human genome by fingerprinting yeast artificial chromosomes. *Cell* 1992, **70**, 1059–1068.
17. Engelstein M, Hudson TJ, Lane JM, *et al.* A PCR-based linkage map of human chromosome 1. *Genomics* 1993, **15**, 251–258.
18. Albertsen HM, Abderrahim H, Cann HM, Dausset J, Le Paslier D, Cohen D. Construction and characterization of yeast artificial chromosome library containing seven haploid genome equivalents. *Proc Natl Acad Sci USA* 1990, **87**, 4256–4260.
19. Amler LC, Schwab M. Amplified N-*myc* in human neuroblastoma cells is often arranged as clustered tandem repeats of differently recombined DNA. *Molec Cell Biol* 1989, **9**, 4903–4913.
20. Caron H, van Sluis P, van Hoeve M, *et al.* Allelic loss of chromosome 1p36 in neuroblastoma is of preferential maternal origin and correlates with *N-myc* amplification. *Nature Genet* 1993, **4**, 187–190.
21. Brodeur GM, Sekhon GS, Goldstein MN. Chromosomal aberrations in human neuroblastomas. *Cancer* 1977, **40**, 2256–2263.
22. Weissenbach J, Gyapay G, Dib C, *et al.* A second-generation linkage map of the human genome. *Nature* 1992, **359**, 794–801.
23. Matise TC, Perlin M, Chakravarti A. Automated construction of genetic linkage maps using an expert system (MultiMap): a human genome linkage map. *Nature Genet* 1994, **6**, 384–390.
24. Gyapay G, Morisette J, Vignal A, *et al.* The 1993–1994 Généthon human genetic linkage map. *Nature Genet* 1994, **7**, 246–339.
25. Laureys G, Versteeg F, Speleman F, *et al.* Characterization of the chromosome breakpoints in a patient with a constitutional translocation 1(1;17)(p36.31-13;q11-2.-12) (*this issue*).
26. Takeda O, Homma C, Maseki N, *et al.* There may be two tumor suppressor genes on chromosome arm 1p closely associated with biologically distinct subtypes of neuroblastoma. *Genes Chrom Cancer* 1994, **10**, 30–39.

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Molecular Cytogenetic Analysis of 1;17 Translocations in Neuroblastoma

N. Van Roy, N.C. Cheng, G. Laureys, G. Opdenakker, R. Versteeg and F. Speleman

Loss of chromosome 1 short arm material, resulting from terminal deletions or unbalanced translocations, is a frequent finding in advanced neuroblastoma. In translocations, often relatively small portions of a second chromosome are translocated to the chromosome 1 short arm. The chromosomal origin of this translocated material could often not be identified using banding analysis only. Recent studies, applying fluorescent *in situ* hybridisation, showed that in the majority of these translocations, chromosome 17 is involved. In this study, the nonrandom occurrence of unbalanced 1;17 translocations is further supported by their presence in 3/7 neuroblastoma cell lines. Analysis of the 1p breakpoints extends our earlier observation of breakpoint heterogeneity. A similar scattering of 17q breakpoints was observed. The 1p and 17q breakpoints of the constitutional 1;17 translocation did not coincide with any of the 1;17 translocation breakpoints found in neuroblastoma cell lines. Cell lines, not containing 1;17 translocations, contained other chromosome 17 rearrangements. As a result, extra copies of 17q are found in all cell lines, suggesting a role for genes on 17q in neuroblastoma development. The possible significance of 1;17 translocations in neuroblastoma is discussed.

Key words: 1;17 translocation, chromosome 17 rearrangement, neuroblastoma cell line
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INTRODUCTION

IN ADVANCED neuroblastomas and neuroblastoma cell lines cytogenetic analyses have revealed frequent occurrence of chromosome 1 rearrangements resulting in loss of distal 1p material [1]. The sizes of the lost chromosome 1p segments have varied considerably [2–4]. Molecular studies on larger series of primary tumours have confirmed the high incidence of allelic loss for 1p loci in neuroblastomas [5–7]. This high incidence of deletions has suggested the presence of a gene with tumour suppressor activity [8]. Recent loss of heterozygosity studies on primary tumours and deletion mapping data in cell lines suggest that at least three distinct loci on the chromosome 1 short arm are involved in different subsets of neuroblastoma: an imprinted distal suppressor locus on 1p36.23-31 in *MYCN* single copy

tumours [9] and two more proximal suppressor loci associated with *MYCN* amplification located on 1p35-36.1 [10] and 1p32-34 [11, 12].

