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# Nonrandom Loss of Maternal Chromosome 11 Alleles in Wilms Tumors

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SUMMARY

A series of gene probes for chromosome 11 has been used to study the genetic events associated with the development of Wilms tumor. Examination of DNA samples from (1) five patients with Wilms tumor in whom the tumors showed loss of chromosome 11 alleles and (2) their parents indicate that alleles lost in the tumors are of maternal origin. These data suggest that the parental derivation of chromosome 11 alleles lost in these Wilms tumors is not random.

## INTRODUCTION

Wilms tumor (WT) is thought to arise when two mutations at homologous loci result in the loss of normal gene function (Knudson and Strong 1972; Riccardi et al. 1978). The first mutation may occur in germ-line or somatic cells—resulting in heritable or nonheritable Wilms tumor, respectively—and the second in somatic tissue, rendering the cell homozygous or hemizygous for the mutant locus (Knudson and Strong 1972). Loss of heterozygosity for gene markers on chromosome 11 has previously been observed in some Wilms tumors (Fearon et al. 1984; Koufos et al. 1984; Orkin et al. 1984; Reeve et al. 1984; Raizis, 1985). This loss may be attributed to chromosome loss or dele-

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tion, mitotic nondisjunction, or recombination events. By analogy to studies on retinoblastoma, it is believed that the chromosome that is retained in the Wilms tumor tissue carries a mutation at the Wilms tumor locus (Cavenee et al. 1983, 1985). In nonhereditary cases of Wilms tumor, we would expect that the initial somatic mutation would occur with equal frequency on the maternally or paternally derived chromosome, which would result in an equal frequency of maternally and paternally derived alleles, respectively, retained in the tumor. To determine the parental derivation of the initial mutation, we examined chromosome 11p markers in parents of patients with Wilms tumor who showed loss of heterozygosity for 11p markers. In five cases in which we could determine which parent had contributed the alleles not present in the tumor, we found that the maternal alleles were absent. These findings suggest that the loss of maternal alleles in Wilms tumors is not a random event.

## MATERIAL AND METHODS

The five informative Wilms tumor patients studied included three with sporadic unilateral Wilms tumor and no aniridia or other congenital anomalies; one phenotypic female with a 46,XY karyotype, bilateral Wilms tumor, and Drash syndrome (Goldman et al. 1981); and one patient with bilateral cryptorchidism and a single Wilms tumor in a horseshoe kidney. Cytogenetic analysis of tumor and constitutional samples has been reported elsewhere (Dao et al., in press). Parental, constitutional, and tumor DNA samples were obtained from leukocytes, lymphoblastoid cell lines transformed by Epstein-Barr virus, and surgical or autopsy tissue samples, as described elsewhere (Gray et al. 1985; Dao et al., in press). The DNA samples were then digested with appropriate restriction enzymes, electrophoresed on 1% agarose gels, transferred to nitrocellulose filters, and hybridized with a number of nick-translated or oligolabeled gene probes ($1–3 \times 10^8$ cpm/$\mu$g) specific for human chromosome 11 (HGM 1985). The gene probes used in this study were c-Harvey *ras* 1 oncogene (11p15) (Shih and Weinberg 1982; HGM 1985), insulin (11p15) (Bell et al. 1981; Harper et al. 1981), parathyroid hormone (PTH) (11p15) (Hendy et al. 1981; HGM 1985), catalase (11p13) (Korneluk et al. 1984; Quan et al. 1985), pepsinogen (11p11-q13) (HGM 1985; Taggart et al. 1985), and apolipoprotein A1 (ApoA1) (11q13) (Schroeder and Saunders 1987).

## RESULTS

In the five Wilms tumor patients studied, a restriction-fragment-length polymorphism was detected constitutionally for at least one marker on 11p, as shown in table 1. Figure 1 displays hybridization patterns of chromosome 11 markers to digested DNA samples. Patient 1 had a normal karyotype, no aniridia, and a typical sporadic Wilms tumor. She was heterozygous for both the PTH and ApoA1 markers in her normal kidney DNA, as demonstrated by 2.7- and 2.1-kb hybridizing bands for PTH and 8.3- and 6.6-kb bands for ApoA1. In her Wilms tumor tissue, only the 2.7-kb PTH and 6.6-kb ApoA1 bands were present. The mother was homozygous for the 2.1-kb PTH allele, and the father was homozygous for the 2.7-kb allele. In addition, the mother

