Linkage of Familial Wilms’ Tumor Predisposition to Chromosome 19 and a Two-Locus Model for the Etiology of Familial Tumors

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Abstract

Familial predisposition to Wilms’ tumor (WT), a childhood kidney tumor, is inherited as an autosomal dominant trait. For most WT families studied, the 11p13 gene WT1 and genomic regions implicated in tumorogenesis in a subset of tumors can be ruled out as the site of the familial predisposition gene. Following a genome-wide genetic linkage scan, we have obtained strong evidence (log of the odds ratio = 4.0) in five families for an inherited WT predisposition gene (FW72) at 19q13.3–q13.4. In addition, we observed loss of heterozygosity at 19q in tumors from individuals from two families in which 19q can be ruled out as the site of the inherited predisposing mutation. From these data, we hypothesize that alterations at two distinct loci are critical rate-limiting steps in the etiology of familial WTs.

Introduction

WT is a childhood kidney tumor that occurs in both sporadic and familial forms. Analysis of WT families has indicated that predisposition is due to an autosomal dominant trait with incomplete penetrance (1, 2). One WT gene, WT1, located at chromosome 11p13, has been isolated, and WT1 mutations have been identified in a subset of tumors (3, 4). However, genetic linkage analyses ruled out WT1 as the WT predisposition gene in several large WT families, demonstrating that the etiology of WT is heterogeneous (5–7). Although genetic linkage at 17q has been reported in one large WT family (8), 17q can be excluded as the site of the predisposing mutation in most families studied (9). Similarly, 11p15 and 16q13–q22, regions at which tumor-specific LOH has been observed in subsets of WTs (10, 11), have also been ruled out (5, 6, 12). To localize a gene conferring predisposition to WT in these families, we undertook an extensive, genome-wide genetic linkage study. From these analyses, we can demonstrate linkage to a ~14-cM region of 19q in five WT families. Additionally, familial tumors were assessed for LOH at this region of 19q.

Materials and Methods

WT Families. The pedigrees of the five WT families are shown in Fig. 1. The families include 23 individuals with a confirmed diagnosis of WT and 2 individuals with a probable diagnosis of WT. The presence of 10 unaffected obligate carriers in the families demonstrates the incompletely penetrant nature of the predisposing gene. The observation of both male and female carriers indicates that the predisposition gene is not subject to strong imprinting effects. Affected individuals displayed none of the congenital anomalies (e.g., aniridia, genitourinary anomalies, renal mesangial sclerosis, stigmata of Beckwith-Wiedemann syndrome) that are occasionally observed in the WT patient population. There is no indication of predisposition to other childhood or adult cancers in the five linked families. Four of the families were Anglo-American, and one (WTX524) was Latino-American.

Genotyping. Tissue samples were obtained following informed consent from 68 family members, including 18 affected and 8 obligate carrier individuals. DNA was isolated from lymphocytes or lymphoblastoid cell lines from the individuals whose identification numbers are underlined in Fig. 1. These individuals were genotyped by PCR at more than 220 highly polymorphic microsatellite loci, spaced an average of 18 cM apart and located on all 22 autosomes. An additional ~70 loci were genotyped for specific genomic regions at which a LOD score of >1.0 was obtained for the largest family (WTX524). LOD scores of >0.5 were obtained for more than one family, or when genotypic data from a family were uninformative. Initially, PCR primers for a genetically defined panel of polymorphic loci spaced ~30 cM apart (Research Genetics, Huntsville, AL) were used. Subsequently, when more closely spaced markers were needed, PCR primers for additional loci at specific genomic regions were also used (Research Genetics; Genosys Biotechnologies, The Woodlands, TX).

Genetic Linkage Analysis. Genotypic data were assessed for genetic linkage by two-point analysis (LIPED algorithm; Ref. 13) and multipoint analysis (FastLink algorithm; Refs. 14 and 15). A penetrance value of 0.30 and a frequency of 0.0001 for the predisposing allele were used. At the marker loci, equal frequencies for each allele were routinely used. The robustness of the linkage data analyzed in this manner was assessed by using published allele frequencies for randomly selected loci. Because most of the obligate carriers in the families were available for genotyping, few genotypes were inferred, and varying marker allele frequencies had no effect on the resultant LOD score.

LOH Analysis. To assess familial tumors for 19q LOH, we obtained tumor tissue, resected before chemotherapy or radiation therapy was administered, from seven children in four of the five 19q-linked families (WTX524, WTX614, WTX637, and WTX917). Tumor tissue was also obtained from two individuals from two additional, small WT families that are discordant at 19q markers. These nine tumor DNA samples were genotyped by PCR at linked 19q loci (Figs. 2 and 3). One of the nine tumors, which was from an individual in family WTX668 (Fig. 4), was also included in part of the genome-wide scan and was, therefore, genotyped and assessed for LOH at more than 114 marker loci, located on all 22 autosomes.

