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# Fine Mapping of the Human Renal Oncocytoma-Associated Translocation (5;11)(q35;q13) Breakpoint

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**ABSTRACT:** *Recent cytogenetic analysis of a series of human renal oncocytomas revealed the presence of a recurring chromosomal translocation (5;11)(q35;q13) as sole anomaly in a subset of the tumors. The molecular characterization of this translocation was initiated using two primary t(5;11)-positive renal oncocytomas and a panel of somatic cell hybrids derived from one of these tumors, in conjunction with fluorescence in situ hybridization (FISH) and Southern blot analysis. The breakpoint in chromosome band 11q13 could be located within a genomic interval of at maximum 400 Kb immediately centromeric to the BCL1 locus. © Elsevier Science Inc., 1997*

## INTRODUCTION

Oncocytomas are benign tumors that occur predominantly in the kidney, accounting for approximately 5% of all primary tumors at that site. Histologically, oncocytomas are characterized by the presence of epithelial cells with a strong granular eosinophilic cytoplasm. Ultrastructural examination revealed that these so-called oncocytic cells are densely packed with mitochondria, many of which show abnormal morphologic characteristics [1]. This phenotype, however, also can be found in some of the malignant neoplasms of the kidney such as the granular and chromophobic variants of renal cell carcinomas [2]. Although long-term follow-up studies have shown that oncocytomas do not metastasize, several reports indicate the invasive potential of these tumors [3–5]. Preliminary cytogenetic studies performed on a limited series of renal oncocytomas have revealed a heterogeneous chromosomal constitution [3, 5–7]. Basically, two subgroups can be distinguished: (1) those with numerical anomalies, including -Y and -1 [8–10]; and (2) those with recurring structural anomalies, in particular t(5;11)(q35;q13) [11, 12] or variants thereof [13–15]. The t(5;11)(q35;q13) has been observed as sole

cytogenetic anomaly in at least 3 independent cases and, as such, should be considered as a primary change. Deletion of 3p material that occurs in the majority of renal cell carcinomas [16, 17] has not been observed in renal oncocytomas. The presence of mitochondrial DNA alterations has been reported by some investigators [18, 19]. These latter observations, however, could not be substantiated by others [20].

In view of the steadily accumulating evidence that chromosomal changes play causal roles in cancer development [21–23], we assume that in this case, too, the chromosomal translocation points at a gene(s) that plays a crucial role in the pathogenesis of renal oncocytomas. Therefore, we initiated the fine mapping of the t(5;11)(q35;q13) breakpoint. Because band 11q13 is involved in a variety of malignant disorders, including mantle cell lymphomas, squamous cell carcinomas, breast cancer, multiple endocrine neoplasia type 1, and extragonadal germ cell tumors [24–30], and because 11q13 is relatively well mapped [24, 31, 32], we selected this region for detailed analysis. To this end, fluorescence in situ hybridization (FISH) with breakpoint region-specific probes was employed. In addition, a panel of oncocytoma-derived interspecies somatic cell hybrids was generated to facilitate our studies. The resulting mapping data are useful (1) to construct a detailed physical map of the breakpoint region; and (2) to provide a basis to isolate the breakpoint itself and the gene(s) involved in the development of renal oncocytomas.

## MATERIALS AND METHODS

### Tumors and Somatic Cell Hybrids

Samples of the t(5;11)(q35;q13)-positive renal oncocytomas T88-1665 and T92-12439 were obtained from 2 different

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patients after radical nephrectomy at surgery. Following disaggregation of tumor cells with collagenase and subsequent short-term culturing, metaphase spreads were prepared using standard procedures. In addition, a panel of somatic cell hybrids was isolated after fusion of the thymidine kinase deficient ( $tk^-$ ) Chinese hamster cell line A3 with T92-12439 cells. Fusion and hybrid selection were performed essentially as described before [33], using Sendai virus as a fusogen and a combined HAT/Ouabain procedure for selection of hybrid cells in which the reciprocally translocated chromosomes segregate. The chromosomal constitution of hybrid cells was evaluated by cytogenetic analysis using R-banding after heat denaturation and by FISH using a combination of centromere-specific probes and whole-chromosome paints.

