Homozygous deletion in Wilms tumours of a zinc-finger gene identified by chromosome jumping

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CYTOGENETIC analysis has identified chromosome 11p13 as the smallest overlap region for deletions found in individuals with WAGR syndrome, which includes Wilms tumour (a recessive childhood nephroblastoma), anirida, genito-urinary abnormalities and mental retardation[1]. The underlying loci have since been resolved into an aniridia (AN2) locus at a telomeric position, and a locus of closely spaced genes or a single pleiotropic gene involved in genito-urinary tract abnormalities and Wilms tumour at a more centromeric position[2-7]. Pulsed-field gel analysis of the 11p13 region has revealed the presence of several putative CpG islands[8,9], structures which are frequently associated with the 5′ ends of

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expressed sequences, mainly housekeeping genes and some tissue-specific genes[10]. Starting from a CpG island, we have now isolated four neighbouring CpG islands, all within 650 kilobases (kb), by means of two consecutive bidirectional jumps in rare-cutting restriction-enzyme jumping libraries[11]. In two instances, flanking sequences were conserved in other species and RNA transcripts were identified. A complementary DNA clone isolated for one of them derives from an RNA highly expressed in fetal kidney, and is predicted to encode a Krüppel-like[12] zinc-finger protein that is probably a transcription factor. The entire cDNA region is included in two partially overlapping homozygous deletions found in Wilms tumour DNA samples. Cloning of the breakpoints in one tumour revealed a deletion size of 170 kb, one-third of which is covered by the cDNA. The expression pattern and sequence of this cDNA could point to an important role for its corresponding gene in the normal development of the renal system as well as in Wilms tumour.

We chose probe 282, located close to the region implicated in Wilms tumour and genito-urinary tract abnormalities (WT-GU region) and near two CpG islands, as a starting point. By chromosome walking, probe A282-EH2.6 containing sites for *Bss*HII, *Sac*II and other rare-cutting enzymes that are not methylated in genomic DNA was isolated. We expected that the 5.1-kb size of the corresponding *Eco*RI fragment would allow jumping in a *Bss*HII-*Eco*RI jumping library in both directions. Screening of ~400,000 clones yielded 11 strongly hybridizing colonies, with the inserts falling into two categories (LFC1 and LFT1), consistent with jumps in both directions (Fig. 1). As chromosome jumping is directional, the type of starting fragment indicated the direction of the jump, which we confirmed by mapping the new end fragments against somatic cell hybrid panels and by PFG analysis to provide correct placement on the pulsed-field (PFG) map (not shown).

To cross the CpG islands, the LFT1 and LFC1 jumping clones were used to isolate lapping λ phage. In the case of LFT1-CP5, the large size of the telomeric *Bss*HII-*Eco*RI fragment (~10 kb) precluded the use of the same *Bss*HII-*Eco*RI jumping library for a consecutive jump. A second *Bss*HII jumping library, recut
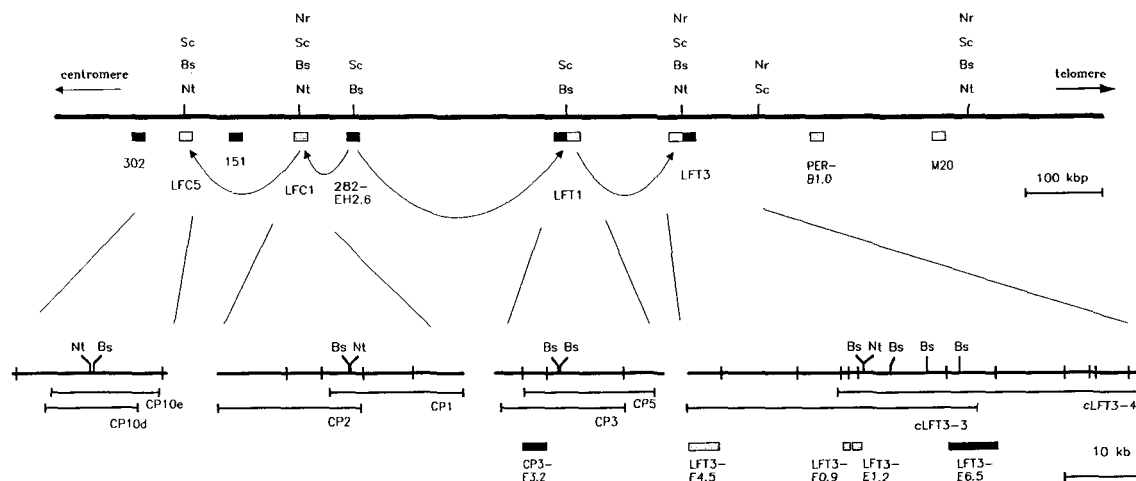

