Pergamon

European Journal of Cancer, Vol. 33, No. 12, pp. 1991–1996, 1997

Printed in Great Britain
0959-8049/97 $17.00+0.00

PII: S0959-8049(97)00279-7

Original Paper

Human Krüppel-Related 3 (*HKR3*): a Candidate for the 1p36 Neuroblastoma Tumour Suppressor Gene?

J.M. Maris,[1] J. Jensen,[1] E.P. Sulman,[1] C.P. Beltinger,[1] C. Allen,[2] J.A. Biegel,[2] G.M. Brodeur[1] and P.S. White[1]

[1]Division of Oncology; and [2]Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia and the Department of Pediatrics, The University of Pennsylvania School of Medicine, ARC 902A, 324 South 34th Street, Philadelphia, Pennsylvania 19104-4318, U.S.A.

Human Krüppel-related 3 (*HKR3*) is a zinc finger gene that maps within chromosome subbands 1p36.2-.3, a region postulated to contain a tumour suppressor gene associated with advanced neuroblastomas. Genomic clones of *HKR3* were isolated from a P1 library and physically mapped to within 40 kb of *D1S214* at 1p36.3. The gene is ubiquitously expressed in human tissues, but esecially high levels are present in human fetal and adult nervous tissues. Hemizygous deletion of *HKR3* in a lymphoblastoid cell line derived from a neuroblastoma patient with a constitutional 1p36 interstitial deletion and in the neuroblastoma cell line SK-N-AS, which also has a small interstitial 1p36 deletion, has been observed. Allelic loss at *D1S214* in 15/15 informative primary neuroblastoma specimens with 1p36 deletions has also been observed. In a panel of 16 neuroblastoma cell lines, no gross genomic DNA rearrangements were noted, the gene was always expressed (albeit at variable levels) and there was no evidence for truncating mutations. Furthermore, there were no mutations detected in the zinc finger coding region in four neuroblastoma cell lines with 1p deletions analysed by direct sequence analysis. We conclude that *HKR3* is a novel zinc finger gene that maps to a region of the genome commonly rearranged or deleted in neuroblastoma and other human cancers. © 1997 Elsevier Science Ltd.

Key words: chromosomes, human, pair 1; chromosome mapping; genes, suppressor, tumour; molecular sequence data; mutation; neuroblastoma (genetics); transcription factors; zinc fingers
Eur J Cancer, Vol. 33, No. 12, pp. 1991–1996, 1997

INTRODUCTION

MOLECULAR GENETIC studies have defined a region at the distal short arm of chromosome 1 (1p) that is postulated to contain a suppressor gene associated with advanced neuroblastomas [1–4]. Our laboratory has undertaken a multifaceted approach directed towards the positional cloning of this gene. However, the common region of deletion remains at least 5 megabases in size [1] and familial predisposition does not map to this region [5]. Therefore, positional cloning approaches, such as loss of heterozygosity (LOH) in primary tumours and linkage analyses in neuroblastoma kindreds, may be of limited value in further narrowing the region. In addition, several proposed candidate neuroblastoma suppressor genes have been mapped outside the commonly deleted region [1]. We therefore adopted a positional candidate approach [6] to identify the neuroblastoma suppressor gene, in parallel with our ongoing efforts to narrow the region of interest. As such, we routinely evaluate genes or expressed sequence tags that we have mapped to the common region of deletion. Any gene that has potential suppressor function based upon primary sequence analysis, tissue expression and/or analogy with known tumour suppressor genes is considered a candidate for further analysis.

The human genome contains several hundred members of the zinc finger multigene family [7]. Zinc finger genes as a group are thought to play various key roles in human development, where they function as DNA-binding transcriptional regulators. Several zinc finger genes have been implicated in malignant human diseases. For example, *WT1* is a Krüppel-related zinc finger gene that is homozygously inactivated in a subset of Wilms' tumours [8–10]. Other human malignancies

Correspondence to J. M. Maris.

in which the altered expression of a zinc finger gene has been implicated in tumorigenesis include leukaemias [11–13], lymphomas [14–17], glial-derived brain tumours [18, 19] and rhabdomyosarcomas [20]. Therefore, genes predicted to encode zinc finger motifs that map within the commonly deleted region at 1p36.2-.3 may be considered candidates for the neuroblastoma suppressor gene.