### Fluorescence in Situ Hybridization

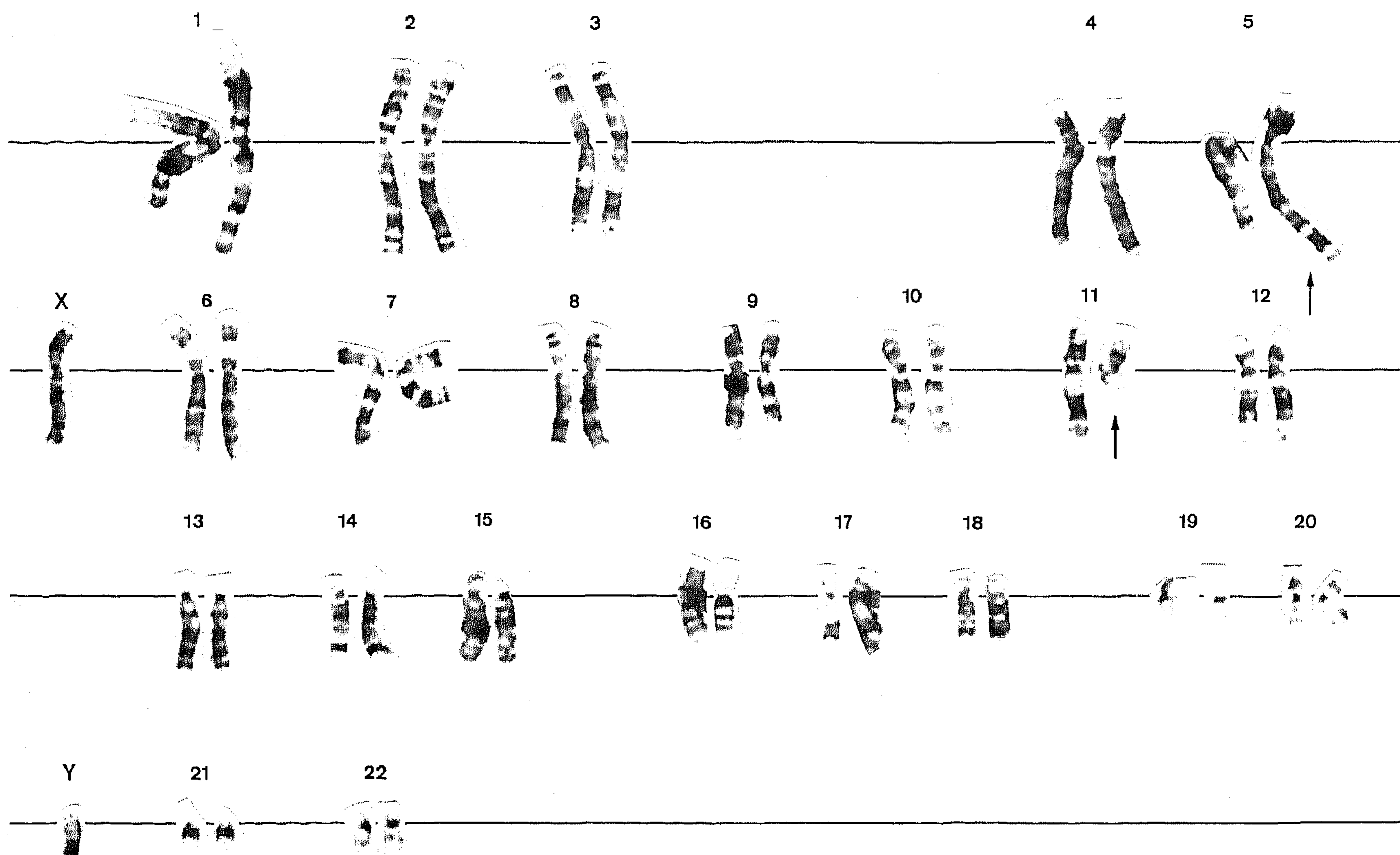
Multicolor FISH experiments were carried out as reported before [28, 34]. In short, probe DNAs were labeled with biotin-14-dATP (Life Technologies, Gaithersburg, MD, USA) or digoxigenin-11-dUTP (Boehringer, Mannheim, Germany) following nick translation. All labeled probes, except the alphoid probe, were subjected to preannealing to compete out repetitive sequences using 25- to 50-fold excess of Cot.1 DNA (Life Technologies, Gaithersburg, MD, USA). For efficient hybridizations, 10 ng labeled alphoid and 200 ng labeled cosmid probe DNAs were applied under a  $20 \times 20$  mm coverslip onto primary tumor or somatic cell

