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Advances in Brief

Familial Predisposition to Neuroblastoma Does Not Map to Chromosome Band 1p36[1]

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Abstract

Familial predisposition to neuroblastoma, a common embryonal cancer of childhood, segregates as an autosomal dominant trait with high penetrance. It is therefore likely that neuroblastoma susceptibility is due to germ line mutations in a tumor suppressor gene. Cytogenetic, functional, and molecular studies have implicated chromosome band 1p36 as the most likely region to contain a suppressor gene involved in sporadic neuroblastoma tumorigenesis. We now demonstrate that neuroblastoma predisposition does not map to any of eight polymorphic markers spanning 1p36 by linkage analysis in three families. In addition, there is no loss of heterozygosity at any of these markers in tumors from affected members of these kindreds. Furthermore, there is strong evidence against linkage to two Hirschsprung disease (a condition that can cosegregate with neuroblastoma) susceptibility genes, *RET* and *EDNRB*. We conclude that the neuroblastoma susceptibility gene is distinct from the 1p36 tumor suppressor and the currently identified Hirschsprung disease susceptibility genes.

Introduction

Neuroblastoma is the most common malignant solid neoplasm of early childhood and usually occurs sporadically. However, like other embryonal tumors, neuroblastoma occasionally segregates in families as an autosomal dominant trait, with the clinical hallmarks of earlier mean age at diagnosis and multiple primary tumors (1). This is thought to be due to a heritable germ line mutation of a tumor suppressor gene, as is predicted from the two-mutation model of oncogenesis first proposed for retinoblastoma (2). Furthermore, like retinoblastoma, it has been estimated that up to 22% of sporadic neuroblastomas may result from a new germinal mutation in a tumor suppressor gene (3). Although the retinoblastoma susceptibility gene (*RB1*) was isolated a decade ago (4), predisposing genes for neuroblastoma and the other embryonal cancers of childhood have not been located.

Cytogenetic (5), molecular (6), functional (7), and clinical (8–10) evidence has implicated chromosomal subbands 1p36.2–.3 as the region most likely to contain a suppressor gene critical in sporadic neuroblastoma tumorigenesis. Furthermore, the identification of two neuroblastoma patients with either a constitutional deletion (11) or translocation (12) involving 1p36 has implicated this suppressor locus as harboring a potential susceptibility gene. We therefore hypothesized that a neuroblastoma susceptibility locus would map to chromosomal band 1p36, and that the responsible gene would be identical to the sporadic neuroblastoma suppressor gene. Alternatively, neuroblastoma susceptibility could be due to germinal mutations in a Hirschsprung disease susceptibility gene, because this condition has been reported to cosegregate with neuroblastoma (13, 14). These hypotheses were tested using linkage and LOH[3] analyses at these candidate regions in neuroblastoma kindreds.

Materials and Methods

Families and Specimens. A protocol for specimen collection and molecular genetic analysis of inherited neuroblastoma has been approved by the Children's Hospital of Philadelphia Institutional Review Board. Families in which more than one first-degree relative had a definitive diagnosis of neuroblastoma, ganglioneuroblastoma, or ganglioneuroma were ascertained. The three largest pedigrees identified are the subject of this study and are displayed in Fig. 1. Note that two of the affected members of family 1 were also diagnosed with Hirschsprung disease, as described previously (14). DNA was extracted from peripheral blood lymphocytes (and tumor specimens, when available) of indicated family members, using standard methodologies (15).

Genotyping. A panel of STRPs at each candidate locus was used for genotyping and LOH studies. The 1p36 STRPs included the dinucleotide repeat polymorphisms *D1S243, D1S468, D1S214, D1S160, D1S489,* and *D1S507* and the tetranucleotide repeats *D1S1646* and *D1S548* (8). The 10q11.2 STRPs at the *RET* locus included the dinucleotide repeats *D10S193, D10S199, D10S1100* (RET A), and *sTCL-2* (RET B) and the tetranucleotide repeat *D10S1217*. Two dinucleotide repeat polymorphisms (*D13S160* and *D13S170*) flank the *EDNRB* gene and were used for genotyping at this locus. All primer sequences, allele sizes, and allele frequencies are available either through the Genome Data Base or upon request.