Further evidence for the presence of a suppressor locus on 1p36 comes from the observation of a constitutional 1;17 translocation [13] and an interstitial deletion involving band 1p36 [14] in two neuroblastoma patients. The breakpoints of the 1;17 translocation have been analysed in detail [15, 16]. Apart from serving as a landmark for identification of a suppressor locus, this translocation also drew our attention to a similar translocation in a neuroblastoma tumour from another patient. The derivative chromosome 1 resembled the der(1) of the constitutional 1;17 translocation. Fluorescent *in situ* hybridisation (FISH) analysis confirmed the chromosome 17 origin of the extra material on the short arm of the derivative chromosome 1 [17]. Subsequent screening of a panel of 8 cell lines showed preferential involvement of chromosome 17 in unbalanced chromosome 1 changes resulting in loss of 1p material, and over-representation of 17q material. Extra 17q material was also found in those cell lines not carrying 1;17 translocations [17].

In this study, we screened 7 additional cell lines for the presence of 1;17 translocations. 1p breakpoints were analysed by FISH using a previously published panel of DNA probes for 1p [18] and three additional 1p-DNA markers. The 17q breakpoints in six cell lines with 1;17 translocations from this and our previous study were analysed with chromosome 17 region specific probes.

MATERIALS AND METHODS

Cell lines

In total, 15 cell lines were studied. These include a first series of 8 cell lines (GI-ME-N, IMR32, SK-N-AS, N206, SK-N-FI, SK-N-SH, TR14, SMS-KCNR) for which a detailed FISH analysis has previously been published [10, 17] and the present series of 7 additional cell lines: UHG-NP, SK-N-BE [19], SJNB-1, SJNB-6, SJNB-8, SJNB-12 [20] and NGP [21]. UHG-NP is derived from the primary tumour of patient 1 published by Van Roy and colleagues [17]. Details of cytogenetic and immunohistochemical characterisation of this new cell line will be reported elsewhere (Van Roy *et al.*, in preparation).

Cytogenetic analysis and fluorescence in situ *hybridisation (FISH)*

At least 19 G-banded metaphases were analysed from each cell line. Chromosome 1 abnormalities were analysed using a chromosome 1 specific plasmid library (pBS-17 [22]) and by combining region specific probes with the pUC1.77 probe for the 1q12 heterochromatic region (for references and chromosomal map position see reference [18]). Screening for 1;17 translocations and other chromosome 17 abnormalities was done by combining pUC1.77 with a chromosome 17 specific library (pBS-17, [22]). For some cell lines, additional chromosome specific libraries were tested. Three additional chromosome 1 region specific probes were included in this study for breakpoint analysis: c102 (CEB15)(1p36.33; [23]) and YAC clones for loci *MYCL* (ICRF209E9) and *D1S62* (ICRF928A10) [24, 25]. Our FISH data and data from Hellsten and associates [24] strongly suggest a more distal position for *MYCL* than the 1p32 position described by Nau and colleagues [26]. Both *D1S62* and *GLUT* map proximal to *MYCL* [27] and were assigned to band 1p34 [18]. We, therefore, propose bands 1p34.3-1p35 as a chromosomal position for *MYCL*. A more detailed description of the gene mapping of the *MYCL* locus will be published elsewhere (Van Roy *et al.*, submitted). For chromosome 17, two short arm and four long arm region specific probes were tested. c197-2 and c197-4 (LL132) are two cosmids from the Miller-Dieker syndrome (MDS) critical region in 17p13.3 [28]. 7G4 is a cosmid for the distal part of the *NF1* gene (17q11.2, [29]). *cMCP3* (SCYA7) encodes the human monocyte chemotactic protein-3 and has been mapped to 17q11.2-q12 [30]. YAC clones A230A7 and 397F9 were used for detection of the *ERBB2* (17q12-21.1)[31]) and *THRA1* (17q11.2-12, GDB 1d GOO-195-323) locus, respectively.

Probe labelling, slide pretreatment, *in situ* hybridisation and immunocytochemical detection were carried out as reported previously [17].

RESULTS

1;17 *translocations*

Cohybridisation of pUC1.77 with pBS-17 (chromosome 17 specific plasmid library) revealed the presence of unbalanced 1;17 translocations in SJNB-8 and SJNB-12. The karyotype of SJNB-8 is pseudodiploid and only one copy of the der(1)t(1;17) is present. SJNB-12 has a near tetraploid karyotype and presents with two copies of the unbalanced 1;17 translocation (Figure 1). Analysis with chromosome 1p region specific probes localises the 1p breakpoint in SJNB-8 and SJNB-12 proximal to the *MYCL* locus (Figure 2a). Cytogenetically, band 1p32 appears to be retained in the 1;17 translocation. Therefore, we propose a breakpoint in 1p33-34. In SJNB-8, cytogenetic analysis and FISH also evidenced the presence of additional material at the distal end of the long arm of the der(1)t(1;17) (Figure 1).

