

## ARTICLES

# Molecular cytogenetic detection of 9q34 breakpoints associated with nail patella syndrome

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**The nail patella syndrome (NPS1) is an autosomal dominant disorder characterised by dysplasia of the finger nails and skeletal abnormalities. NPS1 has been mapped to 9q34, to a 1 cM interval between D9S315 and the adenylate kinase gene (AK1). We have mapped the breakpoints within the candidate NPS1 region in two unrelated patients with balanced translocations. One patient [46,XY,t(1;9)(q32.1;q34)] was detected during a systematic survey of old cytogenetic files in Denmark and southern Sweden. The other patient [46,XY,t(9;17)(q34.1;q25)] was reported previously. D9S315 and AK1 were used to isolate YACs, from which endclones were used to isolate PACs. Two overlapping PAC clones span the 9q34 breakpoints in both patients, suggesting that NPS1 is caused by haploinsufficiency due to truncation or otherwise inactivation of a gene at or in the vicinity of the breakpoints.**

**Keywords:** nail patella syndrome; NPS1; PAC; YAC; extended DNA; FISH; fine mapping

## Introduction

The nail patella syndrome (NPS1) also known as onycho-osteodysplasia, is an autosomal dominant, fully penetrant, pleiotropic disorder associated with nail dysplasia, a variety of skeletal anomalies such as absent or hypoplastic patellae, iliac horns, elbow and hip

abnormalities, and extraosseous symptoms including nephropathy and glaucoma<sup>1</sup> NPS1 is one of the first genetic disorders for which linkage to an autosomal locus, the ABO blood group, was discovered in man.<sup>2</sup> The ABO–NPS1 linkage group, which was later extended to include the adenylate kinase-1 (AK1) locus,<sup>3,4</sup> was also among the first to be regionally mapped to an autosome, at 9q34.<sup>5,6</sup> A recent linkage study has refined the mapping of NPS1 to a 1–2 cM interval, between D9S60 and AK1.<sup>7</sup> We have narrowed this region further by haplotype analysis in two Dutch kindreds, placing the *NPS1* gene in a 1 cM interval between marker D9S315 and AK1.<sup>8</sup>

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Many disease genes have been identified by positional cloning following the identification of disease-associated balanced chromosome rearrangements (DBCRs) that have truncated, deleted or otherwise inactivated the genes of interest.<sup>9,10</sup> In order to optimise the search for DBCRs, a large cytogenetic network, Mendelian Cytogenetics Network (MCN), has been established [N Tommerup, personal observation]. A balanced translocation carrier with NPS1 with the karyotype 46,XY,t(9;17)(q34.1;q25) was recently described.<sup>11</sup> During a systematic re-examination of cytogenetic archives within MCN [Bugge *et al*, in preparation], we have discovered another patient with NPS1 and an apparently balanced 1;9-translocation, with a breakpoint involving the 9q34 region. In the present study, we have isolated YAC and PAC clones from the candidate region, and mapped these with respect to both of these translocations by fluorescence *in situ* hybridisation (FISH).

## Materials and Methods

### Case Histories

**Case 1: t(1;9)** The patient, born in 1978 in Peru, was delivered by Caesarean section by a 16-year-old mother of Indian descent and unknown father. Birthweight was 3200 g. At the age of 4 days he was adopted by Danish foster parents. At the age of 3 months he was admitted to a paediatric ward in Lima with the characteristic presentation of hypoplastic nails, and the diagnosis of nail patella syndrome was established. At the age of 10 months he was admitted to a paediatric ward in Denmark. He was noted to be mentally normal; he could not stand and he had typical hypoplastic nails. X-rays of the knees, pelvis and elbows were all normal for the age, and there was no hip dislocation. Investigations of the kidneys, including pyelograms, did not indicate anything abnormal. There was unilateral, right-sided buphthalmos with increased intraocular pressure (18 mm Hg compared to 13 mm Hg in the left eye), and there was a suspicion of a left-handed reduced anterior angle. Because of the hypoplastic nails and the glaucoma, the diagnosis of nail patella syndrome was confirmed. At the age of 18, he was involved in a traffic accident where he broke his right patella. X-rays showed normally developed patella on both sides. Following this accident he has had a prolonged period with severe walking difficulties. Except for a single blood sample he has refused further clinical examination.

**Case 2: t(9;17)** This recently described NPS1 patient<sup>11</sup> was detected because of cytogenetic and clinical investigation of a son with an unbalanced karyotype, 46,XY,der(17)t(9;17)(q34.1;q25). The patient, who was a balanced carrier with the karyotype 46,XY,t(9;17)(q34.1;q25), displayed several features of NPS1, including dysplastic nails, polygonal patellae, dislocation of the head of the radius and iliac horns. No other cases of NPS1 were known in the family.