FIG. 1 The WT–GU region: Physical map and chromosome jumping. The PFG restriction map (top) from ref. 9 has been refined to include additional probes. The four directional jumps using probe A282-EH2.6 as a starting point are indicated with previously used random probes shown at their approximate positions. Phage or cosmid clones for the new CpG islands are drawn on a larger scale (bottom). Only two representative clones are shown in each case with their *Eco*RI restriction maps (vertical lines) and cutting sites for *Not*I and *Bss*HII. Shaded boxes depict the location of the probes used in Figs 2 and 4; conservation in rodent DNA is indicated by black boxes. Abbreviations: Nr, *Nru*I; Sc, *Sac*II; Bs, *Bss*HII; Nt, *Not*I.
METHODS. Probe A282-EH2.6 was isolated by two chromosome-walking steps in a *Mbo*I partial digest library in vector EMBL3 from probe 282 (ref. 5). The *Bss*HII–*Eco*RI jumping library[11] was plated as a lysogen onto MSI membranes and screened as previously described[30]. Eleven positive clones were picked and phage induced for re-screening. DNA was prepared from pure stocks and inserts released by *Eco*RI–*Sal*I cleavage. Fragments likely to represent the anticipated jump were subcloned into plasmid vectors and their position verified by mapping on somatic cell hybrid panels[5] and comparison of the PFG pattern with that of the starting clone[9]. The new jump fragments were used to isolate longer phage clones from the EMBL3 library and fragments suitable for a consecutive jump were subcloned. The LFC5 and LFT3 jumps were isolated by screening 5,000 phage from a *Bss*HII/*Bam*HI–*Hind*III jumping library with a 1.6-kb *Bss*HII–*Hind*III fragment from CP1 and a 200-bp *Bss*HII–*Hind*III subclone of CP5, respectively. Correct inserts were again used to isolate overlapping EMBL3 or cosmid clones (*Mbo*I partial digest human placental library in pWE15 provided by G. Evans). Restriction maps for these clones were derived from hybridizations of overlapping clones and by partial digest strategies. All subcloning and hybridization was done according to standard procedures[31].

FIG. 2 CpG island probes are conserved and contain expressed sequences. a, Subclones close to the CpG island map to the 11p13–11p14 region and cross-hybridize with rodent DNA (arrows). b, These probes also detect RNA transcripts in fetal RNA (1, kidney; 2, heart; 3, thymus; 4, liver; 5, lung; 6, brain). The CP3E3.2 transcript is expressed in all tissues. Expression of the LFT3-associated transcript was only observed in fetal kidney.
METHODS. a, Somatic cell hybrid panels (EcoRI-digested human DNA (1), hamster DNA (2), human hamster hybrid DNA containing a normal chromosome 11 (3) or WAGR deletion chromosomes (4,5), and human mouse hybrid DNA (6) containing 11pter–q23) described in ref. 5 were hybridized and washed under normal stringency. Bands in hamster (lanes 2–5) and mouse (lane 6) DNA are marked by arrows. b, Fetal RNAs (20–22 weeks) were prepared by the GuSCN–CsCl$_2$ method, 10 μg each separated on a 1.1% agarose-formaldehyde gel and transferred to Genatran membrane. Hybridization was performed under standard conditions (50% formamide, 5 × SSC, 42 °C) with washing in 0.5 × SSC, 0.5% SDS at 55 °C followed by overnight exposure. A 600-base pair (bp) AvaII–HincII fragment of CP3E3.2 and the 1.6-kb EcoRI fragment of the LK1 cDNA clone were used as probes. Size estimates were derived from the positions of ribosomal RNA and a BRL RNA ladder.

