analysis. DNA was extracted by standard techniques. Microsatellite markers for the same loci as used in the fifth National Wilms Tumour Study (NWTSG 5) (Fig. 1) were amplified by PCR using fluorescent labels (HEX, FAM or NED), and run on ABI 3100 automated 16 capillary sequencer. The ABI genotyper software was used for data analysis, and all peaks were checked visually. LOH was defined as a ratio of <0.75 or >1.3 using the formula

$$\frac{\text{Tumour (peak height allele 1/peak height allele 2)}}{\text{Normal (peak height allele 1/peak height allele 2)}}$$

which are typical ratios used when heterogeneous tissue is being assessed.[15] All tumour DNAs regardless of whether LOH was detected were re-amplified from original DNA, and the analysis repeated on new PCR products. In 2%, a discrepant result was obtained, usually as a result of poor or failed PCR. Such samples were only included if a third experiment gave clear results. Samples were collected from a total of 500 Wilms tumour patients. 42 (8.4%) failed to amplify, three were non-informative for all markers and three patients only had available samples taken at relapse, and hence were excluded from the survival analyses. Regions of LOH were demarcated by markers with retention.

2.2. *Statistical considerations*

The frequency of LOH at chromosomes 1p and 16q by stage and age (classified as 0–2-year-old, 2–4 years and greater than 4-year-old) was compared using standard chi-square test. Kaplan–Meier curves of overall survival (OS) and relapse-free



Marker	Band	Mb Position	Product size (bp)
D1S243	1p36.33	2.13	142-170
D1S214	1p36.31	6.88	120-142
D1S1612	1p36.23	8.04	94-130
D1S450	1p36.22	9.51	243-267
D1S244	1p36.22	10.50	285-296

Marker	Band	Mb Position	Product size (bp)
D16S400	16q21	62.00	205-215
D16S421	16q22.1	65.85	206-215
D16S3101	16q23.1	76.25	158-166
D16S518	16q23.1	76.70	271-290
D16S422	16q23.3	81.47	109-177
D16S2621	16q24.2	86.53	263-293

Fig. 1 – Physical location and PCR product size of the 1p and 16q microsatellite markers used in this study. These markers are identical to those used in the fifth trial of the National Wilms Tumour Study Group (NWTSG5).

survival (RFS) were calculated for each locus individually, as well as combined. The relative risk (hazard ratios), together with 95% confidence intervals, for the effect of LOH at chromosomes 1p and/or 16q was estimated using a Cox proportional model. In order to evaluate confounding effect of age, stage and histology, a multivariate model was also included.

3. Results

3.1. *Clinical correlates of LOH 1p/16q in the UKW1–3 trials*

Four hundred and fifty two Wilms tumours sampled at nephrectomy from patients treated in the UKW1-3 trials gave an informative LOH analysis. Four hundred and twenty six (94.2%) were of favourable histology, and 26 (5.8%) were anaplastic tumours. These were representative of the 1472 Wilms tumour patients registered in these trials in terms of stage distribution and histology. The proportion exposed to preoperative chemotherapy increased between the UKW1 and 2 (20.3%) trials and the UKW3 (54.7%) trials as expected from the trial designs. There was no significant difference in the prevalence of LOH according to treatment approach or by trial.

LOH of either 1p and/or 16q was identified in 23.2%, with a significantly higher proportion of 16q LOH detected in anaplastic (9/26, 34.6%) compared to FH Wilms tumours (62/426, 14.6%, $p = 0.01$). LOH 1p was found in a similar proportion of anaplastic (3/26, 11.5%) and FH Wilms tumours (44/426, 10.3%, $p = 0.84$). The remainder of the analyses is restricted to the 426 FH Wilms tumours. Pre-nephrectomy chemotherapy was received by 157 (37%) of the FH Wilms tumours. Those with tumour stages I–III were mainly treated by immediate nephrectomy (247/321, 77%), while stages IV and V were more frequently exposed to preoperative chemotherapy (73/90, 81%). Among tumours with unknown treatment stage, 10/15 (67%) received preoperative chemotherapy.