### Cytogenetic Analysis

Cytogenetic analysis of the chromosomes from PHA-stimulated peripheral lymphocyte cultures and EBV-transformed cell lines from the 1;9-translocation carrier was performed according to standard protocols. GTG banded chromosomes were analysed on a Leica DMRBE microscope equipped with a Grundig FA 87 digital video camera and a Quips karyotyping software (Vysis, USA). For whole chromosome painting, commercial paints 1 and 9 were used (Cambio, USA).

### FISH Mapping with CEPH YACs

Selected YACs from the 9q34 region (Table 1) were obtained from either Foundation Jean Dausset-CEPH (France) or from the Molecular Cytogenetics and Positional Cloning Center (Berlin) (<http://www.mpimg-berlin-dahlem.mpg.de>), labelled with biotin or digoxigenin, and hybridised to metaphase chromosomes as previously described.<sup>11,12</sup>

### Isolation of YAC and PAC Clones

The ICI YAC PCR library<sup>13</sup> was screened using markers D9S315, D9S112 and *AKI*, respectively. The oligonucleotide primers for the markers were retrieved from the genome database (GDB).

Endclones from both ends of YAC 15f-f1 were generated essentially as described previously.<sup>14</sup> These endclones were used as probes to screen the RPCI-5 human genomic PAC library.<sup>15</sup> Each endclone gave 5 positive hits which were initially tested on the 9;17-translocation by FISH analysis.

### FISH Mapping with PACs

FISH on the t(1;9) metaphases was performed as described previously<sup>12</sup> using 200 ng biotin-labelled YAC and 175 ng biotin-labelled PAC clones. For high-resolution FISH mapping, chromatin was released from fixed cells from the 1;9-translocation carrier as described.<sup>14</sup>

All FISH signals were visualised on a Leica DMRB epifluorescence microscope equipped with a Sensys 1400 CCD camera (Photometrics, USA) and an IPLab Spectrum imaging software (Vysis, USA). The FISH studies on the t(9;17) were as described previously.<sup>11</sup> Original pseudocolor images were converted to greyscale images and printed on Epson photo quality glossy paper on an Epson 600 inkjet colour printer.

### Alu-PCR of YAC and PACs

Amplification of DNA from YACs 4i-b3, 15f-f1 and 13f-f1, and PACs 830-M14 with the interspersed repetitive sequence primers Alu-517, A1S, Alu-BK33, and L1H were carried out as previously described.<sup>16</sup>

## Results

### Cytogenetics

The initial cytogenetic analysis of case 1 in 1979 based on QFQ banding resulted in the karyotype: 46,XY,t(1;9)(q41;q34) and in 1997 GTG banding localised the chromosome 1 breakpoint to 1q32.3 (Figure 1A). It is not known whether this translocation has occurred *de novo* as the biological parents were unavailable. Whole chromosome painting (WCP) of

chromosome 1 and 9 confirmed a two-way reciprocal translocation with a breakpoint in the very distal part of 9q. Due to the small size of the translocated 9q34-qter region, this segment was painted very faintly on the derivative chromosome 1 (Figures 1B, C). The mean fractional length of the WCP9 segment on the derivative chromosome 9 (mean F1pter = 0.76) corresponded to a breakpoint within 9q34.1-34.2, compatible with the breakpoints derived from the G band pattern of the translocation chromosomes (Figure 1A).

The cytogenetic analysis of case 2 has been reported recently.<sup>11</sup>

### FISH Mapping with CEPH YACs

The results of the FISH mapping of the two translocation breakpoints using YACs from CEPH are shown in Table 1. None of the YACs spanned the breakpoints and both chromosome 9 breakpoints were localised within the same ≈4 cM region flanked by the proximal YAC 897c1 and the distal YAC 415c11.

### Isolation and FISH Mapping of YAC Clones with Flanking Markers

An informative recombination event in the Dutch NPS1 families allowed us to position D9S112 distal to D9S315 (Figure 2), placing D9S112 within the NPS1 candidate region (Figure 3). Two YAC clones (4i-b3 and 15f-f1) were positive for D9S112 and one YAC clone (13f-f1) was positive for *AK1*. Initially, no YACs

were detected with D9S315, but YAC 15f-f1 turned out to be positive for D9S315 as well as D9S112. Discrepancies in our data, CEPH and Whitehead maps may be due to high recombination rates observed in the subtelomeric and telomeric regions of chromosomes. Alu PCR fingerprinting of the YACs also suggest that this region is enriched with repeats which might result in recombinations and deletions of the YACs.

