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Loss of heterozygosity for the short arm of chromosome 7 in sporadic Wilms tumour

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Cytogenetic analysis of Wilms tumours (WT) have shown that abnormalities involving chromosome 7 occur in approximately 25% of tumours. In some cases, these abnormalities involve deletions of the short arm, and are seen as the sole cytogenetic change, strongly suggesting the presence of a tumour suppressor gene in this location. Since loss of heterozygosity (LOH) studies have been crucial in defining chromosomal regions involved in Wilms tumorigenesis, we have analysed 40 sporadic Wilms tumours using a panel of 10 microsatelite polymorphic markers distributed along the length of the chromosome arm. In our series, four tumours (10%) showed allelic loss for 7p markers which is twice the background rate of LOH in WT. The shortest common region of overlap of LOH was located between markers D7S517-D7S503 in band 7p21-15. In one tumour there was evidence for a homozygous, interstitial deletion at a locus within this region. These findings provide strong evidence for the existence of a tumour suppressor gene involved in Wilms tumorigenesis and defines the critical region of the chromosome involved.

Keywords: Wilms Tumour; LOH 7p; WT tumour suppresskor gene 7p 21-15

Introduction

Cytogenetic analysis has been an important means for the initial sub-localization of genomic regions involved in the development of Wilms tumour (WT). In particular, the presence of constitutional deletions involving chromosome 11p in patients predisposed to WT led directly to the isolation of the WT1 gene (Call *et al*., 1990), although subsequent mutational analysis has indicated that this gene is primarily associated with the well-characterized Wilms tumour predisposition syndromes, WAGR and Denys-Drash (Huff *et al*., 1991; Baird *et al*., 1992a,b; Pelletier *et al*., 1991). Surprisingly, however, mutations in WT1 are only found in approximately 10% of sporadic WT (Coppes *et al*., 1993; Little *et al*., 1992; Varanasi *et al*., 1994). Following on from these observations, loss of heterozygosity (LOH) studies, which are now generally accepted as indicating the sites of tumour suppressor genes (Cavenee *et al*., 1983; Coppes *et al*., 1994), demonstrated that only 25% of sporadic WT showed loss of alleles for markers on 11p and, in some cases this LOH was restricted to the more distal 11p15 region. Thus, it is now clear that there are at least two separate loci on the short arm of chromosome 11 (in 11p13 and 11p15) which are likely to be involved in Wilms tumorigenesis (Mannens *et al*., 1988; 1990; Coppes *et al*., 1992a; Reeve *et al*., 1989; Wadey *et al*., 1990). More recently, LOH along the long arm of chromosome 16 has been identified in approximately 20% of tumours (Austruy *et al*., 1995; Coppes *et al*., 1992; Grundy *et al*., 1994; Maw *et al*., 1986), implicating yet another chromosome locus for WT, although in this case it appears to be involved more in progression rather than initiation, since the LOH was associated with more aggressive tumours (Grundy *et al*., 1994). To complicate matters further, lack of genetic linkage between the familial form of WT and markers from either chromosome 11 or 16, in several large pedigrees, suggests that there is yet another gene which is responsible for the genesis of this subgroup of tumours (Schwartz *et al*., 1991; Huff *et al*., 1988; Grundy *et al*., 1988; Huff *et al*., 1992). Thus, the molecular biology of Wilms tumour is becoming even more complex than originally envisaged, which is perhaps not surprising since the development of such a complex organ as the kidney is likely to involve the orchestration of many different genes involved in many different developmental processes.

Accepting that there may well be still other genetic loci involved in Wilms tumorigenesis, a closer inspection of the cytogenetic literature demonstrates that abnormalities involving chromosome 7 are observed in approximately 23% of tumours. Trisomy 7 is the most common observation although deletions and translocations are also seen (Austruy *et al*., 1995). How genes on chromosome 7 will fit into the picture remains to be seen since trisomy 7 usually occurs together with other chromosomal aberrations (Mitleman *et al*., 1991; Austruy *et al*., 1995; Slater and Mannens, 1992). Interestingly, trisomy 7 has also been reported in normal kidney (Sandberg, 1990). Whilst the significance of this is not clear, it has been suggested that these cells have an increased propensity for abnormal proliferation and thus an increased susceptibility for malignant change (Meloni *et al*., 1992). This hypothesis is endorsed by the description of a tumour with t(7;7) (p13;q21) rearrangement in which there was evidence for trisomy 7 in the tumour prior to the translocation event (Sawyer *et al*., 1993).