Cell line UHG-NP has a near tetraploid karyotype containing three copies of the der(1)t(1;17)(Figure 1, 3a). The chromosome 1p breakpoint in UHG-NP was mapped between *ID-3* (formerly heir-1) and *D1S60* (Figure 2a).

In our previous study, the analysis in TR14 showed the localisation of the 1;17 translocation breakpoint in the most distal band 1p36.33 as evidenced by the presence of *D1Z2* on the derivative chromosome 1. In this study, we included CEB15, a marker which is located telomeric from *D1Z2*. CEB15 was also shown to be retained on the derivative chromosome 1 (Figure 2a).

The chromosome 1 breakpoint in the patient with the constitutional translocation [13] is located in yet another region on chromosome 1 (Figure 2a) [16] compared with the chromosome 1 breakpoints in neuroblastoma cell lines.

Mapping of 17q breakpoints in 1;17 translocations was carried out by testing four region specific probes on SJNB-8, SJNB-12, UHG-NP, GI-ME-N, IMR32 and TR14. Breakpoint positions are as follows: proximal to *NF1* (but distal to the centromere) in GI-ME-N, TR14 and SJNB-12; distal to *SCYA7* but proximal to *THRA1* in SJNB-8; and distal to *THRA1* in UHG-NP and IMR32 (Figure 2b).

Chromosome 1 *and chromosome* 17 *rearrangements other than* 1;17 *translocations*

In the NGP cell line an apparently reciprocal translocation involving 1p was detected. Using chromosome specific libraries,

Correspondence to F. Speleman at the Department of Medical Genetics, University Hosptial Ghent, De Pintelaan, 185, B-9000 Ghent, Belgium. N. Van Roy and F. Speleman are at the Department of Medical Genetics and Pediatric Oncology, University Hospital Ghent, Belgium; N.C. Cheng and and R. Versteeg are at The Institute of Human Genetics, Academic Medical Centre, University of Amsterdam, The Netherlands; and G. Opdenakker is at the Rega Institute for Medical Research, University of Leuven, Belgium.

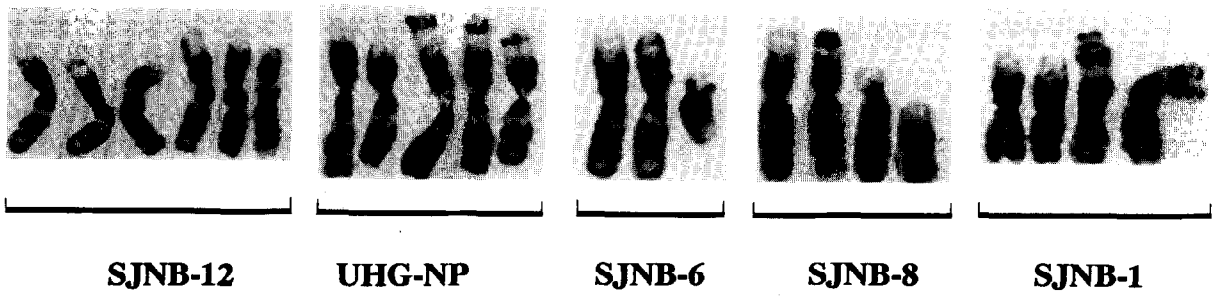

Figure 1. Partial karyotypes of neuroblastoma cell lines SJNB-12, UHG-NP, SJNB-6, SJNB-8 and SJNB-1 showing normal chromosomes 1 (left) and abnormal chromosomes 1 (right).