TABLE 1

RESTRICTION-FRAGMENT-LENGTH POLYMORPHISMS IN WILMS TUMOR PATIENTS

| PATIENT AND TISSUE SOURCE | GENE PROBE (Restriction Endonuclease) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | c-Ha-*ras*1 (*Bam*HI) | Insulin (*Eco*RI) | PTH (*Pst*I + *Hin*dIII) | Catalase (*Kpn*I) | Catalase (*Hae*III) | Catalase (*Taq*I) | Pepsinogen (*Eco*RI) | ApoA1 (*Sst*I) | ApoA1 (*Xmn*I) |
| 1: | | | | | | | | | |
| Father | C/C | C/C | A/A | ... | A/B | ... | ... | A/A | B/B |
| TT | C/C | C/C | A/A | B/B | B/B | B/B | ... | A/A | B/B |
| NK | C/C | C/C | A/B | B/B | B/B | B/B | ... | A/A | A/B |
| Mother | C/C | C/C | B/B | ... | B/B | ... | ... | A/A | A/B |
| 2: | | | | | | | | | |
| Father | A/C | C/C | B/B | A/B | ... | ... | ... | A/A | A/B |
| Blood | A/C | C/C | A/B | ... | ... | ... | ... | A/A | A/B |
| TT | A/A | C/C | B/B | B/B | ... | B/B | ... | A/A | A/B |
| VM | A/A | C/C | B/B | B/B | ... | B/B | ... | A/A | A/B |
| NK | A/C | C/C | A/B | A/B | ... | A/B | ... | A/A | A/B |
| Mother | C/C | A/C | A/A | A/A | ... | ... | ... | A/A | A/B |
| 3: | | | | | | | | | |
| Father | C/C | A/C | A/A | A/A | ... | A/A | ... | ... | A/A |
| TT | C/C | C/C | A/A | A/A | B/B | A/A | ... | A/B | A/A |
| NK | C/C | C/C | A/B | A/A | B/B | A/B | ... | A/B | A/A |
| Mother | C/C | C/C | B/B | A/B | ... | B/B | ... | ... | A/A |
| 4: | | | | | | | | | |
| Father | B/C | A/C | A/A | A/A | ... | ... | ... | A/A | A/A |
| TT | C/C | A/A | A/A | A/A | B/B | ... | B/C | A/A | A/A |
| NK | C/C | A/C | A/A | A/A | A/B | ... | B/C | A/A | A/A |
| Mother | B/C | C/C | A/B | ... | ... | ... | ... | ... | ... |
| 5: | | | | | | | | | |
| TC | C/C | C/C | B/B | ... | B/B | B/B | ... | A/A | ... |
| LC | C/C | B/C | B/B | ... | B/B | A/B | A/A | A/A | A/A |
| Mother | C/C | B/C | B/B | ... | B/B | A/A | ... | A/A | ... |

NOTE.—Father's, mother's, tumor-tissue (TT), normal kidney (NK), blood, vaginal metastasis (VM), lymphocyte-culture (LC), and tumor-culture (TC) DNAs were digested with appropriate restriction enzymes, electrophoresed on 1% agarose gels, transferred to nitrocellulose filters, and hybridized with nick-translated or oligo-labeled probes ($1–3 \times 10^8$ cpm/μg) as previously described (Goldman et al. 1981). Alleles for each locus are lettered with A as the longer allele and B and C as shorter alleles. Loss of heterozygosity in tumor tissues is indicated by enclosed boxes. An ellipsis indicates that the samples were not digested with that restriction enzyme. Patient 1, 197830-000; patient 2, 198871-000; patient 3, WT-5100-00; patient 4, 84-WT-181; and patient 5, LCS-150.