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In contradistinction to those seen as secondary changes, sole cytogenetic abnormalities are usually considered to represent primary events that have a role in tumorigenesis (Mitleman *et al*., 1991). Monosomy for 7p and trisomy for 7q, as the only apparent cytogenetic abnormality in Wilms tumour, have been reported in four separate tumours (Peier *et al*., 1995; Sawyer *et al*., 1993; Wang-Wuu *et al*., 1990; Wilmore *et al*., 1994). In addition, a number of reports describe more complex rearrangements that have the same end result. For example, in two cases, the presence of an isochromosome 7q, in conjunction with a del (11), was reported in one case and an *ins*(1;7)(q32;p12p22) and a stem line variation of 7p was reported in the other (Slater *et al*., 1985; Solis *et al*., 1988; Wang-Wuu *et al*., 1990). Tumours with breakpoints at 7p13 have also been independently reported by a number of groups (Sawyer *et al*., 1993; Slater *et al*., 1985; Solis *et al*., 1988; Kaneko *et al*., 1991; Wang-Wuu *et al*., 1990). However, band 7p15 has also been implicated, firstly in a patient with a congenital t,(1;7) translocation who subsequently developed a Wilms tumour (Wilmore *et al*., 1994; Hewitt *et al*., 1991), and secondly from a Wilms tumour with the sole t,(7;7)(p13;q21) cytogenetic change which resulted in monosomy for 7p13-pter (Sawyer *et al*., 1993). The recent description of cytogenetic abnormalities involving the short arm chromosome 7 in 2/11 Wilms tumours, provides further evidence supporting the existence of a 'tumour suppressor gene' in this chromosomal region (Miozzo *et al*., 1996). One of these tumours had a complex karyotype including a t(1;7)(p36;p15) and a deletion spanning 7p12-pter. Interestingly, this tumour had no detectable normal chromosome 7p material. The other tumour had an isochrome 7q in addition to trisomy for chromosomes 8, 9 and 13 (Miozzo *et al*., 1996). Further evidence for a Wilms tumour predisposition gene on 7p is provided by two children with a constitutional translocation involving the short arm of chromosome 7 and Wilms tumour. The first child had a de novo t(1;7) (q42;p22) translocation. He had bilateral radial aplasia other skeletal abnormalities and transient thrombocytopenia (Hewitt *et al*., 1991). At 5 years of age he presented with a unilateral favourable histology Wilms tumour. At laparotomy the contralateral kidney looked abnormal and biopsy revealed a nephrogenic rest, suggesting this child had a predisposition to the development of Wilms tumour (Hewitt *et al*., 1991). A second child with sporadic unilateral Wilms tumour had a constitutional t(2;7)(q33;p22) translocation (Rivera *et al*., 1985). A constitutional translocation involving the long arm of chromosome 7 has also been reported in a boy with cryptorchidism who developed Wilms tumour at 14 months of age and had moderate mental retardation (Barnard *et al*., 1984). Cytogenetic evidence, therefore, indicates the presence of a tumour suppressor gene(s) on the short arm of chromosome 7 and perhaps a secondary role for 7q in the genesis of Wilms tumour.

Because of the strong circumstantial cytogenetic evidence for the involvement of 7p in Wilms tumorigenesis, we have undertaken an LOH analysis of sporadic Wilms tumours using polymorphic microsatellite markers and demonstrate evidence for a tumour suppressor gene in this region.

Results

In this study, 40 Wilms tumours and corresponding normal samples from peripheral blood lymphocytes were used for LOH analysis. The patients presented to the Hospitals for Sick Children, Great Ormond Street (GOS), London, over the past 15 years. Following the clinical diagnosis of a 'Wilms tumour', the patients underwent nephrectomy and DNA was prepared from the tumours. Since it has become important to correlate genetic changes with tumour phenotype, in WT in particular, the clinicopathologic details of these 40 Wilms tumours are summarized in Table 1. Two of the tumours analysed in this study were of a very unusual histologic subtype, showing monomorphous epithelial changes. These tumours arose in a brother and sister, their mother had been cured of a Wilms tumour by nephrectomy 20 years previously. In view of this history of familial Wilms tumour, GOS 149 and 399 alone cannot be classified as sporadic Wilms tumours, but were still analysed for LOH on 7p.

