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Nail patella syndrome in a cytogenetically balanced t(9;17)(q34.1;q25) carrier

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Nail patella syndrome (NPS) is an autosomal dominant disorder characterized by dysplasia of the nails and patella, decreased mobility of the elbow, iliac horns and in some cases nephropathy. Linkage studies have localized the NPS locus to chromosome 9q34 within a 1–2 cM interval between *D9S60* and the *adenylate kinase gene* (*AK1*), but the gene has remained elusive. We have identified a balanced t(9;17)(q34.1;q25) associated with NPS. By using FISH with probes from 9q the breakpoint region was narrowed to a 17.0 cM interval between *D9S262* and *ABL*, which includes the NPS critical region. The patient showed the typical clinical features of NPS such as hypoplastic, deep-set nails, a dislocated elbow, iliac horns, and a polygonal patella. This suggests that the translocation has resulted from a break within or near the *NPS* gene, causing defective expression. The translocation in our patient may aid in the identification of the *NPS* gene.

Keywords: NPS; nail patella syndrome; balanced translocation

Introduction

Nail patella syndrome (NPS),[1] also known as hereditary onycho-osteodysplasia, is an autosomal dominant, pleiotropic disorder characterized by nail hypoplasia or dysplasia, absent or hypoplastic patella, subluxation of the radius resulting in impaired mobility of the elbow, iliac horns and, in about 30% of cases, nephropathy. Additional deformities of the skeletal system are associated with NPS, at lower frequencies.[2] NPS was first recognized as an inherited disorder by Little (1897),[3] who reported a four-generation pedigree with 18 affected members who had absent patellae and thumb nails. In further publications the phenotype was expanded and the dominant inheritance of the disorder was clarified.[4–9]

Linkage of NPS to the ABO blood group locus and the *adenylate kinase 1* (*AK1*) gene on chromosome 9 were among the first autosomal linkages for a genetic disorder to be established in humans.[10] Linkage of NPS to loci on 9q has been confirmed in all informative families that have been studied.[11–18] Campeau *et al*[19] localized the *NPS* gene to an interval on 9q34.1, distal to *D9S60* and proximal to *ASS*, comprising a genetic distance of about 9 cM. Recently McIntosh *et al*[20] performed linkage analysis with 13 polymorphic markers in five families with a total of 69 affected persons. Informative recombination events place the *NPS* locus within a 1–2 cM interval between *D9S60* and the *AK1* gene.

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Here we present a patient with NPS and a balanced chromosomal translocation t(9;17)(q34.1;q25). As the breakpoint in our patient might be within or near the *NPS* gene and causing defective expression, we have performed fluorescence *in situ* hybridization (FISH) to further characterize the breakpoint. Hybridization results place the breakpoint within a 17 cM region on chromosome 9q which includes the critical gene region defined by linkage analysis.

Materials and Methods

Propositus

The index patient was referred to our laboratory for chromosome analysis because of dysmorphic signs. The boy was born in the 40th week of gestation [weight 2290 g (< 10th percentile), height 50 cm (50th percentile), head circumference 37 cm (90th percentile), APGAR 6/8/9). Clinical investigation revealed a dolichocephalus, dysplastic ears, an asymmetric face, arachnodactyly, camptodactyly, mental retardation (state of mental development at the age of 21 months: 7–9 months), high prominent nasal bridge, microstomia, ptosis of eye lids and hypotonia. The boy is now 21 months old.

Father

The clinical, radiological and ultrasound examination of the father showed dysplastic nails (thumbs and index fingers), a dislocation of the head of the radius with decreased pronation and supination, iliac horns (Figure 1) and polygonal patellae. There was no nephropathy documented (normal renal ultrasound examination, normal urine analysis, and normal urea (12 mg/dl, range 10–50 mg/dl) and creatinine (0,8 mg/dl, range 0,64–1,05 mg/dl) concentrations in blood). No other cases with NPS were known in the family.