a

CP3E3.2

cLFT3E6.5

b

CP3E3.2

LK1E1.6
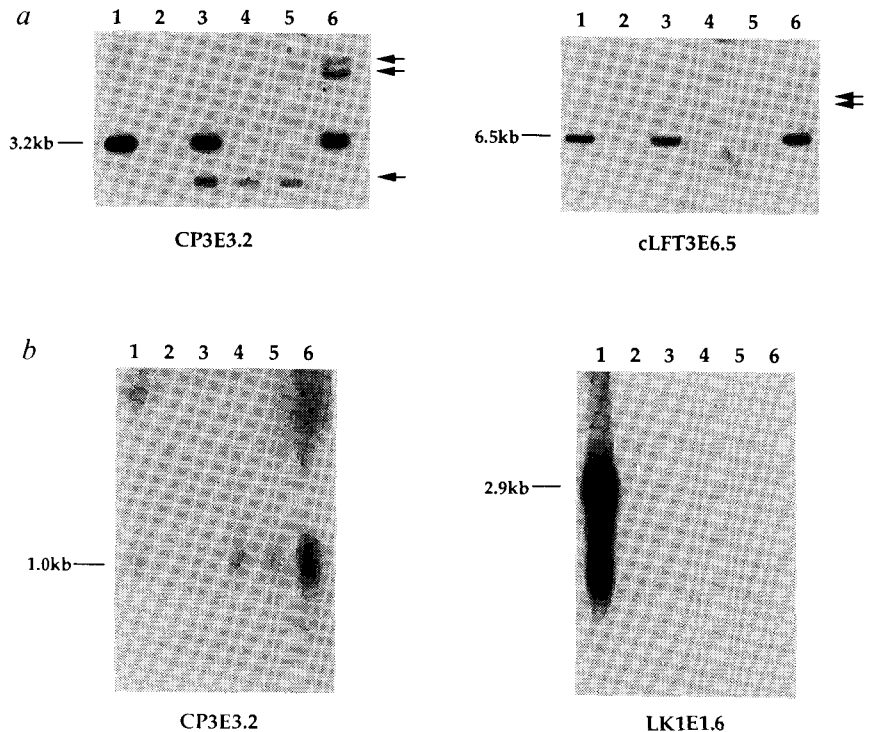
with the enzymes HindIII and BamHI instead of EcoRI, circumvented this problem[13]. Two identical clones (LFT3) from this library mapped to the correct chromosomal position (Fig. 1). Screening of a human cosmid library yielded four overlapping clones, spanning ~60 kb. Hybridization with probes from these cosmid clones confirmed their expected position on the PFG map. We used the BssHII/BamHI-HindIII jumping library to isolate another jump clone (LFC5), centromeric to LFC1 (Fig. 1). Again, overlapping λ clones (CP10) were isolated to encompass the entire CpG island. We therefore isolated four new CpG islands together with their flanking genomic regions.

Several genomic fragments on each side of every CpG island were assayed for cross-hybdrization with rodent DNA on somatic cell hybrid panels under high stringency. At two of the four CpG islands we detected conserved fragments (Fig. 2a). On northern blots, a subclone of CP3E3.2 detected a transcript of ~1 kb (Fig. 2b) expressed in all fetal tissues examined, consistent with the characteristics of a housekeeping gene. With the 6.5-kb EcoRI fragment of cLFT-6, a hybridization signal was seen only with fetal kidney RNA (Fig. 2b) among the tissues examined. We investigated this transcript further, because it maps in the WT–GU subregion of the WAGR locus.

A total of 15 cDNA clones for the LFT3-associated transcript were isolated from a human fetal kidney cDNA library. The longest clone (3 kb), designated LK15 (Fig. 3a), is similar in size to the RNA transcript, estimated to be 2.9 kb. The transcript probably originates from the CpG island, as the 5' end of LK15 maps within 4 kb of the island. Analysis of genomic DNA and overlapping cosmid clones indicated that the corresponding locus is spread over ~60 kb.

Sequence analysis of the cDNA clones revealed a combined length of 3,020 nucleotides with a single long open reading frame of 1,725 nucleotides starting at the 5' end of LK15 (Fig. 3b). The first ATG codons (positions 381 and 759) in the cDNA sequence do not fit the consensus sequence for initiation codons[14]. This observation, taken together with that of an open reading frame extending to the end of the clone, implies that the translation start site of the messenger RNA has not yet been cloned.

The predicted protein contains four tandemly repeated sequence motifs (Fig. 3c) related to the zinc-fingers of the product of Krüppel, a Drosophila segmentation gene[12]. By sequence comparison, the murine Krox-20-encoded polypeptide and its human homolog EGR2 were most similar to that encoded by clone LK15 with 63.1% amino-acid identity in the zinc-finger domain[15,16]. Krox-20 is an early growth response gene activated during G0–G1 transition in cultured cells[15].

Another feature of the translated cDNA sequence is the unusually high proline content, with two regions between amino acids 5 and 46 and 153 and 209 containing 33% proline. The latter region includes a stretch of nine prolines, reminiscent of the cluster of seven prolines near the N terminus of the protein encoded by Krox-20[15]. Proline-rich regions have also been found in several other putative DNA-binding proteins[17], including the Krüppel gene product, and could represent transcriptional activation domains as described for the CTF/NF-1 family of closely related polypeptides[17]. From these structural features, it is reasonable to infer that the product of the LK15 cDNA clone is a DNA-binding protein that could be a transcriptional regulator.

We also tested all new CpG islands against a panel of DNAs from 65 Wilms tumours to detect possible deletions or rearrangements. The centromeric clones as well as LFT1 detected no homozygous deletions on Southern blots. But, the telomeric LFT3 probe identified homozygous deletions in two tumours (Fig. 4a, b). Both deletions start between the LFT1 and LFT3 CpG islands and encompass all fragments detected by the LK15 cDNA clone, but do not include the more distal probe M20 (Fig. 4c). The proximal breakpoint in tumour PER is located only ~2 kb centromeric to the CpG island on the 500-kb NotI fragment. We cloned the rearranged EcoRI fragment of 10 kb and mapped the PER B1.0 subclone to the 375-kb NotI fragment between the LK15 cDNA and probe M20. More detailed mapping (not shown) revealed a deletion size of ~170 kb with both alleles having apparently identical deletions.