Among tumours with LOH 16q, 39/62 (63%) showed loss at all informative markers, with the remainder showing varying patterns of partial LOH (Fig. 2). Only four tumours had LOH restricted to markers telomeric of D16S518, which lies outside the previously described common region of 16q LOH.[16] For chromosome 1p, 36/44 (82%) tumours had loss of all informative markers, 11% had loss of all markers except D1S450 and D1S244 and 7% had loss of these markers but retention of the others.

LOH was identified in all age groups and in all stages (Table 1A). There was a trend towards higher prevalence of LOH among older patients. Correlation of LOH with tumour stage was analysed as two groups reflecting initial treatment intensity: stages I and II (who received only vincristine +/– actinomycin D) versus stages III and IV (who received additional doxorubicin and usually radiotherapy). There was no significant association between LOH on either chromosome 1p (odds ratio: 1.21, 95%CI: 0.64–2.3, $p = 0.55$) or chromosome 16q (odds ratio: 1.14, 95%CI: 0.67–1.9, $p = 0.62$) and stages III and IV versus stages I and II.

Among 426 favourable histology tumours, there were 65 relapses and 39 deaths with a 4-year relapse-free (RFS) of 84.6% (95%CI: 80.8, 87.7) and an overall survival (OS) of 92.0% (95%CI: 88.9, 94.2). The distribution of events by LOH and stage is shown in Table 1B. In univariate analysis, any LOH 16q was associated with a significantly worse relapse-free survival with a hazard ratio of 2.64 (Table 2). There was a non-significant trend towards a similar adverse impact on

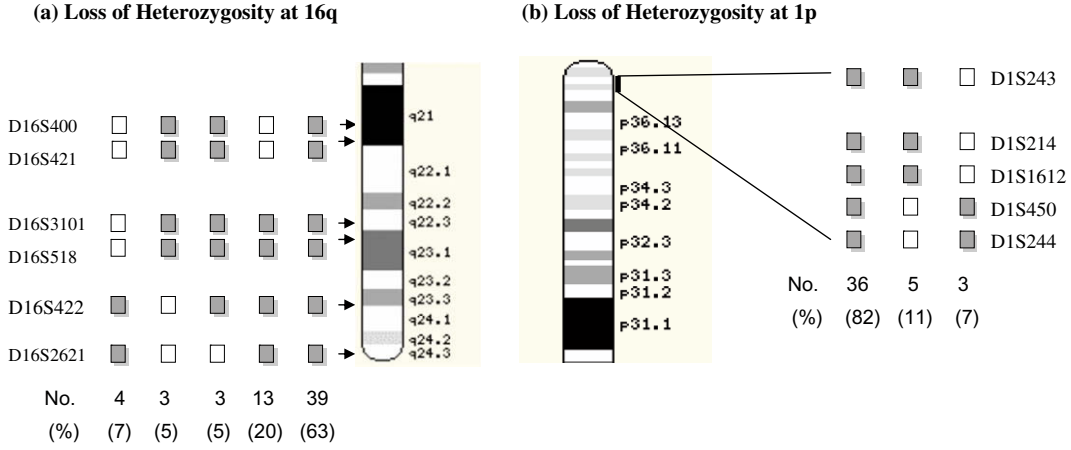


Fig. 2 – Summary of patterns of loss of heterozygosity (LOH) for the microsatellite markers on chromosome 16q (A) and chromosome 1p36 (B) found among 426 favourable histology Wilms tumours treated in the UKW1–3 trials. Numbers (%) below each column indicate the number and proportion of tumours with each pattern of LOH. Key: ■ = LOH and □ = no LOH.

Table 1A – Clinical parameters and prevalence of LOH 1p and 16q among 426 patients with favourable histology Wilms tumour treated in the UKW1–3 trials.

