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Evidence for susceptibility genes to familial Wilms tumour in addition to *WT1, FWT1* and *FWT2*

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Summary Three loci have been implicated in familial Wilms tumour: *WT1* located on chromosome 11p13, *FWT1* on 17q12-q21, and *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

Keywords: Wilms tumour; *FWT1*; *FWT2*; familial predisposition

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. *WT1* is a tumour suppressor gene on chromosome 11p13. Constitutional *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 *WT1* mutation was associated with congenital malformations, either urogenital abnormalities in males and/or aniridia. *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 *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 *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 *FWT1* allele, inherited from the non-mutation carrying parent, does not occur (Rahman et al, 1997). Therefore, *FWT1* is unlikely to be a classical tumour suppressor gene.

Recently, an additional familial WT susceptibility gene (*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 *FWT2*, data at *WT1* and *FWT1* were not provided (McDonald et al, 1998). It is currently unclear whether *WT1*, *FWT1* and *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 *FWT2* and have assessed the likelihood of the existence of additional WT susceptibility genes.

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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 $\gamma[^{32}P]$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	Multipoint LOD score at θ = 0		
	WT1 ***D11S904*-6 cM-*D11S907***	***FWT1*** ***D17S250*-12.5 cM-*D17S1820***	***FWT2*** ***D19S921*-9.0 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[a]	–4.27[b]	1.00[c]

[a]Multipoint analysis using *D11S4154*-3.5 cM-*D11S907*. [b]Multipoint analysis using *D17S946*-0.0 cM-*D17S250*-10.5 cM-*D17S588* [c]Multipoint analysis using *D19S921*-6.0 cM-*D19S254*-4.0 cM-*D19S891*

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-*D17S800*1 cM-*D17S579*-2.0 cM-*D17S806*-4 cM-*D17S588*- 2 cM-*D17S1820*-telomere. At least six markers were examined to determine linkage to *FWT2*. The marker ordered 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-*D19S891*-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

