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Characterisation of the Chromosome Breakpoints in a Patient with a Constitutional Translocation t(1;17)(p36.31-p36.13;q11.2-q12) and Neuroblastoma

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Cytogenetic and molecular studies in neuroblastoma suggest the presence of a tumour suppressor gene at the distal chromosome band 1p36. Previously, we hypothesised that a constitutional translocation involving the region 1p36 [t(1;17)(p36;q12-q21)] in a patient with neuroblastoma predisposed him to tumour development. Here we report the molecular delineation of the translocation breakpoints. Somatic cell hybrids containing the derivative chromosomes were used to determine the position of chromosome 1p and 17q DNA probes respective to the breakpoints using fluorescence *in situ* hybridisation. The 1p breakpoint was localised between the PND and *D1S56* loci. The chromosome 17q breakpoint is flanked by *NF1* and *SCYA7*, as proximal and distal marker, respectively. We redefined the translocation as t(1;17)(p36.31-13;q11.2-q12). The identification of flanking markers of the breakpoints is a prerequisite for breakpoint cloning and identification of a putative neuroblastoma suppressor gene.

Key words: neuroblastoma, constitutional chromosome 1;17 translocation, suppressor gene
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INTRODUCTION

CONSTITUTIONAL CHROMOSOME abnormalities in patients with a specific tumour were the first indications for the chromosomal location of tumour suppressor genes. The association of a constitutional deletion syndrome at the chromosomal band 13q14 and the retinoblastoma ultimately led to the cloning of the retinoblastoma gene *RB1* [1]. A constitutional deletion at chromosomal band 11p13 was found in the WAGR (Wilms' tumour, aniridia, genito-urinary malformations, retardation) syndrome and from this region the *WT1* gene was cloned [2]. Knudson suggested a similar two-hit mechanism as in retinoblastoma and Wilms' tumour for the origin of neuroblastoma [3], but a constitutional predisposing deletion syndrome was not described. Loss of heterozygosity studies in neuroblastoma tumours pointed to the chromosome 1p36 band as the candidate region for harbouring a neuroblastoma tumour suppressor gene [4, 5]. We have described a reciprocal constitutional chromosome translocation involving the same band 1p36 in a patient with neuroblastoma [6]. We hypothesised that this constitutional chromosome abnormality predisposed the patient to neuroblastoma development by disruption, dysregulation or deletion of one or more genes located at the translocation

breakpoints. This hypothesis can be tested by the molecular cloning of the translocation breakpoints and the isolation of a neuroblastoma related gene. Here we describe fluorescent *in situ* hybridisation analysis of a panel of 1p and 17q markers on somatic cell hybrids containing the patient's translocation chromosomes. For both breakpoints, close flanking DNA markers were identified.

MATERIALS AND METHODS

Fusion experiment

The patient's fibroblasts FB 412 and a thymidine kinase-deficient Chinese hamster cell line (a3) were fused by polyethylene glycol (MW 6000, 45% in serum free medium) as described [7]. Hybrid cell lines were selected in a medium containing HAT (hypoxanthine 0.1 mM, aminopterin 0.02 mM, thymidine 0.01 mM) and ouabain (0.2 μM).

FISH experiments

Fluorescent *in situ* hybridisation (FISH) experiments were performed as described [8]. Chromosome 1 and 17 region specific probes were hybridised on metaphases obtained from the hybrid lines. To facilitate detection of the normal and derivative chromosomes 1 and 17, cohybridisation with a 1q12 probe (pUC1.77) and a chromosome 17 centromeric probe (p17H8) was performed. Detailed probe information, references, and the FISH map position of the chromosome 1 markers used for the characterisation of the breakpoints have been described previously [8]. Two markers were added to the FISH chromosome 1 map: EFZ13 (*D1S64*) [9] and CRI-L1039 (*D1S71*) [10] by hybridisation to high resolution R-banded human chromosomes and dual colour hybridisation with 1p36.2 markers, respectively [8].

RESULTS

Two hybrid cell lines were selected: 32-2F, which contained the normal human chromosomes 1 and 17 and the derivative human chromosome 17 [der(17)] and 32-7A, which retained the human derivative 1 [der(1)] chromosome and the normal chromosome 17.

Two anonymous DNA probes EFZ13 (*D1S64*) and CRI-L1039 (*D1S71*) were added to the chromosome 1 FISH map [8]. Multiple hybridisation sites on chromosome 1 were observed. With *D1S64*, the strongest signal was seen at 1p36.2, whereas additional weaker spots were visible at 1p36.33, 1p21.1, 1p12 and 1q21.1 (Figure 1a). With CRI-L1039 (*D1S71*), a faint hybridisation signal was seen at the distal part of 1p and at 1p12, whereas strong signals were seen at 1q21.1 (Figure 1b). The relative order of *D1S64* and *D1S71* with regard to *TRE*, *D1S31* and *D1S149* could not be determined by dual colour hybridisation experiments.