Table 1 Wilms tumour samples used in 7p LOH analysis

No	*Tumour sample (GOS no.)*	*Disease stage*	*Histopathology*	*Status*
1	249	1	Mononorphous Epithelial	Alive
2	145	3	FH	Alive
3	16	3	FH	Alive
4	54	3	FH	Alive
5	55	2	FH	Alive
6	101	1	UH	Relapsed/Alive
7	185	1	FH	Alive
8	120	4	FH	Alive
9	132	1	FH	Alive
10	90	1	FH	Alive
11	244	3	FH	Alive
12	219	1	FH	Alive
13	129	3	FH	Alive
14	231	5	FH	Alive
15	100	2	FH	Alive
16	207	1	FH	Alive
17	44	4	FH	Alive
18	89	3	FH	Alive
19	135	3	FH	Alive
20	146	1	FH	Alive
21	218	5	FH	Alive
22	126	3	FH	Alive
23	51	3	FH	Alive
24	360	1	FH	Alive
25	270	4	FH	Alive
26	399	1	Mononorphous Epithelial	Alive
27	407	3	UH	Relapsed/died
28	446	1	FH	Alive
29	206	4	FH	Alive
30	234	3	FH	Alive
31	169	4	FH	Alive
32	439	3	FH	Alive
33	178	1	FH	Alive
34	66	Bilateral	FH	Alive
35	542	3	FH	Alive
36	505	5	FH	Alive
37	119	1	FH	Alive
38	96	4	FH	Relapsed/Died
39	198	Bilateral	FH	Relapsed/Died
40	358	4	FH	Relapsed/Alive

*In the absence of anaplastic nuclear changes the term favourable histology (FH) is used due to the generally good outcome for these patients

Loss of constitutional polymorphism for 7p microsatellite markers

All of the polymorphic microsatellite markers tested had a frequency of heterozygosity above 0.73 and all 40 patients were heterozygous for at least three of the 10 polymorphic loci tested. The physical location of the microsatellite markers used is shown in Table 2, and is derived from published data (Green *et al*., 1994; Tsui *et al*., 1995) as well as that presented at the most recent chromosome 7 workshop (SCW7.2). Not all of the markers used in this study have been assigned to a cytogenetic or radiation hybrid (RH) map (Green *et al*., 1994; Tsui *et al*., 1995). The optimal PCR conditions for each of the microsatellite primer pairs is also detailed in Table 2.

LOH for markers on the short arm of chromosome 7 was found in 4 (10% of the 40 tumours; GOS 44, 96, 146 and 231. The autoradiographs from these microsatellite repeats are shown in Figure 1 and summarized diagrammatically in Figure 2. Although the extent of the allelic loss varied between each of the four tumours, in all but one case, LOH resulted in hemizygosity.

Allelic loss in tumour GOS 44 extended from 7pter to a position distal to the D7S502 marker in 7p11.2. Since marker D7S679 (the next most telomeric marker tested to D7S502) was not informative in this tumour, we could only assign the extent of the allelic loss in this case to between the D7S485 and D7S502 loci (Figure 2). In this same tumour, allelic loss was shown for polymorphic markers, D7S517, D7S503, D7S493, D7S673, D7S528 and D7S485 (Figure 1). Using primer pairs for the D7S507 locus, comparison of tumour and normal DNA from this patient, showed a homozygous deletion at this locus (Figure 1). Flanking markers D7S517 and D7S503 were both still hemizygous in this tumour thereby defining the maximum extent of this deletion. The presence of very faint bands at the position of the lost alleles (Figure 1), especially D7S507, most likely represent the presence of residual normal kidney cells within the tumour. This data is perhaps best explained by the presence of an homozygous deletion for a region including D7S507 in GOS 44. The most recent chromosome 7 workshop (SCW 7.2) places D7S507 centromeric to D7S517 with a LOD score of 1000 : 1, and with a physical location of 7p15-21 (Green *et al*., 1994; Tsui *et al*., 1995). It would be unlikely for there to be retention of the D7S507 locus, when markers either side (D7S517 and D7S503) unequivocally show LOH, unless this tumour had a very complex and unusual chromosomal rearrangement. The physical distance between D7S517 and D7S503 is approximately 21.3 cM (Green *et al*., 1994; Tsui *et al*., 1995). Clearly, further polymorphic loci mapping between D7S517 and D7S503 should be tested against tumour GOS 44 to define the extent of the deleted region further.

