

Evidence for Genetic Heterogeneity in Familial Wilms' Tumor¹

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Abstract

Wilms' tumor (WT), a childhood kidney cancer, occurs both sporadically and, less frequently, in a familial context. Genetic linkage studies of several large WT families have excluded the one cloned WT gene, *WT1*, as the locus responsible for familial predisposition. These data demonstrate the existence of a familial predisposition gene distinct from *WT1* and, more broadly, imply that the genetic etiology of WT is heterogeneous. However, it has been unknown whether the predisposition observed in large WT families is also heterogeneous or perhaps is due to mutations at a single locus. Recently, examination of a large French-Canadian WT family has demonstrated genetic linkage to 17q12–q21. We report here the results from a genetic linkage study of six WT pedigrees. Analyses of genotype data from eight loci within the 17q12–q21 region in these families resulted in cumulative lod scores of < -4.0 through the region, thereby excluding linkage. The ability to rule out the 17q region as the site of a predisposition gene in several of these pedigrees individually demonstrates the existence of more than one gene that predisposes to WT in large pedigrees and again emphasizes that the etiology of WT is genetically heterogeneous.

Introduction

WT³ is a childhood tumor of the kidney that occurs predominantly as a sporadic disease with an incidence of 1 in 10,000 live births. However, 1–2% of patients have affected relatives, with siblings and cousins most often affected (1). Segregation analysis, lack of parental consanguinity despite the rarity of familial WT, observations of multiple affected sibships within large pedigrees, and vertical transmission of tumor predisposition all indicate that familial WT is the result of an autosomal dominant trait that is only partially penetrant (1–3). One gene involved in WT tumorigenesis, *WT1*, is located a chromosomal band 11p13, and mutational analyses have demonstrated both somatic and germ-line intragenic *WT1* mutations in some WT patients (4–7). However, genetic linkage analyses ruled out *WT1* as the gene conferring familial predisposition of three large families (8–10). These data demonstrated the existence of a familial predisposition gene distinct from *WT1* and, more broadly, implied that the genetic etiology of WT is heterogeneous.

Observations of tumor loss of heterozygosity and loss of imprinting at 11p15 loci in WTs have suggested that alterations at an 11p15 gene(s) are important in tumor etiology (reviewed in Ref. 11), further supporting the notion of genetic heterogeneity in WT. However, it is unclear whether the predisposition observed in large WT families is

also heterogeneous or perhaps is due to mutations at a single non-11p locus. Recently, examination of a large French-Canadian WT family has demonstrated genetic linkage to 17q12–q21 between marker loci *D17S933* and *D17S787*, a genetic interval of 18 cM (12). This observation strongly suggests the presence of a gene in this region that predisposes to WT and that may or may not be involved in WT predisposition in other WT pedigrees. We report here our genetic linkage analysis of 17q markers in six WT families.

Materials and Methods

WT Families. After informed consent, blood samples were obtained from individuals from six WT families. DNA was isolated from blood lymphocytes and/or established lymphoblastoid cell lines. The six families, whose pedigrees are shown in Fig. 1, have 29 individuals with confirmed cases of WT. Additionally, in pedigree 524, there are two unconfirmed cases of WT; one child (102) in the second generation was operated on for a kidney tumor at 1 year of age and died a few months later of pneumonia. A younger sibling (109) died at 6 months of age of pneumonia, and an abdominal tumor was diagnosed at autopsy. Both unilateral and bilateral tumors were observed in five of the families; in one family (WTX593), only unilateral tumors were diagnosed. Age of onset ranged from 1 month (individual 700 in family WTX637) to 17 years (individual 004 in family WTX614). Multiple obligate carriers are observed in the four larger families, which implies the segregation of a predisposing mutation with incomplete penetrance (1). Aside from slight hemihypertrophy observed in the unaffected mother in the first generation of family 637, there are no congenital anomalies observed in these families, nor is there any indication of predisposition to other childhood or adult cancers.