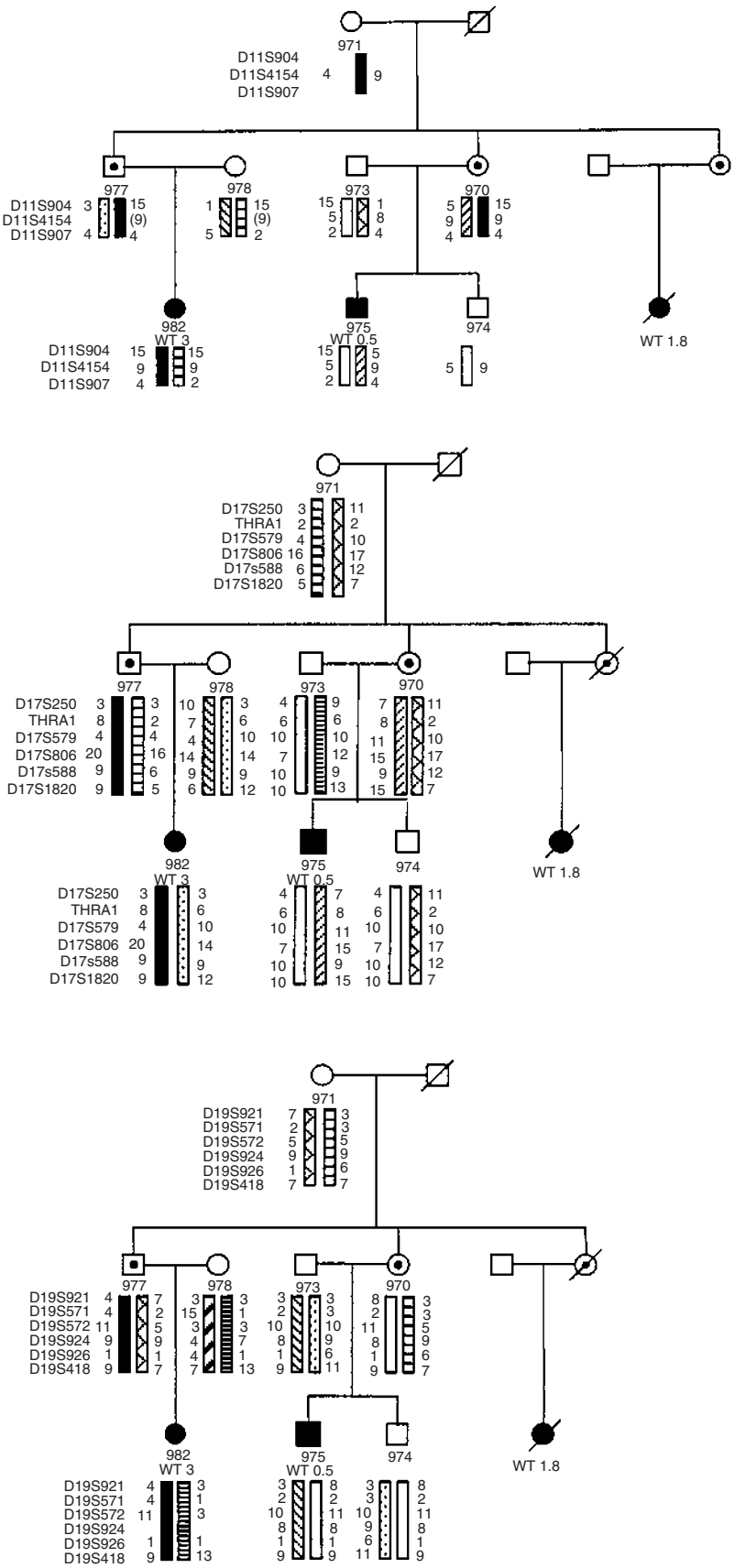
Of 13 previously published families with two or more cases of WT, two families (WILMS 7 (Figure 1*A*) and FAMILY M (Figure 1*D*) are highly unlikely to be due to either *FWT1* or *WT1* mutations (Rahman et al, 1998). Both families are unlinked at *FWT1*. WILMS 7 is unlinked at *WT1* (Rahman et al, 1998). 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 θ = 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).