Ruppert and colleagues [21] initially cloned six partial human Krüppel-related (*HKR*) zinc finger genes, which were isolated because of their homology to the *GLI* proto-oncogene. One of these clones, *HKR3*, was mapped to human chromosome 1. Sugawara and colleagues [22] independently cloned a full-length cDNA termed clone 18, which is predicted to encode a 77 kDa protein with 11 Krüppel-like zinc finger motifs. Furthermore, a conserved N-terminal region, termed the TAB (tramtrack-associated box), as well as an acidic activation domain, were identified and characterised. We have recently demonstrated that this clone 18 cDNA is *HKR3*, refined the mapping to 1p36.3 and reported on the details of its genomic organisation and expression [23]. We now briefly review the relevant aspects of the genomic characteristics of *HKR3* and present our preliminary mutational analysis of this gene in neuroblastomas.

MATERIALS AND METHODS

Details of the materials and methods used for the cloning, mapping, genomic structure definition and expression of *HKR3* have been reported previously [23].

Allelic loss analysis

Fluorescence *in situ* hybridisation (FISH) was performed with a P1 clone containing human genomic *HKR3* labelled with digoxigenin-11-dUTP (Oncor) as a probe. A lymphoblastoid cell line derived from a neuroblastoma patient with a constitutional 1p36 deletion [1, 24] and the neuroblastoma cell line SK-N-AS [1, 25] were hybridised with 300 ng of labelled probe. Hybridisation, signal detection and imaging were performed as described [23, 26]. Analysis of primary neuroblastoma and paired constitutional DNA specimens for LOH was performed using the *D1S214* dinucleotide repeat polymorphism (Genome Database). PCR amplification, separation of alleles and data analysis were performed as previously described [2].

Neuroblastoma cell lines

A panel of 16 neuroblastoma cell lines was used to assay for *HKR3* mutations. These include: CHP 126A, CHP 134, LA-N-5, LA-N-6, NAB, Nb 69, NBL-S, NGP, NLF, NMB, SK-N-AS, SK-N-SH, SMS-KAN, SMS-KCN, SMS-LHN and SMS-SAN. Details of the cyto- and molecular-genetic characteristics of these cell lines as well as culture conditions have been previously described [27] or are available upon request. In terms of 1p deletion status, 10 of these cell lines are known to have 1p36 hemizygous deletions, the NGP cell line harbours a complicated 1p36 rearrangement [1] and the remaining five cell lines (Nb-69, NBL-S, SK-N-SH, LA-N-5 and LA-N-6) have not been completely characterised by us, although the SK-N-SH cell line probably retains both copies of 1p36 [25].

Southern and Northern analyses

Southern hybridisation of restriction digested genomic DNA isolated from human tissues or neuroblastoma cell lines was performed following separation by standard gel electrophoresis (5 μg of *Pvu*II digested DNA separated in 0.8% agarose). 20 ng each of two overlapping 1 kb *HKR3* RT–PCR products [23] were random primer labelled with ^{32}P-αdCTP (Amersham, Cleveland, Ohio, U.S.A.) to a specific activity of $> 10^8$ dpm/μg and used in combination as a probe. DNA transfer and hybridisation were performed as described [28]. Expression of *HKR3* mRNA was determined by Northern hybridisation. Briefly, 10 μg of total cellular RNA was separated in 1% agarose/1X MOPS [3-(*N*-morpholino)-propanesulphonic acid] and RNA quality was assessed with ethidium bromide staining. The RNA was transferred overnight and hybridised with the same RT–PCR generated probes as used in the Southern analyses. All conditions were as previously described [23, 29].

