Evidence for susceptibility genes to familial Wilms tumour in addition to \textit{WT1}, \textit{FWT1} and \textit{FWT2}

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Summary Three loci have been implicated in familial Wilms tumour: \textit{WT1} located on chromosome 11p13, \textit{FWT1} on 17q12-q21, and \textit{FWT2} on 19q13. Two out of 19 Wilms tumour families evaluated showed strong evidence against linkage at all three loci. Both of these families contained at least three cases of Wilms tumour indicating that they were highly likely to be due to genetic susceptibility and therefore that one or more additional familial Wilms tumour susceptibility genes remain to be found. © 2000 Cancer Research Campaign

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Wilms tumour (WT) is an embryonal tumour of the kidney that affects 1 in 10 000 children and accounts for 8% of all childhood cancers (Stiller and Parkin, 1990). In 1–2% of cases the disease clusters in families in which susceptibility to WT appears to be predominantly inherited as an autosomal dominant trait with incomplete penetrance (Breslow et al, 1996).

The genetics of familial WT is complex and at least three loci predisposing to familial WT have been proposed. \textit{WT1} is a tumour suppressor gene on chromosome 11p13. Constitutional \textit{WT1} mutations have been documented in four families with more than one case of WT (Yunis and Ramsay, 1980; Pelletier et al, 1991; Kaplinsky et al, 1996; Pritchard-Jones et al, 2000). In all but one (Kaplinsky et al, 1996), the \textit{WT1} mutation was associated with congenital malformations, either urogenital abnormalities in males and/or aniridia. \textit{WT1} has been excluded as the susceptibility gene in several WT families in which no congenital abnormalities were observed (Grundy et al, 1998; Huff et al, 1988; Schwartz et al, 1991; Baird et al, 1994).

We have mapped a familial WT susceptibility gene on chromosome 17q12-q21, designated \textit{FWT1}, by genetic linkage analysis of a large family of French-Canadian descent (MON 480) (Rahman et al, 1996). The existence of this locus has been confirmed by analysis of additional affected members from MON 480 and a second unrelated pedigree (K1104) with seven cases of WT (Rahman et al, 1998). WT cases in \textit{FWT1}-linked pedigrees tend to be diagnosed at a later age than non-familial cases (Rahman et al, 1998) and analyses of WT from MON 480 have demonstrated that loss of the wild-type \textit{FWT1} allele, inherited from the non-mutation carrying parent, does not occur (Rahman et al, 1997). Therefore, \textit{FWT1} is unlikely to be a classical tumour suppressor gene.

Recently, an additional familial WT susceptibility gene (\textit{FWT2}) located on chromosome 19q13, has been proposed (McDonald et al, 1998). The evidence in favour of this locus is not conclusive. Furthermore, in families that were unlinked to the putative \textit{FWT2}, data at \textit{WT1} and \textit{FWT1} were not provided (McDonald et al, 1998). It is currently unclear whether \textit{WT1}, \textit{FWT1} and \textit{FWT2} account for all familial WT predisposition, or whether additional familial WT susceptibility genes are likely to exist. In this study we have evaluated a set of WT families for the contribution of \textit{FWT2} and have assessed the likelihood of the existence of additional WT susceptibility genes.

**MATERIALS AND METHODS**

**WT families**

Families with two or more verified cases of WT were identified from the UK, Canada, France, Germany, Switzerland, New Zealand and USA. Permission for the study was given by the Review Boards/Ethics Committee and informed consent was obtained from the patient or parent as appropriate.

**Microsatellite analysis**

Genomic DNA was prepared from whole blood, from immortalized lymphoblastoid cell lines and from fixed paraffin-embedded tumour sections using standard techniques. Genotyping using polymorphic microsatellite repeats was performed by standard polymerase chain reaction (PCR) amplification with one primer end-labelled using T4 polynucleotide kinase and $\gamma[32P]ATP$. PCR products were electrophoresed through 6% denaturing polyacrylamide gels and the gel was exposed to autoradiography film for 1–16 h.
Table 1  Multipoint LOD scores for three familial WT loci, WT1, FWT1 and FWT2

| Family     | WT1 D11S9046-cM-D11S907 | FWT1 D17S250-12.5-cM-D17S1820 | FWT2 D19S921-8.0-cM-D19S926 |
|------------|--------------------------|--------------------------------|-----------------------------|
| WILMS 7    | -4.84                    | -4.85                          | -4.77                       |
| WILMS 12   | -5.42                    | -5.43                          | -5.43                       |
| WILMS 13   | -0.21                    | -5.17                          | 0.16                        |
| FAMILY M   | 0.30                     | -5.32                          | 0.25                        |
| HPN12      | -4.35\*                  | -4.27b                         | 1.00\*                      |

*Multipoint analysis using D11S4154-3.5 cM-D11S907.  *Multipoint analysis using D17S946-0.0 cM-D17S250-10.5 cM-D17S588
*Multipoint analysis using D19S921-6.0 cM-D19S254-4.0 cM-D19S91