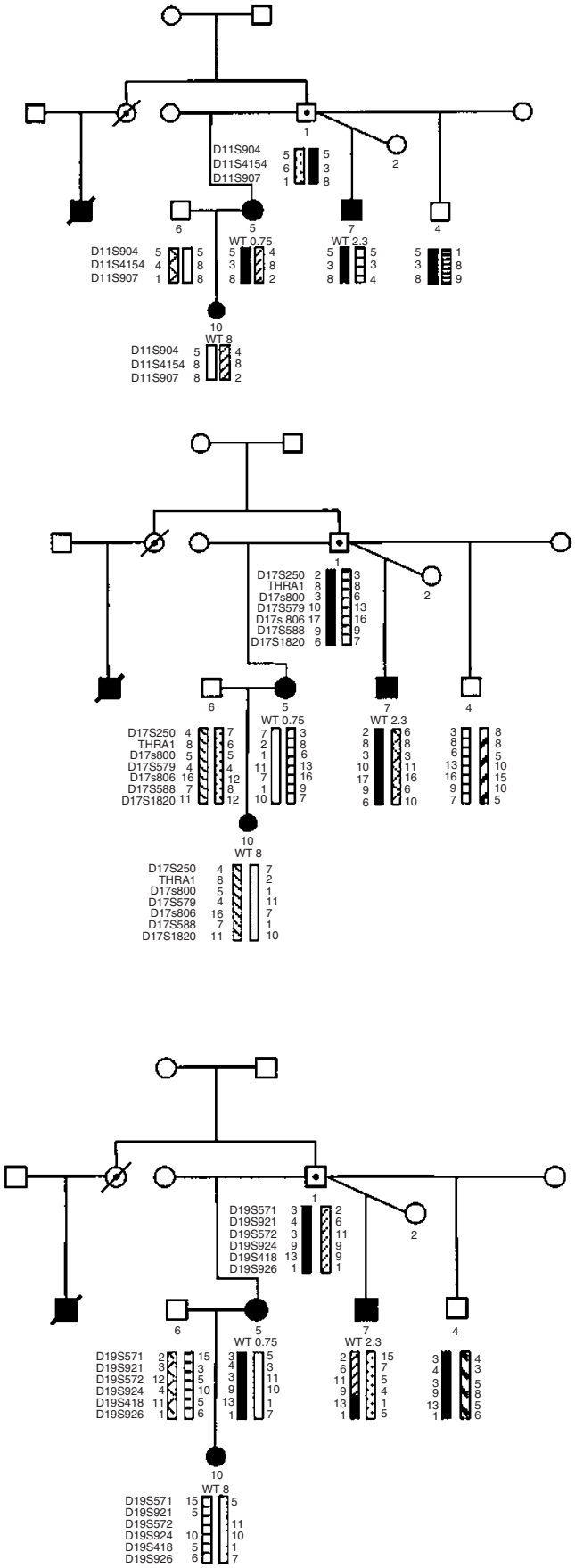
DISCUSSION

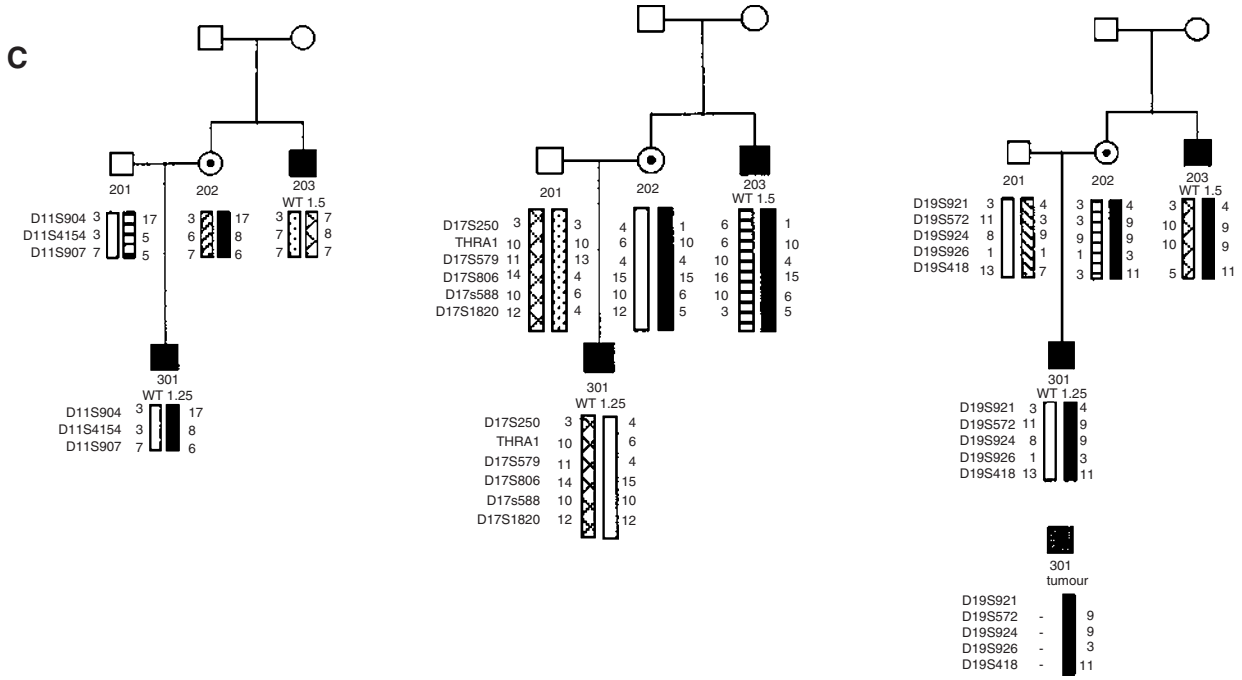
Of 19 families with two or more individuals affected by WT, five are unlikely to be due to mutation of either *WT1* or *FWT1*. One of these five families, HPN12 generated a multipoint LOD score of 1.00 at chromosome 19q13. Whilst not providing unambiguous confirmation of its existence, this result suggests 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.