The homozygous deletion in Wilms tumour 8A terminates proximal to that in PER, as is also the case for the WiT-13 deletion that includes the CpG island and part of the 375-kb NotI fragment[18,19]. The distal end of the Wilms tumour 8A deletion has not been identified but must be located in the PER deletion. The two deletions therefore overlap by <170 kb, with 60 kb covered by the LK15 locus (Fig. 4c). It seems quite feasible to isolate the remaining region of overlap of both homozygous deletions to determine whether other genes adjacent to LK15
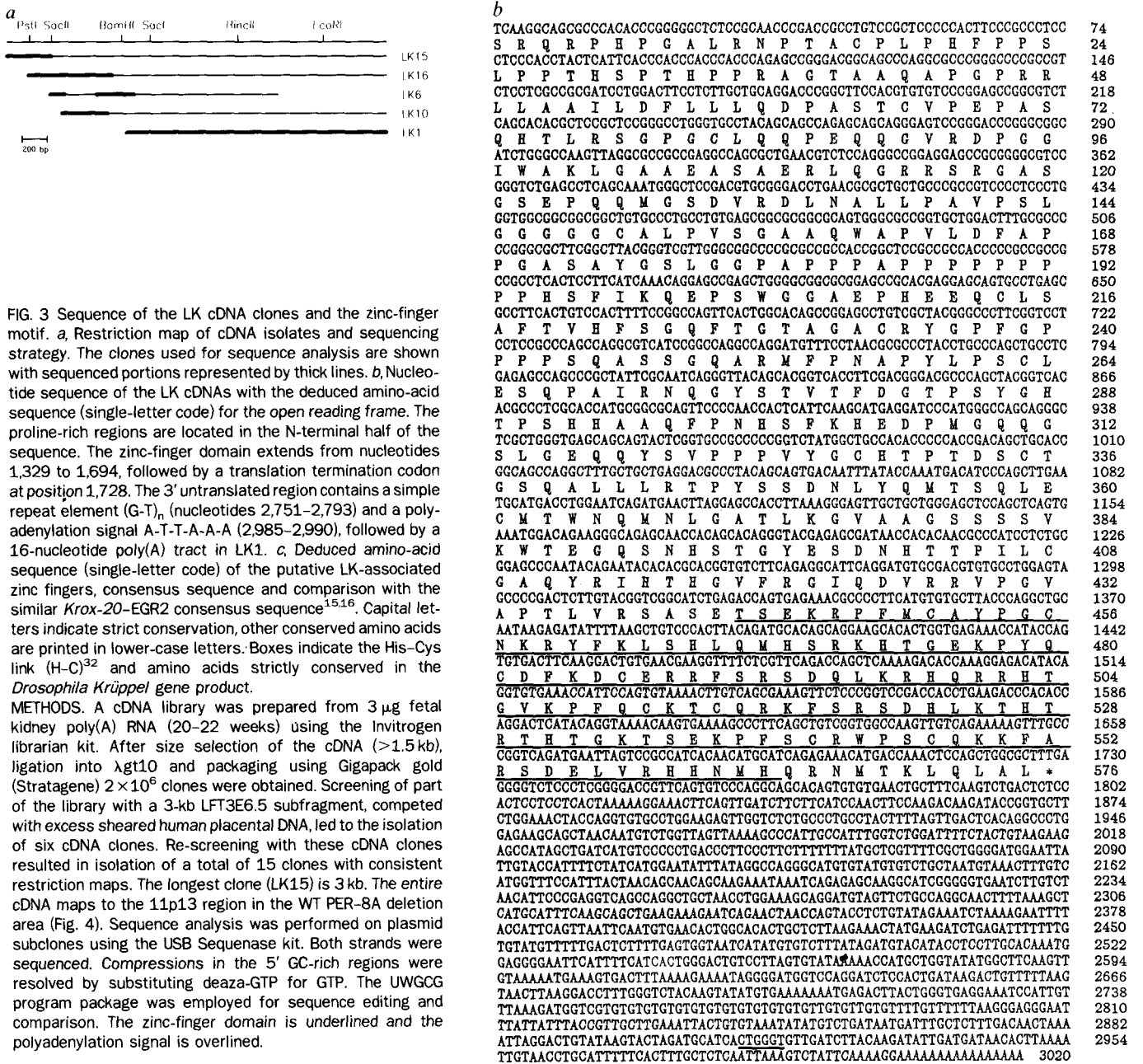
FIG. 3 Sequence of the LK cDNA clones and the zinc-finger motif. *a*, Restriction map of cDNA isolates and sequencing strategy. The clones used for sequence analysis are shown with sequenced portions represented by thick lines. *b*, Nucleotide sequence of the LK cDNAs with the deduced amino-acid sequence (single-letter code) for the open reading frame. The proline-rich regions are located in the N-terminal half of the sequence. The zinc-finger domain extends from nucleotides 1,329 to 1,694, followed by a translation termination codon at position 1,728. The 3′ untranslated region contains a simple repeat element $(G-T)_n$ (nucleotides 2,751–2,793) and a polyadenylation signal A-T-T-A-A-A (2,985–2,990), followed by a 16-nucleotide poly(A) tract in LK1. *c*, Deduced amino-acid sequence (single-letter code) of the putative LK-associated zinc fingers, consensus sequence and comparison with the similar *Krox-20*–EGR2 consensus sequence[15,16]. Capital letters indicate strict conservation, other conserved amino acids are printed in lower-case letters. Boxes indicate the His–Cys link (H–C)[32] and amino acids strictly conserved in the *Drosophila Krüppel* gene product.