In vitro *transcription and translation (IVTT) assay*

Detection of *HKR3* gene termination mutations was performed with the TNT T7 coupled reticulocyte lysate system (Promega, Madison, Wisconsin, U.S.A.). Briefly, RNA was extracted from the neuroblastoma cell lines as well as two neuroblastoma primary tumour specimens and control human tissues (adult and fetal brain and adult adrenal gland) using standard methods [28]. First-strand cDNA synthesis was performed with a gene-specific primer (5′-CAAAGCCACACGGTTTATTGA-3′ corresponding to cDNA 2280 → 2261 and therefore mapping to the 3′ portion of the cDNA) using the SuperScript pre-amplification system according to the manufacturer's recommendations for transcripts with a high GC content (Gibco BRL, Gaithersburg, Maryland, U.S.A.). Following the degradation of any remaining RNA with *E. coli* RNase H (Gibco BRL), nested PCR was performed to generate a 2145 bp product that contained the entire coding region. The second PCR was performed with an upstream sense primer that contained a T7 promoter and translation initiation sequence 5′ of the gene-specific sense oligonucleotide which allowed for efficient *in vitro* transcription and translation [30]. The IVTT was performed using 5 μl of the RT–PCR product as a template with 40 μCi ^{35}S-methionine (Amersham) in a rabbit reticulocyte lysate (total volume 50 μl) according to the manufacturer's recommendations (Promega). The labelled protein products were separated by SDS–polyacrylamide gel electrophoresis (PAGE) with a separating gel concentration of 10%. Autoradiography was performed at –70°C for 1–4 h.

DNA sequencing for mutational analysis

A 1335 bp RT–PCR product was generated from total cellular RNA extracted from the neuroblastoma cell lines CHP 134, NLF, NMB and SMS-KCN. This RT–PCR product was designed to span the entire zinc finger encoding region where a high degree of conservation in primary structure is expected (Figure 1). Bidirectional sequencing was performed on an Applied Biosystems DNA sequencer model 373A with the ABI Taq DyeDeoxy Terminator Cycle Sequencing kit. Sequencing data were analysed with the Sequencher analysis program (Gene Codes, Ann Arbor, Michigan, U.S.A.).

RESULTS

Cloning, mapping and genomic characteristics of HKR3

Three genomic *HKR3* clones (HKR-4184, 4185, 4186) were isolated by screening a human genomic bacteriophage P1 library. One of these clones, HKR-4185, was used to