Characteristic	No. of pts (*n*)	Percentage (%)	1p LOH			16q LOH			Both loci		
			n	%	χ^2 *p*-Values	*n*	%	χ^2 *p*-Values	*n*	%	χ^2 *p*-Values
All patients	426		44	10.3		62	14.6		11	2.6	
Age (years)					0.04			0.29			0.92
0–2	130	30.5	14	10.8		14	10.8		3	2.3	
2–4	165	38.7	10	6.1		25	15.2		4	2.4	
4+	131	30.8	20	15.2		23	17.6		4	3.0	
Stage					0.89			0.64			0.10
I	132	31.0	14	10.6		17	12.9		0	–	
II	69	16.2	6	8.7		14	20.3		3	4.3	
III	120	28.1	12	10.0		17	14.2		3	2.5	
IV	64	15.0	9	14.1		11	17.2		4	6.2	
V	26	6.1	3	11.5		3	11.5		1	3.8	
Missing	15	3.5	0	0		0	0		0	–	
Treatment approach					0.80			0.74			0.22
Preoperative chemo	157	36.9	17	10.8		24	15.3		6	3.8	
Immediate nephrectomy	269	63.1	27	10.0		38	14.1		5	1.9	

Table 1B – Numbers of relapses and deaths by stage and LOH at chromosomes 1p and 16q among 426 patients with favourable histology Wilms tumour treated in the UKW1–3 trials.

Stage	No. of pts.	No. of relapses	No. of deaths	No. of relapses				No. of deaths			
				1p LOH		16q LOH		1p LOH		16q LOH	
				Yes	No	Yes	No	Yes	No	Yes	No
I and II	201	26	4	3	23	8	18	0	4	2	2
III and IV	184	34	30	6	28	11	23	4	26	7	23
V or Missing	41	5	5	0	5	0	5	0	5	0	5
Total	426	65	39	9	56	19	46	4	35	9	30

overall survival (Table 3). LOH 1p showed no clear association with either RFS or OS, though the power of this study would not reliably detect a hazard ratio of <3.0 (Tables 2 and 3). The small number of cases ($n = 11$) with combined LOH for 1p and 16q showed similar associations to those with only 16q LOH for adverse RFS.

Table 2 – Univariate analysis of relapse-free survival (RFS) by LOH at chromosomes 1p and 16q for 426 favourable histology wilms tumours treated in the UKW1–3 trials.

LOH status	No. of patients	No. of relapses	Four year RFS (%)	RFS			
				Hazard ratio (HR)	*p*-Value	95%CI	
None	382	56	85.2	1	–	–	–
1p LOH	44	9	79.6	1.47	0.28	0.73	2.98
None	364	46	87.2	1	–	–	–
16q LOH	62	19	69.3	2.64	<0.001	1.55	4.50

Table 3 – Univariate analysis of overall survival (OS) by LOH at chromosomes 1p and 16q for 426 favourable histology wilms tumours treated in the UKW1–3 trials.

LOH status	No. of patients	No. of deaths	Four year OS (%)	OS			
				Hazard ratio	*p*-Value	95%CI	
None	382	35	92.1	1	–	–	–
1p LOH	44	4	91	1.02	0.97	0.36	2.88
None	364	30	92.8	1	–	–	–
16q LOH	62	9	87	1.86	0.10	0.88	3.93

For multivariate analysis, performed to control for the known prognostic variables of tumour stage and patient age, tumours were grouped into mutually exclusive groups of only LOH 1p, only LOH 16q and combined LOH at both loci (Table 4 and Fig. 3). This confirmed the adverse impact of 16q LOH on relapse-free survival (hazard ratio of 2.69, 95%CI: 1.47–4.92, $p = 0.001$), and revealed a significant association with overall survival (hazard ratio 2.67, 95%CI: 1.17–6.06, $p = 0.02$). The hazard ratio increases slightly with age at diagnosis, but this does not reach significance. Advanced tumour stage at diagnosis is the most important factor determining survival, with more than 9-fold increased risk of death for children with initial stage III and IV tumours compared to those with stage I or II (Table 5).