Sizing of these YAC clones by pulsed-field gel electrophoresis indicated that 4i-3b was 90 kb, 15f-f1 was 100 kb and 13f-f1 was 300 kb. FISH analyses suggested that none of the YACs spanned the translocation breakpoints. YACs 4i-b3 and 15f-f1 gave signals on der(9) in both cases, thus mapping both YACs proximal to the translocation breakpoints. YAC13f-f1 gave signals on der(1) in the t(1;9) case, and on der(17) in the t(9;17) case, mapping it distal to the 9q34 breakpoints. In addition, YAC 13f-f1 was found to be chimeric giving signals on chromosome 17.

### Mapping and Characterisation of the Identified PACs

End clones of YAC 15f-f1 were used to screen the RPCI5 human genomic PAC library.<sup>15</sup> Each endclone detected five positive PAC clones and FISH analyses showed that two of these PACs (830-M14 and 1195-M2) were spanning the 9q34 breakpoints in both patients (Figure 4).

On metaphase chromosomes of patient t(1;9), intensity of the FISH signals generated by PAC 830-M14

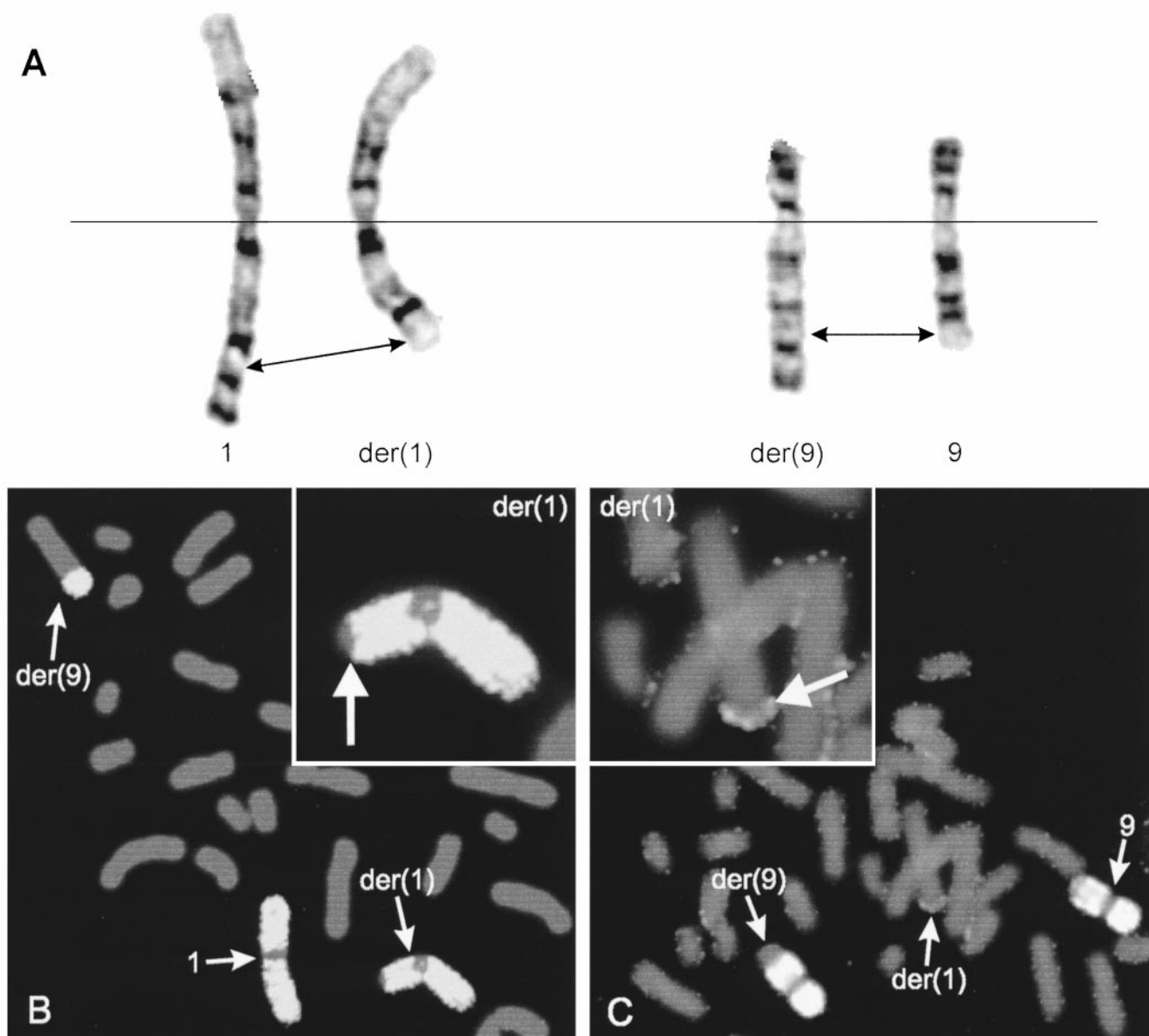
**Table 1** FISH mapping of two nail patella translocations with CEPH YACs