hybrid-derived metaphase spreads. Visualization of the hybridization signals was accomplished using Texas Red-conjugated avidin and biotinylated goat antiavidin antibodies (Vector Laboratories, Burlingame, CA, USA) for biotin-labeled probes and fluorescein-conjugated sheep antidigoxigenin (Boehringer, Mannheim, Germany) and fluorescein-conjugated donkey antisheep antibodies (Jackson ImmunoResearch, West Grove, PA, USA) for digoxigenin-labeled probes. Slides were immersed in antifade-solution, supplemented with 4,6-diamino-2-phenylindole (DAPI, 0.5  $\mu\text{g}/\text{mL}$ ; Sigma, St. Louis, MO, USA) for counterstaining of the chromosomes. Evaluation of the FISH results was performed using a Zeiss Axiophot epifluorescence microscope, equipped for the visualization of fluorescein isothiocyanate (FITC), Texas Red, and DAPI fluorescence. Digital images were captured using a high-performance CH250/A cooled CCD-camera (Photometrics, Tucson, AZ, USA) interfaced onto a Macintosh Quadra 950 computer. Acquisition, processing, and display were accomplished using the BDS-image™ FISH software package (Oncor Imaging, Gaithersburg, MD, USA). Photographs were made using a Polaroid Quick-Print with Kodak EPP 100 Plus color slide film.

### Southern Blot Analysis

High-molecular-weight DNAs were isolated as described before [35], digested to completion, electrophoresed through 0.8% agarose gels, and blotted onto Genescreen Plus nylon

**Figure 1** Renal oncocytoma-derived karyotype exhibiting  $t(5;11)(q35;q13)$  as the sole cytogenetic abnormality (arrows).



membranes (Dupont) using standard protocols. Blots were hybridized with probes (labeled with  $\alpha$ - $^{32}$ P-dCTP by random priming) in 0.5 M phosphate, 1 mM Na<sub>2</sub>-EDTA, and 7% SDS (w/v) at 65°C overnight and subsequently washed in 40 mM or 10 mM phosphate 0.1% SDS at 65°C.