Genotyping. Ninety-six individuals, including 20 affected individuals and 15 obligate carriers, were genotyped for 7 dinucleotide repeat polymorphisms in the 17q region of interest (centromeric to telomeric): *D17S933*, *D17S250*, *D17S855* (intragenic to *BRCA1*), *D17S579*, *D17S806*, *D17S809*, and *D17S808*. Additionally, individuals from four of the families were genotyped at an RFLP marker, *D17S74*, which lies between *D17S809* and *D17S808*. These eight marker loci span an estimated 26 cM (13).

Genetic Linkage Analysis. The genotype data were analyzed by multi-point linkage analyses (FastLink algorithm; Refs. 14 and 15) using a penetrance of 0.3 for the predisposing allele. The frequency of familial WT can be roughly estimated at one in a million (1–2% of all WT, which occurs in 1 in 10,000 live births), and the expected frequency of sporadic disease in a family member is 1 in 10,000. For the linkage analysis, the mutant allele frequency was set at 0.0001. The analysis therefore takes into account the possibility (0.0001) of a confounding sporadic WT case occurring in a family and also the unlikely possibility (0.000001) of a second gene carrier entering the pedigree by marriage. At each marker locus, equal frequencies for each allele were used.

Results and Discussion

Because of the possibility of genetic heterogeneity between the six pedigrees, lod scores were examined separately for each family. As presented in Table 1, the resultant lod scores at each of the eight loci in the region were negative throughout most of the region for four of the six pedigrees. For three families (524, 593, and 614), these data are statistically sufficient to exclude linkage for these families indi-

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³ The abbreviation used is: WT, Wilms' tumor.