METHODS. A cDNA library was prepared from 3 μg fetal kidney poly(A) RNA (20–22 weeks) using the Invitrogen librarian kit. After size selection of the cDNA (>1.5 kb), ligation into λgt10 and packaging using Gigapack gold (Stratagene) 2×10^6 clones were obtained. Screening of part of the library with a 3-kb LFT3E6.5 subfragment, competed with excess sheared human placental DNA, led to the isolation of six cDNA clones. Re-screening with these cDNA clones resulted in isolation of a total of 15 clones with consistent restriction maps. The longest clone (LK15) is 3 kb. The entire cDNA maps to the 11p13 region in the WT PER–8A deletion area (Fig. 4). Sequence analysis was performed on plasmid subclones using the USB Sequenase kit. Both strands were sequenced. Compressions in the 5′ GC-rich regions were resolved by substituting deaza-GTP for GTP. The UWGCG program package was employed for sequence editing and comparison. The zinc-finger domain is underlined and the polyadenylation signal is overlined.

```
a
   PstI SacII  BamHI SacI      HincII      EcoRI
   |  |  |     |     |         |           |
  ──────────────────────────────────────────  LK15
  ────────────────────                        LK16
      ──────────────                          LK6
      ─────────                               LK10
          ──────────────────────             LK1
  ├───┤
  200 bp
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```
b
TCAAGGCAGCGCCCACACCCGGGGGCTCTCCGCAACCCGACCGCCTGTCCGCTCCCCCACTTCCCGCCCTCC   74
  S   R   Q   R   P   H   P   G   A   L   R   N   P   T   A   C   P   L   P   H   F   P   P   S    24
CTCCCACCTACTCATTCACCCACCCACCCACCCAGAGCCCGGACGGCAGCCCAGGCGCCCGGGCCCCGCCGT  146
  L   P   P   T   H   S   P   T   H   P   P   R   A   G   T   A   A   Q   A   P   G   P   R   R    48
CTCCTCGCCGCGATCCTGGACTTCCTCTTGCTGCAGGACCCGGCTTCCACGTGTGTCCCGGAGCCGGCGTCT  218
  L   L   A   A   I   L   D   F   L   L   L   Q   D   P   A   S   T   C   V   P   E   P   A   S    72.
CAGCACACGCTCCGCTCCGGGCCTGGGTGCCTACAGCAGCCAGAGCAGCAGGGAGTCCGGGACCCGGGCGGC  290
  Q   H   T   L   R   S   G   P   G   C   L   Q   Q   P   E   Q   Q   G   V   R   D   P   G   G    96
ATCTGGGCCAAGTTAGGCGCCGCCGGAGGCCAGCGCTGAACGTCTCCAGGGCCGGAGGAGCCGCGGGCGTCC  362
  I   W   A   K   L   G   A   A   E   A   S   A   E   R   L   Q   G   R   R   S   R   G   A   S   120
GGGTCTGAGCCTCAGCAAATGGGCTCCGACGTGCGGGACCTGAACGCGCTGCTGCCCGCCGTCCCCTCCCTG  434
  G   S   E   P   Q   Q   M   G   S   D   V   R   D   L   N   A   L   L   P   A   V   P   S   L   144
GGTGCCGGCGGCGGCTGTGCCCTGCCTGTGAGCGGCGGCGGCGCAGTGGGCGCCGGTGCTGGACTTTGCGCCC  506