Cytogenetic Analysis

Peripheral blood samples were obtained from the patient and his parents. For high resolution chromosomal analysis, whole blood lymphocyte cultures were stimulated by phytohaemagglutinin (PHA) and synchronized by methotrexate by means of standard protocols. Metaphase chromosomes were routinely analysed by standard trypsin-giemsa (GTG) and quinacrine (QFQ) banding.

Microdissection and Reverse Painting

Five q-arm fragments of the aberrant chromosome 17 were microdissected, amplified by DOP-PCR, and hybridized to normal control metaphases.[21]

FISH Analysis

For confirmation of the partial trisomy 9q34.1 FISH with a directly labelled LSI™ bcr/abl translocation probe from

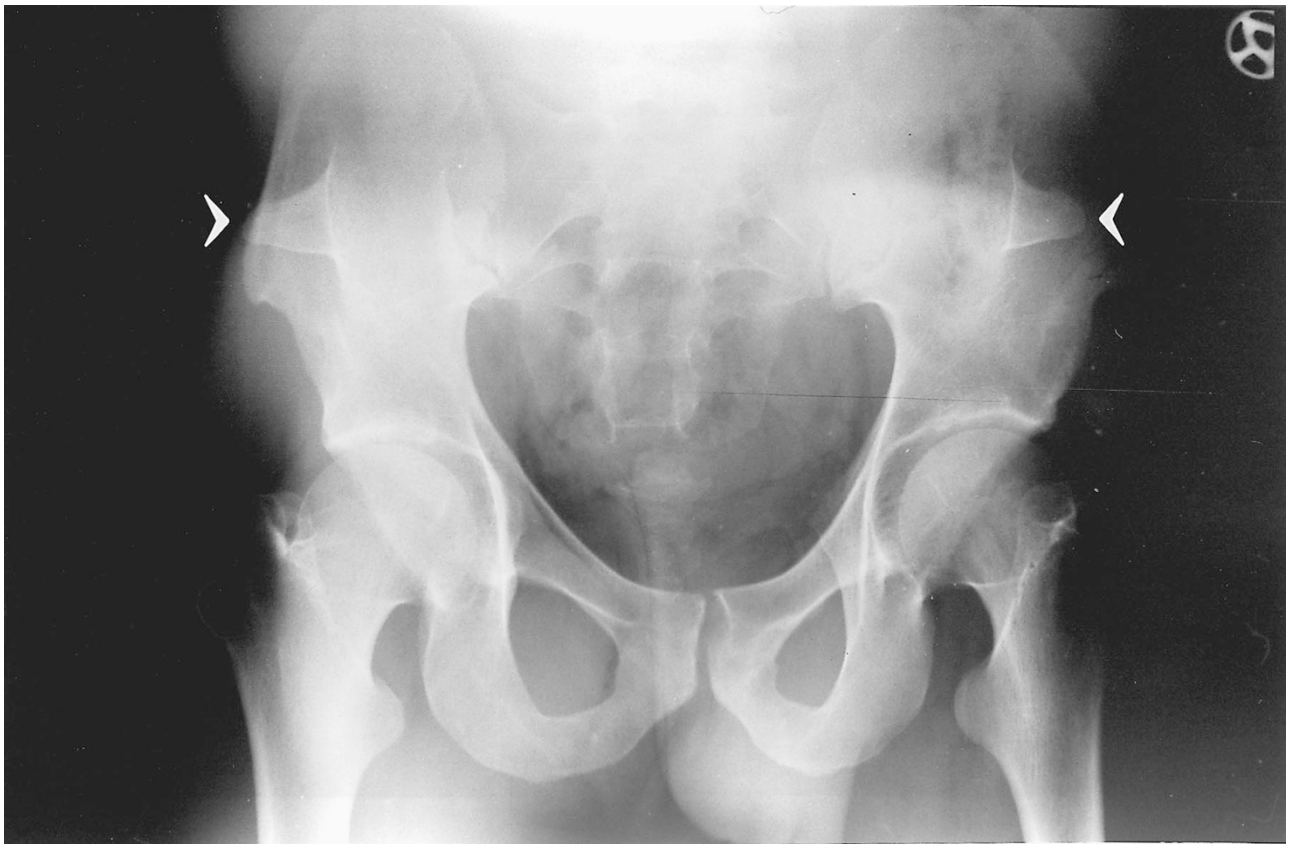

Figure 1 *Pelvioradiography showing iliac horns (white arrows) in the patient with t(9;17)(q34.1;q25)*