YAC	cM	cR	STS	t(1;9) <sup>a</sup>	t(9;17) <sup>a</sup>
933C5	120		D9S262,D9279	Proximal	Proximal <sup>11</sup>
641F4	126/129		D9S170	ND	Proximal
754E10	130	450	D9S195,D9S1872	Proximal	Proximal
761F1	130	450	D9S258,D9S195	Proximal	Proximal
755G12	130/133		D9S258,D9S1823	Proximal	Proximal
811B1	128/132		D9S1811,D9S1864	ND	Proximal
765b11	133		D9S1823	Proximal	ND
727C7		454	FB24A12	Proximal	ND
946B5	136	458	D9S282,D9S1881	Proximal	ND
792G11	136/140		D9S1840,D9S1881	ND	Proximal
824H1		459	WI-10044	Proximal	ND
905D9		461	NIB1929	Proximal	ND
905D7		463	NIB1486	Proximal	ND
920C12	137		D9S1825,D9S1789	Proximal	ND
771H1	148/141		D9S164,D9S260	Proximal	ND
946F7	141/143		D9S260,D9S159	Proximal	ND
954D10	143/145		D9S159,D9S179	Proximal	ND
897E1	144		D9S1863	Proximal	ND
415C11	148		ASS,ABL	Distal	Distal <sup>11</sup>
147E10	148		ABO,TSCI	ND	Distal <sup>11</sup>

<sup>a</sup>Localisation of the FISH signals relative to the breakpoint on chromosome 9. cR: centiRad; ND: not determined.

were almost equally strong on both derivative chromosomes, whilst the signals of PAC 1195-M2 were consistently stronger on der(1) than on der(9) (Figure 4a,b). This was confirmed by the length of the FISH signals on extended chromatin from the t(1;9) case. In each nucleus, PAC 1195-M2 generated two long and one shorter signal, whereas PAC 830-M14 generated one long and two shorter, almost identical signals (Figure 5a,b). The measurements suggested that the

t(1;9) breakpoint corresponded to a split of PAC 1195-M2 in a ratio of 1:2 (proximal:distal), whereas PAC 830-M14 was split in a ratio of almost 1:1. Furthermore, there was no significant difference when the combined length of the two shorter PAC 830-M14 signals presumed to correspond to the derivative chromosomes were compared with the length of the longest signal presumed to represent the normal chromosome 9. This finding supports the absence of a



**Figure 1** The reciprocal apparently balanced translocation  $t(1;9)(q31;q34)$ . **A** G-banded metaphase chromosomes, 1,9, and derivative translocation chromosomes, with arrows indicating the likely breakpoints on the derivative chromosome. **B** and **C** Partial metaphases with chromosome 1 (**B**) and 9 (**C**) painting. Inserts showing the tiny 9q34→qter fragment on the derivative chromosomes (large arrows).

larger deletion at the 9q34 breakpoint in the t(1;9) case. In contrast, the FISH signals on the 9;17-translocation showed an opposite pattern of signal distribution on the two derivative chromosomes. PAC 830-M14 gave weaker signals on der(17) than on der(9), whereas PAC 1195-M2 gave almost equal signals on both derivative chromosomes.

Alu-PCR fingerprinting<sup>16</sup> also suggested that PACs 830-M14 and 1125-M2 were overlapping and PAC 830-M14 also overlapped with YACs 4i-b3 and 15f-f1 which are located proximal to the breakpoints (Figure 3).

Taken together, these results are consistent with a map of the region, where the 1;9-translocation breakpoint is located proximal to the 9;17-translocation breakpoint (Figure 3).