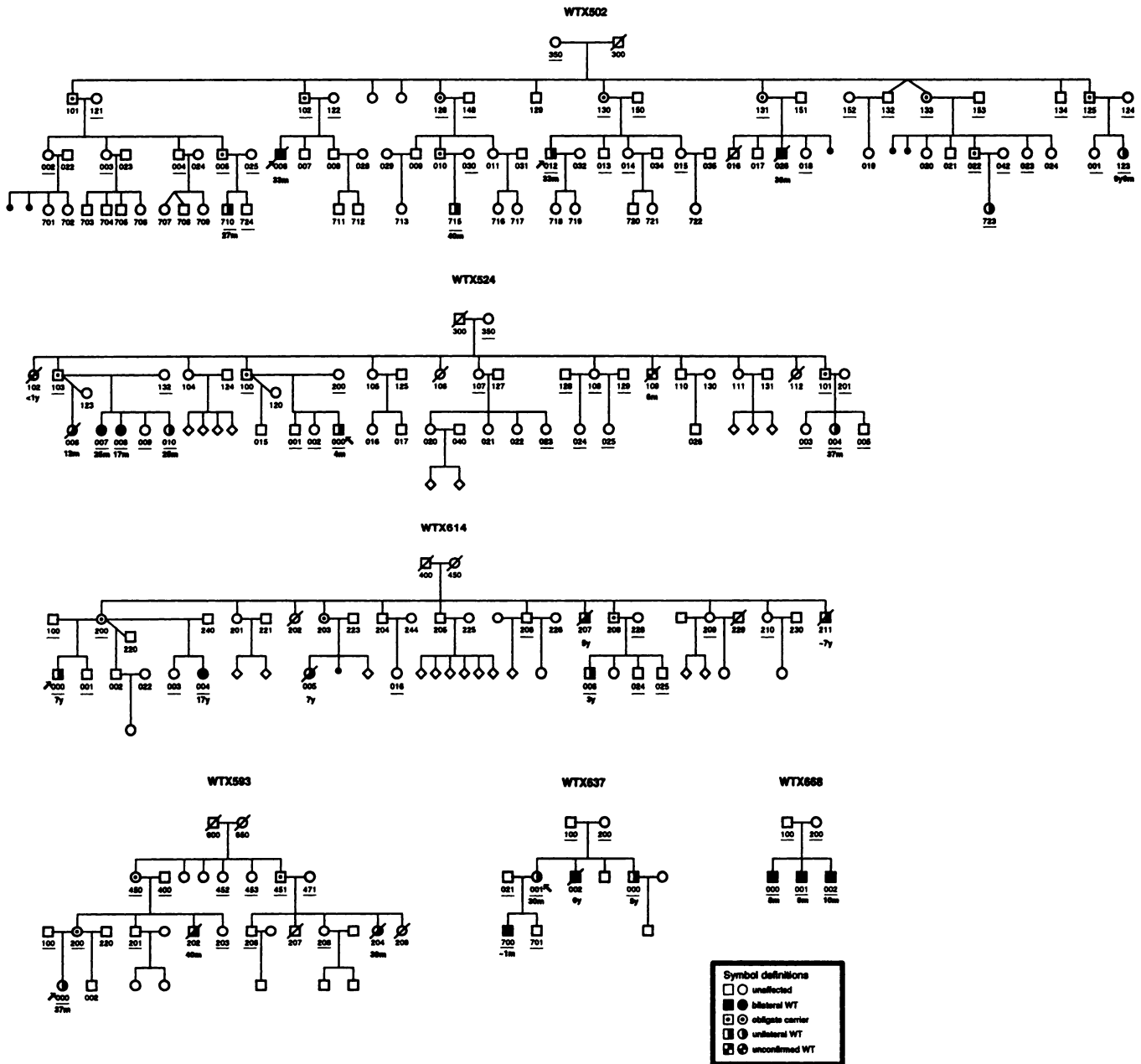


Fig. 1. Pedigrees of six WT families. 4m, age (months) at diagnosis. 350, identification number, individual genotyped; 300, identification number, individual not genotyped. Previous publications on these families include Refs. 8 (WTX524), 9 (WTX502), 23 (WTX502, WTX524, WTX637, and WTX593), 24 (WTX502), and 25 (WTX637).

vidually. The small size of pedigree 668 affects the power to rule out linkage in this family alone at several of the marker loci. Analysis of data from another small pedigree, 637, results in positive lod scores at markers throughout the region. By chance alone, marker genotype

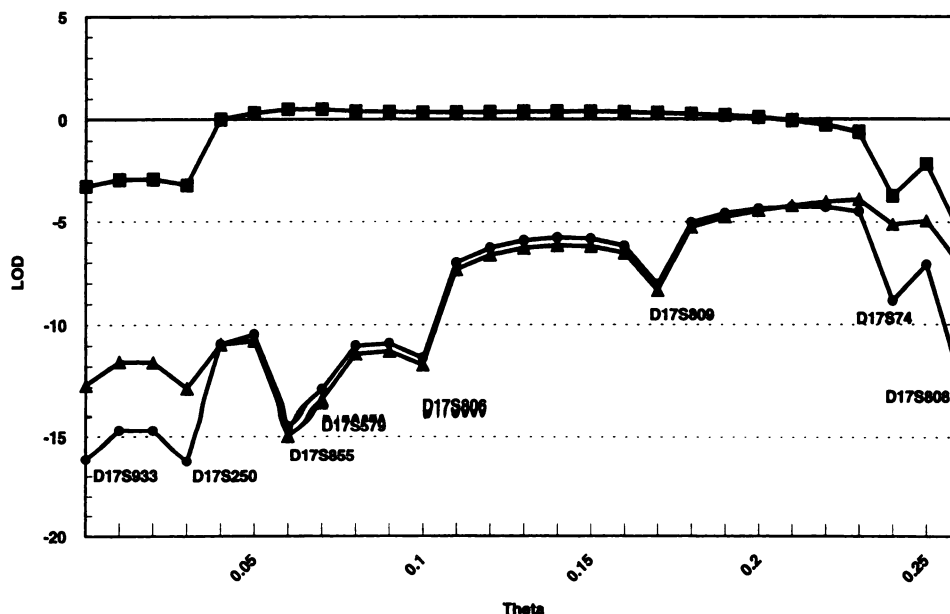
data from this family will result in a maximum score of 0.301 one in eight times; meaningful assessment of lod score data from this family in isolation is not possible. Interestingly, for pedigree 502, a large pedigree, positive lod scores were obtained for five of the markers,