PCR amplification was performed in 25-μL volumes with 40 ng of human genomic DNA as a template. Each reaction contained 0.2 μM of each primer, 0.2 mM of each deoxynucleotide triphosphate, 1.5 mM $MgCl_2$, 1 × PCR buffer II, and 0.2 units of AmpliTaq DNA polymerase (Perkin Elmer Cetus, Branchburg, NJ). The sense primer was end-labeled with [γ-^{33}P]dATP using T4 polynucleotide kinase (Promega, Madison, WI) before PCR. Amplification was performed with a touchdown procedure consisting of a 1-cycle denaturation at 95°C for 2 min; 15 cycles of 45-s denaturation at 95°C and an annealing/extension temperature starting at 70°C for 1 min and decreasing by 0.7°C each cycle; 25 cycles of 95°C for 45 s, 55–60°C for 30 s, and 72°C for 1 min; and 1 cycle of a 7-min final extension at 72°C. Radiolabeled PCR products were separated in an 8% polyacrylamide/7 M urea sequencing gel and were autoradiographed at room temperature for 2–4 h.

Linkage Analysis. Maximum likelihood linkage analyses were undertaken to compute two-point lod scores for the 1p36, *RET*, and *EDNRB* marker loci and the putative neuroblastoma disease locus. These analyses were undertaken using a model that assumed neuroblastoma was attributable to a single diallelic autosomal dominant gene. The population disease allele frequency was assumed to be 0.0001. Published allele frequencies at each of the polymorphic

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[3] The abbreviations used are: LOH, loss of heterozygosity; STRP, simple tandem repeat polymorphism; lod, logarithm of the odds for linkage.