## Discussion

So far, apparently balanced translocations associated with congenital genetic disorders have either truncated,

deleted or otherwise inactivated the function of the corresponding disease gene,<sup>10</sup> supporting the theory that these disorders are caused by haploinsufficiency. A patient with an unbalanced translocation resulting in monosomy of 9q32→qter with dysplastic nails but normal patellas has been described.<sup>17</sup> This, together with the identification of apparently balanced translocations associated with various features of NPS1<sup>11</sup> support the theory that NPS1 can be added to the growing list of haploinsufficiency syndromes.<sup>18</sup>

Our data indicate that both the translocation breakpoints map within the same < 100 kb region of chromosome 9q34, within the closest flanking markers (D9S112 and *AK1*) of NPS1. The extended FISH data on the 1;9-translocation also indicate that if deletions are involved they must be very small, ie well below the assumed PAC sizes of 100–120 kb. Furthermore, the opposite pattern of intensities of FISH signals of the two breakpoint-spanning PACs on the derivative chromosomes in the two translocations support the theory that the two 9q34 breakpoints are slightly different, with the 1;9 breakpoint located more proximal than the 9;17 breakpoint. The tentative assumption is that one or

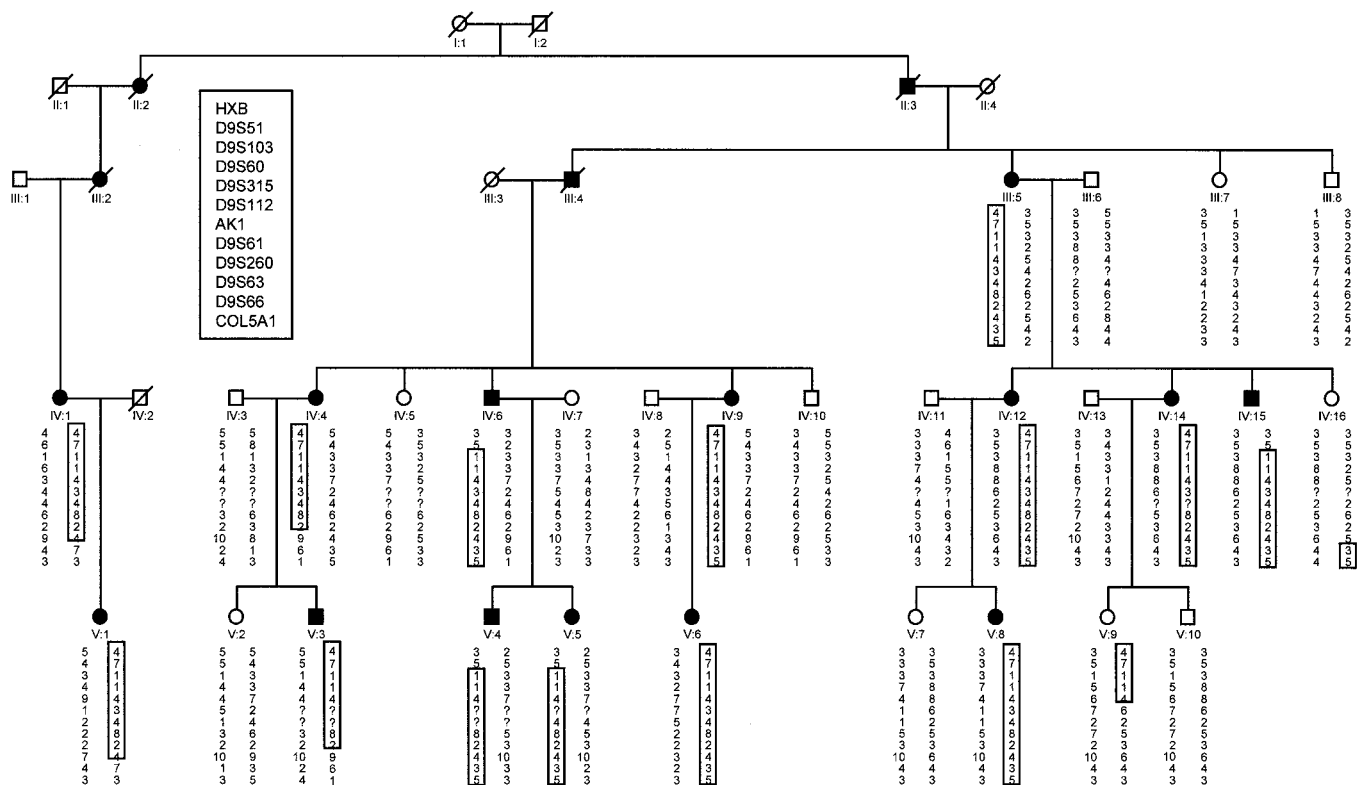


Figure 2 Haplotype analysis of the Dutch family localising D9S112 distal to D9S315.