### Probes

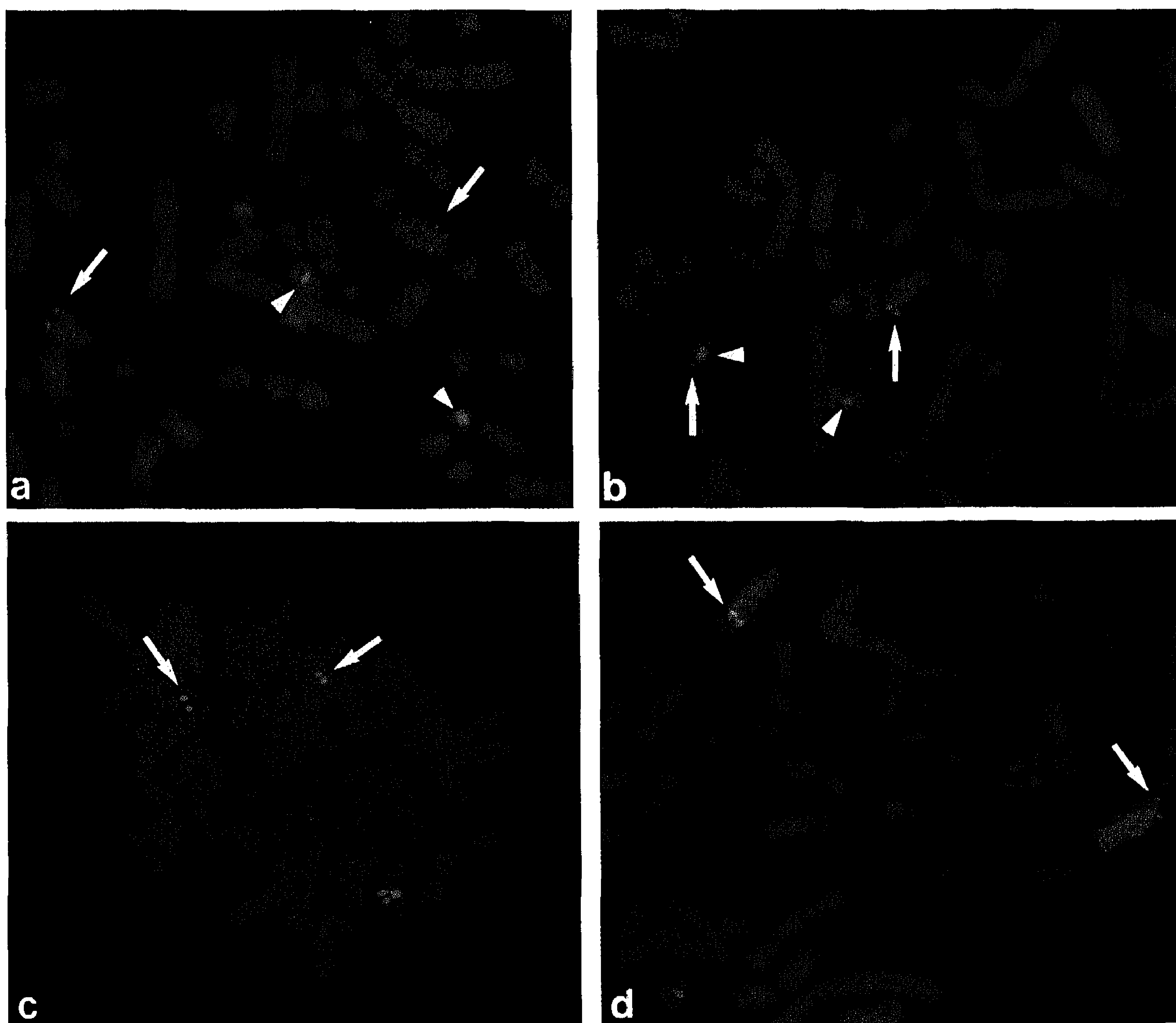
For the FISH and Southern blot analyses we used 27 cosmid and/or plasmid probes specific for chromosomes 5 and 11. As references in our FISH experiments we used a plasmid probe specific for the centromeric (alphoid) region of chromosome 11 (pCL11A), and whole-chromosome painting 5- and 11-specific plasmid libraries pBS5 and pBS11, respectively [36]. A summary of all the probes used is listed in Table 1 [37–39].

## RESULTS AND DISCUSSION

### Characterization of t(5;11)(q35;q13) in Primary Tumors

Cytogenetic analysis of two primary renal oncocytomas (T88-1665 and T92-12439) revealed the presence of t(5;11)(q35;q13) as sole anomaly (Fig. 1). These primary tumors were used for a more detailed characterization of the translocation. Starting from the 11q13 region, FISH analyses were performed on metaphase spreads obtained from short-term cultured primary tumor cells. Double hybridizations with combinations of 11q13-specific cosmids (Table 1) and the alphoid probe (pCL11A) specific for the centromeric region of chromosome 11 were carried out to verify the location of the translocation breakpoint within this cytogenetic band. In both primary tumors, the cosmid probes for PGA (cgHGP18) and BCL1 (cCLBCL1) mapped proximal and distal to the