  G   G   G   G   G   C   A   L   P   V   S   G   A   A   Q   W   A   P   V   L   D   F   A   P   168
CCGGGCGCTTCGGCTTACGGGTCGTTGGGCGGCCCGGCGCCGCCCACCGGGCTCCGCCGCCACCCCCGCCGCCG  578
  P   G   A   S   A   Y   G   S   L   G   G   P   A   P   P   P   A   P   P   P   P   P   P       192
CCGGCTCACTCCTTCATCAAACAGGAGCCGAGCTGGGGCGGCGGCGGCGGAGCCGCACGAGGAGCAGTGCCTGAGC  650
  P   P   H   S   F   I   K   Q   E   P   S   W   G   G   A   E   P   H   E   E   Q   C   L   S   216
GGCCTTCACTGTCCACTTTTCCGGCCAGTTCACTGGCACAGCCGGAGCCTGTCGCTACGGGCCCTTCGGTCCT  722
  A   F   T   V   H   F   S   G   Q   F   T   G   T   A   G   A   C   R   Y   G   P   F   G   P   240
CCTCCGCCCAGCCAGGCGTCATCCGGCCAGGCCAGGATGTTTCCTAACGCGCCCTACCTGCCCAGCTGCCTC  794
  P   P   P   S   Q   A   S   S   G   Q   A   R   M   F   P   N   A   P   Y   L   P   S   C   L   264
GAGAGCCAGCCCGCTATTCGCAATCAGGGTTACAGCACGGTCACCTTCGACGGGACGCCCAGCTACGGTCAC  866
  E   S   Q   P   A   I   R   N   Q   G   Y   S   T   V   T   F   D   G   T   P   S   Y   G   H   288
ACGCCCTCGCCACCATCGGCGCAGTTCCCCAACCACTCATTCAAGCATGAGGATCCCATGGGCCAGCAGGGC  938
  T   P   S   H   H   A   A   Q   F   P   N   H   S   F   K   H   E   D   P   M   G   Q   Q   G   312
TCGCTGGGTGAGCAGCAGTACTCGGTGCCGCCCCCGGTTCTATGGCTGCCACACCCCCACCGACAGCTGCACC 1010
  S   L   G   E   Q   Q   Y   S   V   P   P   V   Y   G   C   H   T   P   T   D   S   C   T       336
GGCAGCCAGGCTTTGCTGCTGAGGACGCCCTACAGCAGTGACAATTTATACCAAATGACATCCCAGCTTGAA 1082
  G   S   Q   A   L   L   L   R   T   P   Y   S   S   D   N   L   Y   Q   M   T   S   Q   L   E   360
TGCATGACCTGGAATCAGATGAACTTAGGAGCCACCTTAAAGGGAGTTGCTGCTGGGAGCTCCAGCTCAGTG 1154
  C   M   T   W   N   Q   M   N   L   G   A   T   L   K   G   V   A   A   G   S   S   S   V       384
AAATGGACAGAAGGGCAGAGCAACCACAGCACAGGGTACGAGAGCGATAACCACACAACGCCCATCCTCTGC 1226
  K   W   T   E   G   Q   S   N   H   S   T   G   Y   E   S   D   N   H   T   T   P   I   L   C   408
GGAGCCCAATACAGAATACACACGCACCGTGTCTTCAGAGGCATTCAGGATGTGCGACGTGTGCCTGGAGTA 1298
  G   A   Q   Y   R   I   H   T   H   G   V   F   R   G   I   Q   D   V   R   R   V   P   G   V   432
GCCCCGACTCTTGTACGGTCGGCATCTGAGACCAGTGAGAAACGCCCCTTCATGTGTGCTTACCCAGGCTGC 1370
  A   P   T   L   V   R   S   A   S   E   T   S   E   K   R   P   F   M   C   A   Y   P   G   C   456
AATAAGAGATATTTTAAGCTGTCCCACTTACAGATGCACAGCAGGAAGCACACTGGTGAGAAACCATACCAG 1442
  N   K   R   Y   F   K   L   S   H   L   Q   M   H   S   R   K   H   T   G   E   K   P   Y   Q   480
TGTGACTTCAAGGACTGTGAACGAAGGTTTTCTCGTTCAGACCAGCTCAAAAGACACCAAAGGAGACATACA 1514