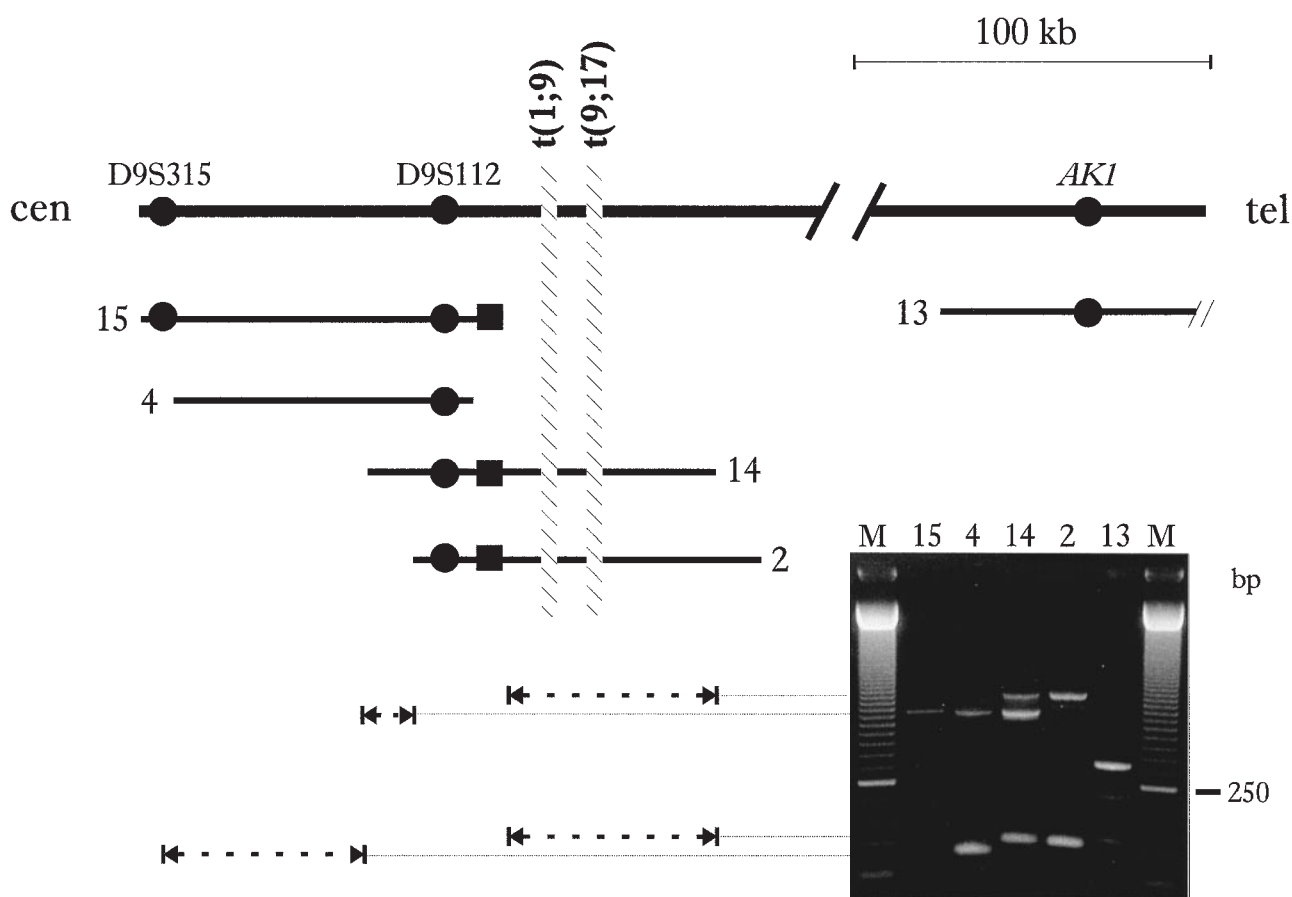
both of these 9q34 breakpoints either interrupt the *NPS1* gene itself or that position effects could be involved.

At present, we can only speculate about the possible involvement of position effects in NPS1. The likely involvement of position effects has been shown to be associated with a growing number of balanced translocations associated with congenital disorders, including aniridia,<sup>19,20</sup> X-linked deafness,<sup>21</sup> holoprosencephaly<sup>22</sup> and campomelic dysplasia<sup>23-26</sup> with the common theme that dosage-sensitive transcription factors are involved.<sup>27</sup> In campomelic dysplasia, all studied translocation breakpoints have so far been mapped at considerable distances (up to at least 300 kb) from the target gene *SOX9*.<sup>23-26</sup> An interesting point is that the clinical features of campomelic dysplasia in most of these translocation cases are less severe than observed

for intragenic *SOX9* mutations. With regard to the present study, this could have implications for the 1;9-translocation patient who only showed a few of the features of NPS1.