9q34.1 (Vysis, USA) was performed on the propositus' lymphocytes according to the manufacturer's protocols. In order to define the breakpoint region in the father, FISH was performed with cos abl 8, a cosmid probe from within the *ABL* gene,[22] and with the YAC clone 933c05 (*D9S279*, *D9S262*), the YAC clone 147e10 (*ABL*, *D9S213*) and the YAC clone 415c11 (*ABO*, *TSCI*) from the 9q33-34 region. The genetic distance of the STS markers with respect of the short arm telomere (cMpter) are 124 cM for YAC 933 c05, 148 cM for YAC 415 c11 and 157 cM for YAC 147 e10. The cosmid clone cos-abl 8 contains a 40 kB fragment representing 3' coding and 3' flanking sequences of the human *ABL* gene on chromosome 9. The YAC clones were amplified by the Alu polymerase chain reaction.[23] The cosmid and the YAC clones were biotin-labelled by nick translation. Chromosomal *in situ* suppression hybridization and detection of the biotinylated probes was carried out as described by Lichter and Cremer.[24]

Results

Clinical and Cytogenetic Analysis

A 6.5 months old boy was referred for chromosomal analysis because of dysmorphic signs and malformations (Table 1). Evaluation of GTG and QFQ banded chromosome preparations from PHA-stimulated lymphocytes of the proband revealed a 46,XY,add(17)(q25) karyotype (Figure 2a). In order to clarify the origin of the chromosomal aberration and for appropriate genetic counselling, chromosomal analysis from peripheral blood lymphocytes was performed on both parents. The father's karyotype showed an apparently identical add(17)(q25) chromosome (Figure 2b), whereas the mother's karyotype was normal. Assuming a balanced translocation in the father, the other translocation chromosome could not be identified unequivocally by GTG and QFQ banding analysis. Inspection of the father by the genetic counsellor and subsequent clinical and radiological examinations resulted in the diagnosis of NPS.

Microdissection and Reverse Painting

A fragment from the aberrant chromosome 17q was isolated by microdissection and the DNA was amplified by DOP-PCR. Reverse painting with the amplified q-arm fragment of the aberrant chromosome 17 showed hybridization signals on chromosomes 9q and 17q (Figure 2d). Given the results of GTG banding the father's karyotype was defined as t(9;17)(q34.1;q25) (Figure 2b and Figure 2c). The karyotype in the boy was defined as 46,XY,der(17)t(9;17)(q34.1;q25), respectively.

FISH Analysis

The clinical diagnosis NPS in the father suggested that the translocation breakpoint on 9q34 was disrupting a putative NPS gene. Therefore the breakpoint was further characterized by FISH. Hybridization with the bcr/abl translocation probe on the proband's metaphases showed three red abl signals on both the normal chromosomes 9 and the derivative chromosome 17, confirming the partial trisomy 9q34.1 (Figure 2e). Hybridization with the YAC 933c5 on the father's metaphases showed signals on both chromosomes 9 (Figure 2f), while hybridization with cos abl 8, YAC 415c11, and YAC 147e10 showed one signal on chromosome 9 and one signal on the derivative chromosome 17 (Figure 2g). We conclude from this data that the breakpoint is located between YAC 933c5 and cos abl 8 and YAC 415c11 (Figure 3).