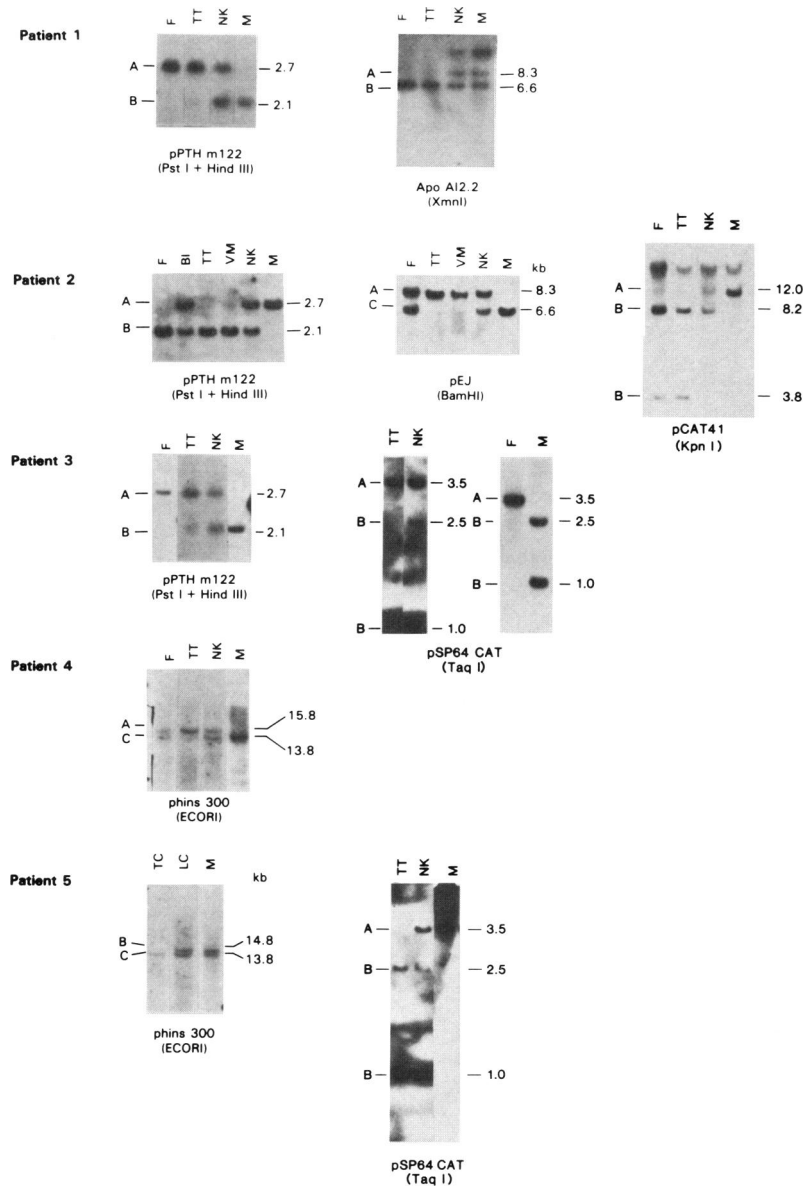


FIG. 1.—Autoradiograph of Southern blots containing digests of DNAs from Wilms tumor patients and their parents, hybridized with probes from chromosome 11. Alleles at each locus are labeled A, B, or C, according to decreasing length. Numbers to the right indicate the molecular size in kilobases.

was heterozygous for the 8.3- and 6.6-kb ApoA1 alleles, whereas the father was homozygous for the 6.6-kb allele. Therefore, according to Mendelian genetics, the 2.7- and 6.6-kb bands retained in the tumor DNAs were inherited from the father.

Patient 2 was constitutionally heterozygous at the c-Ha-*ras*-1, PTH, catalase, and ApoA1 loci (table 1). Hybridizing bands of 8.3 and 6.6 kb, 2.7 and 2.1 kb, and 8.3 and 6.6 kb represent the presence of two alleles each for c-Ha-*ras*-1, PTH, and ApoA1, respectively, whereas a band of 12.0 kb represents one catalase allele and bands of 8.2 and 3.8 kb represent a second catalase allele in *Kpn*I-digested DNA. In the tumor, the presence of only the 8.3-kb allele for c-Ha-*ras*-1, the 2.1-kb allele for PTH, and the 8.2/3.8-kb allele for catalase could be detected. Since the mother was homozygous for the 6.6-kb allele for c-Ha-*ras*-1 and the 12.0-kb allele for catalase, the 8.3-kb c-Ha-*ras*-1 and the 8.2/3.8-kb catalase alleles were inherited from the father. Both alleles for ApoA1 were detected in the tumor tissue, however, indicating that loss of maternal loci occurred on the short arm of chromosome 11 from the catalase gene to the telomere.

Hybridization of the PTH probe to *Pst*I + *Hin*dIII-digested DNA from patient 3 demonstrated heterozygosity for alleles of 2.7 and 2.1 kb, as displayed in normal kidney DNA (fig. 1). In Wilms tumor DNA, however, only the 2.7-kb allele was visible. Since the mother was homozygous for the 2.1-kb allele and the father was homozygous for the 2.7-kb allele, the 2.7-kb allele was paternally derived, which again demonstrated the loss of the maternal allele in the tumor. Wilms tumor tissue from patient 3 retained both alleles for the ApoA1 gene tissue, again demonstrating that loss of maternal loci was limited to the short arm of chromosome 11.

The two remaining Wilms tumor patients were heterozygous for both the insulin and catalase genes. Normal kidney DNA from patient 4 displayed bands of 15.8 and 13.8 kb on hybridization with the insulin probe, but his Wilms tumor DNA displayed only a hybridizing band of 15.8 kb. The mother was homozygous for the 13.8-kb allele; therefore, the 15.8-kb allele remaining in the tumor tissue was the allele inherited from the father. Tumor DNA retained both alleles for pepsinogen, a gene located in 11p11-q13, again revealing the specificity of the 11p region for tumor-specific alterations. Similarly, leukocyte DNA from patient 5 showed hybridizing bands of 14.8 and 13.8 kb with the insulin probe and of 3.5, 2.5, and 1.0 kb with the catalase probe. Although the father's DNA was unavailable in this case, the 2.5/1.0-kb allele for catalase remaining in the tumor tissue had to be of paternal origin, since the mother was homozygous for the 3.5-kb allele.