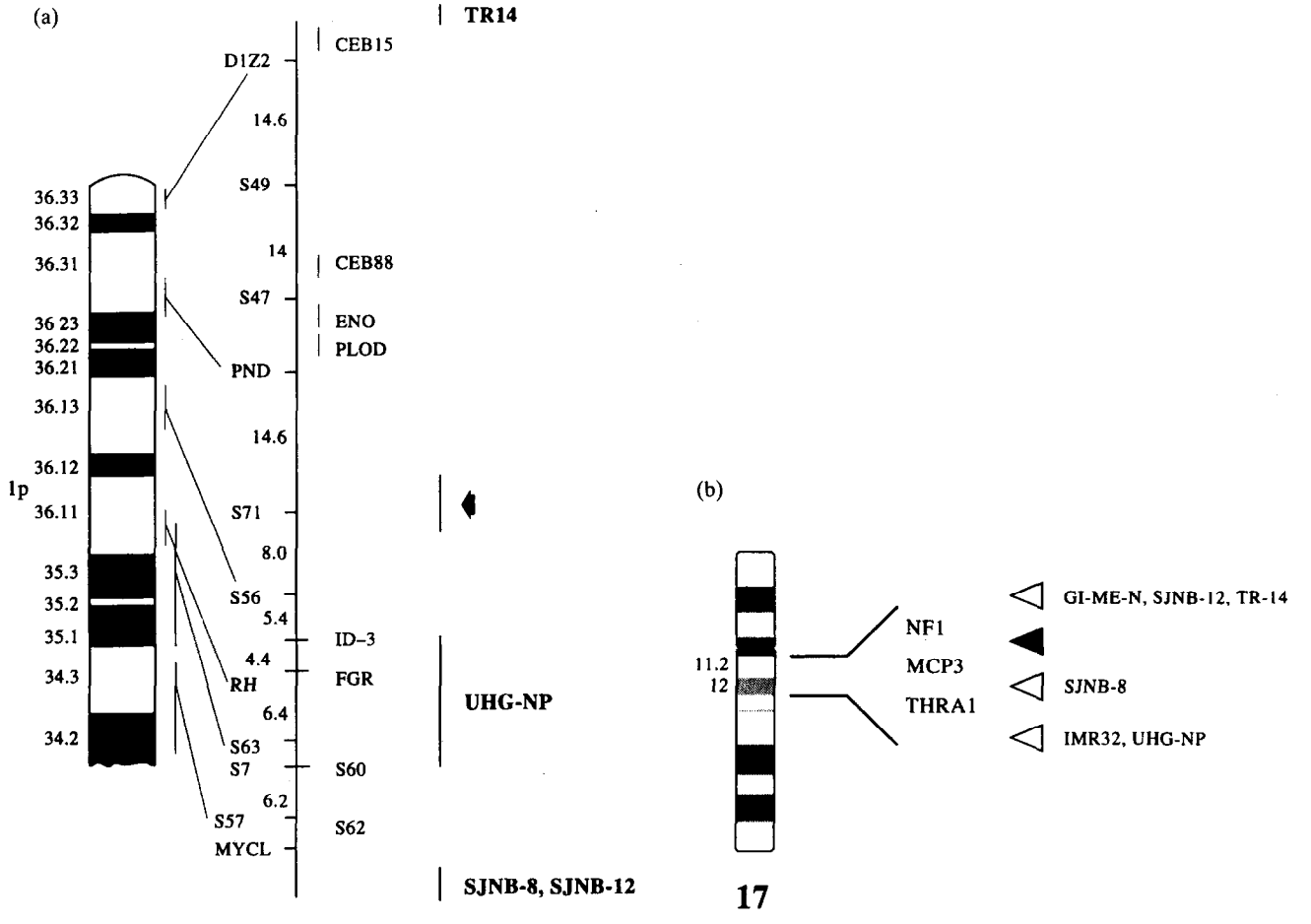

Figure 2. (a) Schematic representation of chromosome 1. The markers are ordered along chromosome 1 according to their FISH map position. The breakpoints in the cell lines are indicated by vertical bars on the right. The filled arrowhead gives the position of the constitutional chromosome 1 breakpoint. (b) Schematic representation of chromosome 17. Open arrowheads point to the position of the chromosome 17 breakpoints in the cell lines. The filled arrowhead points to the constitutional chromosome 17 breakpoint.

the reciprocal translocation could be identified as a t(1;15)(p36;q24). The breakpoint on 1p appeared to be located within the *ENO1* gene as a 14.5 kb phage clone for the gene hybridised to both the derivative chromosome 1 and 15 (Figure 3d). PFGE analysis showed no rearrangement for the *ENO1* gene. An extensive molecular study of this translocation breakpoint is necessary to explain our findings and to evaluate the significance of this translocation.

In two other cell lines, SJNB-6 and SJNB-8, FISH showed translocation of distal chromosome 1 short arm material to another chromosome (Figure 3e). The breakpoints on 1p were mapped proximal to *MYCL*. For both cell lines, the second chromosome involved could not yet be identified. Based on banding analysis of SJNB-6, chromosomes 16, 19, 20 and 22 were selected as the most plausible candidates, but they were excluded by FISH analysis. Banding and FISH analysis suggest that the marker chromosomes containing distal 1p material in SJNB-6 and SJNB-8 are probably the result of a more complex rearrangement than a simple reciprocal translocation (Figure 1).