  C   D   F   K   D   C   E   R   R   F   S   R   S   D   Q   L   K   R   H   Q   R   R   H   T   504
GGTGTGAAACCATTCCAGTGTAAAACTTGTCAGCGAAAGTTCTCCCGGTCCGACCACCTGAAGACCCACACC 1586
  G   V   K   P   F   Q   C   K   T   C   Q   R   K   F   S   R   S   D   H   L   K   T   H       528
AGGACTCATACAGGTAAAACAAGTGAAAAGCCCTTCAGCTGTCGGTGGCCAAGTTGTCAGAAAAAGTTTGCC 1658
  R   T   H   T   G   K   T   S   E   K   P   F   S   C   R   W   P   S   C   Q   K   K   F   A   552
CGGTCAGATGAATTAGTCCGCCATCACAACATGCATCAGAGAAACATGACCAAACTCCAGCTGGCGCTTTGA 1730
  R   S   D   E   L   V   R   H   H   N   M   H   Q   R   N   M   T   K   L   Q   L   A   L   *   576
GGGGTCTCCCTCGGGGACCGTTCAGTGTCCCAGGCAGCACAGTGTGTGAACTGCTTTCAAGTCTGACTCTCC 1802
ACTCCTCCTCACTAAAAAGGAAACTTCAGTTGATCTTCTTCATCCAACTTCCAAGACAAGATACCGGTGCTT 1874
CTGGAAACTACCAGGTGTGCCTGGAACAGTTGGTCTCTGCCCTGCCTACTTTTAGTTGACTCACAGGCCCTG 1946
GAGAAGCAGCTAACAATGTCTGGTTAGTTAAAAGCCCATTGCCATTTGGTCTGGATTTTCTACTGTAAGAAG 2018
AGCCATAGCTGATCATGTCCCCCTGACCCTTCCCTTCTTTTTTTATGCTCGTTTTCGCTGGGGATGGAATTA 2090
TTGTACCATTTTCTATCATGGAATATTTATAGGCCAGGGCATGTGTATGTGTCTGCTAATGTAAACTTTGTC 2162
ATGGTTTCCATTTACTAACAGCAACAGCAAGAAATAAATCAGAGAGCAAGGCATCGGGGGTGAATCTTGTCT 2234
AACATTCCCGAGGTCAGCCAGGCTGCTAACCTGGAAAGCAGGATGTAGTTCTGCCAGGCAACTTTTAAAGCT 2306
CATGCATTTCAAGCAGCTGAAGAAAGAATCAGAACTAACCAGTACCTCTGTATAGAAATCTAAAAGAATTTT 2378
ACCATTCAGTTAATTCAATGTGAACACTGGCACACTGCTCTTAAGAAACTATGAAGATCTGAGATTTTTTTG 2450
TGTATGTTTTTGACTCTTTTGAGTGGTAATCATATGTGTCTTTTATAGATGTACATACCTCCTTGCACAAATG 2522
GAGGGGAATTCATTTTCATCACTGGGACTGTCCTTAGTGTATA*AAACCATGCTGGTATATGGCTTCAAGTT 2594
GTAAAAATGAAAGTGACTTTAAAAGAAAATAGGGGATGGTCCAGGATCTCCACTGATAAGACTGTTTTTAAG 2666
TAACTTAAGGACCTTTGGGTCTACAAGTATATGTGAAAAAAATGAGACTTACTGGGTGAGGAAATCCATTGT 2738
TTAAAGATGGTCGTGTGTGTGTGTGTGTGTGTGTGTGTGTTGTGTTGTGTTTTGTTTTTAAGGGAGGGAAT 2810
TTATTATTTACCGTTGCTTGAAATTACTGTGTAAATATATGTCTGATAATGATTTGCTCTTTGACAACTAAA 2882
ATTAGGACTGTATAAGTACTAGATGCATCACTGGGTGTTGATCTTACAAGATATTGATGATAACACTTAAAA 2954
TTGTAACCTGCATTTTTCACTTTGCTCTCAATTAAAGTCTATTCAAAAGGAAAAAAAAAAAAAAAA       3020
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c
443  T S E KRP F │M│C│A Y P G│C│N K R│Y│F K L S H│L│Q M│H│S R K│H│  ⎫
474  T G E K P Y│Q│C│D F K D│C│E R R│F│S R S D Q│L│K R H│Q R R│H│  ⎪
504  T G V K P F│Q│C│K T - -│C│Q R K│F│S R S D H│L│K T H│T R T│H│   ├ LK15
532  T G K T S E K P F│S│C│R W P S│C│Q K K│F│A R S D E│L│V R H│H N M│H│ 564 ⎭

     T q/s e K P f │x│C│x x x x│C│x r/k r/k │f│x r s d x│L│x x│H│x r x│H│   consensus LK15
     T G x K P F│x│C│x x x x│C│x R x│F│s R S D e│l│t r│H│i r i│H│        consensus Krox-20
```