Results and Discussion

Linkage Analysis. Initial two-point linkage analysis resulted in summed LOD scores for the five WT families of >3.0 at two consecutive loci on 19q (D19S206 and D19S572), and multipoint analyses resulted in maximal scores of 4.0 for the interval between D19S572 and D19S589 (Fig. 2). Examination of the segregating, linked haplotypes indicated that the common region of concordancy in the five families was between markers D19S801 and D19S926, which span a genetic distance of ~14 cM (Fig. 3). Analysis of all of the genotype data from the genome-wide linkage scan demonstrated that
19q was the only region in the genome at which all affected individuals in the largest family, WTX524, were concordant for linkage. Furthermore, the LOD scores obtained at 19q for the other large families equaled or surpassed the mean LOD score that would be expected to be obtained for a locus under the assumption of linkage (θ = 0), as estimated by the SIMLINK program (16). Simulation studies also estimated that the cumulative odds of obtaining false-positive LOD scores for the largest three families at the same locus was less than 1 in 100,000. Our linkage data, then, indicate that WT predisposition in these five families is the result of inheritance of a mutant allele at a 19q locus, FWT2.

The five families differed with respect to the ages at which affected members were diagnosed and the occurrence of bilateral tumors. In family WTX524, WT was uniformly diagnosed at young ages (4–37 months), and two children had bilateral tumors. In contrast, all of the affected children in family WTX614 had unilateral tumors, which, in
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Five of the six children, were diagnosed at ages older than the average age for familial cases (35 months) and sporadic cases (43 months; Ref. 17). These families all showed evidence for linkage to 19q, which suggests that the differences noted between families may be due to differences in the genetic background of modifying genes and/or differences in the particular FW T2 mutation segregating in the five families.

19q LOH in Tumors. Tumor tissue from seven children in four of the 19q-linked families (WTX524, WTX614, WTX637, and WTX917) was available for LOH analysis. None of the seven displayed LOH for markers in the linked region (data not shown). However, tumors from children from two other small families did display 19q LOH. As shown in Fig. 4, tumor DNA from one individual in family WTX668 showed 19q LOH, although affected family members were discordant at 19q loci, thereby ruling out 19q as the site of the family’s inherited predisposing gene. Because DNA from this tumor was included, along with autologous blood DNA, in part of the genome-wide scan, it was genotyped and assessed for LOH at more than 114 markers located on all 22 autosomes. In the entire genome, 19q was the only region at which LOH was detected in this tumor, which strengthens the significance of the observed 19q LOH. Similarly, in a second small WT family (WTX149), tumor DNA from an affected individual displayed 19q LOH, although 19q could be ruled out as the site of the inherited predisposition gene because the affected child and his affected uncle were discordant for 19q alleles (Fig. 4). The 19q LOH observed in these two cases (WTX668 and WTX149) suggests that, although 19q did not harbor the inherited predisposition gene in these two families, somatic alterations at the 19q FW T2 locus were important in tumorigenesis.

Two-Locus Mutational Model for the Etiology of Familial WT. From the above data, we speculate that critical rate-limiting steps in the etiology of familial tumors involve at least two separate genes, one of which is altered by a germ-line predisposing mutation and the other of which is altered somatically. Depending upon the WT family, the germ-line mutation occurs at either of these two loci, with the tumor-specific somatic alteration(s) occurring at the other locus. This model is reminiscent of that proposed for tumor development in hereditary multiple exostoses, an autosomal dominant disorder in which individuals with these bony overgrowths are at a greatly increased risk of developing chondrosarcoma. This disorder is genetically heterogeneous; three exostosis genes have been identified by genetic linkage studies of families. Molecular analyses of these tumors have indicated that tumorigenesis involves a combination of germ-line and somatic mutations at any two of these loci (18). We propose a similar multilocus model for WT. Additional molecular analyses of familial WTs and genetic analyses of other WT families will provide further data for assessing this model.

Genetic Heterogeneity of Familial WT Predisposition. Not all WT families display genetic linkage to 19q markers, consistent with the known heterogeneity in WT predisposition (9). Chromosome 19q can be ruled out as the site of the predisposition gene in two small families, although two tumors from these families display tumor-specific LOH at markers within the 19q-linked region. Interestingly, both families are African-American, whereas the five 19q-linked families are Anglo-American (four) or Latino-American (one). These data suggest a possible ethnic difference in the genes conferring WT predisposition in the general population, similar to possible ethnic differences in prostate cancer predisposition (19). Molecular analyses of a larger sample of WT families from different ethnic groups will be required to assess this hypothesis.

In summary, we have obtained significant evidence (LOD = 4.0) for genetic linkage of a familial WT susceptibility locus to 19q in five families. We have also observed 19q LOH in two tumors from two families whose predisposition is not due to FW T2 at 19q. From these data, we hypothesize that critical, rate-limiting steps in the etiology of familial tumors involve the interaction of a germ-line predisposing mutation and a somatic alteration at a second locus. Genetic analysis of additional WT families and LOH studies of tumors will aid in the ultimate identification of the FW T2 gene and of the biochemical pathways that are abrogated in tumorigenesis.

Acknowledgments

The cooperation of family members is greatly appreciated. We thank Drs. A. Meadows and J. Wilimas for bringing two WT families to our attention; L. Zharkikh for excellent technical assistance; and P. Begin and J. Cook for sample collection. We also thank the Utah Genome Center for advice and assistance in the initial phase of this project.
References


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