Three markers were used to evaluate linkage to WT1. The marker order determined from LDB (Collins et al, 1996) is centromere-D11S904-2.5 cM-D11S4154-3.5 cM-D11S907-telomere. WT1 is located between D11S4154 and D11S907. At least six markers were used to evaluate linkage to FWT1. Additional markers were analysed to generate informative data when required. The marker order determined from LDB is centromere-D17S946/D17S250-2.5 cM-THRA1-1 cM-D17S8001 cM-D17S879-2.0 cM-D17S806-4 cM-D17S588-2 cM-D17S1820-telomere. At least six markers were examined to determine linkage to FWT2. The marker order determined from Genethon marker map (Dib et al, 1996) is centromere-D19S571-4 cM-D19S921-2.0 cM-D19S572-1.0 cM-D19S924-3.0 cM-D17S254/D19S418-3.0 cM-D19S926-1.0 cM-D19S91-telomere.

Statistical analysis

Genetic linkage analysis was performed using the FASTLINK program (Cottingham et al, 1993). Familial WT was modelled as a rare dominant (\(q = 0.000001\)) with a penetrance of 30% (Rahman et al, 1996). Allele frequencies were calculated from 15 unrelated individuals. Multipoint LOD scores were generated using two informative markers from each chromosome haplotype. When analysing family HPN12 the marriage loop was broken at ID1460, using the makeped component of the LINKAGE package.

RESULTS

Of 13 previously published families with two or more cases of WT, two families (WILMS 7 (Figure 1A) and FAMILY M (Figure 1D) are highly unlikely to be due to either FWT1 or WT1 mutations (Rahman et al, 1998). Both families are unlinked at FWT1. WILMS 7 is unlinked at WT1 (Rahman et al, 1998). FAMILY M generates a small positive LOD score of 0.3 at WT1 (Table 1), but mutational screening by a combination of single strand conformation polymorphism and direct sequencing did not detect a predisposing WT1 mutation in this family (Baird et al, 1994). These two families were included in the current study. Six previously unpublished families were also included. Three of these (WILMS 12, WILMS 13 and HPN12) also show evidence against linkage to FWT1 and WT1, and are illustrated in Figures 1B, C and E respectively. The remaining new families F2655 (uncle and nephew affected), F1124 (affected sib pair) and MON 948 (affected sib pair) were linked at either/both FWT1 and WT1 and are not shown. Therefore, five of our total series of 19 families, WILMS 7, FAMILY M, WILMS 12, WILMS 13 and HPN12 are highly unlikely to be due to either WT1 or FWT1 mutations (Figure 1, Table 1).

To evaluate the contribution of FWT2 and to assess the possibility of additional familial WT susceptibility genes, the five families that showed evidence against WT1 and FWT1 acting as predisposition genes, were selected for analysis of markers in the vicinity of FWT2. Multipoint LOD scores for these five families at markers from the WT1, FWT1 and FWT2 regions are shown in Table 1, and the segregating haplotypes of marker alleles in each family are shown in Figure 1. Two of the five families (WILMS 7 and WILMS 12) show no evidence of a shared haplotype between affected members at FWT2. The evidence against linkage is reflected in the negative multipoint LOD scores (Table 1). In three families (HPN12, WILMS 13 and FAMILY M) a chromosome 19q marker haplotype is shared by the affected individuals. HPN12 has a complex structure with the two affected individuals being related through both parents. The multipoint analysis yields a maximum LOD score of 1.00 at \(\theta = 0\). WILMS 13 is an uncle/nephew pedigree and generates a LOD score of 0.16. FAMILY M contains an affected mother and two affected children and generates a LOD score of 0.25. The WT from ID301 in WILMS 13 and ID302 in FAMILY M showed loss of heterozygosity (LOH) at all markers tested on chromosome 19q. In each tumour, the haplotype lost was the one not linked to the disease in the family (Figure 1C, D).

DISCUSSION

Of 19 families with two or more individuals affected by WT, five are unlikely to be due to mutation of either WT1 or FWT1. One of these five families, HPN12 generated a multipoint LOD score of 1.00 at chromosome 19q13. Whilst not providing unambiguous confirmation of its existence, this result suggests that the previous localization of FWT2 to chromosome 19q may be correct. Two further small families (WILMS 13 and FAMILY M) are consistent with linkage to FWT2. In both families one WT showed somatic loss of the haplotype that is not linked to the disease in the family. Although this would be consistent with the conventional model of a tumour suppressor gene, previous analyses of WT from families putatively linked to FWT2 revealed that none of seven tumours showed wild-type allele loss (McDonald et al, 1998). The significance of the allele loss in WILMS 13 and FAMILY M is therefore unclear. Moreover, as both families consist of few, closely related individuals, linkage to chromosome 19q13 may have occurred by chance and the WT predisposition gene in these families may well be located elsewhere in the genome.
Evidence for a further familial WT gene