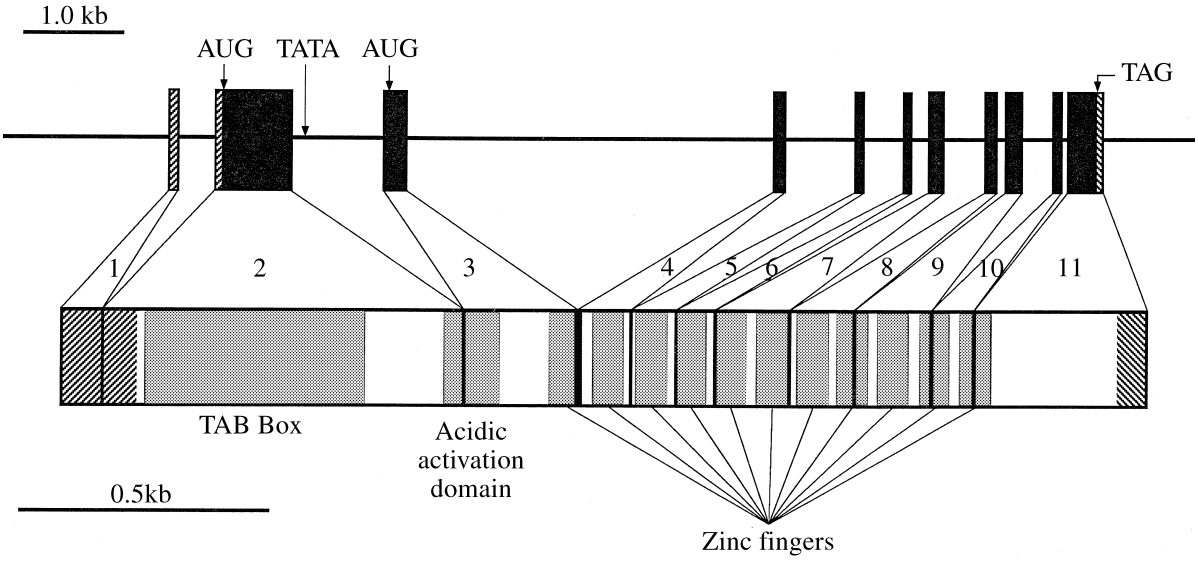

Figure 1. Genomic structure of the *HKR3* gene. The 11 exons of *HKR3* are distributed within 9.5 kb of genomic DNA (top). The AUG translation initiation codons for the two alternate transcripts are indicated and are in frame (see text). The smaller alternate transcript is initiated following the TATA motif within intron 2. The 2289 bp of cDNA are displayed in the middle, with the predicted functional protein motif encoding regions indicated. The bottom solid line indicates the position of the 1335 bp RT–PCR product used for the sequencing experiments. Hatched boxes indicate flanking non-coding regions, black boxes indicate coding regions (top) and grey boxes indicate protein motif regions (middle).

further sublocalise *HKR3* by FISH analysis of normal metaphases derived from a lymphoblastoid cell line. Specific hybridisation was detected at chromosome 1p36.2-.3 in all 50 metaphases examined. Subsequent identification of four *HKR3*-containing YACs allowed for the physical mapping of the gene to within 40 kb of *D1S214*, a dinucleotide repeat polymorphism that is a genetic framework marker at 1p36.3 [31].

The *HKR3* gene spans 9.5 kb and is contained in 11 exons (Figure 1). The AUG translation initiation site for the largest open reading frame, predicted from the published cDNA sequence, is contained in exon 2. The 11 C-terminal C_2H_2 zinc fingers are distributed from exons 3–11. There is no apparent conservation of motifs within exons, as many of the consensus finger domains and the putative acidic activation domain are interrupted by introns. However, the conserved N-terminal TAB motif is completely located within exon 2. The 5′ region of *HKR3* (684 bp of sequenced 5′ untranslated region, exon 1, intron 1 and most of exon 2) fulfill the criteria for a CpG island with a moving average of > 50% C + G and a value of > 0.6 observed/expected CpG dinucleotides [32, 33]. In addition, a TATA consensus sequence (TATAATA) was identified at the 5′ end of intron 2 followed by a full-length (290 bp) Alu-repeat element of the J subfamily. This TATA box was shown to be located 31 bp upstream of an alternative transcription start site, which predicts a variant protein of 51 kDa. This alternate transcript was shown to be expressed at high steady-state levels and to have the major open reading frame initiated within exon 3 in frame with the full-length transcript. Interestingly, this transcript would not include coding information for the TAB [23]. Finally, *HKR3* was shown to be ubiquitously expressed in human adult and fetal tissues as a 2.3 kb transcript.

Hemizygous deletion of HKR3 *in neuroblastomas*

Two of the smallest interstitial deletions overlapping the commonly deleted region at 1p36 in neuroblastomas are defined by a lymphoblastoid cell line derived from a patient with a constitutional 1p36 deletion who subsequently developed neuroblastoma [1, 24] and the neuroblastoma cell line SK-N-AS [1, 25]. *HKR3* was hemizygously deleted in each of these cell lines, as demonstrated by FISH (data not shown). To extend these findings, our previously assayed panel of 156 primary neuroblastoma specimens matched with constitutional DNAs were assayed for LOH at *D1S214* [2]. Specimens on 95 patients were analysed at *D1S214* and 77 were informative (observed heterozygosity of 0.81). Fifteen patients had LOH at *D1S214* (19%) in an overall sample slightly biased towards lower stage tumours [2]. All patients who had LOH at 1p36 had allelic loss at *D1S214*, and we did not observe LOH at other 1p loci when heterozygosity was retained at *D1S214*.

