

Erwin Petek · Christian Windpassinger  
Burkhard Simma · Thomas Mueller  
Klaus Wagner · Peter M. Kroisel

## Molecular characterisation of a 15 Mb constitutional de novo interstitial deletion of chromosome 3p in a boy with developmental delay and congenital anomalies

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**Abstract** We describe the case of a 22-month-old boy with developmental and psychomotor retardation as well as craniofacial dysmorphism, including a cleft lip. Analysis of G-banded chromosomes of the propositus showed a de novo interstitial deletion of the short arm of chromosome 3, del(3)(p13p11). Fine mapping of the deletion was performed using fluorescence in situ hybridisation analysis with region-specific BAC clones. Eight BACs were absent from one chromosome 3 from the patient. Molecular analyses of eleven polymorphic DNA markers helped to narrow down the breakpoints and demonstrated that the derivative chromosome 3 is of paternal origin. The deleted segment encompasses about 15 Mb between marker D3S3551 and the centromere. Only a small number of known genes, including *PROK2*, *GPR27*, *RYBP*, *PPP4R2*, *ROBO1*, and *GBE1*, which map in the 3p13-p11 region are included in the deletion.

**Keywords** Chromosome 3p · Cleft lip · Developmental delay · Interstitial deletion · Mental retardation

1981; Hertz et al. 1988) and four further patients with deletions involving band 3p12 have been described in the literature (Pfeiffer et al. 1998; Wieczorek et al. 1997; Naritomi et al. 1988; Neri et al. 1984). Cytogenetic and clinical data are summarised by Schinzel (2001).

In these cases, the deletion has been characterised by the analysis of G-banded chromosomes. In the present study, we describe a male child with a de novo interstitial deletion del(3)(p13p11). We determined the extent of the deletion by high-resolution chromosome studies and fluorescence in situ hybridisation (FISH) with mapped BAC clones. To clarify the parental origin of the derivative chromosome 3, we also performed molecular analyses using microsatellite markers and compared the phenotypic features of the propositus with the patients described in previous studies. Using the genomic sequence between markers D3S3551 and the centromere, we have constructed a transcription map of the genomic interval deleted in our patient.

### Introduction

Interstitial deletions of the proximal short arm of chromosome 3 occurring as constitutional aberrations are infrequent and a defined clinical phenotype is not established yet.

So far, three patients with interstitial deletions involving band 3p11 (Crispino et al. 1995; Sichong et al.

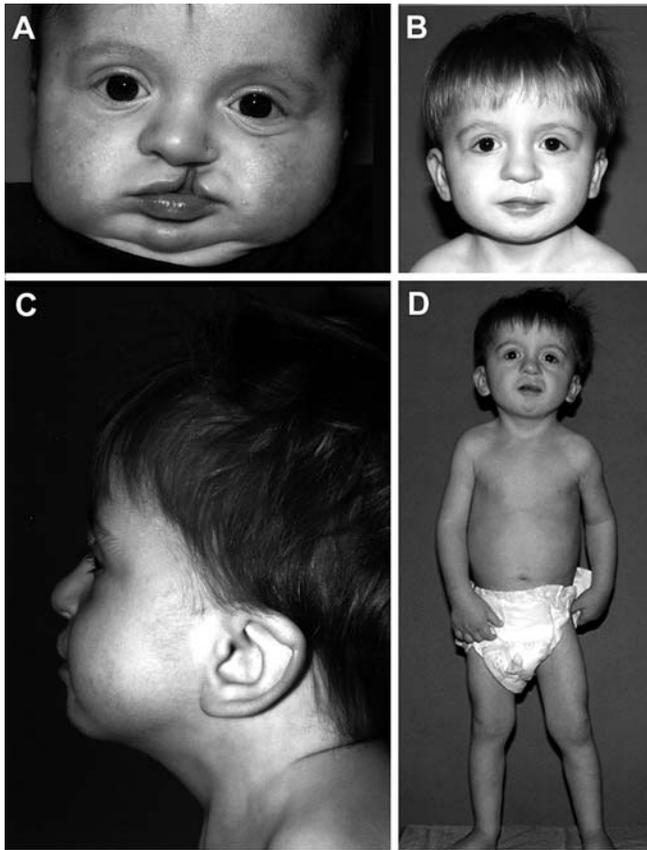
### Clinical report

The patient is a Bosnian boy born at 41 weeks of gestation, by spontaneous vaginal delivery. He is the second child of a 29-year-old mother and a 32-year-old father. Both parents were healthy and non-consanguineous. There is no family history of birth defects, mental or growth retardation, multiple miscarriages or infertility. No teratogenic exposures or maternal illnesses during pregnancy were reported. The pregnancy was uneventful.