Seven patients had a successful LOH analysis performed on paired samples from diagnosis and first relapse. One patient showed no LOH in either sample, one of each had either 1p or 16q LOH present at both diagnosis and relapse with no change in the extent of loss. In four patients, LOH was detected in the relapse samples only, and was not present at diagnosis (these cases were classified as 'no LOH' for the survival analysis). All four of these samples displayed LOH of 16q with one of these showing additional LOH for 1p, providing evidence that 1p and 16q LOH can arise as part of clonal evolution. Of interest, in one of these patients, LOH 16q was only present in the hepatic metastasis and not in the local recurrence.

4. Discussion

Despite long-term survival of over 85%, challenges remain in the management of Wilms tumour. The current approach to stage-adapted therapy in favourable histology Wilms tumour treated with immediate nephrectomy means that tumour stage for localised tumours is no longer a prognostic marker.[1] Hence, new approaches to risk stratification are required to further refine the intensity of first line therapy for this generally good prognosis group of tumours. The Childrens' Oncol-

Table 4 – Multivariate Cox proportional model for relapse-free survival for FH Wilms tumours treated on the UKW1–3 trials.

Variables	No. of patients	Hazard ratio	*p*-Value	95%CI	
LOH					
None	331	1	–	–	–
1p only	33	1.44	0.447	0.56	3.72
16q only	51	2.69	0.001	1.47	4.92
Both loci	11	3.86	0.011	1.35	11.01
Age (years)					
0–2	130	1	–	–	–
2–4	165	1.56	0.22	0.77	3.19
4+	131	1.28	0.51	0.61	2.68
Stage					
I–II	201	1	–	–	–
III–IV	184	1.35	0.28	0.78	2.31

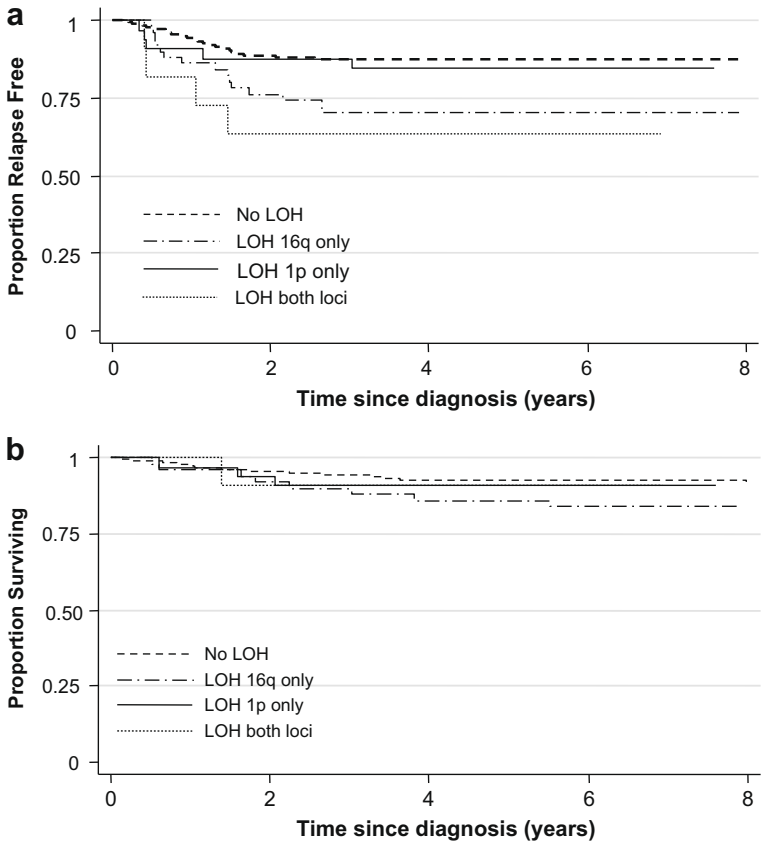


Fig. 3 – Kaplan–Meier estimates of relapse-free (A) and overall (B) survival for 426 favourable histology Wilms tumours treated in the UKW1–3 trials according to the presence or absence of loss of heterozygosity at either 1p alone, 16q alone or combined loss at both loci (univariate analysis).