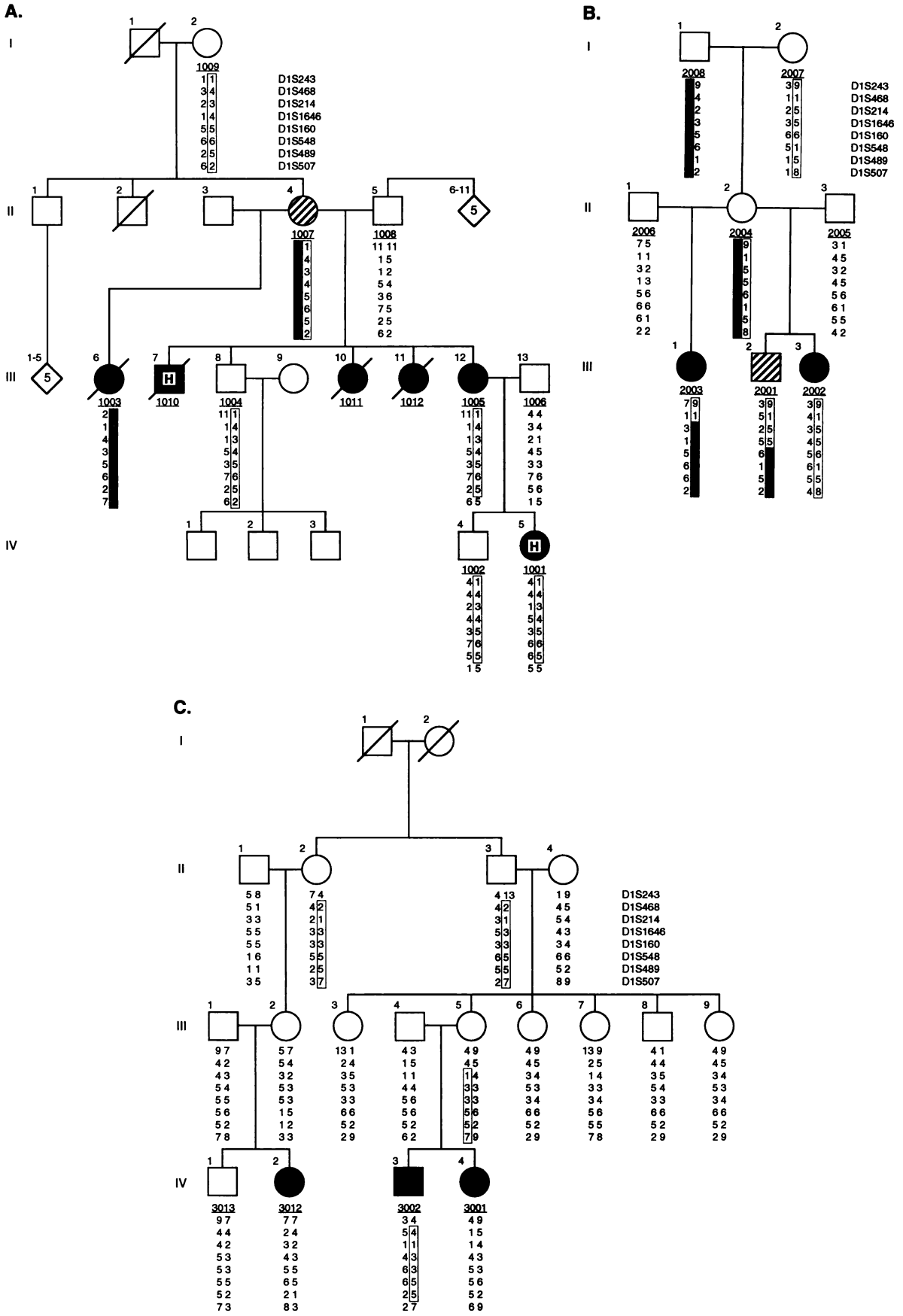

Fig. 1. Chromosome 1p36 haplotype analysis of neuroblastoma in families 1–3 (*A-C*). The inferred 1p36 haplotypes are displayed beneath each individual that was available for analysis. The polymorphic markers are listed in telomeric to centromeric order and cover approximately 38 cM (17). Boxes are indicated to emphasize the inheritance of haplotypes and the absence of cosegregation. *Solid symbol*, affected/neuroblastoma or ganglioneuroblastoma; *hatched symbol*, affected/ganglioneuroma; *unfilled symbol*, unaffected; *H*, Hirschsprung disease.

Table 1 *Clinical characteristics of affected patients*[a]

UPN	Pedigree no.	Age	Stage	Primary site(s)	Pathologic Dx	*MYCN*
1001	A-IV-5	1 mo	3_M	Adrenal/PM/CA	NBL	I
1003	A-III-6	4 yr	4	CA	NBL	NA
1010	A-III-7	1 mo	1	Adrenal	NBL (*in situ*)	NA
1011	A-III-10	15 mo	2_M	Bil Adrenal/PM	NBL	NA
1012	A-III-11	4 mo	1_M	Bil Adrenal/PM	NBL	NA
1005	A-III-12	5 mo	1	PM	NBL	NA
1007	A-II-4	30 yr	1	PM	GN[b]	NA
2001	B-III-2	7 yr	1	Cervical	GN	NA
2002	B-III-3	6 mo	1	Adrenal	NBL	NA
2003	B-III-1	18 mo	1	CA	GNB	NA
3001	C-IV-4	2 yr	2	Adrenal	NBL	I
3002	C-IV-3	6 yr	2	Pelvis	GNB	I
3012	C-IV-2	7 yr	4	Pelvis	NBL	I

[a] Clinical characteristics of all affected members of kindreds 1–3 including age, stage by the International Neuroblastoma Staging System (31), primary site or sites, histopathology, and *MYCN* copy number at diagnosis. UPN, unique patient number M, multifocal disease; PM, posterior mediastinum; CA, celiac axis; Bil, bilateral; NBL, neuroblastoma; GNB, ganglioneuroblastoma; GN, ganglioneuroma; NA, specimen not available; Dx, diagnosis.
[b] Diagnosis of GN presumptive (14).

loci (Genome Data Base) were used for all computations. We also assumed an incomplete penetrance function, relating the probability of having developed disease to inheritance of the genetic variant. This penetrance function assumed a 90% probability of manifesting the disease by age 15 in variant allele carriers and no chance of developing the disease in noncarriers. To further strengthen the argument against linkage, we computed lod scores with various combinations of these inferences (disease allele frequency estimates of 0.0001 and 0.001 and disease penetrances of 0.5 and 0.9). All likelihood computations were accomplished using the MLINK and ILINK program packages as implemented in FASTLINK v. 2.3P (16).

LOH Analysis. Corresponding tumor and constitutional DNA pairs were run in parallel for all available tumor specimens, as described (8). LOH was determined when there was at least a 60% decrease in the intensity of one allele in the tumor specimen compared to the constitutional DNA.