Physical parameters at birth were weight 4,120 g (<90th percentile), body length 54 cm (90th percentile), and head circumference (OFC) 37 cm (<90th percentile). Apgar scores were 8/10/10 at 1/5/10 min after birth, respectively. Postnatal examination revealed an isolated lateral cleft lip, downslanting palpebral fissures, square facies with wide forehead, hypoplastic supra-orbital ridges, arched eyebrows, short nose, low-set posteriorly angulated ears, short neck, narrow shoulders, mild pectus carinatum, mild rhizomelia, and hypospadias

E. Petek (✉) · C. Windpassinger · K. Wagner · P. M. Kroisel  
Institute of Medical Biology and Human Genetics,  
Karl-Franzens University of Graz, Harrachgasse 21/8,  
A-8010 Graz, Austria  
E-mail: petek@kfunigraz.ac.at  
Tel.: +43-316-3804116  
Fax: +43-316-3809605

B. Simma · T. Mueller  
Department of Pediatrics, Academic Teaching Hospital,  
Feldkirch, Austria



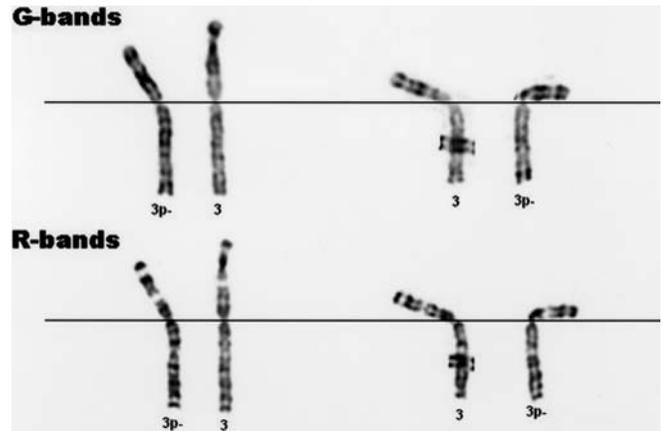
**Fig. 1** **A** Frontal view of the propositus at six months old (before surgical correction of the cleft lip). **B** Frontal (after surgical correction of the cleft lip), **C** lateral, and **D** total view of the propositus at an age of 22 months

(Fig. 1). A surgical correction of the cleft lip was performed at an age of 4 months.

Karyotype analysis of cultured peripheral blood lymphocytes revealed an interstitial deletion of the short arm of chromosome 3 [46,XY,del(3)(p13p11)]. Chromosome analysis of the parents showed a normal karyotype, indicating a de novo deletion in the patient.

Due to developmental retardation, a follow-up examination was performed at 16 months. His weight was 8,55 kg (<3rd percentile), length was 75,5 cm (3rd percentile), and OFC was 47 cm (10th–25th percentile). Psychomotor retardation, including aggressive behaviour, was becoming increasingly obvious. His language development was significantly delayed and consisted of babbling, although an occasional single word utterance was reported. He sat unsupported at 9 months and did not walk until the age of 18 months.

Additional diagnostic evaluations included echocardiography, which showed a patent foramen ovale due to an aneurysm of the atrial septum. On ophthalmologic examination, his left eye showed absent lacrimal puncta on the upper and lower eyelid. Brain MRI scan noted an enlargement of the subarachnoid space bilaterally particular in the frontal lobe. No further brain anomalies were present. Standard laboratory parameters at an age



**Fig. 2** Partial G- and R-banded high resolution karyotypes of the normal and the derivative chromosomes 3

of 16 months were in the normal range except a leukocytosis ( $2.48 \times 10^4/\mu\text{l}$ ) and lymphocytosis (77%) due to a viral infection since control investigation two months later revealed values entirely in the normal range.