In tumour GOS 231, LOH was observed for polymorphic markers D7S517, D7S507, D7S673 and D7S528 (Figure 1). The physical extent of the LOH in this tumour, therefore, extended from 7pter to 7p15 (Green *et al*., 1994; Tsui *et al*., 1995). Comparison of the PCR product from the constitutional *versus* the tumour DNA for locus D7S493 apparently reveals three bands in the constitutional DNA and two in the tumour DNA (Figure 1). Because there is clear evidence for allelic loss at markers above and below D7S493 (Figure 1), it is likely that the region of LOH in GOS231 includes D7S493 and that loss of the lowermost band represents tumour-specific allele loss. However, interpretation of the PCR product for locus D7S493 in this tumour could only be scored as 'tentative' (Figure 1).

In tumour GOS 96, the D7S517 marker was not informative but there was evidence for LOH at D7S507, whereas markers centromeric to, and including, D7S503 showed retention of heterozygosity (Figure 1). The region of LOH in GOS 96 therefore extends only from the telomere to D7S507 at the p15-p21 border (Green *et al*., 1994; Tsui *et al*., 1995). There was evidence for LOH at D7S517 and D7S503 in GOS 146, while marker D7S507 was not informative (Figure 1). Retention of heterozygosity is shown at marker

Table 2 Microsatellite repeat primer pairs for chromosome 7p LOH analysis

Locus symbol	*Physical location*	*Degree of heterozygosity*	*CA strand primer*	*GT strand primer*	*Size range (base pairs)*	*(Mg^{2+}) m.mol*	*Annealing temperature*
			TGGAGAAGC	AGCTGTAATTA			
D7S517	7p22	0.83	CATGTGAGT	GTTGCTGGTTTGA	239–257	1.5	56°C
D7S507	7p21-p15	0.89	TGCCCAATT	CTACGTACA	148–168	2	58°C
			CTCAGTGTT	TGCCTGCAA			
D7S503	7p21-p15	0.87	ACTTGGAGTA	GTCCCTGAAAA	148–180	2	58°C
			ATGGGAGCAG	CCTTTAATCAG			
D7S493	7p15	0.88	GGAAGTTCCC	GAAAGCACTTAC	194–224	2	60°C
			AGCCATAGTT	CTACTGAGGATTT			
D7S673	7p15-p14	0.86	GGGGNCCTT	TCCCAGTCC	118–148	1.5	58°C
			GAGAAGT	TGTGGCTAC			
D7S528	7p14	0.73	TCAACTTGAA	CATGTGCG	108–116	1.5*	52–56°C‡
			TTTCACTTTCAG	TGCTTGTGT			
D7S485	7p14	0.78	TCTGTCATGT	CTACAGGATTCT	244–256	2	55°C
			GAGGATGCAG	ATTCAGGATACG			
D7S510	7p14	0.79	CAGTGTGGA	ACCCAGCC	252–264	2	52–56°C‡
			GGTTCCCAA	GCAAGATT			
D7S679	7p14–p13	0.81	TCACCAGCA	TACAGGCGTG	118–130	1.5	54°C
			GGCTTCCATA	AGGCACCAT			
D7S502	7p13	0.84	GGAAGGTA	TAAGCCACC	273–291	2	58°C
			TGTTGCGG	AAGAACACC			

*Also required 1% DMSO. ‡Touch down program initial annealing temperature 56°C final annealing temperature 52°C