Southern hybridisation of HKR3 *to neuroblastoma cell line DNAs*

DNA extracts from 13 of the full panel of 16 neuroblastoma cell lines (LA-N-5, LA-N-6 and SMS-LHN excluded) and a control lymphoblastoid cell line were digested with *Pvu*II and hybridised with two overlapping *HKR3* cDNA probes spanning the entire coding region. Both 1.7 and 2.2 kb fragments were detected in the control specimen as well as each neuroblastoma cell line. There were no additional or rearranged bands detected in the neuroblastoma cell line DNA samples.

HKR3 *expression in neuroblastoma cell lines*

RNA was extracted from a panel of normal tissues [23] and eight of the neuroblastoma cell lines (CHP 134, LA-N-5, NGP, NLF, NMB, SK-N-SH, SMS-KAN and SMS-KCN). An approximate 2.3 kb transcript was detected in various quantities in all control and each of the eight neuroblastoma cell line samples (highest in NLF and NMB, lowest in CHP 134 and SMS-KAN). The messages encoded by the alternate transcripts differ by only 127 nucleotides and therefore would not be resolved by Northern blot analysis.

IVTT assay

To screen for the presence of subtle mutations rapidly, the IVTT assay was used to detect the presence of nonsense (truncating) codon alterations. RNA was extracted from each of the 16 neuroblastoma cell lines and two primary tumour specimens, with RNA from fetal brain, adult brain and adult adrenal gland tissues serving as controls. A 79.5 kDa protein product was predicted from the primary cDNA sequence data and was present in all neuroblastoma cell lines, primary tumours and control tissues assayed and no truncated protein products were detected (Figure 2).

Direct sequence analysis of the zinc finger region

As another rapid screen for subtle mutations within the *HKR3* coding region, four neuroblastoma cell lines known to contain 1p36 deletions were chosen for sequence analysis. A 1335 bp RT–PCR product was generated from the total RNA extracts of CHP 134, NLF, NMB and SMS-KCN to serve as a sequencing template. Bidirectional sequencing was performed and compared to the wild-type *HKR3* cDNA sequence. No mutations or polymorphisms were detected.

DISCUSSION

There are several reports of altered expression of zinc finger genes in human cancers. Indeed, each of the three major genetic mechanisms known to be involved in tumorigenesis have been implicated, including: (1) activation or amplification of a proto-oncogene; (2) development of a novel (and abnormally regulated) chimeric gene and protein through chromosomal translocation; and (3) homozygous inactivation of a tumour suppressor gene. For example, the *GLI* proto-oncogene is amplified in a subset of human brain tumours [18]. *PLZF*, *BCL5*, *TTG-1* and *LAZ3* are examples of zinc finger genes that have been cloned from chromosomal translocation breakpoints in a variety of haematopoietic malignancies [12–15]. *WT1* is a prototypic tumour suppressor gene that is homozygously inactivated in 10–30% of Wilms' tumours [8, 9]. Thus, it appears that several members of the large zinc finger gene family have an important role in human tumorigenesis.

HKR3 was originally identified because of its homology with the *GLI* proto-oncogene [21]. We have subsequently shown that *HKR3* is a novel zinc finger gene located within chromosomal subband 1p36.3. *HKR3* encodes carboxy-terminal finger repeats and amino-terminal transcriptional regulatory motifs, which is typical of other Krüppel-related zinc finger genes [34], including the tumour suppressor gene *WT1* [9, 10]. *HKR3* is highly expressed in both fetal and adult nervous tissues and two separate mRNA transcripts may be produced, the shorter of which excludes a potential transcriptional repressing (TAB) motif. Therefore, based