Table 1 LOD scores at 17q marker loci

Marker locus	Pedigree					
	502	524	593	614	637	668
D17S933	-3.243	-3.172	-2.941	-6.469	0.301	-0.611
D17S250	-3.183	-3.170	-2.807	-6.462	0.301	-0.909 ^a
D17S855	0.489	-3.163	-2.368	-6.498	0.219	-3.168
D17S579	0.489	-3.089	-2.811	-6.501	0.216	-1.395 ^a
D17S806	0.334	-3.013	-2.757	-6.501	0.222	0.137
D17S809	0.297	-3.168	-2.719	-2.928	0.301	0.176
D17S74	0.297	-2.509	-1.876	0.054 ^a	0.301	-1.095 ^a
D17S808	-5.487	-3.505	-0.821	0.117	0.284	-3.168

^a LOD score obtained using genotype data from flanking loci.

Fig. 2. Graph of multipoint lod scores for 17q markers in the region between *D17S933* and *D17S808*. θ values on the X axis are relative to locus *D17S933*. ●, lod scores for all pedigrees combined; ▲, lod scores for all pedigrees except WTX502; ■, lod scores for pedigree WTX502 alone.



suggestive of possible linkage and also of heterogeneity between the families. Therefore, as shown in Fig. 2, lod scores at the eight loci and the seven intervals were summed for all pedigrees (*circles*), for all pedigrees except pedigree 502 (*triangles*), and pedigree 502 alone (*squares*). As shown, summing lod scores from all families resulted in significant exclusion of this region as the site of the familial predisposing locus; lod scores less than -2.0 are observed throughout the 26-cM interval (*circles*). If family 502 is excluded due to the observation of slightly positive lod scores and the possibility of interfamily heterogeneity, even more negative lod scores are obtained (*triangles*). Analyses of genotype data for family 502 resulted in lod scores of ~ 0.5 throughout an interval from marker *D17S855* to proximal of marker *D17S74*; from these data, genetic linkage to 17q markers in this family can be neither demonstrated nor ruled out. In summary, these data demonstrate that the 26-cM region bounded by *D17S933* and *D17S808* can be ruled out as the site of the predisposing gene in at least four of the six families. This region encompasses the 17q12–q21 18-cM region that is linked to predisposition in one large WT family (12), thereby demonstrating for the first time heterogeneity for WT predisposition in large WT families and further underscoring the genetic heterogeneity that is becoming a notable feature in WT in general.

That such heterogeneity exists for WT familial predisposition is supported by three different lines of data: (a) the demonstration by comparative genome hybridization of chromosomal regions 3q, 4q, 9p, and 20p that are lost in tumors from several familial cases but not in tumors from sporadic WT cases suggests that these regions may harbor predisposition genes (16); (b) in rare sporadic WT patients, germ-line genomic alterations have been observed, most notably in individuals with trisomy 18, but also in individuals with constitutional karyotypic rearrangements involving chromosomes 1q, 2q, 3p, 7, 11q, and 13 (17–21); and (c) WT is occasionally observed in the context of syndromes characterized by the presence of developmental anomalies and/or an increased cancer risk such as Beckwith-Weidemann syndrome, neurofibromatosis, hyperparathyroidism-jaw tumor syndrome, and Li-Fraumeni syndrome (reviewed in Ref. 22). This, in addition to the fact that many of the WT individuals with karyotypic rearrangements cited above have developmental anomalies, suggests that a generalized perturbation of development and/or growth control processes can be predisposing to WT (22) and implies that several loci may predispose to WT. In this regard, the WT families reported here

are notable in that growth or developmental anomalies are not observed (with the possible exception of family 637). Thus, predisposition in these families may involve a gene(s) with only a subtle, if any, effect on overall growth.

In summary, we have demonstrated that familial WT is genetically heterogeneous; in each of three large families, we can exclude genetic linkage to markers that have been reported to be linked in one large family (12). This underscores the complexity of the genetic etiology of WT and suggests that alteration of a number of genes may ultimately be determined to play a role in WT etiology.

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