In SJNB-1, two apparently normal chromosomes were found in addition to two abnormal chromosomes 1 with a similar structural rearrangement (Figure 1). Additional 1q-material

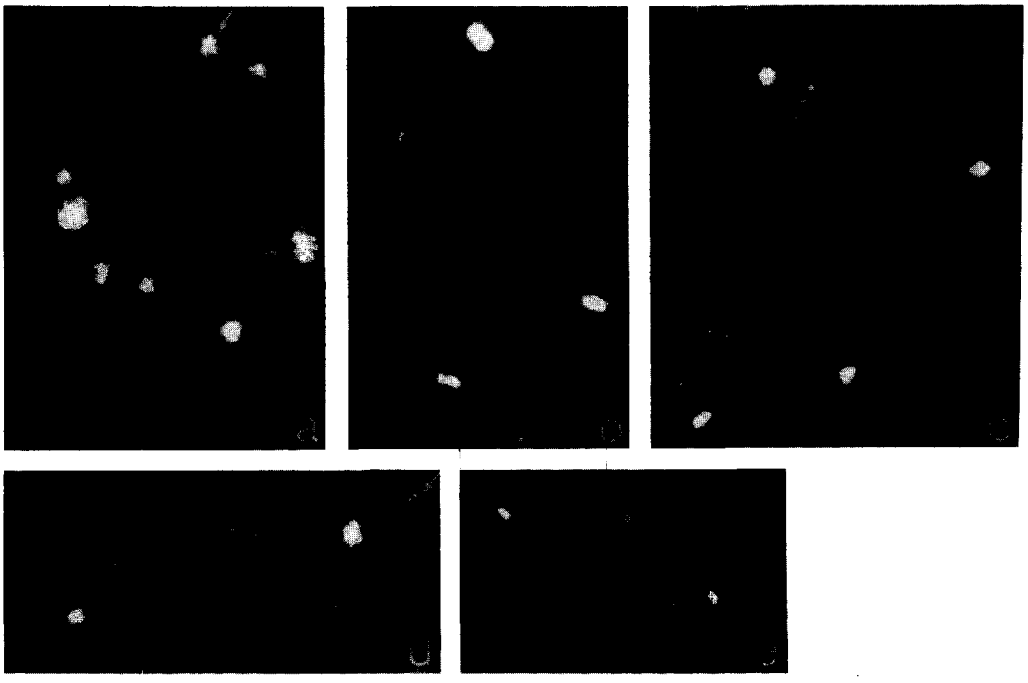

Figure 3. FISH analysis on metaphases of neuroblastoma cell lines. The probe pUCI.77 was used in combination with (a) pBS-17 in UHG-NP, (b) *D1S62* in SJNB-8, (c) *NF1* in TR14, (d) *ENO1* in NGP and (e) p1-79 in SJNB-6. Arrows point to the hybridisation signals of the chromosome 17 specific library (a) and of the region specific probes (b–e). The signals which are not indicated by arrows are the result of hybridisation of the pUC1.77 probe.

(1q21-qter) was translocated to the short arm resulting in loss of 1p material, the breakpoints being mapped between *ID-3* and *D1S60*.

SJNB-12 contains, in addition to three normal chromosomes 1, three rearranged chromosomes 1 (Figure 1). Next to the two 1;17 translocations, the third abnormal chromosome 1 has lost most of its short arm, according to banding analysis (most proximal probe tested *MYCL*) due to a translocation with a second thus far unidentified chromosome.

In SK-N-BE, no normal chromosomes 1 were found. One chromosome 1 shows a tandem duplication of the distal part of the short arm. The duplicated region comprises a region which is located proximal to *D1S57*. A terminal 1p-deletion is found in the other chromosome 1 with the breakpoint being proximal to *D1S57* (not shown).

In the present series, cell lines not containing 1;17 translocations exhibited other chromosome 17 rearrangements, resulting in extra 17q material. These rearrangements were not explored further in this study.

DISCUSSION

In this study, we have identified unbalanced 1;17 translocations in 3 of 7 cell lines. Our previous studies [10, 17] and data reported by Savelyeva and associates [32] bring the number of cell lines with 1;17 translocations to 12 of a total number of 29 analysed cell lines. In addition, three primary tumours with a 1;17 translocation were reported [17, 33], and we are aware of two additional cases (data not shown). All 1;17 translocations are unbalanced due to loss of the derivative chromosome 17. This results in loss of distal 1p material and at least one extra copy of a 17q segment distal to the translocation breakpoint. In two primary tumours, both chromosomes 17 were present. The chromosomes 17 from which the 1;17 was derived were still present and intact, suggesting an S/G2 phase event for the formation of the 1;17 translocation [33].