ogy Group has introduced combined LOH at 1p and 16q to select patients for increased chemotherapy. The SIOP group uses histological response to preoperative chemotherapy to define a new high-risk subtype, where a large proportion of viable blastema remains.[10,17] By contrast, viable tumours in which the predominant cell type is epithelial or stromal appear to have a better than average prognosis, and may be candidates for future reductions in therapy. Stage III after preoperative chemotherapy remains an adverse risk factor, despite use of radiotherapy.

Table 5 – Multivariate Cox proportional model for overall survival for FH Wilms tumours treated on the UKW1–3 trials.

Variables	No. of patients	Hazard ratio	*p*-Value	95%CI	
LOH					
None	331	1	–	–	–
1p only	33	1.18	0.80	0.34	4.04
16q only	51	2.67	0.02	1.17	6.06
Both loci	11	0.92	0.94	0.12	6.88
Age (years)					
0–2	130	1.00	–	–	–
2–4	165	0.86	0.79	0.29	2.54
4+	131	1.55	0.40	0.55	4.36
Stage					
I–II	201	1.00	–	–	–
III–IV	184	9.13	0.00	3.12	26.76

This study aimed to determine the prognostic impact and the clinical correlations of LOH at 1p and 16q in Wilms tumour patients treated in the United Kingdom (UK), where treatment has evolved over 20 years from a predominantly immediate nephrectomy approach to one of preoperative chemotherapy. The results should be applicable to the United Kingdom Wilms tumour population as a whole as these trials enrolled a very high percentage of all cases diagnosed nationally, and samples included in this study represented all those retrievable from several CCLG centres. The prevalence of tumour-specific LOH for markers on 16q (14.6%) and 1p (10.3%) in favourable histology Wilms tumours in UK patients is consistent with those found previously, though the prevalence of combined loss of both loci was only 2.6%. Of note, there was no difference in prevalence of LOH for either chromosomal region between chemo-naive tumours or those exposed to preoperative chemotherapy, suggesting that this adverse molecular factor is present throughout the tumour at diagnosis and not just in a chemoresistant subclone.

LOH 16q was associated with a 2.7-fold increased risk of relapse and of death in FH Wilms tumour. Tumours with combined LOH had an similar outcome to those with LOH 16q

alone. Although LOH 1p showed no significant association with relapse-free or overall survival, due to the size of this study, it was not possible to exclude hazard ratios of the magnitude seen in the fifth trial of the National Wilms Tumour Study Group (NWTGS5).

These data, suggesting that genes on 16q are of greater importance for Wilms tumour outcome, are not inconsistent with those of the NWTSG 5 study. This larger study recruited over 2000 patients treated with immediate nephrectomy and a similar stage and histology-adapted use of anthracyclines and radiotherapy as was used in the UKW trials.[9] Among patients with FH Wilms tumour, the relative risk of relapse was similar for LOH for 1p (RR 1.56) or 16q (1.49) when each was considered in isolation. When the possibility of LOH at both loci was taken into account, for 1656 tumours stratified by stage, these RRs became 1.25, 1.28 and 2.59, respectively, for stage I–IV tumours with LOH 1p only, 16q only or both compared to those without any LOH. Even with the large numbers in this trial, the investigators felt that only tumours with LOH at both loci justified selection for increased treatment intensity, though they could not completely rule out a clinically significant effect of LOH at either locus, particularly in low stage disease. While our study did not have the power to rule out a hazard ratio of <3.0 for association of LOH with relapse, the results presented here add weight to the possibility considered by the NWTSG investigators that isolated 16q LOH is an adverse prognostic factor. We performed a meta-analysis to formally compare the results of this study with those of the NWTSG5 trial (Table 6). The results are consistent with a model, whereby LOH at either locus has an independent effect that is simply multiplicative when combined.