**Figure 2** FISH analysis of primary renal oncocytoma T88-1665 (c) and oncocytoma (T92-12439)-derived somatic cell hybrid A3ON17B (a,b,d) metaphase spreads. (a) Cosmid clone cCI5-20 (green; arrows) in combination with the chromosome 11 centromere-specific probe pCL11A (red; arrowheads); cCI5-20 remains on der(5). (b) Cosmid clone cCI5-12 (red; arrows) in combination with pCL11A (green; arrowheads); cCI5-12 translocates to der(11). (c) Cosmid clone cgHGP18 (red) in combination with cosmid clone cCLBCL1 (green); split signals on der(11) and der(5) (cgHGP18 and cCLBCL1, respectively) are marked by arrows. (d) Cosmid clone cCI5-20 (red; arrow) in combination with whole chromosome 11 paint probe pBS11 (green); cCI5-20 remains on der(5).





**Table 1** List of chromosome 5 and 11 specific DNA probes used

Locus symbol	Probe name	Type	Chromosomal localization.	Reference
D5S22	J0205H-C	P	5q34-qter	[38]
PDGFRB	RP41	P	5q33-q35	[38]
CSF1R	P19F7	P	5q33-q35	[38]
ADRB2	966	P	5q31-q32	[38]
	cCI5-12	C	5q35.2-q35.3	[39]
	cCI5-14	C	5q35.1-q35.2	[39]
	cCI5-20	C	5q34-q35.1	[39]
	cCI5-30	C	5q35.2-q35.3	[39]
	cCI5-37	C	5q35.1-q35.2	[39]
pBS5		P	5pter-qter	[36]
D11Z1	pCL11A	P	11cen	[38]
PGA	cgHGP18	C	11q13	[38]
D11S449	cCI11-219	C	11q13.1-q13.2	[31]
PYGM	cCL15	C	11q12-q13.2	[38]
D11S546	cCI11-363	C	11q13.1-q13.3	[31]
	363S2	P		[28]
D11S807	cCLGW39	C	11q12-q13.2	[38]
D11S460	cCI11-254	C	11q13.1-q13.3	[31]
	254.3	P		[28]
D11S443	cCI11-44	C	11q13.1-q13.4	[31]
	no.30	P		Schuuring et al., unpublished results
D11S146	cCL59	C	11q12-q13.2	Schuuring et al., unpublished results
	pHBI59	P		[32]
BCL1	cCLBCL1	C	11q13.3	Schuuring et al., unpublished results
	Bcl1b	P		[32]
CCND1	B31.1	P	11q13	[32]
	pBS11	P	11pter-qter	[36]

Abbreviations: C, cosmid; P, plasmid.

breakpoint in 11q13, respectively (Fig. 2), confirming the position of the breakpoint within this genomic interval.