Results and Discussion

Of 17 families ascertained in which neuroblastoma seems to be segregating as an autosomal dominant trait, 3 kindreds with 13 affected individuals were chosen for these studies (Fig. 1). The clinical characteristics of the affected members from these pedigrees are shown in Table 1. The clinical heterogeneity is highlighted by the variation in age, stage, and primary site (or sites) at diagnosis as well as the degree of differentiation of the initial biopsy specimen. Evaluation of *MYCN* amplification status was available in only a few patients, because many were diagnosed decades ago, and/or the tumor specimens were unavailable.

Affected and unaffected family members were genotyped with a panel of eight STRP markers spanning 1p36. Two-point linkage analysis was performed at each marker locus. These polymorphisms are concentrated at 1p36.2–.3 and were chosen because they roughly define the consensus region of hemizygous deletion in neuroblastomas established by LOH studies (6). The linear order of the STRPs is depicted in Fig. 1 and covers 38 cM of genomic DNA (17). These STRPs are uniformly spaced throughout 1p36, and no contiguous markers are more than 7 cM apart. *D1S507* is 4 cM proximal to the region of consistent loss (6), and *D1S243* is the most telomeric STRP on most genetic maps (17).

There was no evidence to support linkage at any 1p36 locus (Table 2). Furthermore, there was strong evidence against linkage (lod < -2) at six of the eight chromosome 1p36 markers for θ values of 0 and 0.01 (*e.g.*, lod $= -8.797$ for $\theta = 0$ at *D1S214*). Evidence against linkage was observed using a variety of allele frequency estimates and penetrance models. In addition, linkage analyses using pairs of affected relatives were consistent with the lod score results (data not shown).

Tumor specimens were available for analysis from patients 1001, 1003, 2001, 2002, and 2003 (Fig. 1). Each tumor specimen was assayed for LOH at all 1p36 polymorphic loci. At least six of the eight STRPs were informative for each case studied. LOH was not detected in the tumor DNA of any patient, and there was no evidence for a replication error phenotype (Fig. 2). Taken together with the linkage results, the current data strongly suggest that the neuroblastoma susceptibility gene does not map to 1p36.

Congenital aganglionosis of the colon or Hirschsprung disease is also a disorder of neural crest-derived cells and is known to occasionally cosegregate with neuroblastoma, as in our family 1 (13, 14). Familial Hirschsprung disease is a polygenic trait in which two susceptibility genes have been identified thus far: the *RET* proto-oncogene at 10q11 (18) and the endothelin-B receptor gene (*EDNRB*) at 13q22 (19). Therefore, we tested both of these loci for genetic linkage to neuroblastoma predisposition, using the same families and models as described for 1p36. lod scores of < -4.0 at both loci were found, arguing strongly against linkage of these Hirschsprung disease predisposition genes to neuroblastoma susceptibility (*e.g.*, lod $= -10.069$ and -8.183 for $\theta = 0$ at *D10S193* and *D13S170*, respectively; data not shown, but available upon request). In addition, there was no LOH in any of

Table 2 *Combined results of two-point lod score analysis for chromosome 1p36 markers and neuroblastoma*

	Recombination fraction (θ)							
Marker	0	0.01	0.05	0.1	0.2	0.3	0.4	lod_{max} (θ)
D1S243	−7.797	−2.403	−1.109	−0.476	−0.069	0.034	0.026	0.038 (0.332)
D1S468	−7.798	−2.403	−1.019	−0.475	−0.067	0.035	0.026	0.039 (0.327)
D1S214	−8.797	−2.993	−1.555	−0.940	−0.383	−0.132	−0.022	−0.002 (0.479)
D1S1646	−3.604	−0.927	−0.316	−0.121	−0.015	−0.001	−0.001	−0.001 (0.314)
D1S160	−7.626	−3.164	−1.707	−1.064	−0.458	−0.167	−0.030	0.002 (0.485)
D1S548	0.535	0.525	0.482	0.427	0.313	0.199	0.093	0.535 (0.000)
D1S489	−0.174	−0.149	−0.074	−0.018	0.028	0.031	0.018	0.033 (0.264)
D1S507	−7.438	−2.430	−1.044	−0.495	−0.081	0.027	0.023	0.032 (0.338)