play a part in the development of the genito-urinary system or in Wilms tumour.

The low incidence of homozygous deletions that are detected by our probes, which can be at most 50 kb away from a Wilms tumour locus in this region, and the presence of an apparently normal-sized transcript for LK15 in 12 tumours (data not shown), contrasts with the frequent deletions and internal rearrangements of *Rb-1*, another tumour suppressor gene, seen in retinoblastomas and other tumours[20-24]. It is possible that the presence of another important gene in this region favours more-subtle alterations in these tumours, or loci at other sites in the genome[25-29] could have a larger role in sporadic Wilms tumours than previously believed.

The expression pattern of the LK15 transcript indicates that its corresponding gene could be involved in normal kidney development, especially because the genito-urinary anomaly component of the WAGR syndrome maps to this genomic region[6,7]. The presence of the Krüppel-like[12] zinc-finger domain in the LK15 cDNA and its potential function as a regulatory protein is consistent with this model.

Our identification of this zinc-finger gene demonstrates the power of the combined approach of mapping CpG islands by

FIG. 4 The LFT3–LK1 region is homozygously deleted in two Wilms tumours. *a*, Probe LFT3E4.5 detects a band in DNA from Wilms tumour PER, whereas Probe E0.9 detects a fragment of altered size, and the adjacent probe E1.2 is homozygously deleted. All bands detected by the LK1 cDNA are also deleted. The PER B1.0 probe, isolated from the other side of the cloned deletion junction, detects the same rearranged band in PER DNA as probe E0.9. *b*, In Wilms tumour 8A, the proximal boundary of the homozygous deletion region is located between probes CP5E3.2 and probe LFT3E4.5. The entire LK1 cDNA region including the 3' end (not shown) is deleted in this DNA. The PER B1.0 probe, however, is not included in the homozygous deletion. The extremely weak 1.8 kb band in both PER and 8A that is seen with LK1E1.6 is probably due to a small percentage of normal cells in the tumour samples. *c*, Schematic representation of the extent of homozygous deletions in tumours PER and 8A. Dashed lines indicate uncertainty of the exact breakpoint location in 8A. The extent of the LK15 locus is derived from the analysis of additional overlapping cosmid clones (not shown). The size of the PER deletion was measured by PFG mapping studies not shown here. METHODS. DNA (4 μg) from tumours and normal control DNA (C) were digested with *Eco*RI (or *Bam*HI for probe PER B1.0 in *b*), and filters were prepared and hybridized as described[5]. Before using filters again they were stripped in 10^{-3} M Tris-HCL, pH 8, 10^{-4} M disodium EDTA, 0.1% SDS at 75 °C. To clone the rearranged fragment in PER, *EcoRI*-digested tumour DNA was ligated into EMBL4 and packaged using Gigapack gold (Stragagene). Screening with LFT3E0.9 gave eight independent isolates. After restriction mapping of the 10-kb deletion junction, suitable fragments were subcloned into plasmid vectors. The PER B1.0 clone correctly maps to chromosome 11p13 and is located ~170 kb telomeric to the LFT3 CpG island as determined by PFG analysis. Abbreviations as in Fig. 1.
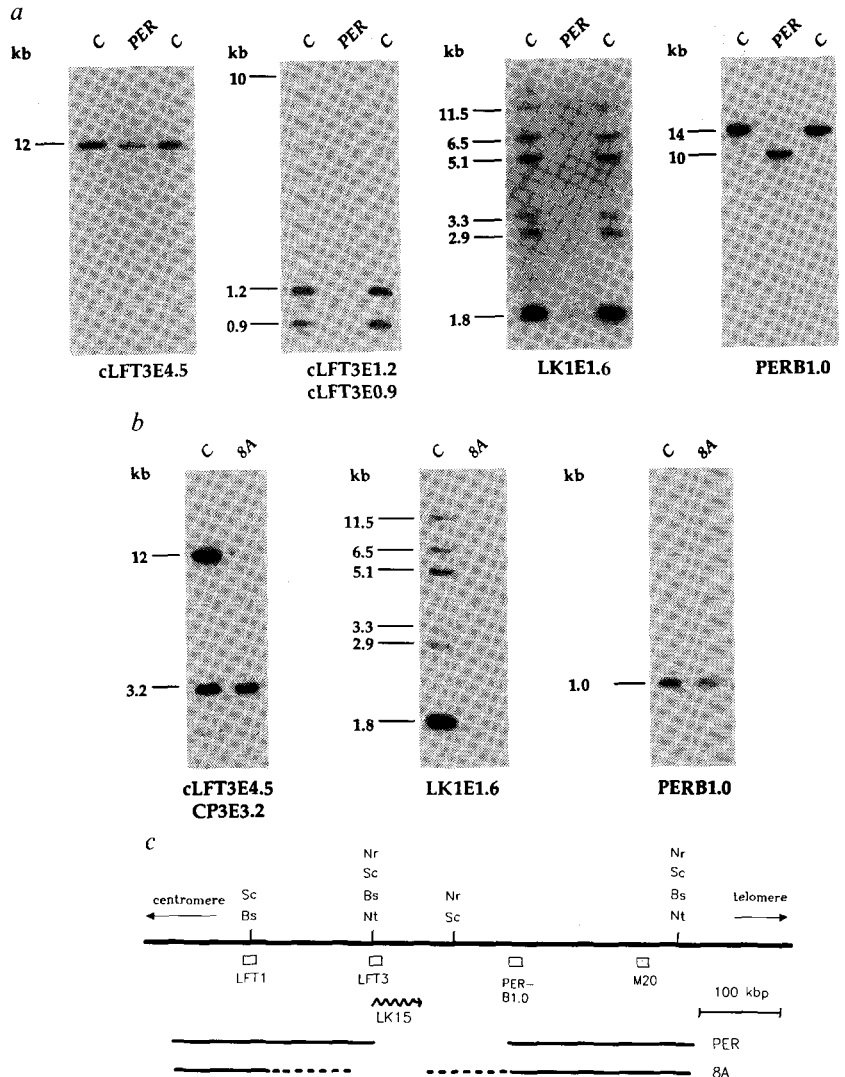
PFG analysis and subsequent cloning by consecutive jumps from one island to the next using rare-cutting restriction-enzyme jumping libraries. Molecular probes for island-associated genes should access a significant fraction of expressed sequences, and, especially in chromosomal regions rich in CpG islands, provide a framework for a gene map. □

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