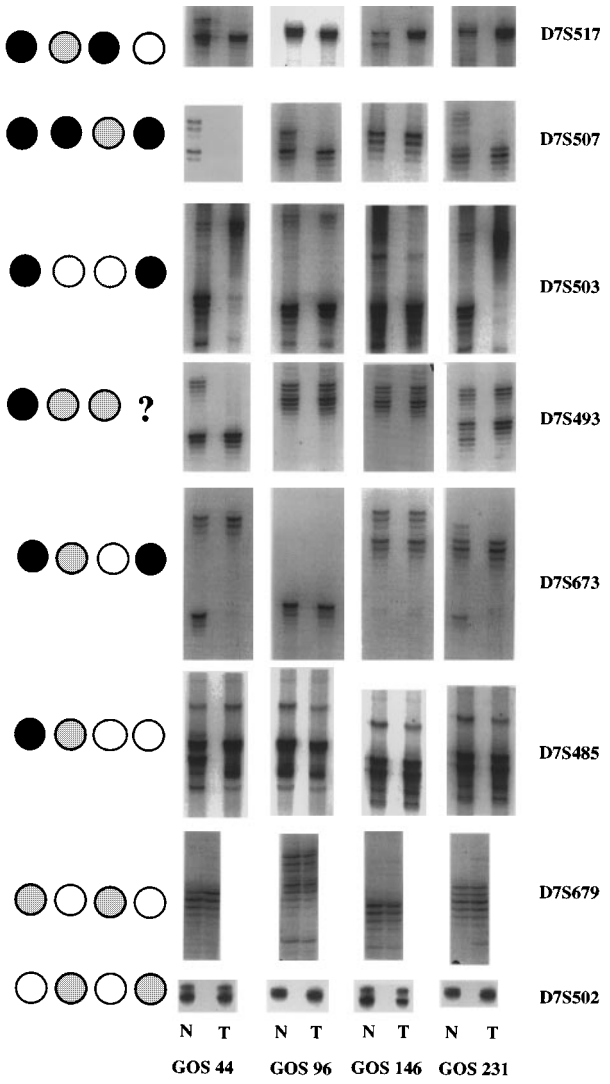

Figure 1 Examples of microsatellite analysis for the four Wilms tumours showing loss of heterozygosity for markers on chromosome 7. An interpretation of the status of alleles at individual loci is shown in the right for each of the four tumours where; the filled circles indicate LOH; the hatched circles indicate that the patients were constitutionally homozygous at these loci; the open circles indicate no loss of heterozygosity. In GOS 231 at locus D7S493, there were apparently three alleles detected and, although the lowermost allele shows LOH, the two upper bands are retained in the tumour

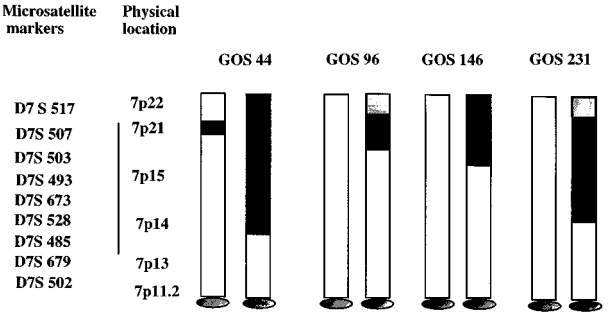

Figure 2 Diagrammatic representation of the extent of LOH from the four tumours showing allele loss on the short arm of chromosome 7

D7S493 in Figure 1, the deletion in this tumour therefore also extended to the 7p15-21 border.