Table 1 Clinical findings in our patient with partial trisomy 9q and in reported patients with 9q34 syndrome

	Our patient with partial trisomy 9q34.1-qter	*Present in described cases with trisomy 9q34.1-qter*[a]	
		n	*%*
Dolichocephaly	+	7	100
Dysplastic ears	+	7	100
Asymmetric face	+	7	100
Arachnodactyly	+	7	100
Camptodactyly	+	7	100
Metal retardation	+	6	85.7
High/prominent nasal bridge	+	6	85.7
Strabismus/gaze palsy	–	5	71.4
Microstomia	+	5	71.4
Ptosis of eyelids	+	4	57.1
Hypotonia	+	4	57.1

[a]Ref. [25, 26]; $n=7$; +presence of a feature, –absence of a feature or not mentioned in the clinical description.

Discussion

Partial Trisomy 9q34.1

Partial trisomy 9q34.1 has often been described and represents a well defined phenotype.[25] A comparison of the patient's phenotype with those of previously reported cases shows similarities in 10 out of 11 features which are present in more than 50% of the patients described[26] (Table 1). Our patient therefore helps to delineate further the partial trisomy 9q34.1 syndrome.

NPS Locus

The more important result of our analysis is that the proband's father has NPS and an apparently balanced t(9;17) (q34.1;q25). No other NPS patient with a chromosomal translocation has yet been reported. Previous linkage studies have placed the NPS locus to the 9q34 region suggesting that the translocation in our patient may have caused a break within or near the *NPS* gene, causing defective expression. Although intensive linkage analysis with informative recombination events place the *NPS* locus within a 1–2 cM interval between *D9S60* and the *AK1* gene, the gene itself has not yet been identified. Our FISH analysis maps the breakpoint region to a 17 cM interval, which includes the NPS critical region, being distal closer to the critical region (1.1 cM) than proximal (15.9 cM) (Figure 3). Further investigations of the breakpoint region, especially the search for a breakpoint spanning probe may expedite the identification of the gene locus for nail patella syndrome.