### Southern Blot Analysis of Oncocytoma-Derived Somatic Cell Hybrids

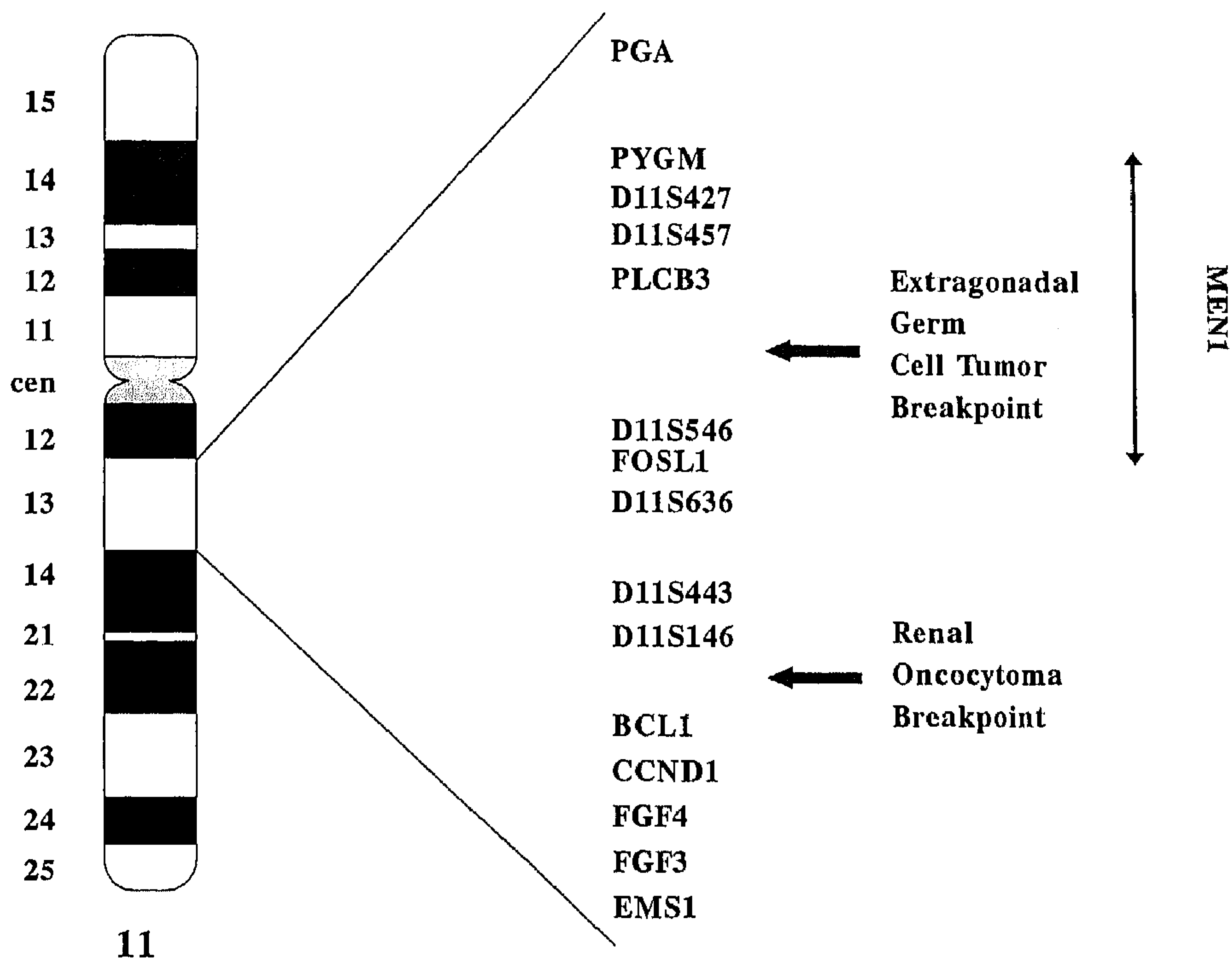
To characterize t(5;11)(q35;q13) in further detail, a panel of somatic cell hybrids was constructed in which the reciprocally translocated chromosomes segregate. After fusion, 86 independent hybrid clones were isolated, 4 of which were selected for further mapping purposes (Table 2): A3ON4A and A3ON16A containing der(5), A3ON11A containing der(5) and der(11), and A3ON17B containing the normal cop-

ies of chromosomes 5 and 11 in combination with both der(5) and der(11). These clones were screened for the presence and/or absence of a series of chromosomes 5- and 11-specific probes using Southern blot analysis (not shown). The markers D5S22, PDGFRB, CSF1R, and ADRB2 were found to map proximal to the breakpoint on chromosome 5 (positive in A3ON4A and A3ON16A). Similarly, the markers D11S546, D11S460, D11S443, and D11S164 could be located proximal to the breakpoint on chromosome 11, and the markers BCL1 and CCND1 distal to the breakpoint (Table 2). These results agree with and substantiate the above FISH results localizing BCL1 (and the more distal marker CCND1) telomeric to the 11q13 breakpoint.