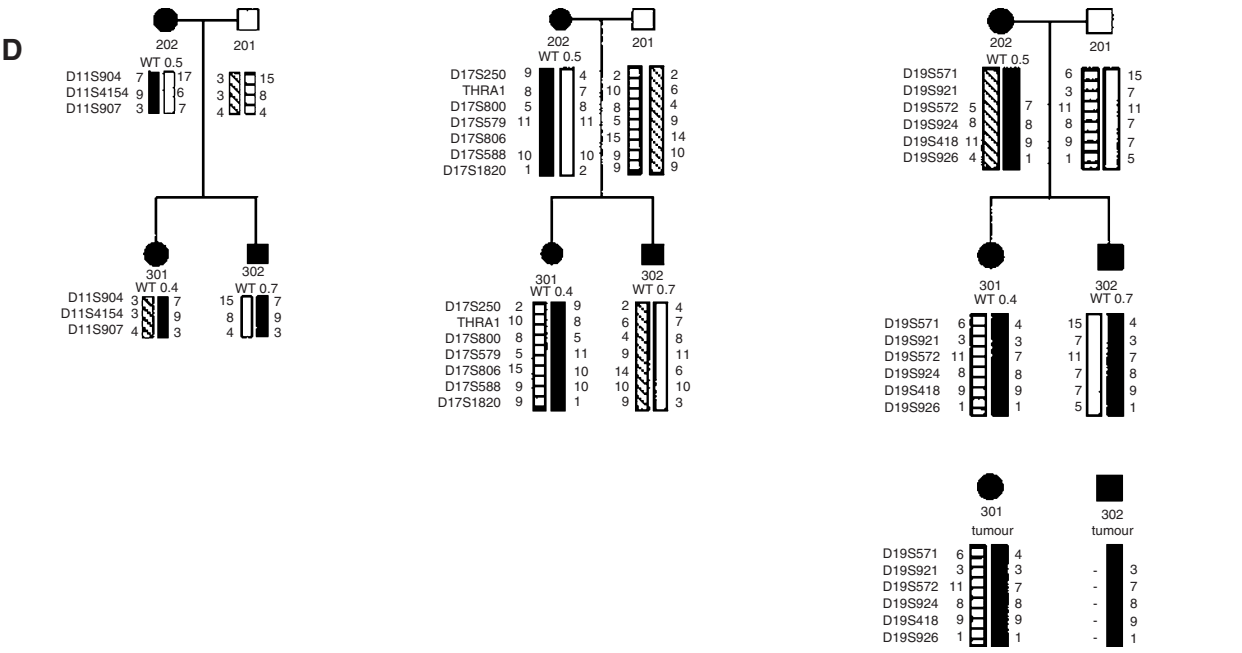
A


971
D11S904
D11S4154
D11S907
977
978
973
970
982
WT 3
975
WT 0.5
974
WT 1.8
D17S250
THRA1
D17S579
D17S806
D17s588
D17S1820
D19S921
D19S571
D19S572
D19S924
D19S926
D19S418