However, although there are reports of familial aggregation of some of the non-skeletal features in NPS1, such as renal manifestations<sup>28</sup> and glaucoma,<sup>29</sup> it is still premature to make predictions about genotype/phenotype relationships in NPS1. Despite the disparity of clinical manifestations between the two translocation cases, it is noteworthy that in the reported case with deletion of 9q32→qter,<sup>17</sup> with a presumed total loss of the *NPS1* region, the symptoms only included nail dysplasia, whereas normal patellas were present, as in the present 1;9 translocation case.

Apart from providing compelling evidence for the localisation of *NPS1*, the present study also illustrates

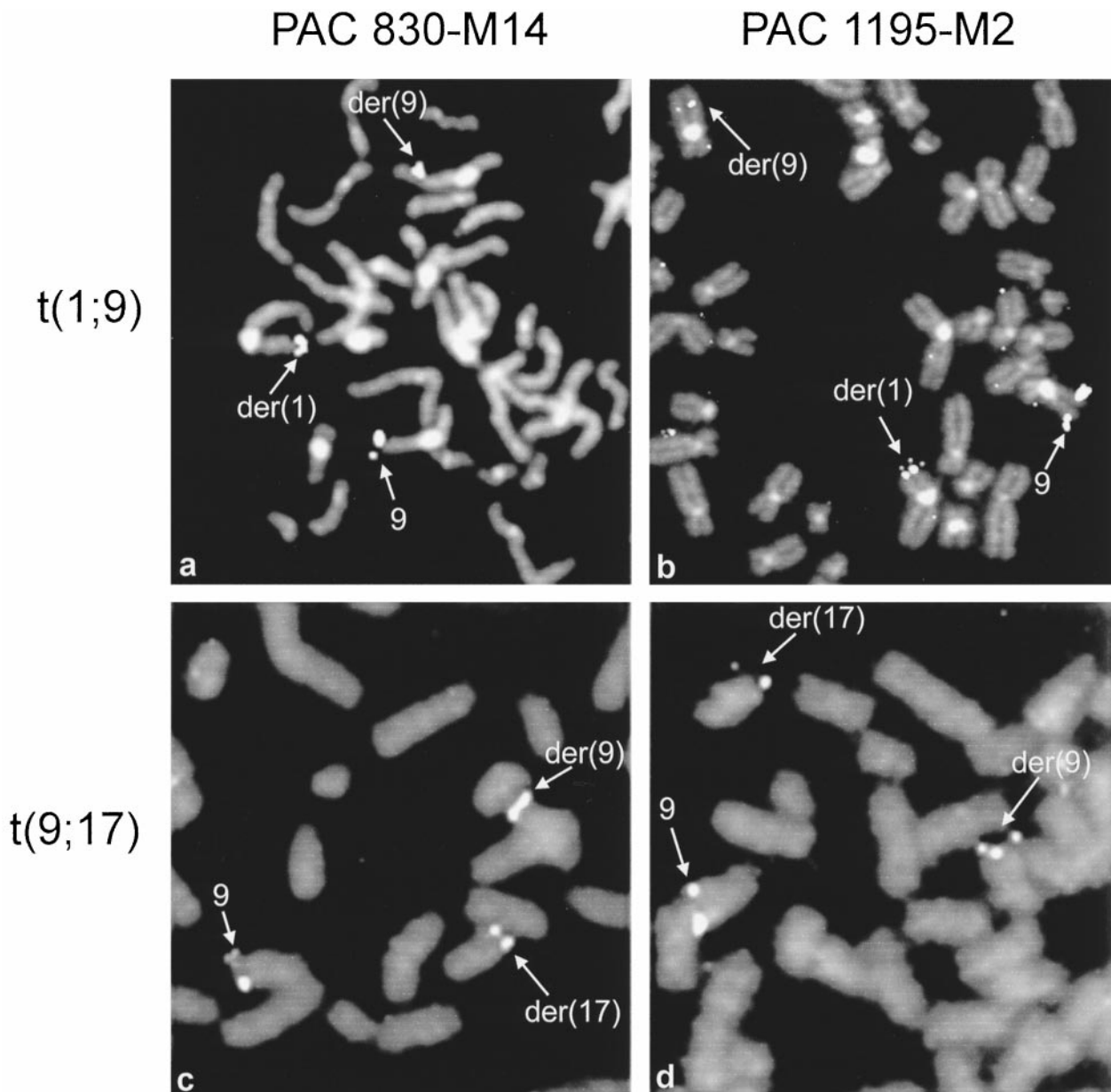


**Figure 3** Map of the *NPS1* region, showing the likely relationship between the three YACs 15 (15f-f1), 4 (4f-b3) and 13 (13f-f1), and the two overlapping PAC clones 14 (83-M14) and 2 (1195-M2) which span the translocation points in both patients. The approximate localisation of the two translocation breakpoints, t(1;9) and t(9;17), based on the FISH data, are indicated with hatched bars. Alu-PCR fingerprinting of the YACs and PACs using the primers Alu-BK33 and A1S are shown in the gel picture. The common PCR fragments for the PACs and YACs, and their rough localisations at the overlapping regions are indicated by the dotted lines. Solid black squares indicate the distal end clone of YAC 15. Size marker (M), 50 base-pair ladder (Pharmacia).

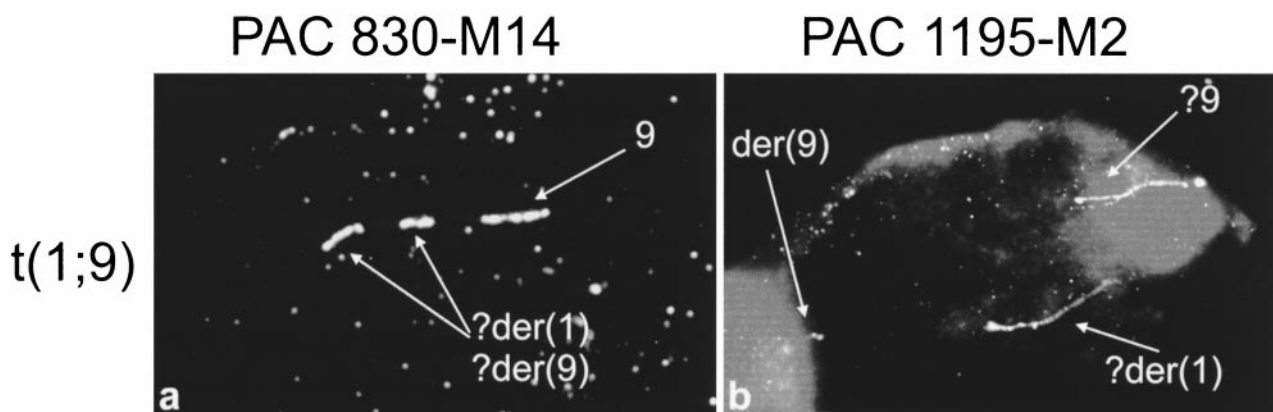
that crucial chromosome rearrangements associated with even well-known and mapped genetic entities can be (re)discovered by systematic screening of old cytogenetic files. We have previously documented this with respect to Prader-Willi syndrome,<sup>30</sup> suggesting that a more wide-scale re-examination of old cytogenetic files would be a very cost-efficient way to identify novel disease genes.