**Table 2** Presence and/or absence of the normal and t(5;11)-derived chromosomes and their markers in different Chinese hamsters x renal oncocytoma somatic cell hybrids

Hybrid clone	Chromosomes				Markers <sup>a</sup> chromosome 5				Markers chromosome 11					
	5	11	der(5)	der(11)	D5S22	PDGFRB	CSF1R	ADRB2	D11S546	D11S460	D11S443	D11S146	BCL1	CCND1
A3ON4A	-	-	+	-	+	+	+	+	-	-	-	-	+	+
A3ON11A	-	-	+	+	+	+	+	+	+	+	+	+	+	+
A3ON16A	-	-	+	-	+	+	+	+	-	-	-	-	+	+
A3ON17B	+	+	+	+	+	+	+	+	+	+	+	+	+	+

<sup>a</sup>For details of the locus-specific probes, see Table 1.



**Figure 3** Chromosome 11 showing 11q13 DNA markers together with the relative positions of the MEN1 locus and the extragonadal germ cell tumor—and the renal oncocytoma-associated translocation breakpoints.

### FISH Analysis of Oncocytoma-Derived Somatic Cell Hybrids

Because of lack of primary tumor-derived metaphase spreads we subsequently made use of hybrid cell line A3ON17B (Table 2), containing the normal copies of chromosomes 5 and 11 in addition to its translocation derivatives der(5) and der(11), respectively, for further FISH analysis. To this end, five 5q34-q35-specific cosmid probes (Table 1) were used. Of these, only cCI5-20 hybridized to the der(5) chromosome, whereas the other four cosmids, cCI5-12, cCI5-14, cCI5-30, and cCI5-37, hybridized to der(11), that is map distal to the breakpoint (Fig. 2). These results confirm our previous cytogenetic localization of the t(5;11) breakpoint within band 5q35. By using an additional series of chromosome 11-specific cosmids (Table 1), the localization of the 11q13 breakpoint between the markers D11S146 and BCL1 could, again, be confirmed (i.e., cosmids cCI11-219, cCL15, cCI11-363, cCLGW39, cCI11-254, cCI11-44, and cCL59 map proximal, and cCLBCL1 maps distal to the breakpoint).

Taken together, our results indicate that the oncocytoma-specific 11q13 breakpoint is flanked by two chromosomal regions that are frequently affected in (1) multiple endocrine neoplasia type 1 (MEN1) and extragonadal germ cell tumors (proximal) and (2) mantle cell lymphomas (BCL1; distal) (Fig. 3). A physical map encompassing the markers D11S443, D11S146, BCL1, and CCND1 has been constructed using pulse field gel electrophoresis [31] and somatic cell hybrid [32] analyses. Based on these mapping data, the physical distance between D11S443/D11S146 and BCL1 was estimated to measure between 350 and 550 kb.

Recently, this distance between these loci was more precisely determined using Fibre-FISH (380–400 kb) [Schuur-ing et al., unpublished results]. Based on these compiled mapping data, several candidate genes located in the 11q13 region, that is, PPP1 $\alpha$ , PLC $\beta$ 3, FOSL1, SEA, PYGM, FGF3, FGF4, EMS1, and GST $\gamma$ , [27, 34, 36] can be excluded from being involved in the development of t(5;11)(q35;q13)-positive oncocytomas. It remains to be established whether the expression of CCND1 is affected by the translocation.

In conclusion, we have located the renal oncocytoma-specific chromosomal breakpoint in band 11q13 within an interval of at maximum 400 kb flanked by the markers D11S443/D11S146 and BCL1. This information should (1) pave the way for the positional cloning of this specific translocation breakpoint and the identification of the gene(s) involved in the development of these tumors; and (2) provide molecular tools for the discrimination between oncocytomas and other (malignant) renal neoplasms. Furthermore, because rearrangements of 11q13 also can be encountered in other RCCs, the availability of breakpoint-specific probes will be instrumental in establishing to what extent relationship(s) do exist among these neoplasms at the molecular level [7].

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