#### Cytogenetic and molecular-cytogenetic analysis

Analysis of G-banded metaphase chromosomes from the propositus showed the presence of an interstitial deletion of the short arm of chromosome 3. On the basis of the G-banding pattern, the karyotype was determined as 46,XY,del(3)(p13p11) in 30 metaphases analysed (Fig. 2). FISH with a commercial painting probe for whole chromosome 3 (Vysis, Ill., USA) excluded a translocation of the deleted segment to another chromosome (data not shown).

In order to refine the extent of the 3p deletion and to define the breakpoints in more detail we hybridised digoxigenine-labelled DOP-PCR products of 13 bacterial artificial chromosomes (BACs) (RP11-9A1, RP11-24O17, RP11-154H23, RP11-648C16, RP11-522N9, RP11-20B7, RP11-550F7, RP11-314M24, RP11-539J20, RP11-450I19, RP11-8M11, RP11-81P15, RP11-21I16) from the 3p14-3q11 region to metaphase chromosomes of the proband. All probes were labelled by a modified DOP-PCR protocol (Petek et al. 1997). FISH was performed according to standard protocols. The results of these hybridisations are summarised with additional information on the position of these BACs on chromosome 3 according to the November 2002 freeze of the Genome Browser of the UC Santa Cruz (UCSC database; <http://genome.ucsc.edu/>) (Table 1). After co-hybridisation of BAC clone RP11-154H23 and the differentially labelled BAC RP11-648C16, only BAC RP11-154H23 hybridised to both chromosomes 3 of the propositus, whereas BAC RP11-648C16 did not give a hybridisation signal on the deleted chromosome (Fig. 3). Therefore, the distal breakpoint can be allocated to the

**Table 1** Summary of the FISH results with BAC clones to determine the deletion extent on chromosome 3p

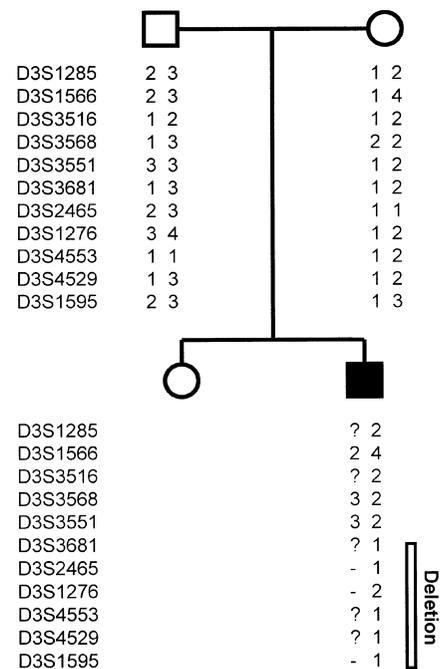
BAC clone	GenBank accession no.	Markers anchored to the BAC probes used for FISH	Relative position in bp according to the human genome assembly, Build 31 <sup>a</sup>	Chromosomal band on chromosome 3	FISH signal present (+) or absent (-) on the deleted chromosome 3
RP11-9A1	AC103557	D3S3635	63626317-63785382	p14.1	+
RP11-24O17	AC098969	D3S1217	66247684-66406243	p14.1	+
RP11-154H23	AC097634	D3S3516	71153572-71347124	p13	+
RP11-648C16	AC107623	D3S1434	71497390-71674290	p13	-
RP11-522N9	AC104330	WI-6148	71984508-72162726	p13	-
RP11-20B7	AC108702	D3S3039	73386298-73557403	p13	-
RP11-550F7	AC026877	D3S2539	76074613-76273436	p12.3	-
RP11-314M24	AC108752	SHGC-82374	77943992-78111744	p12.3	-
RP11-539J20	AC123565	D3S2509	78786539-78942701	p12.3	-
RP11-450I19	AC106726	D3S3508	79790866-79991224	p12.2	-
RP11-8M11	B72415	D3S1595	85764059-85862269	p11.2	-
Centromere			86596812-89696811		
RP11-81P15	AC108709	D3S3671	89743056-89922278	q11.1	+
RP11-21I16	AC016955	SHGC-62945	93564191-93733533	q11.2	+

<sup>a</sup>UCSC Human Genome Project Working Draft, November 2002 assembly (hg13)