Discussion

Loss of heterozygosity for polymorphic markers in tumour compared to constitutional DNA implies that the loss of such genetic material has a role in tumorigenesis. By contrast to adult malignancies, such as breast and colon cancer, where multiple chromosomal regions show LOH, allelic loss in Wilms tumour is restricted to a relatively small number of loci (Devilee *et al*., 1991). Indeed, of 33 autosomal arms analysed for LOH, only two regions, the short arm of chromosome 11 and the long arm of chromosome 16, showed a significant degree of allelic loss in one study (Maw *et al*., 1986). Excluding loci on chromosomes 11 and 16, LOH was observed at less than 5% of the total number of informative loci analysed in these same tumours (Maw *et al*., 1986). These findings suggest that LOH in Wilms tumour is a non-random event occurring only in specific chromosomal regions. Cytogenetic studies have also implicated chromosome 7 in Wilms tumorigenesis (Rivera *et al*., 1985) and, in particular, suggest the presence of a tumour suppressor gene at 7p15 (Hewitt *et al*., 1991; Wilmore *et al*., 1994; Sawyer *et al*., 1993; Miozzo *et al*., 1996). We therefore analysed a series of sporadic Wilms tumours from a single institution for allelic loss on the short arm of chromosome 7. Four (10%) of 40 tumours showed LOH for polymorphic markers on 7p which is twice the reported background frequency of LOH in Wilms tumours (Maw *et al*., 1986). Molecular analysis revealed a common region of LOH in all four tumours extending from D7S503-pter. In two of the tumours, GOS 96 and GOS 146, LOH was limited to this region, whilst the other two, GOS 231 and GOS 44, showed allelic loss extending proximally to 7p15 and 7p12 respectively. The shortest region of overlap is further defined by the discovery of a putative homozygous deletion including marker D7S507 in tumour GOS 44 which was not present in the constitutional DNA. In this same tumour, GOS 44, there was evidence for hemizygous allelic loss at marker D7S517, which lies distal to D7S507, and at D7S503 which lies proximal to the homozygously deleted marker. Marker D7S664, which maps between D7S517 and D7S503, was not informative in this patient (data not shown). According to the most recent mapping data, D7S517 and D7S503 are approximately 21.3 cM apart. The evidence presented here, therefore, supports the presence of a tumour suppressor gene located between D7S517 and D7S503, the physical location of which is 7p15-21 (Green *et al*., 1994; Tsui *et al*., 1995). These observations provide further evidence for the existence of a tumour suppressor gene on chromosome 7p that is causally involved in Wilms tumorigenesis. Other supporting evidence is provided by a molecular study of a patient with a Wilms tumour and bilateral nephrogenic rests (Wilmore *et al*., 1994). Nephrogenic rests are considered, by some, to be precursor lesions for Wilms tumour (Beckwith *et al*., 1990). This patient had a constitutional t(1;7) translocation interrupting the 7p15 region (Hewitt *et al*., 1991). Cytogenetic analysis of the tumour revealed the retention of the translocation and an isochromosome of the long arm of 7 (Wilmore *et al*.,

1994). It is possible that the constitutional translocation breakpoint interrupts a critical gene on 7p15 representing the first hit, the remaining normal copy of the gene at 7p15 is then lost in the tumour resulting in the inactivation of both copies of a tumour suppressor gene at this locus. The constitutional translocation breakpoint from this patient has now been localized within a YAC contig and lies between microsatellite markers D7S795 proximally and D7S683 distally (Reynolds *et al*., 1996). The constitutional breakpoint in this patient therefore lies distal to the homozygous deletion in GOS 44.

LOH for markers on 7p have also been reported in other sporadic Wilms tumours (Wilmore *et al*., 1994; Miozzo *et al*., 1996). Wilmore *et al*. (1994) analysed a series of 23 sporadic Wilms tumours for LOH of markers on 7p by Southern blot analysis, reporting LOH for the 7p14-pter region in a bilateral tumour and trisomy for chromosome 7 in two other unilateral tumours (Wilmore *et al*., 1994). Miozzo *et al*. (1996) reported cytogenetic abnormalities involving chromosome 7 in three of 11 (27%) sporadic Wilms tumours. Molecular characterization of these 11 tumours using a panel of microsatellite markers revealed allelic losses or imbalance in four tumours. Three of 27 tumours (27%) showed LOH for microsatellite markers on 7p and, although the extent of the allelic loss varied in length between these tumours, a common region of allele loss was identified between loci D7S528 (proximal) and D7S510 (distal) in 7p14-13 (Miozzo *et al*., 1996). The physical distance between these markers was estimated to be 25 cM (Miozzo *et al*., 1996). However, the proximal and distal extent of LOH in several of these tumours is not clearly defined; in one tumour (case 3) the distal extent of the region of allele loss has not been determined and in two other tumours (cases 6 and 8) the proximal and distal extent of LOH was inferred from cytogenetic data rather than by molecular analysis (Miozzo *et al*., 1996). Therefore, the true extent of the reported deletions in these tumours remains unclear at present and it is uncertain whether there is any overlap with the region defined in our study.