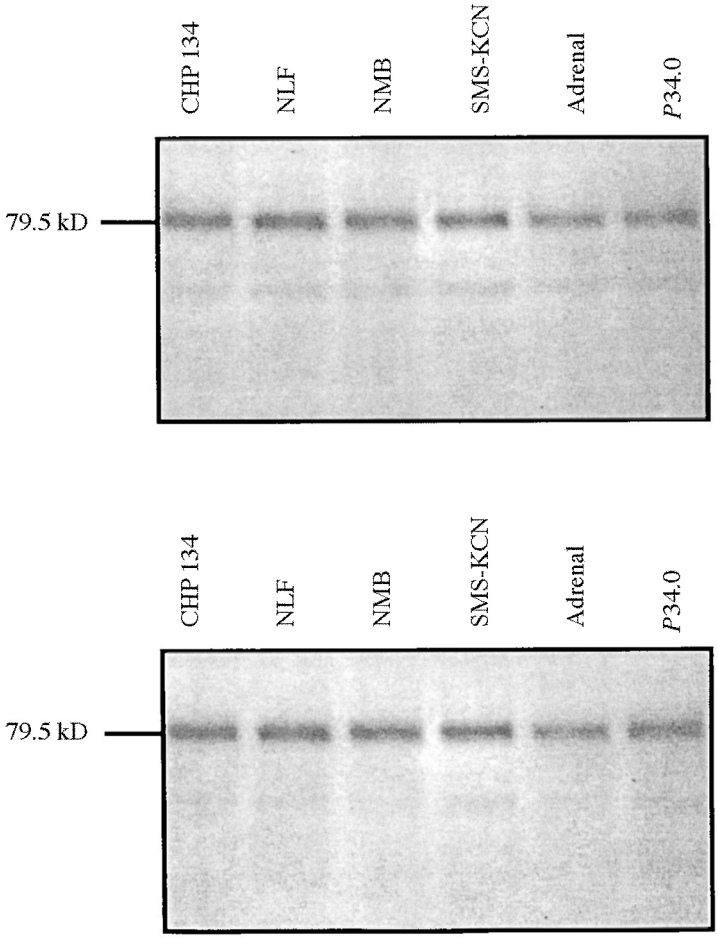

Figure 2. No truncated protein products by IVTT. A representative autoradiogram of the IVTT produced protein products from RNAs extracted from four 1p deleted neuroblastoma cell lines (CHP-134, NLF, NMB, SMS-KCN), normal adrenal gland and a 1p deleted, *MYCN* amplified primary neuroblastoma (P34.0). A protein product of the predicted size is present in each sample.

upon mapping, coding information, expression and analogy with another paediatric embryonal cancer, *HKR3* is a candidate for a neuroblastoma suppressor gene.

HKR3 was hemizygously deleted in all neuroblastomas assayed with 1p36 deletions, confirming our mapping data. However, we were unable to find gross rearrangements at the *HKR3* locus in neuroblastoma tissues by Southern blotting. Furthermore, all neuroblastoma tissues assayed produced an appropriate sized transcript, although the quantity of mRNA varied substantially. Finally, subtle mutations were not identified by either the IVTT assay or direct sequencing of the zinc finger encoding region. The evidence, therefore, is substantial that *HKR3* is not directly involved in neuroblastoma tumorigenesis.

A common problem confronting those involved with positional cloning is how to 'rule out' a gene as a viable candidate. Mutational analysis is an evolving art in the field of molecular biology. Certainly, detection of gross rearrangements or homozygous deletions at the genomic DNA level, or altered expression at the mRNA level, will remain the first-line screen. Conventional methods to detect subtle mutations, such as single-strand conformation polymorphism (SSCP) and denaturing gradient gel electrophoresis (DGGE), require information about exon–intron structure and are labour intensive. Methods that rely solely on cDNA sequence, such as IVTT and automated sequencing, are desirable, but have the disadvantages of requiring mRNA and missing intronic mutations that may interfere with mRNA processing (splice site mutations). Although there have been many advances in the field of mutation detection, there remains no universally applicable method [35]. Therefore, in the absence of a perfect genetic screen to assay for a candidate gene's involvement in neuroblastoma, a functional approach may be more appealing. We plan to transfect stably an *HKR3* cDNA clone into 1p deleted neuroblastoma cell lines to assay for reversion of the malignant phenotype and/or loss of tumorigenicity. This latter methodology would also address the problems raised if haploinsufficiency and/or imprinting of the neuroblastoma suppressor gene are mechanisms involved in tumorigenesis.