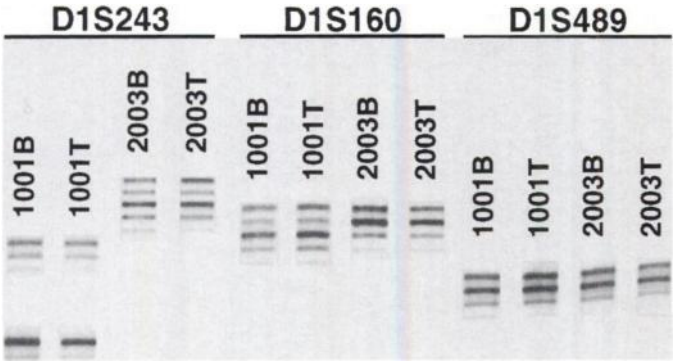

Fig. 2. LOH analysis in patients 1001 and 2003 at three dinucleotide repeat polymorphisms. *T*, tumor DNA; *B*, constitutional DNA from peripheral blood. Each patient is informative at all three loci, and there is no reduction of allelic intensity that would be consistent with LOH.

the five tumor specimens at either Hirschsprung disease susceptibility locus (average observed heterozygosity for all seven STRPs = 0.77).

Retinoblastoma provides the best-defined example in which inactivating mutations in a tumor suppressor gene are responsible for familial and sporadic tumors of a specific tissue type. Deletion of 13q14 is the most common genetic change in retinoblastoma tumor cells, and identification of constitutional 13q14 deletions in the germ line of retinoblastoma patients was an important initial step in isolating the *RB1* gene (20). Germ line mutations in one allele of the *RB1* gene are heritable, and somatic mutations in the second allele are strongly implicated in the development of both retinoblastoma and osteogenic sarcoma within families (4). Two somatic mutations in *RB1* account for the cases of sporadic retinoblastomas. This paradigm of one tumor suppressor gene being responsible for both familial and sporadic tumors is in accord with the Knudson hypothesis of tumorigenesis (2).

However, it seems that predisposition to the majority of human cancers is more complex and genetically heterogeneous. For example, more than one susceptibility gene is implicated in each of the hereditary forms of several adult cancers, including cutaneous malignant melanoma/dysplastic nevus syndrome (21), breast cancer (22, 23), and nonpolyposis colon cancers (24). Furthermore, the Wilms' tumor predisposition gene does not map to the site of the most frequent hemizygous deletion at 11p13–15 (25, 26) or the *WT1* gene (27). Taken together with recent studies suggesting the involvement of more than one suppressor locus in sporadic tumors (28, 29), our results indicate that neuroblastoma tumorigenesis will also be complex.

In summary, we present strong evidence that the neuroblastoma susceptibility locus is distinct from the putative 1p36 tumor suppressor gene as well as the two known Hirschsprung disease susceptibility genes. However, 1p36 is clearly important in the development and/or progression of sporadic neuroblastoma (8–10), and it may be involved in some familial cases due to genetic heterogeneity. We are currently extending our linkage analyses to additional families as well as other candidate regions such as 14q32 and 11q13 (30). Identification of a neuroblastoma predisposition gene should lend insight not only into the molecular pathogenesis of familial neuroblastoma but also into the development of sporadic disease.