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**Figure 4** Detection of the 9q34 breakpoints by the presence of signals on both *der(1)* and *der(9)* in (a,b) the 1;9-translocation and (c,d) the 9;17-translocation. In (a) notice the almost equal signals on *der(1)* and *der(9)* with PAC830–M14, both of lesser intensity than the signal on the normal chromosome 9, compared with the signals produced by PAC 1195–M2 (b) with a small signal on *der(9)* and a much larger signal on *der(1)*, which is almost of similar intensity as the signal on the normal chromosome 9.



**Figure 5** FISH mapping in two nuclei with extended chromatin from the 1;9-translocation carrier. **a** FISH with PAC 830-M14, with three signals, one large (the normal chromosome 9) and two smaller of comparable size [*der(1)* and *der(9)*]. **b** FISH with PAC 1195-M2, with three signals, two of which must correspond to the normal 9 and the *der(1)* chromosome. The third, much shorter signal, corresponding to *der(9)*, is slightly out of focus.

the Dutch Kidney Foundation (C97-1698), the Austrian Fond zur Förderung der wissenschaftliche Forschung (to GU)(P12792-GEN), the German Genome Programme/Deutsche Forschungsanstalt für Luft- und Raumfahrt eV (4763), and the EU-Commission (BMH4-CT97-2268). The gene (*LMX1B*) defective in NPS was cloned by Dreyer *et al.*<sup>32</sup> after the present manuscript had been submitted for publication. We have analysed the two overlapping PACs spanning the chromosome 9 breakpoints of both patients with PCR and direct sequencing using two sets of gene specific primers.<sup>32</sup> Both PACs include sequences corresponding to exon 3 and exon 8 of the *LMX1B* gene, suggesting that these clones include the gene defective in NPS1.

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