Chromosome 1p markers with a FISH map position ranging from 1p36.33 to 1p35 were hybridised to the der(1) and der(17). Most probes could be mapped either to the der(1) (proximal to the 1p36 breakpoint), or to the der(17) (distal to the 1p36 breakpoint). We located the 1p breakpoint in the region with *PND* and *D1S56* as distal and proximal boundaries, respectively (Figure 2a). The chromosomal break occurred in a cluster of genes because probes for *D1S149*, *TRE* and *D1S64* hybridised to both the der(1) and the der(17). For *D1S31* and *D1S71* weak signals could only be observed on the der(1).

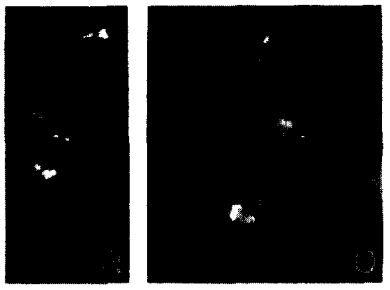

Figure 1. Fluorescent *in situ* mapping of (a) EFZ13 (*D1S64*) (arrow), (b) CRI-L1039 (*D1S71*) (arrow) on normal human metaphase chromosomes.

The relative order of 17q markers was determined in previous studies [11–14]. The recently isolated *MCP-3* gene (*SCYA7*), encoding the human monocyte chemotactic protein-3 [15], was mapped between the 7G4 (*NF1*) and A230A7 (*ERBB2*) [16]. The *NF1* and the *SCYA7* loci were identified as proximal and distal flanking marker, respectively, for the chromosome 17q breakpoint at subband 17q11.2 (Figure 2b).

DISCUSSION

We hypothesised that a patient with a constitutional translocation, redefined as t(1;17)(p36.31-p36.13;q11.2-q12), was predisposed to neuroblastoma development [6], since the chromosome 1p breakpoint was located at the 1p36 band to which a putative neuroblastoma suppressor gene has been attributed. We identified *PND* and *D1S56* as the distal and proximal boundaries, respectively, of the chromosome 1 breakpoint region. Recently, a constitutional interstitial deletion was described at chromosome 1 in a patient with neuroblastoma [17], with as the largest possible boundaries of the deleted region, the *D1Z2* and *APNH* loci. The markers *PND–D1S56*, identified as flanking markers in our patient, are located within the *D1Z2–APNH* region. Both patients with neuroblastoma and constitutional abnormalities at 1p36.2-1 strongly suggest the presence of a neuroblastoma related gene at these chromosomal subbands.

In our patient with a constitutional 1;17 translocation, the break at chromosome 1 occurred in a complex and repetitive DNA region encoding transfer RNA genes for glutamic acid (*TRE*) and asparagin (*TRN*), *RNU1* genes, and the anonymous loci *D1S31*, *D1S64* and *D1S71*. Analysis of the genomic structure of this region is described by van der Drift and associates [18]. The presence of this gene cluster at 1p36.2 explains the hybridisation pattern observed with probes for *TRE*, *D1S64* and *D1S149*. These markers hybridised to the derivative chromosome 1 and to the derivative chromosome 17. Previous studies demonstrated that this region at 1p36.31-p36.13 shares sequence homology with other parts of chromosome 1: 1p36.33, 1p12, 1q21.1 and 1q42 [8, 18–20]. The present FISH mapping of *D1S64* and *D1S71* provides additional evidence for these observations. Intriguingly, the 1q21.1 region might be the breakpoint region involved in a patient with ganglioneuroblastoma and a constitutional reciprocal translocation, cytogenet-