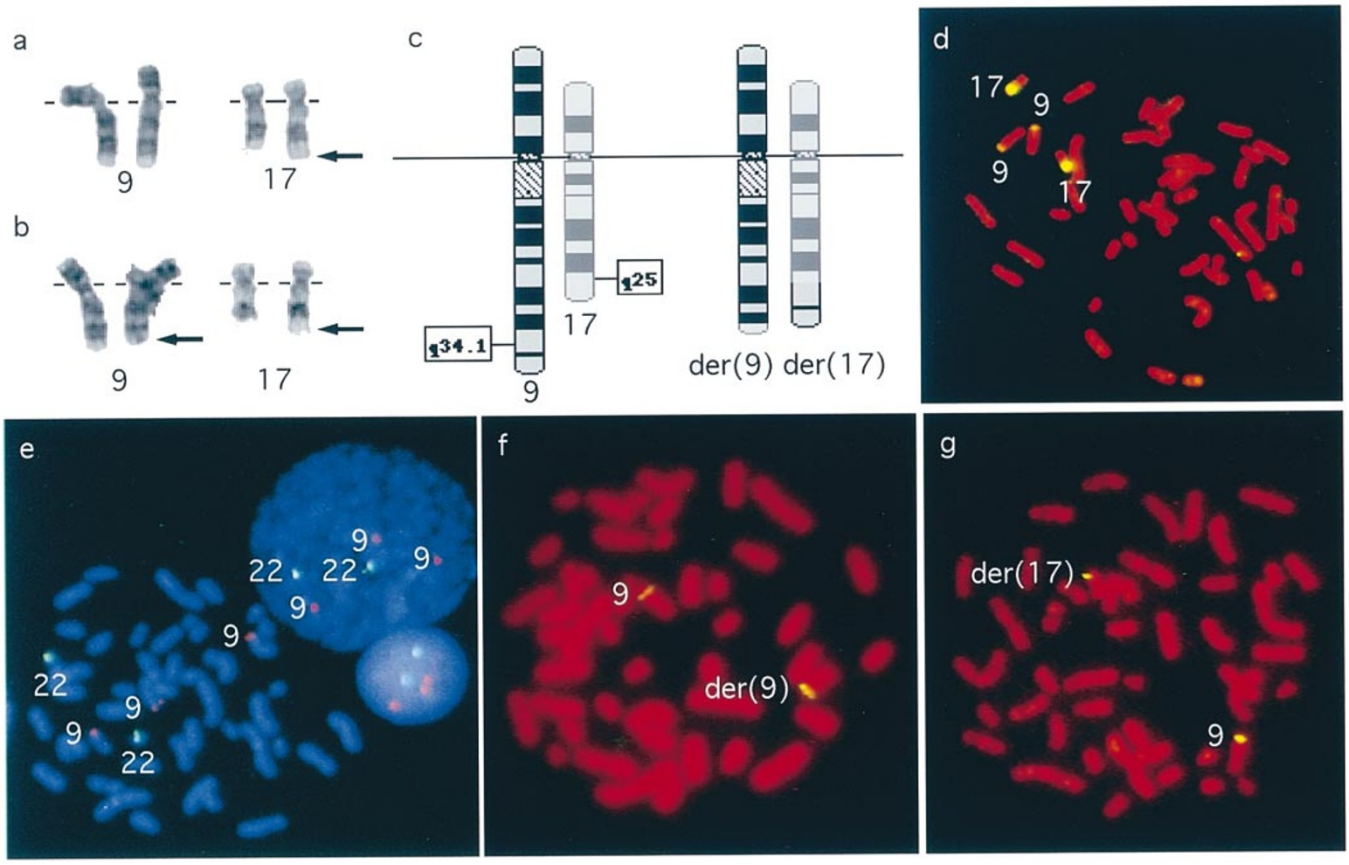

Figure 2 *Cytogenetic and molecular cytogenetic analysis of the proband and his father.*
(**a**) Partial karyotype of the boy with partial trisomy 9q34.1 showing normal chromosomes 9 and 17 and a derivative chromosome 17 (arrow); (**b**) Partial karyotype of the father with balanced t(9;17)(q34.1;q25) showing normal and derivative (arrows) chromosomes 9 and 17; (**c**) Idiogram of t(9;17)(q34.1;q25); (**d**) Reverse painting of a normal metaphase with microdissected add (17)(q25) material showing signals on both chromosomes 9 and both chromosomes 17; (**e**) Metaphase and interphase FISH analysis of the proband with the bcr/abl translocation probe showing three signals on 9q34.1 and two signals on chromosomes 22; (**f**) Hybridization results from YAC 933c5 to the NPS patient's metaphases showing a signal on the normal and the derivative chromosome 9; (**g**) Hybridization results from cos abl 8 to the NPS patient's metaphases showing a signal on normal chromosome 9 and derivative chromosome 17.

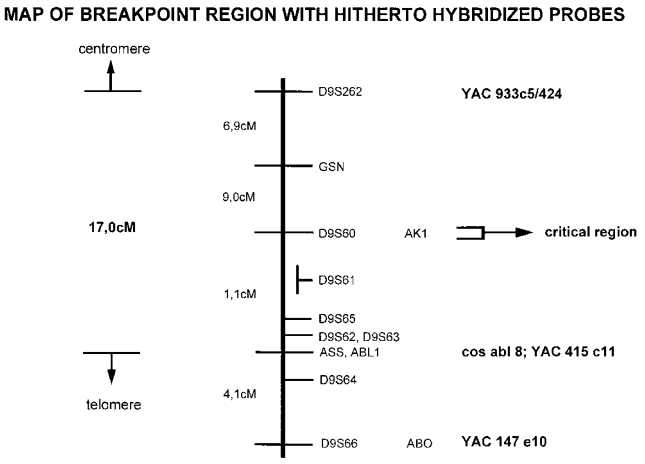

Figure 3 *Map of the breakpoint region[19,20,27] indicating the position of the hybridized probes (right)*

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