ELECTRONIC LETTER

Molecular characterisation of a 3.5 Mb interstitial 14q deletion in a child with several phenotypic anomalies

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J Med Genet 2003;**40**:e47(http://www.jmedgenet.com/cgi/content/full/40/4/e47)

Interstitial deletions of the long arm of chromosome 14 are infrequent. Molecular and clinical studies on patients with deletions involving 14q11.2-q21 have recently been reported.[1] Most of these deletion patients share common clinical signs, such as midline defects of the central nervous system, feeding problems, growth abnormalities, hypotonia, developmental delay, mental retardation, and craniofacial anomalies.[1] Here we present the phenotypic, cytogenetic, and molecular genetic findings of a 2½ year old boy with a 14q12-q13.1 deletion. To our knowledge this is the second case described of a patient with a deletion of less than 3.5 Mb within chromosome bands 14q12-q13.1. Using the genomic sequence between markers D14S1060 and D14S286, we have constructed a transcription map of the genomic interval deleted in our patient.

Key points

- By using region specific BAC clones for FISH analysis and polymorphic markers, we characterised a 3.5 Mb interstitial 14q12-q13.1 deletion in a 2½ year old boy with microcephaly, psychomotor retardation, bilateral optic atrophy, hypodontia, and several minor dysmorphic features. Marker analyses showed that the derivative chromosome 14 is of maternal origin.
- We have also generated a transcription map of the contiguous genomic interval deleted in our patient, linking the regions near markers D14S1060 and D14S286.
- Several known genes, including *TITF1*, *PAX9*, and *MIPOL1*, were identified in this genomic segment and are thus potential candidates for some of the phenotypic features observed in our patient.

CASE REPORT

Our proband (fig 1) is the 40 week product of a second, uncomplicated pregnancy and delivery. Maternal age was 27 years. Birth weight was 4435 g (>90th centile), length 55 cm (>90th centile), and head circumference 34 cm (10th-25th centile). Apgar score was 7/9/10. The neonatal period was complicated by pneumonia, treated with parenteral antibiotic therapy over 10 days. Besides relative microcephaly, hypertelorism, epicanthic folds, a long and flat philtrum, hypodontia, laterally placed, hypoplastic mamillae, second degree hypospadias, bifid scrotum, and bilateral cryptorchidism were noticed. Cranial ultrasound was normal; ultrasound of the kidneys and pelvic region showed bilateral second degree hydronephrosis, and both testes were visible in the inguinal region. Screening for connatal infections (toxoplasmosis, rubella, cytomegalovirus, and parvovirus B19) was negative. Psychomotor development was severely impaired from early infancy. Lack of vision was evident at 3 months and, apart from head control, no gain of motor milestones or social contact was achieved until the age of 29 months. Muscle tone of the trunk was decreased while it was increased and dystonic in the upper and lower extremities with exaggerated deep tendon reflexes.

During the second year of life, high arched, bushy eyebrows and plantar hyperkeratosis developed. Weight (11.2 kg) and length (89 cm) were on the 3rd and 10th-25th centile at the age of 29 months, respectively, while the head circumference, 43.9 cm, was far below the 3rd centile (fig 1). Fundoscopy at the age of 4 months showed bilateral optic atrophy. Normal bilateral hearing was assessed using a commercial infant hearing screening device (ALGO, Natus Medical, Foster City, CA). Cranial MRI at the age of 9 months showed no signs of cerebral malformation but did show reduced brain mass and bilaterally dilated ventricles and widened subarachnoidal spaces. Myelination was moderately delayed for his age. EEG, performed at the age of 23 months, showed low voltage (20-100 μV), mildly slowed background activity without epileptic discharges.

RESULTS AND DISCUSSION

G banded metaphase chromosomes from lymphocyte culture of the patient showed an apparent de novo interstitial deletion on the long arm of chromosome 14 in 50 metaphases analysed. His karyotype was interpreted as 46,XX, del(14)(q12q13.1) (fig 2). To exclude translocation of the deleted segment to another chromosome, we performed fluorescence in situ hybridisation (FISH) with a commercial painting probe for chromosome 14 (VYSIS Inc, Illinois, USA; data not shown).

In order to define the breakpoints of the deletion on the long arm of chromosome 14 in more detail, we hybridised digoxigenin labelled DOP-PCR products of eight bacterial artificial chromosomes (BACs RP11-1078I14 (GenBank accession No AL161851), RP11-1007I4 (AL358340), RP11-26M6 (B84609, B84610), RP11-81F13 (AL162464), RP11-1149E6 (AQ749750, AQ749839), RP11-410J3 (AQ549717, AQ535528), RP11-356O6 (AL121790), RP11-91H1 (AQ283374, AQ283377)) to metaphase spreads of the patient.[2] Of eight BAC clones analysed, BAC clones RP11-26M6 and RP11-81F13 showed signals on only one copy of chromosome 14q (fig 3A). The signal for the remaining six BAC clones was detected on both copies of chromosome 14q (fig 3B).