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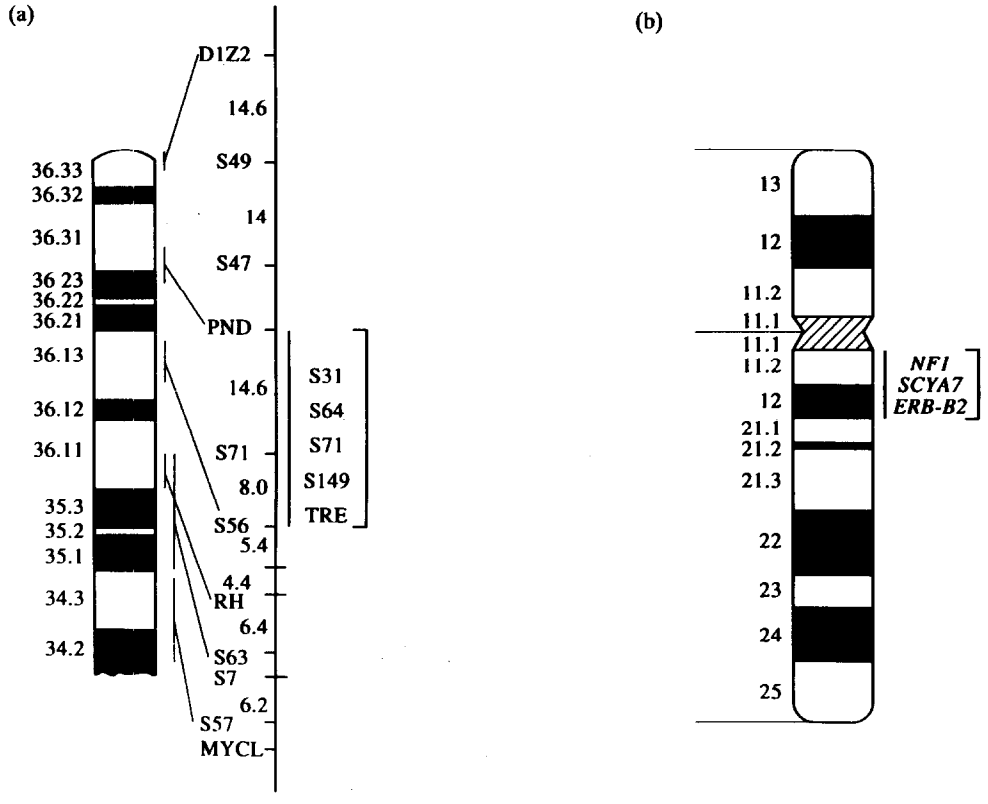

Figure 2. (a) Schematic representation of chromosome 1. The constitutional 1p breakpoint region is indicated by brackets. (b) Schematic representation of chromosome 17. The constitutional 17q breakpoint region is indicated by brackets.

ically defined at subband 1q22 [21]. Further studies are necessary to determine if the breakpoint region of the latter patient can be located in the 1q21.1 region, which shares homologous sequences with the 1p36.31-13 region.

Recently, it was suggested that at least two regions on chromosome 1p harbour neuroblastoma related genes [22–26]. At present, there is no uniform delineation of the chromosomal regions of interest. The constitutional chromosome 1p breakpoint region is located outside the boundaries of two regions, to which neuroblastoma suppressor genes were putatively assigned. One region, telomeric to the breakpoint, is delineated distal by *CEB15* and proximal by *PND* [25]. It is hypothesised that this region harbours an imprinted suppressor gene, which is not associated with *MYCN* amplification [22, 27]. The second region neuroblastoma R2 was defined in neuroblastoma tumours with interstitial deletions, and tentatively assigned to 1p35-p32, since the flanking markers *D1S211* and *D1S209* were linked with the *GLUT* locus [23]. This region is located centromeric of the constitutional 1p breakpoint.

We evaluated the position of the 1p breakpoint region, defined by *PND–D1S56*, compared with interstitial deletions reported in neuroblastoma. Confusion exists about the presence and the boundaries of the interstitial deletions. Indeed, molecular studies were performed with different markers. There is no consensus on the relative marker position, in particular of the position of the *D1S47* marker relative to the *D1S94*, *D1S95*, *D1S96* and *D1S97* markers. Given the relative position of 1p markers acccording to the CEPH linkage map [28] and to the FISH map [8], literature data suggest that 12 interstitial deletions (11 tumours and one cell line) have been observed in neuroblastoma [5, 24, 25, 29, 30], besides the three used to define the neuroblastoma R2 region [23]. The largest possible boundaries of the deleted regions, assuming a position of *D1S94*, *D1S95* and *D1S96* proximal to *D1S50* and distal to *D1S47*, encompassed the constitutional chromosome 1p breakpoint in 10 of 12 neuroblastoma samples with interstitial deletions. However, the paucity of informative markers used in the region of interest prevents further conclusions being drawn.

It is highly probable that the involvement of chromosome 17 as partner chromosome of the constitutional chromosome translocation in our patient is not coincidental, as a number of observations suggest the involvement of genes on chromosome 17 in neuroblastoma genesis. Chromosome 17 abnormalities were described in neuroblastoma tumours and cell lines [31, 32]. We and others found unbalanced 1;17 translocations as nonrandom findings in neuroblastoma karyotypes [32–34]. Introduction of chromosome 17 into a neuroblastoma cell line completely suppressed the tumour-forming ability of the neuroblastoma cells [35]. In this study, we identified 7G4 (*NF1*) as the proximal, cMCP-3 (*SCYA7*) as the distal flanking marker of the constitutional chromosome 17q breakpoint region. Interestingly, an association between the Neurofibromatosis 1 disease and neuroblastoma was described [36], and recent studies reported aberrant mRNA and inactivation of *NF1* in neuroblastoma [37, 38]. Further studies are necessary to clarify the possible role of chromosome 17 in neuroblastoma genesis.

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