In view of the nonrandom involvement of unbalanced 1;17 translocations, we propose a specific biological role for this chromosome change in the development of neuroblastoma. Three other observations support this view. A *de novo* constitutional 1;17 translocation was reported in a patient with neuroblastoma [13]. The derivative chromosome 1 of this 1;17 translocation cytogenetically resembles the der(1)t(1;17) found in neuroblastoma tumours and cell lines. As this constitutional 1;17 translocation does not occur with high incidence in the normal population, such as 13q14q or 11;22 translocations, the association with development of neuroblastoma is very unlikely to be coincidental. A second line of evidence for a specific role for 1;17 translocations in neuroblastoma comes from our finding of frequent occurrence of chromosome 17 aberrations in those cell lines not carrying 1;17 translocations. These chromosome 17 changes lead to over-representation of 17q material. Therefore, genes located on 17q, possibly through a mechanism of gene dosage effect, may also be implicated in neuroblastoma development. This could explain the preferential involvement of chromosome 17 in translocations involving distal 1p. Finally, unbalanced 1;17 translocations have been reported in Merkel cell carcinoma [34–36]. Like neuroblastoma, Merkel cell carcinoma is a tumour of neuroendocrine origin in which loss of the terminal region of 1p also occurs frequently [33, 37]. The observation of 1;17 translocations in Merkel cell carcinoma, in our opinion, points at a role for this chromosome rearrangement in a larger subset of neuro-endocrine tumours than neuroblastoma alone.

Many examples exist of leukaemias or solid tumours associated with recurrent translocations. In an increasing number of these malignancies, the translocation breakpoints and genes involved have been cloned, revealing different mechanisms for deregulation of control of cell growth and differentiation. Clustering of translocation breakpoints within relatively small genomic regions is a constant feature in these tumour specific chromosome

aberrations. Most frequently, these translocations result in fusion of two genes forming a hybrid gene or in deregulation of a gene. These mechanisms are unlikely to occur in recurrent 1;17 translocations in neuroblastoma in view of the scattering of 1p and 17q breakpoints in neuroblastoma over a region of several megabases. Other explanations must be sought for the functional significance of 1;17 translocations in neuroblastoma. An alternative possibility is that these rearrangements combine two genetic events which are favourable for tumour development or progression i.e. loss of a suppressor locus or loci on 1p and extra copies of genes on 17q. The question as to how these 1;17 translocations, with widely differing breakpoints, arise remains open. One possible explanation is the co-localisation of different genes or gene clusters on 1p and 17q which are transcriptionally active within the nucleus during neuroblast differentiation, increasing the chance of illegitimate recombination. Cloning of 1;17 translocation breakpoints should shed more light on these issues.