References

1. Kushner, B. H., Gilbert, F., and Helson, L. Familial neuroblastoma: case reports, literature review, and etiologic considerations. Cancer (Phila.), *57:* 1887–1893, 1986.
2. Knudson, A. G., Jr. Mutation and cancer: statistical study of retinoblastoma. Proc. Natl. Acad. Sci. USA, *68:* 820–823, 1971.
3. Knudson, A. G., and Strong, L. C. Mutation and cancer: neuroblastoma and pheochromocytoma. Am. J. Hum. Genet., *24:* 514–532, 1972.
4. Friend, S. H., Bernards, R., Rogelj, S., Weinberg, R. A., Rapaport, J. M., Albert, D. M., and Dryja, T. P. A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature (Lond.), *323:* 643–646, 1986.
5. Brodeur, G. M., Green, A. A., Hayes, F. A., Williams, K. J., Williams, D. L., and Tsiatis, A. A. Cytogenetic features of human neuroblastomas and cell lines. Cancer Res., *41:* 4678–4686, 1981.
6. White, P. S., Maris, J. M., Beltinger, C., Sulman, E., Marshall, H. N., Fujimori, M., Kaufman, B. A., Biegel, J. A., Allen, C., Hilliard, C., Valentine, M. B., Look, A. T., Enomoto, H., Sakiyama, S., and Brodeur, G. M. A region of consistent deletion in neuroblastoma maps within human chromosome 1p36.2–36.3. Proc. Natl. Acad. Sci. USA, *92:* 5520–5524, 1995.
7. Bader, S. A., Fasching, C., Brodeur, G. M., and Stanbridge, E. J. Dissociation of suppression of tumorigenicity and differentiation *in vitro* effected by transfer of single human chromosomes into human neuroblastoma cells. Cell Growth & Differ., *2:* 245–255, 1991.
8. Maris, J. M., White, P. S., Beltinger, C. P., Sulman, E. P., Castleberry, R. P., Shuster, J. J., Look, A. T., and Brodeur, G. M. Significance of chromosome 1p loss of heterozygosity in neuroblastoma. Cancer Res., *55:* 4664–4669, 1995.
9. Gehring, M., Berthold, F., Edler, L., Schwab, M., and Amler, L. C. The 1p deletion is not a reliable marker for the prognosis of patients with neuroblastoma. Cancer Res., *55:* 5366–5369, 1995.
10. Caron, H., van Sluis, P., de Kraker, J., Bokkerink, J., Egeler, M., Laureys, G., Slater, R., Westerveld, A., Voute, P. A., and Versteeg, R. Allelic loss of chromosome 1p as a predictor of unfavorable outcome in patients with neuroblastoma. N. Engl. J. Med., *334:* 225–230, 1996.
11. Biegel, J. A., White, P. S., Marshall, H. N., Fujimori, M., Zackai, E. H., Scher, C. D., Brodeur, G. M., and Emanuel, B. S. Constitutional 1p36 deletion in a child with neuroblastoma. Am. J. Hum. Genet., *52:* 176–182, 1993.
12. Laureys, G., Speleman, F., Opdenakker, G., Benoit, Y., and Leroy, J. Constitutional translocation t(1;17)(p36;q12–21) in a patient with neuroblastoma. Genes Chromosomes & Cancer, *2:* 252–254, 1990.
13. Clausen, N., Andersson, P., and Tommerup, N. Familial occurrence of neuroblastoma, von Recklinghausen's neurofibromatosis, Hirschsprung aganglionosis, and jaw-winking syndrome. Acta Paediatr. Scand., *78:* 736–741, 1989.
14. Maris, J. M., Chatten, J., Meadows, A. T., Biegel, J., and Brodeur, G. M. Familial neuroblastoma: new affected members and a further association with Hirschsprung disease. Med. Pediatr. Oncol., in press, 1996.
15. Wright, D. K., and Manos, M. M. Sample preparation from paraffin-embedded tissues. *In:* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (eds.), PCR Protocols, pp. 159–168. San Diego, CA: Academic Press, Inc., 1990.
16. Cottingham, R. W., Jr., Idury, R. M., and Schaffer, A. A. Faster sequential genetic linkage computations. Am. J. Hum. Genet., *53:* 252–263, 1993.
17. Dib, C., Faure, S., Fizames, C., Samson, D., Drout, N., Vignal, A., Millasseau, P., Marc, S., Hazan, J., Seboun, E., Lathrop, M., Gyapay, G., Morissette, J., and Weissenbach, J. A comprehensive genetic map of the human genome based on 5264 microsatellites. Nature (Lond.), *380:* 152–154, 1996.
18. Edery, P., Lyonnet, S., Mulligan, L. M., Pelet, A., Dow, E., Abel, L., Holder, S., Nihoul-Fekete, C., Ponder, B. A., and Munnich, A. Mutations of the *RET* proto-oncogene in Hirschsprung disease. Nature (Lond.), *367:* 378–380, 1994.
19. Puffenberger, E. G., Hosoda, K., Washington, S. S., Nakao, K., deWit, D., Yanagisawa, M., and Chakravarti, A. A missense mutation of the endothelin-B receptor gene in multigenic Hirschsprung disease. Cell, *79:* 1257–1266, 1994.
20. Yunis, J. J., and Ramsay, N. Retinoblastoma and subband deletion of chromosome 13. Am. J. Dis. Child., *132:* 161–163, 1978.
21. MacGeoch, C., Bishop, J. A., Bataille, V., Bishop, D. T., Frischauf, A. M., Meloni, R., Cuzick, J., Pinney, E., and Spurr, N. K. Genetic heterogeneity in familial malignant melanoma. Hum. Mol. Genet., *3:* 2195–2200, 1994.
22. Miki, Y., Swensen, J., Shattuck-Eidens, D., Futreal, P. A., Harshman, K., Tavtigian, S., Liu, Q., Cochran, C., Bennett, L. M., Ding, W., Bell, R., Rosenthal, J., Hussey, C., Tran, T., McClure, M., Frye, C., Hattier, T., Phelps, R., Haugen-Strano, A., Katcher, H., Yakumo, K., Gholami, Z., Shaffer, D., Stone, S., Bayer, S., Wray, C., Bogden, R., Dayananth, P., Ward, J., Tonin, P., Narod, S., Bristow, P. K., Norris, F. H., Helvering, L., Morrison, P., Rosteck, P., Lai, M., Barrett, J. C., Lewis, C., Neuhausen, S., Cannon-Albright, L., Goldgar, D., Wiserman, R., Kamb, A., and Skolnick, M. H. A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA1*. Science (Washington DC), *266:* 66–71, 1994.
23. Wooster, R., Bignell, G., Lancaster, J., Swift, S., Seal, S., Mangion, J., Collins, N., Gregory, S., Gumbs, C., Micklem, G., Barfoot, R., Hamoudi, R., Patel, S., Rice, C., Biggs, P., Hashim, Y., Smith, A., Connor, F., Arason, A., Gudmundsson, J., Ficenec, D., Kelsell, D., Ford, D., Tonin, P., Bishop, D. T., Spurr, N. K., Ponder, B. A. J., Eeles, R., Peto, J., Devilee, P., Cornelisse, C., Lynch, H., Narod, S., Lenoir, G., Egilsson, V., Barkadottir, R. B., Easton, D. F., Bentley, D. R., Futreal, P. A., Ashworth, A., and Stratton, M. R. Identification of the breast cancer susceptibility gene *BRCA2*. Nature (Lond.), *378:* 789–792, 1995.
24. Froggatt, N. J., Koch, J., Davies, R., Evans, D. G., Clamp, A., Quarrell, O. W., Weissenbach, J., Hodgson, S. V., Ponder, B. A., Barton, D. E., and Maher, E. R. Genetic linkage analysis in hereditary nonpolyposis colon cancer syndrome. J. Med. Genet., *32:* 352–357, 1995.
25. Grundy, P., Koufos, A., Morgan, K., Li, F. P., Meadows, A. T., and Cavanee, W. K. Familial predisposition to Wilms' tumour does not map to the short arm of chromosome 11. Nature (Lond.), *336:* 374–376, 1988.
26. Huff, V., Compton, D. A., Chao, L., Strong, L. C., Geiser, C. F., and Saunders, G. F.