B







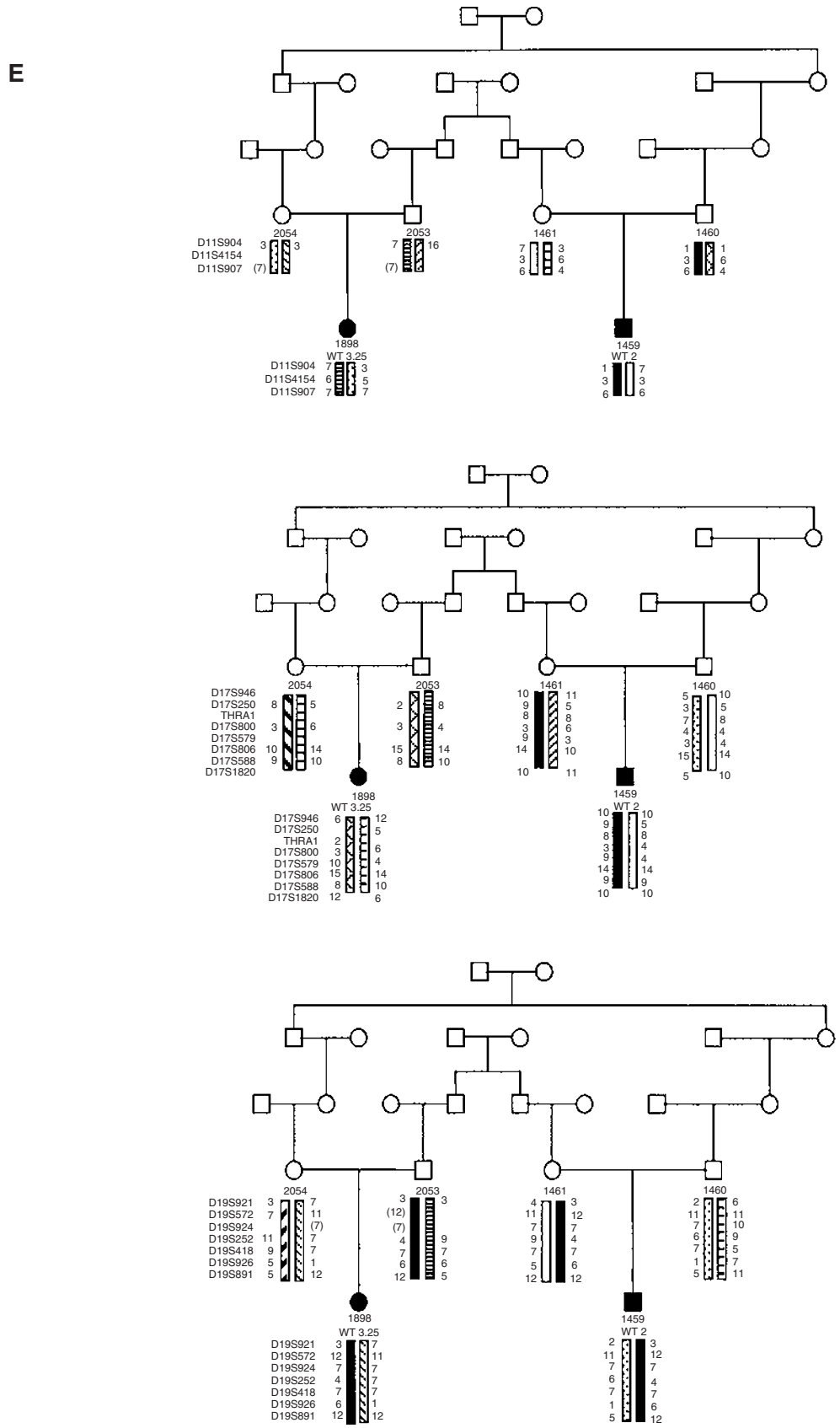


Figure 1 Pedigrees of five WT families in which the disease is unlikely to be due to mutations in either *FWT1* or *WT1*. Closed symbol WT, open symbol with dot obligate carrier. The number after WT is the age at diagnosis. Haplotypes are shown by patterned bars. (**A**) WILMS 7; (**B**)WILMS 12; (**C**)WILMS 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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