1. Brodeur GM, Fong CT. Molecular biology and genetics of human neuroblastoma. *Cancer Genet Cytogenet* 1989, **41**, 153–174.
2. Brodeur GM, Green AA, Hayes FA, Williams KJ, Williams DL, Tsiatis AA. Cytogenetic features of human neuroblastomas and cell lines. *Cancer Res* 1981, **41**, 4678–4686.
3. Gilbert F, Feder M, Balaban G, *et al.* Human neuroblastomas and abnormalities of chromosome 1 and 17. *Cancer Res* 1984, **44**, 5444–5449.
4. Hunt JD, Tereba A. Molecular evaluation of abnormalities of the short arm of chromosome 1 in neuroblastoma. *Genes Chromosom Cancer* 1990, **2**, 137–146.
5. Fong C, Dracopoli NC, White PS, *et al.* Loss of heterozygosity for the short arm of chromosome 1 in human neuroblastomas: correlation with N-*myc* amplification. *Proc Natl Acad Sci USA* 1989, **86**, 3753–3757.
6. Fong C, White PS, Peterson K, *et al.* Loss of heterozygosity for chromosomes 1 or 14 defines subsets of advanced neuroblastomas. *Cancer Res* 1992, **52**, 1780–1785.
7. Ritke MK, Shah R, Valentine M, Douglass EC, Tereba A. Molecular analysis of chromosome 1 abnormalities in neuroblastoma. *Cytogenet Cell Genet* 1989, **50**, 84–90.
8. Schwab M. Is there a neuroblastoma anti-oncogene? In Evans A, Seeger RS, D'Angio G, Knudson A, eds. *Advances in Neuroblastoma Research 3*. Wiley-Liss, New York, 1991, 1–9.
9. Caron H, Peter M, van Sluis P, *et al.* Evidence for 2 tumour suppressor loci on chromosomal bands 1p35-36 involved in neuroblastoma: one probably imprinted, another associated with *N-MYC* amplification. *Hum Mol Genet* (in press).
10. Cheng NC, Van Roy N, Chan A, *et al.* Deletion mapping in neuroblastoma cell lines suggests two distinct tumor suppressor genes in the 1p35-36 region, only one of which is associated with *N-myc* amplification. *Oncogene* 1995, **10**, 291–297.
11. Schleiermacher G, Peter M, Michon J, *et al.* Two distinct deleted regions on the short arm of chromosome 1 in neuroblastoma. *Genes Chromosom Cancer* 1994, **10**, 275–281.
12. Takeda O, Homma C, Maseki N, *et al.* There may be two tumor suppressor genes on chromosome arm 1p closely associated with biologically distinct subtypes of neuroblastoma. *Genes Chromosom Cancer* 1994, **10**, 30–39.
13. Laureys G, Speleman F, Opdenakker G, Benoit Y, Leroy JG. Constitutional translocation t(1;17)(p36;q12-21) in a patient with neuroblastoma. *Genes Chromosom Cancer* 1990, **2**, 252–254.
14. Biegel JA, White PS, Marshall HN, *et al.* Constitutional 1p36 deletion in a child with neuroblastoma. *Am J Hum Genet* 1993, **52**, 176–182.
15. Laureys G, Speleman F, Versteeg R, *et al.* Constitutional translocation t(1;17)(p36.31-36.21;q11.2-12.1) in a neuroblastoma patient. Establishment of somatic cell hybrids and identification of PND/A12M2 on chromosome 1 and NF1/SCYA7 on chromosome 17 as breakpoint flanking single copy markers. *Oncogene*, in press.
16. Laureys G, Versteeg R, Speleman F, *et al.* Characterization of the chromosome breakpoints in a patient with a constitutional translocation t(1;17)(p36.31-21;q11.2-12) and neuroblastoma. *Eur J Cancer* 1995.
17. 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.
18. Van Roy N, Laureys G, Versteeg R, Opdenakker G, Speleman F. High resolution mapping of 46 DNA markers to the short arm of human chromosome 1. *Genomics* 1993, **18**, 71–78.
19. Biedler JL, Spengler BA. A novel abnormality in human neuroblastoma and anti-folate-resistant Chinese hamster cell lines in culture. *J Natl Cancer Inst* 1976, **57**, 683–695.
20. Johnson MR, Look AT, Declue JE, Valentine MB, Lowy DR. Inactivation of the *NF1* gene in human melanoma and neuroblastoma cell lines without impaired regulation of GTP.Ras. *Proc Natl Acad Sci USA* 1993, **90**, 5539–5543.
21. Brodeur GM, Sekhon GS, Goldstein MN. Chromosomal aberrations in human neuroblastomas. *Cancer* 1977, **40**, 2256–2263.
22. Collins C, Kuo WL, Segraves R, Fuscoe J, Pinkel D, Gray JW. Construction and characterization of plasmid libraries enriched in sequences from single human chromosomes. *Genomics* 1991, **11**, 997–1006.
23. Lauthier V, Mariat D, Vergnaud G. CEB15 detects a VNTR locus (Het:92%) on chromosome 1p. *Hum Mol Genet* 1992, **1**, 63.
24. Hellsten E, Vesa J, Heiskanen M, *et al.* Identification of YAC clones for human chromosome 1p32 and physical mapping of the infantile neuronal ceroid lipofuscinosis (INCL) locus. *Genomics* (in press).
25. Lehrach *et al.* In Davies KE, Tilghman SM, eds. *Genome Analysis Volume 1: Genetic and Physical Mapping*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1990, 39–81.
26. Nau MM, Brooks BJ, Battey J *et al.* L-*myc*, a new *myc*-related gene amplified and expressed in human small cell lung cancer. *Nature* 1985, **318**, 69–73.
27. Dracopoli NC, O'Connell P, Elsner TI, *et al.* The CEPH consortium linkage map of human chromosome 1. *Genomics* 1991, **9**, 686–700.
28. Ledbetter SA, Kuwano A, Dobyns WB, Ledbetter DH. Microdeletions of chromosome 17q13 as a cause of isolated lissencephaly. *Am J Hum Genet* 1992, **50**, 182–189.
29. Jorde LB, Watkins WS, Viskochil D, O'Connell P, Ward K. Linkage disequilibrium in the neurofibromatosis 1 (NF1) region: implications for gene mapping. *Am J Hum Genet* 1993, **53**, 1038–1050.
30. Opdenakker G, Fiten P, Nys G, *et al.* The human *MCP-3* gene (SCYA7): cloning, sequence analysis, and assignment to the C-C chemokine gene cluster on chromosome 17q11.2-q12. *Genomics* 1994, **21**, 403–408.
31. Lengauer C, Riethman HC, Speicher MR *et al.* Metaphase and interphase cytogenetics with Alu-PCR-amplified yeast artificial chromosome clones containing the BCR gene and the protooncogenes *c-raf-1*, *c-fms*, and *c-erbB-2*. *Cancer Res* 1992, **52**, 2590–2596.
32. Savelyeva L, Corvi R, Schwab M. Translocation involving 1p and 17q is a recurrent genetic alteration of human neuroblastoma cells. *Am J Hum Genet* 1994, **55**, 334–340.
33. Caron H, Van Sluis P, Van Roy N, *et al.* Recurrent 1;17 translocations in human neuroblastoma reveal nonhomologous mitotic recombination during the S/G2 phase as a novel mechanism for loss of heterozygosity. *Am J Hum Genet* 1994, **55**, 341–347.
34. Rosen ST, Gould VE, Salwen HR, *et al.* Establishment and characterization of a neuroendocrine skin carcinoma cell line. *Lab Invest* 1987, **56**, 302–312.
35. Gibas Z, Weil S, Chen ST, McCue PA. Deletion of chromosome arm 1p in a Merkel cell carcinoma (MCC). *Genes Chromosom Cancer* 1994, **9**, 216–220.
36. Ronan SG, Green AD, Shilkaitis A, Huang TSW, Das Gupta TK. Merkel cell carcinoma: *in vitro* and *in vivo* characteristics of a new cell line. *J Am Acad Dermatol* 1993, **29**, 715–722.
37. Harnett PR, Kearsley JH, Hayward NK, *et al.* Loss of allelic heterozygosity on distal chrosome 1p in Merkel cell carcinoma: a marker of neural crest origins? *Cancer Genet Cytogenet* 1991, **54**, 109–113.