In summary, we have embarked on a positional candidate [6] approach as one method to clone the neuroblastoma suppressor gene located at 1p36.2-.3. Further refinement of the common region of deletion will allow for more efficient screening for candidate genes. We have characterised *HKR3*, a unique zinc finger candidate gene that maps to the middle of the currently defined region commonly deleted in neuroblastomas. Although no mutations or altered transcripts were found in a panel of neuroblastoma cell lines, we plan to determine the functional consequence of stable *HKR3* expression in neuroblastoma cell lines with and without 1p36 deletions.

1. White PS, Maris JM, Beltinger C, *et al.* A region of consistent deletion in neuroblastoma maps within human chromosome 1p36.2-36.3. *Proc Natl Acad Sci USA* 1995, **92**, 5520–5524.
2. Maris JM, White PS, Beltinger CP, *et al.* Significance of chromosome 1p loss of heterozygosity in neuroblastoma. *Cancer Res* 1995, **55**, 4664–4669.
3. Gehring M, Berthold F, Edler L, Schwab M, Amler LC. The 1p deletion is not a reliable marker for the prognosis of patients with neuroblastoma. *Cancer Res* 1995, **55**, 5366–5369.
4. Caron H, van Sluis P, de Kraker J, *et al.* Allelic loss of chromosome 1p as a predictor of unfavorable outcome in patients with neuroblastoma. *N Engl J Med* 1996, **334**, 225–230.
5. Maris JM, Kyemba SM, Rebbeck TR, *et al.* Familial predisposition to neuroblastoma does not map to chromosome band 1p36. *Cancer Res* 1996, **56**, 3421–3425.
6. Collins FS. Positional cloning moves from perditional to traditional. *Nat Genet* 1995, **9**, 347–350.
7. Bellefroid EJ, Lecocq PJ, Benhida A, Poncelet DA, Belayew A, Martial JA. The human genome contains hundreds of genes coding for finger proteins of the Kruppel type. *DNA* 1989, **8**, 377–387.
8. Haber DA, Buckler AJ, Glaser T, *et al.* An internal deletion within an 11p13 zinc finger gene contributes to the development of Wilms' tumor. *Cell* 1990, **61**, 1257–1269.
9. Gessler M, Poustka A, Cavenee W, Neve RL, Orkin SH, Bruns GA. Homozygous deletion in Wilms' tumours of a zinc-finger gene identified by chromosome jumping. *Nature* 1990, **343**, 774–778.
10. Call KM, Glaser T, Ito CY, *et al.* Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 Wilms' tumor locus. *Cell* 1990, **60**, 509–520.
11. Chen Z, Brand NJ, Chen A, *et al.* Fusion between a novel Kruppel-like zinc finger gene and the retinoic acid receptor-alpha locus due to a variant t(11;17) translocation associated with acute promyelocytic leukaemia. *EMBO J* 1993, **12**, 1161–1167.
12. McGuire EA, Hockett RD, Pollock KM, *et al.* The t(11;14)(p15;q11) in a T-cell acute lymphoblastic leukemia cell line activates multiple transcripts, including Ttg-1, a gene encoding a potential zinc finger protein. *Mol Cell Biol* 1989, **9**, 2124–2132.
13. Goddard AD, Borrow J, Freemont PS, Solomon E. Characterization of a zinc finger gene disrupted by the t(15;17) in acute promyelocytic leukemia. *Science* 1991, **254**, 1371–1374.
14. Miki T, Kawamata N, Hirosawa S, Aoki N. Gene involved in the 3q27 translocation associated with B-cell lymphoma, BCL5, encodes a Kruppel-like zinc-finger protein. *Blood* 1994, **83**, 26–32.
15. Kerckaert JP, Deweindt C, Tilly H, *et al.* LAZ3, a novel zinc-finger encoding gene, is disrupted by recurring chromosome 3q27 translocations in human lymphomas. *Nat Genet* 1993, **5**, 66–70.
16. Bastard C, Deweindt C, Kerckaert JP, *et al.* LAZ3 rearrangements in non-Hodgkin's lymphoma: correlation with histology, immunophenotype, karyotype, and clinical outcome in 217 patients. *Blood* 1994, **83**, 2423–2427.
17. Otsuki T, Yano T, Clark HM, *et al.* Analysis of LAZ3 (BCL-6) status in B-cell non-Hodgkin's lymphomas: results of rearrangement and gene expression studies and a mutational analysis of coding region sequences. *Blood* 1995, **85**, 2877–2884.
18. Kinzler KW, Bigner SH, Bigner DD, *et al.* Identification of an amplified, highly expressed gene in a human glioma. *Science* 1987, **236**, 70–73.
19. Wong AJ, Bigner SH, Bigner DD, *et al.* Increased expression of the epidermal growth factor receptor gene in malignant gliomas is invariably associated with gene amplification. *Proc Natl Acad Sci USA* 1987, **84**, 6899–6903.
20. Roberts WM, Douglass EC, Peiper SC, Houghton PJ, Look AT. Amplification of the gli gene in childhood sarcomas. *Cancer Res* 1989, **49**, 5407–5413.
21. Ruppert JM, Kinzler KW, Wong AJ, *et al.* The GLI-Kruppel family of human genes. *Mol Cell Biol* 1988, **8**, 3104–3113.
22. Sugawara M, Scholl T, Ponath PD, Strominger JL. A factor that regulates the class II major histocompatibility complex gene DPA is a member of a subfamily of zinc finger proteins that includes a Drosophila developmental control protein. *Mol Cell Biol* 1994, **14**, 8438–8450.
23. Maris JM, Jensen SJ, Sulman EP, *et al.* Cloning, chromosomal localization, physical mapping, and genomic characterization of *HKR3*. *Genomics* 1996, **35**, 289–298.
24. Biegel JA, White PS, Marshall HN, *et al.* Constitutional 1p36 deletion in a child with neuroblastoma. *Am J Hum Genet* 1993, **52**, 176–182.
25. Van Roy N, Laureys G, Cheng NC, *et al.* 1;17 Translocations and other chromosome 17 rearrangements in human primary neuroblastoma tumors and cell lines. *Genes Chromosom Cancer* 1994, **10**, 103–114.
26. Nakagawara A, Liu X, Ikegaki N, *et al.* Cloning and chromosomal localization of the human TRK-B Tyrosine Kinase Receptor Gene (NTRK2). *Genomics* 1995, **25**, 538–546.