## DISCUSSION

We have demonstrated that in these five cases in which normal, tumor, and parental DNA samples were obtainable and informative, the loss of heterozygosity for 11p markers in Wilms tumors was due to loss of maternal alleles. Review of the literature revealed that parental samples have been studied in two other cases of sporadic Wilms tumor in which the tumor showed loss of

heterozygosity for 11p markers. In both cases only the paternal alleles were present in the tumor (Reeve et al. 1984). In addition, in one case of sporadic multifocal retinoblastoma in which loss of an allele on chromosome 13 had occurred, it was the paternal allele that was retained in the tumor (Dryja et al. 1984). If the genetic events on 11p13 leading to Wilms tumor are analogous to those on 13q14 leading to retinoblastoma (Cavenee et al. 1985), then these findings imply that an initial mutation consistently occurred on the paternal chromosome 11p and that subsequent loss of the maternal wild-type allele was associated with tumor development.

According to the "two-hit" mutation model for Wilms tumor (Knudson and Strong 1972), the first mutation may occur in a germinal or somatic cell. When the first mutation occurs in the germ line, tumors are more often bilateral and may be associated with aniridia or genitourinary anomalies (Knudson and Strong 1972; Bond 1975). All bilateral tumors are assumed to arise from initial germinal mutations, whereas only 15% of sporadic unilateral Wilms tumors are estimated to be due to germinal mutations (Strong 1984). The present series includes one patient with bilateral Wilms tumor and Drash syndrome (patient 3) and one patient (patient 4) with multiple genitourinary anomalies and a fetal rhabdomyomatous Wilms tumor, a rare histologic type often associated with bilateral tumors, young age at onset, and, occasionally, aniridia (Wigger 1976; Gonzales-Crussi et al. 1981). For these two patients and one reported multifocal retinoblastoma patient (Dryja et al. 1984), the initial mutation may be germinal—and its occurrence in one paternally derived chromosome could be attributed to an increased risk of new mutations during spermatogenesis (Vogel and Rathenberg 1985). However, the three other patients in this series and two cases reported elsewhere (Reeve et al. 1984) had sporadic unilateral Wilms tumor and no congenital anomalies. The overwhelming majority of such cases is expected to be nonhereditary and the result of two somatic events. We expected that there would be an equal probability of the initial mutation occurring on one paternally or maternally derived chromosome—and, hence, an equal probability of observing the loss of the paternal or maternal allele in the tumors. However, in all cases it was the maternal alleles that were absent in the tumors, implying that the initial mutation consistently occurred on the paternally derived chromosomes. Although our test sample, which includes the five cases studied here and two others previously reported (Reeve et al. 1984), is small, the probability that all tumors would retain the chromosome derived from the father by chance (i.e., that the same parental chromosome would always undergo the initial mutation) is small ($\chi^2 = 7$; $P < .008$). The probability of all seven patients losing the maternal allele in their Wilms tumor tissues, if the loss is indeed random, is <1%.

All cases observed here as well as those previously reported (Dryja et al. 1984; Reeve et al. 1984) were sporadic, so the finding of tumor-specific nonrandom loss of maternal alleles implies that the initial germinal or somatic mutation occurred on the paternal chromosome. Since reports of familial Wilms tumor indicate that transmission of a presumed Wilms tumor mutation occurs equally in males and females (Strong 1984), we anticipate that tumors in familial

cases would selectively retain the inherited mutant paternal or maternal allele, as has been observed in the case of retinoblastoma (Cavenee et al. 1985). Given the rarity of familial Wilms tumor, we have not had the opportunity to test this hypothesis.

To investigate paternal factors that might be associated with an increased risk of new mutations, we surveyed both the paternal occupation prior to the patients' births and maternal age. No father had been involved in work associated with hydrocarbon or lead exposure, paternal occupations previously associated with an increased risk of Wilms tumor (Kantor et al. 1979; Wilkens and Sinks 1984). The paternal age at the patients' births ranged from 20 to 40 years with a mean of 29.5 years, less than that observed in new autosomal dominant mutations associated with advanced paternal age (Vogel and Rathenberg 1985).

In this series as well as in a review of cases reported in the literature, we have consistently observed tumor-specific retention of the paternally derived alleles for chromosome 11 or 13 markers in all Wilms tumors or retinoblastomas studied, including tumors in which the initial mutation was presumably germinal (multifocal retinoblastoma and Wilms tumor) and somatic. The data imply that, both in germinal and somatic cells, it is the paternal chromosome on which the initial mutation has occurred. Further studies of Wilms tumor etiology using combined molecular-epidemiologic approaches are needed to confirm the unexpected finding of nonrandom selection of paternal 11p alleles in tumors and to understand the significance of this finding.

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