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A Multiplex PCR Assay for Routine Evaluation of Deletion of the Short Arm of Chromosome 1 in Neuroblastoma

G. Schleiermacher, M. Peter, J. Michon, J.-M. Zucker, G. Thomas, H. Magdelénat and O. Delattre

Deletions of the short arm of chromosome 1 (1p) are frequent alterations in neuroblastoma. Although a consensus region of deletion has been mapped to chromosome subband 1p36, recent studies suggest that several distinct loci on this chromosome may be involved in neuroblastoma. Moreover, different patterns of deletion might be associated with different clinical and biological characteristics of the tumours. These findings emphasise the importance of assessing the localisation and the extent of the deletions in neuroblastoma. We developed a technique which allows analysis of loss of heterozygosity at multiple loci on 1p in a single step, making use of a multiplex PCR method. Primers specific for six microsatellite loci mapped in the different regions of interest on 1p were used for simultaneous amplification of DNA, and loss of heterozygosity was determined after separation of the alleles by denaturing polyacrylamide gel electrophoresis. This technique enables a simple analysis of the position and extent of 1p deletions, and can be used for routine evaluation of 1p status in neuroblastoma.

Key words: neuroblastoma, chromosome 1, short arm, deletion, loss of heterozygosity, PCR, microsatellite, prognosis

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INTRODUCTION

NEUROBLASTOMA, a tumour derived from neural crest tissue, is the most frequent extracranial solid tumour in childhood and presents a wide variability of its clinical course. It is characterised by two main genetic alterations: amplification of the oncogene *MYCN* is found in 25–30% of the cases and deletion of the short arm of chromosome 1 (1p), as detected by molecular studies, is observed in 30–40% of tumours [1–3]. Both alterations are detected more frequently in advanced stages of disease and have been associated with a poor outcome [4–7].

Initial molecular studies suggested that deletions encompassed a single region of overlap located in the 1p36.2–3 subband [2, 8]. However, recent observations indicate that, in addition to this locus, other loci on 1p might be involved in the tumorigenesis of neuroblastoma. Indeed, deletions limited to the 1p36.2–3 subband are not associated with *MYCN*, whereas this association is observed for larger deletions [9, 11]. Moreover, preferential loss of maternal alleles has been demonstrated in the former deletions, but not in the latter [12]. These observations suggest that at least two different loci on 1p are involved in tumours. Indication of an additional locus is provided by interstitial deletions which define a second short region of overlap located more proximally on 1p [9]. Finally, preliminary clinical reports suggest that the prognostic information provided by 1p deletion might depend on its size and localisation [10]. These findings emphasise the importance of the determination of the extent and of the chromosome position of deletions in neuroblastoma.

We have developed a method which allows detection of a deletion on 1p in neuroblastoma, determination of its localisation and estimation of its size. This method, which is based on the multiplex PCR amplification of microsatellite loci, enables the determination of these parameters, in a single step, on small tumour samples.

Correspondence to Olivier Delattre.
G. Schleiermacher, M. Peter and H. Magdelénat are at the Laboratoire de Transfert; G. Schleiermacher, J. Michon and J.-M. Zucker are at the Service de Pédiatrie; and G. Thomas and O. Delattre are at the Laboratoire de Génétique des Tumeurs, INSERM 434. Institut Curie, 26 rue d'Ulm, 75231 Paris Cedex 01, France.