**Fig. 3** Two-colour FISH, using BAC clones RP11-154H23 [green one signal on normal chromosome 3 and one signal on der(3)] and RP11-648C16 [red only one signal on normal chromosome 3], both mapped to chromosome 3p13

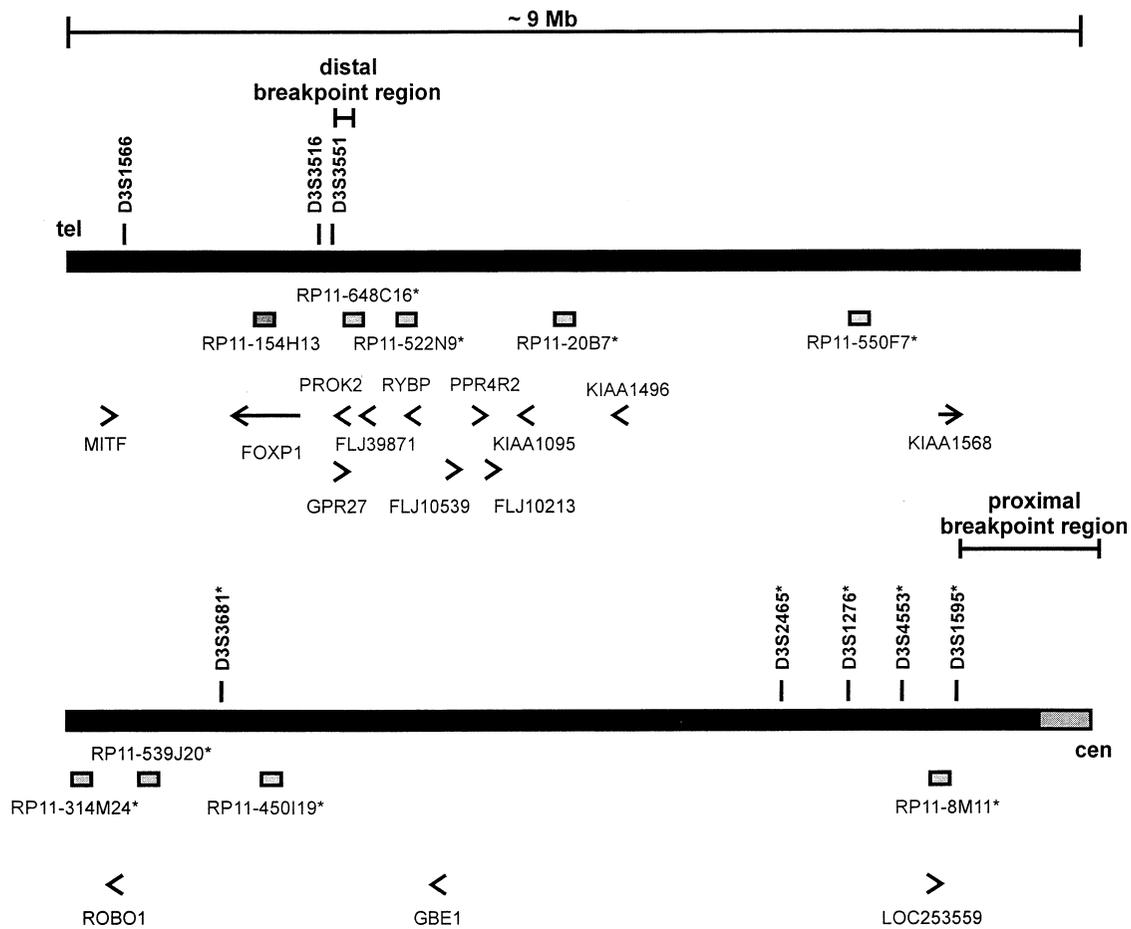
150-kb region between both BACs or the ends of the BAC clones. Co-hybridisation of BAC RP11-8M11 and the centromeric BAC RP11-81P15 helped to narrow down the proximal breakpoint of the deletion. BAC RP11-81P15 hybridised to both the normal and the deleted chromosome 3, but BAC RP11-8M11 was present only on the normal chromosome 3 and deleted from the del(3) chromosome (data not shown). Therefore, the proximal breakpoint can be allocated to the region between both BACs. Our FISH results demonstrate that the deletion on chromosome 3 spans the region flanked by BACs RP11-154H23 and the centromeric BAC clone RP11-81P15.