Attempts have been made to identify a common region of loss at both 1p and 16q in order to identify the key genes involved. These attempts have been hampered by the gene-rich nature of the 1p36 region and the very extensive regions of loss identified on 16q. Thus, to date, no such Wilms tumour genes have been identified though several candidates have been examined.[18,19,16] This study did not set out to finely map potential common regions of loss, but confirms that the majority of Wilms tumours with LOH at either 1p or 16q show loss at all markers examined. For 16q, the most common region of loss, found in 93% of tumours, encompasses the 6.7 Mb region defined by Safford et al.[16] Evidence for a separate region of loss, telomeric to D16S518, relies on only four tumours and could be a random event.

More recent genome wide studies have shown an association between the loss of 16q and the gain of 1q, which itself is known to correlate with adverse outcome.[20,21] The question therefore remains as to whether there are genes on 16q involved in Wilms tumourigenesis or whether this loss serves as a surrogate marker for other events occurring within the genome, such as *t*(1;16) with simultaneous loss of 16q and gain of 1q. The markedly higher prevalence of 16q but not 1p LOH in anaplastic Wilms tumour, seen in this study, the NWTSG5 study and others, suggests that changes on 16q may be of direct relevance to Wilms tumourigenesis.[6,9] Furthermore, we have seen evolution of 16q LOH in seven paired samples analysed at diagnosis and relapse, four of which had acquired 16q LOH in the relapse specimen only, suggesting its association with tumour progression.

The current approaches to defining high-risk non-anaplastic Wilms tumour, i.e. LOH 1p/16q or 'blastemal type' after preoperative chemotherapy, identify only the minority of children who ultimately relapse or die. In this study, 41/69 (59%) of relapses and 27/39 (69%) of deaths were seen in children whose tumours showed no evidence of LOH at either 1p or 16q. This is similar to the 142/213 (67%) of relapses and 52/77 (68%) of deaths seen in the NWTSG5 study, who lacked either marker.[9] The 'blastemal type' shows no better predictive value in SIOP studies, where 35/45 (78%) of relapses and 21/25 (84%) of deaths among unilateral non-anaplastic WTs were not of this high-risk subtype.[17] Hence, there is a need

Table 6 – Meta-analysis of combined NWTSG5 and UKW1–3 data for impact of LOH 1p/16q on relapse-free survival in FH Wilms tumour.

Relapse-free survival	LOH status	No.	Hazard ratio	95%CI		*p*-Value
				Lower limit	Upper limit	
Stage I/II	None	903	–	–	–	–
	1p only	77	1.97	1.09	3.55	0.0243
	16q only	142	2.07	1.33	3.24	0.0014
	Both loci	49	2.99	1.62	5.53	0.0005
Stage III/IV	None	642	–	–	–	–
	1p only	70	0.91	0.46	1.80	0.7839
	16q only	121	1.21	0.78	1.88	0.3877
	Both loci	37	2.67	1.46	4.87	0.0014
Stages I–IV combined	None	1545	–	–	–	–
	1p only	147	1.42	0.91	2.21	0.1270
	16q only	263	1.58	1.15	2.16	0.0043
	Both loci	86	2.82	1.83	4.34	0.0000

A fixed effect meta-analysis model was used to combine the hazard ratios for LOH at 1p, 16q and both loci relative to the No LOH group across the NWTSG5 and UKW1–3 studies. This was initially done separately for stages I–II and III–IV, and the hazard ratios were then compared between the two-stage groupings, a comparison that was found to be significant only within the NWTSG5 study. However, no significant difference was found in the combined data from the two trials. Overall hazard ratios were therefore calculated by combining the hazard ratios from the two-stage groupings.

to discover new molecular prognostic markers in Wilms tumour and to understand the biological relationship between the two currently used risk stratification systems. Such studies are being undertaken in the current SIOP WT 2001 multinational clinical trial.

Conflict of interest statement

None declared.

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