27. Reynolds CP, Tomayko MM, Donner L, *et al.* Biological classification of cell lines derived from human extra-cranial neural tumors. *Prog Clin Biol Res* 1988, **271**, 291–306.
28. Ausubel FM, Brent R, Kingston RE, *et al.* (eds) *Current Protocols in Molecular Biology*. New York, Wiley, 1995.
29. Kroczek RA, Siebert I. Optimization of Northern analysis by vacuum-blotting, RNA-transfer visualization, and ultraviolet fixation. *Anal Biochem* 1990, **184**, 90–95.
30. Heim RA, Kam-Morgan LN, Binnie CG, *et al.* Distribution of 13 truncating mutations in the neurofibromatosis 1 gene. *Hum Mol Genet* 1995, **4**, 975–981.
31. Dib C, Faure S, Fizames C, *et al.* A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 1996, **380**, 152–154.
32. Larsen F, Gundersen G, Lopez R, Prydz H. CpG islands as gene markers in the human genome. *Genomics* 1992, **13**, 1095–1107.
33. Gardiner-Garden M, Frommer M. CpG islands in vertebrate genomes. *J Mol Biol* 1987, **196**, 261–282.
34. Abrink M, Aveskogh M, Hellman L. Isolation of cDNA clones for 42 different Kruppel-related zinc finger proteins expressed in the human monoblast cell line U-937. *DNA Cell Biol* 1995, **14**, 125–136.
35. Forrest S, Cotton R, Landegren U, Southern E. How to find all those mutations. *Nat Genet* 1995, **10**, 375–376.

Acknowledgements—This work was supported by grants from the National Institutes of Health CA-9132 (JMM) and CA-39771 (GMB).