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Figure 1  Pedigrees of five WT families in which the disease is unlikely to be due to mutations in either FWT1 or WT1. Closed symbol WT, open symbol with dot obligate carrier. The number after WT is the age at diagnosis. Haplotypes are shown by patterned bars. (A) WILMS 7; (B) WILMS 12; (C) WILMS 13; (D) FAMILY M; (E) HPN12.
Of the five families highly unlikely to be due to WT1 or FWT1, two also showed strong evidence against linkage to markers in the vicinity of FWT2. As there are at least three cases of WT in each of these families, they are highly likely to be due to an underlying genetic predisposition and therefore strongly suggest the existence of at least one further familial WT susceptibility gene. Although only two of the 19 families showed evidence against linkage at all three known loci, many of the small familial clusters (such as affected sib pairs) in the series of 19 families could have been linked by chance to one or other locus. Indeed, of five families with at least three cases of WT in our series, only one (FAMILY M) is consistent with linkage at FWT2. Two (WILMS 7 and WILMS 12) were unlinked at WT1, FWT1 and FWT2 and the two remaining families showed clear evidence of linkage to FWT1 (MON 480 and K1104). It is thus possible that a substantial proportion of susceptibility to familial WT that is not attributable to WT1 or FWT1 is also not attributable to FWT2.

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REFERENCES

Baird PN, Pritchard J and Cowell JK (1994) Molecular genetic analysis of chromosome 1p in familial Wilms tumours. Br J Cancer 69: 1072–1077
Breslow NE, Olson J, Mokness J, Beckwith JB and Grundy P (1996) Familial Wilms' tumour a descriptive study. Med Pediatr Oncol 27: 396–403
Collins A, Frezel J, Teague J and Morton NE (1996) A metric map of humans: 23 500 loci in 850 bands. Proc Natl Acad Sci USA 93: 14771–14775
Cortingham RW, Idury RM and Schaffler AA (1993) Faster sequential genetic linkage computations. Am J Hum Genet 53: 252–263

Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, Marc S, Hazan J, Seboun E, Lathrop M, Gypay G, Morissette J and Weissenbach J (1996) A comprehensive genetic map of the human genome based on 5264 microsatellites. Nature 380: 152–154
Grundy P, Koufos A, Morgan K, Li FP, Meadows AT and Cavenee WK (1988) Familial predisposition to Wilms' tumour does not map to the short arm of chromosome 11. Nature 336: 374–376
Huff V, Compton DA, Chao LY, Strong LC, Geiser CF and Saunders GF (1988) Lack of linkage of familial Wilms' tumour to chromosomal band 11p13. Nature 336: 377–378
Kaplinsky C, Ghahremani M, Frishberg Y, Rechavi G, Pelletier J (1996) Familial Wilms' tumour associated with a WT1 zinc finger mutation. Genomics 38: 451–453
McDonald JM, Douglass EC, Fisher R, Geiser CF, Krill CE, Strong LC, Virshup D and Huff V (1998) Linkage of familial Wilms' tumour predisposition to chromosome 19 and a two-locus model for the etiology of familial tumors. Cancer Res 58: 1387–1390
Pelletier J, Bruening W, Li FP, Haber DA, Glaser T, Housman DE (1991) WT1 mutations contribute to abnormal genital system development and hereditary Wilms' tumour. Nature 353: 431–434
Pritchard-Jones K, Rahman N, Gerrard M, Varenius D and King-Underwood L (2000) Familial Wilms tumour due to WT1 mutation: intronic polymorphism causing artefactual constitutional homozygosity. J Med Genet (in press)
Rahman N, Arbour L, Tonin P, Renshaw J, Pelletier J, Baruchel S, Pritchard-Jones K, Stratton MR, Narod SA (1996) Evidence for a familial Wilms' tumour gene (FWT1) on chromosome 17q12-q21. Nat Genet 13: 461–463
Rahman N, Arbour L, Tonin P, Baruchel S, Pritchard-Jones K, Narod SA and Stratton MR (1997) The familial Wilms' tumour susceptibility gene, FWT1, may not be a tumour suppressor gene. Oncogene 14: 3099–3102
Rahman N, Abidi F, Ford D, Arbour L, Rapey E, Tonin P, Barton D, Batcup G, Berry J, Cotter F, Davison V, Gerrard M, Gray E, Grundy R, Hanafy M, King D, Lewis I, Ridolfi Luebthy A, Madiensky L, Mann J, O'Meara A, Oakhill T, Skolnick M, Strong L, and Stratton MR (1998) Confirmation of FWT1 as a Wilms' tumour susceptibility gene and phenotypic characteristics of Wilms' tumour attributable to FWT1. Hum Genet 103: 547–556
Schwartz CE, Haber DA, Stanton VP, Strong LC, Skolnick MH and Housman DE (1991) Familial predisposition to Wilms tumor does not segregate with the WT1 gene Genomics 10: 927–930
Stiller CA and Parkin (1990) International variations in the incidence of childhood renal tumours. Br J Cancer 62: 1026–1030
Yunis JJ and Ramsay NK (1980) Familial occurrence of the aniridia-Wilms tumor syndrome with deletion 11p13–14.1. J Pediatr 96: 1027–1030