One of the four tumours found to have LOH for 7p in the molecular study reported here, GOS 231, had previously undergone cytogenetic analysis. Solis *et al*. (1988) reporting a t(7;11)(p15;q32) in this tumour. Molecular characterization of this same tumour showed LOH for 11p15 markers but retention of heterozygosity at 11p13 (Wadey *et al*., 1990). LOH for 11p markers in GOS 231 was unequivocal and consistent (Wadey *et al*., 1990), whereas, the cytogenetic abnormality was a frequent but not a universal finding (Solis *et al*., 1988). These observations suggest that loss of genetic material from 11p preceded the cytogenetic abnormality (Solis *et al*., 1988; Wadey *et al*., 1990). The data presented here shows unequivocal LOH for 7p markers in GOS 231, extending from 7p15 to 7pter. By the same reasoning, LOH for markers from 7p15-pter must have arisen before the cytogenetic abnormality on the long arm of 7q-t(7;11)(p15;q32). This observation is consistent with the cytogenetic and molecular analysis of a number of Wilms tumours in which the 7q abnormalities were considered to be a progression event (Wang-Wuu *et al*., 1990; Kaneko *et al*., 1991; Sawyer *et al*., 1993; Peier *et al*., 1995; Wilmore *et al*., 1994; Rivera, 1995; Miozzo *et al*., 1996).

Table 3 Cytogenetic abnormalities of chromosome 7 in patients with Wilms tumour; clinical correlates

Author	*Age (months)*	*Stage*	*Histology*
This paper	28	4	FH
This paper	22	4	FH
This paper	12	1	FH
Solis + This paper	18	3	FH
Solis	48	4	FH
Hewitt	60	1	FH
Wilmore	–	5	
Pier	48	2	FH
Wang-Wu	54	4	FH
Wang-Wu	60	3	FH
Wang-Wu	28	4	FH
Kaneko	24	1	FH
Kaneko	24	3	FH
Sawyer	11	1	FH
	33.6	St 1 = 4 (29%)	
		St 2 = 1 (7%)	
		St 3 = 3 (21%)	
		St 4 = 5 (36%)	
		St 5 = 1 (7%)	

The possibility of an association between cytogenetic and/or molecular changes on the short arms of chromosomes 7 and 11 is raised by the extensive study of tumour GOS 231 and by two other reports in the literature (Wang-Wuu *et al*., 1990; Wilmore *et al*., 1994). A bilateral tumour analysed by Wilmore *et al*. (1994) was noted to have LOH for markers on the short arms of chromosomes 7 and 11p15. Wang-Wuu *et al*. (1990) reported an isochromosome 7q and a del 11(p12;p13). One other tumour, GOS 21, karyotyped in our laboratory by Solis *et al*. (1988) was found to have a cytogenetic abnormality involving chromosome 7. Unfortunately, there was no constitutional DNA available for LOH studies as this patient had died of disease.

Clinical details are available on 14 patients with abnormalities of 7p and are summarized in Table 3. The average age at onset of Wilms tumour in this group is 34 months, considerably younger than expected for unilateral cases, raising the possibility of a Wilms tumour predisposition gene in this locus (Breslow *et al*., 1993). The distribution of stage-of-disease at presentation is also skewed towards presentation with higher stage disease, 36% of patients having stage 4 disease compared to around 14% in the National Wilms tumour studies and the United Kingdom trials (Pritchard *et al*., 1995; D'Angio *et al*., 1989). Although the numbers in this review are very small, the possibility of an association between LOH for markers on 7p with earlier onset and more advanced disease is worth further investigation in a larger number of patients.

Materials and methods

Microsatelite polymorphic markers for 7p

DNA was prepared from tumour tissue and lymphocytes using standard phenol/chloroform extraction techniques as described previously (Wadey *et al*., 1990). The CA strand primer was radiolabelled with gamma ^{32}P (0.4 μCi) using polynucleotide kinase (NBL). The PCR was performed using a Biometra thermal cycler in a reaction volume of 10 μl containing 50–100 ng of tumour or constitutional

DNA, dNTP's at a final concentration of 0.2 mM, 1.5–2 mmol Magnesium chloride, 1–2 pmol labelled and unlabelled primer, Taq DNA polymerase 0.5 U and 1 × Buffer (Bioline). The step-cycle file comprised 3 min denaturation at 94°C, 30 cycles of 94°C for 30 s, annealing temp for 30 s and 72°C for 30 s. The annealing temperature varied with each primer system (Table 2). On completion, 6 μl of stop solution (Gibco BRL) was added to each sample and denatured at 95°C for 3 min. 3 μl of the resultant solution was loaded onto a 6% denaturing polyacrylamide gel. The gel was run at 60 watts constant power in 1 × TAE buffer; the length of time depended on the fragment size. Gels are then fixed in 10% methanol, 10% acetic acid prior to drying and exposure to XAR-5 film (Kodak) for 12–72 h without an intensifying screen.

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