**Fig. 4** Haplotypes with 11 polymorphic markers from 3p encompassing the del(3p) critical region are indicated below each investigated individual in the family. Microsatellite analysis was not performed on the healthy sister of the patient. A dark square indicates the carrier of the deletion. Markers D3S1285, D3S3516, D3S3681, D3S4553 and D3S4529 were uninformative

### Molecular analysis

Genotyping of the proband and his parents was performed with chromosome sequence tagged site (STS) microsatellite markers D3S1285, D3S1566, D3S3516, D3S3568, D3S3551, D3S3681, D3S2465, D3S1276, D3S4553, D3S4529, and D3S1595 by PCR-amplification using dye-labelled primers. Genomic DNA for



**Fig. 5** Transcription map of the genomic interval between marker D3S1566 and the centromere on human chromosome 3p. The proximal and distal breakpoint region of the deleted interval in our patient is shown. Genes are indicated by *arrows* and *arrowheads*. Markers and BAC clones deleted on the der(3) in our patient are indicated by an *asterisk*

microsatellite analysis was extracted from peripheral blood lymphocytes using standard techniques (Sambrook et al. 1989). The amplified products were separated and analysed on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Calif., USA).

The results indicated that the deletion of chromosome 3p in this patient was on the chromosome inherited from the father. STS markers D3S2465, D3S1276, and D3S1595, all three within the region of the deletion, showed one maternal allele (Fig. 4). Markers D3S1285, D3S3516, D3S3681, D3S4553 and D3S4529 were uninformative. According to the map of markers in the UCSC database, the deletion is flanked by marker D3S3551 and the centromere.

## Discussion

The seven previously reported cases of proximal interstitial deletions including chromosome bands 3p12-p11

have only been determined by G-banding of metaphase chromosomes. Here we report the molecular characterisation of a male infant with multiple congenital anomalies and a de novo interstitial deletion on the short arm of chromosome 3, del(3)(p13p11). DNA studies have determined that the deletion is paternal in origin. To our knowledge no other patient with an identical interstitial deletion has been reported. Among the reported cases of chromosome 3p deletions, only three had deletions proximal to 3p11.2 (Crispino et al. 1995; Sichong et al. 1981; Hertz et al. 1988). In all of the three reports the distal breakpoint was assigned to chromosome band 3p14. We compared the phenotype of these patients with that of our patient. Some of the previously described patients with interstitial 3p deletions had a number of clinical symptoms in common with those of our patient, like psychomotor retardation, dysmorphic facial features, and dysplastic, low-set ears. A cleft lip was only found in the present case. Diverse cardiovascular and brain anomalies were also observed in almost all of these cases. In general the clinical phenotype in our patient was less severe. Several of these clinical manifestations are unspecific and can also be observed in other chromosomal unbalances or aneuploidy syndromes. In addition, the different age of the patients and the divergent extent of the deleted segment make it difficult to delineate a proximal 3p phenotype.

To identify clinical features with a genetic basis or genetic component mapped within the deleted region in 3p we constructed an annotated gene map of the interval D3S3551–centromere. The November 2002 freeze of the Genome Browser of the UC Santa Cruz revealed nine ‘reference sequence genes (known genes)’ in the genomic region of the deletion (*PROK2*, *GPR27*, *RYBP*, *FLJ10539*, *PPP4R2*, *FLJ10213*, *ROBO1*, *GBE1* and *LOC253559*). Mutations responsible for dominant inherited clinical features are not reported for these genes. By a combination of EST database searches and *in silico* detection of UniGene clusters within genomic sequence generated from this template map, we have mapped four additional transcripts (*FLJ39871*, *KIAA1095*, *KI AAI496*, and *KIAA1568*) within this interval (Fig. 5). Many of these are novel or hypothetical proteins and further investigation is needed to determine if any of these sequences are actively expressed and their potential contribution to the phenotype of our 3p deletion patient. Beside these transcripts, the large number of spliced expressed sequence tags (ESTs) in this region will probably lead to the identification of additional genes not yet characterised. As in other clinical cytogenetic syndromes, further molecular studies on patients with similar or overlapping deletions or rearrangements in the region, as well as functional gene maps will be necessary to delineate genotype/phenotype correlation.

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