Lack of linkage of familial Wilms' tumour to chromosomal band 11p13. Nature (Lond.), *336:* 377–378, 1988.

27. Schwartz, C. E., Haber, D. A., Stanton, V. P., Strong, L. C., Skolnick, M. H., and Housman, D. E. Familial predisposition to Wilms' tumor does not segregate with the *WT1* gene. Genomics, *10:* 927–930, 1991.
28. Schleiermacher, G., Peter, M., Michon, J., Hugot, J. P., Vielh, P., Zucker, J. M., Magdelenat, H., Thomas, G., and Delattre, O. Two distinct deleted regions on the short arm of chromosome 1 in neuroblastoma. Genes Chromosomes & Cancer, *10:* 275–281, 1994.
29. Takeda, O., Homma, C., Maseki, N., Sakurai, M., Kanda, N., Schwab, M., Nakamura, Y., and Kaneko, Y. There may be two tumor suppressor genes on chromosome arm 1p closely associated with biologically distinct subtypes of neuroblastoma. Genes Chromosomes & Cancer, *10:* 30–39, 1994.
30. Srivatsan, E. S., Ying, K. L., and Seeger, R. C. Deletion of chromosome 11 and of 14q sequences in neuroblastoma. Genes Chromosomes & Cancer, *7:* 32–37, 1993.
31. Brodeur, G. M., Pritchard, J., Berthold, F., Carlsen, N. L., Castel, V., Castleberry, R. P., De Bernardi, B., Evans, A. E., Favrot, M., Hedborg, F., Kaneko, M., Kemshead, J., Lampert, F., Lee, R. E. J., Look, A. T., Pearson, A. D. J., Philip, T., Roald, B., Sawada, T., Seeger, R. C., Tsuchida, Y., and Voute, P. A. Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. J. Clin. Oncol., *11:* 1466–1478, 1993.