Genomic DNA was extracted from peripheral blood lymphocytes using standard techniques.[3] Genotyping of the proband and his parents was performed with chromosome sequence tagged site (STS) microsatellite markers D14S1041, D14S275, D14S80, D14S1042, D14S262, D14S1021, D14S1060, D14S70, D14S1049, D14S988, D14S253, D14S1017, D14S69, D14S75, D14S286, and D14S1039 by PCR amplification. This analysis showed that the deletion of chromosome 14q in this patient was maternal in origin. STS markers D14S70, D14S1049, D14S988, D14S253, D14S1017, D14S69, and D14S75, all six within the region of the deletion, showed one

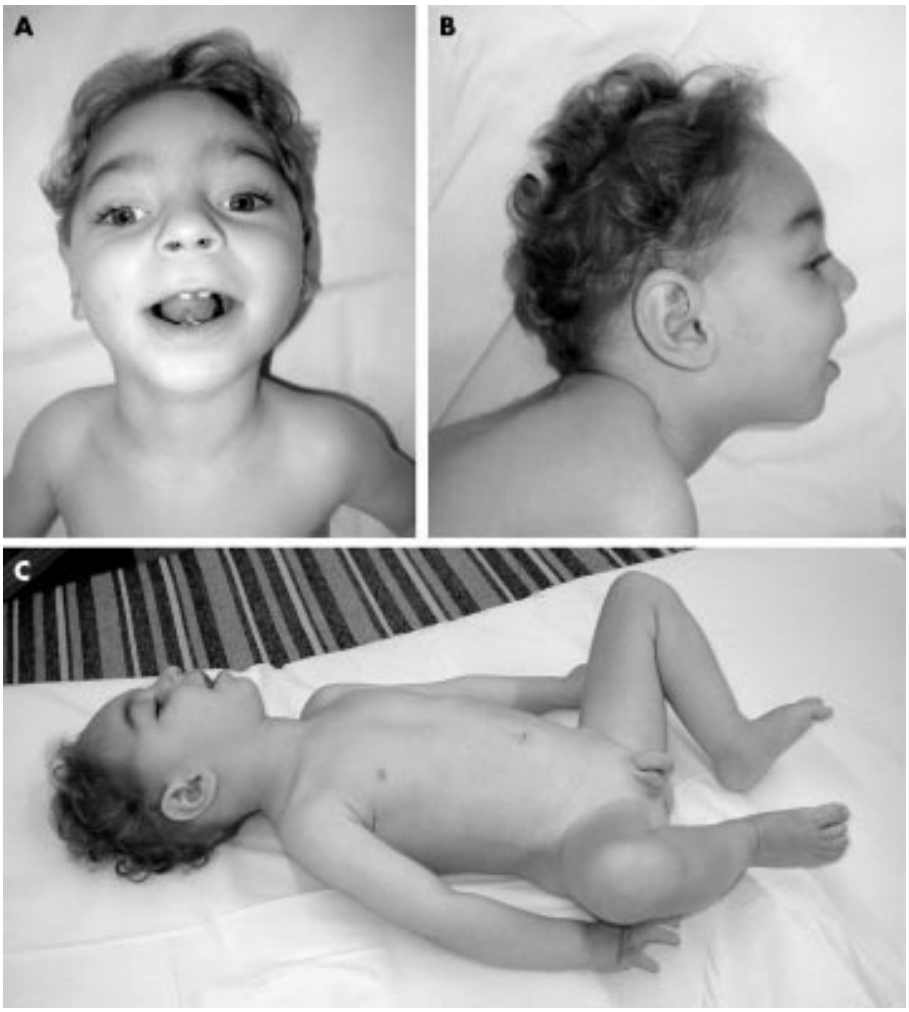

Figure 1 (A) Frontal, (B) lateral, and (C) total view of the proband.

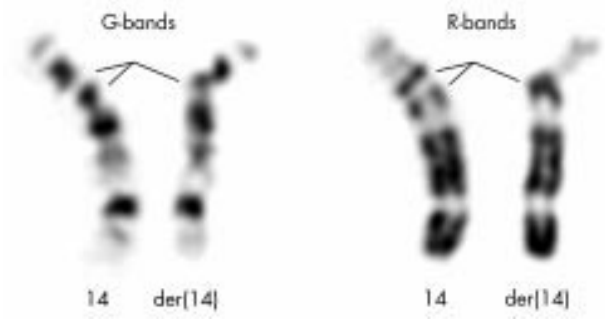

Figure 2 Partial G and R banded high resolution karyotype of the normal and the derivative chromosomes 14.

paternal allele but no maternal allele (data not shown). Markers D14S1041 and D14S1039 were uninformative.

To identify clinical features with a genetic basis or genetic component mapped within the deleted region in 14q we constructed an annotated gene map of the interval D14S1060-D14S286. The April 2002 freeze of the Genome Browser of the UC Santa Cruz (http://genome.ucsc.edu/)[4] showed 20 "Reference Sequence genes (known genes)" in the genomic region of the deletion that corresponds to mouse chromosome 12. 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 five additional transcripts (PSMA6, DKFZp566D133, KIAA0884, MIPOL1, and LOC115669) within this interval (table 1, fig 4). 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 14q deletion patient. Besides 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.

Mutations responsible for dominant inherited clinical features are reported for *PAX9* (hypodontia, OMIM 106600),[5] *MIPLO1* (mirror image polydactyly of hands and feet, OMIM 135750),[6] and *TITF1* (benign hereditary chorea, OMIM 118700).[7] Whereas hypodontia can also be found in our patient, no hand and foot abnormality was diagnosed in the present case or in the previously published 14q deletion patients.[1] Gene mutations and breakpoint junctions, however, cannot always be used to explain affected phenotypes in deletion cases. In addition, *MIPLO1* is located several kbs distal to the deleted segment in our patient. Haploinsufficiency for the *MIPOL1* gene was suspected to be responsible for limb anomalies owing to its disruption in a t(2;14) translocation case.[6] Other genes close to the breakpoints on chromosomes 2 and 14, like the death box gene *DDX1* (GenBank accession No X70649) and the neuroblastoma amplified protein gene *NAG* (GenBank accession No NM015909), both located proximal to the breakpoint on 2p23.3, and the *PAX9* gene on 14q13 cannot formally be excluded. The locus on 14q13 is supported by an additional translocation patient with mesomelic bone dysplasia (case 9 of Kamnasaran *et al*[1]).

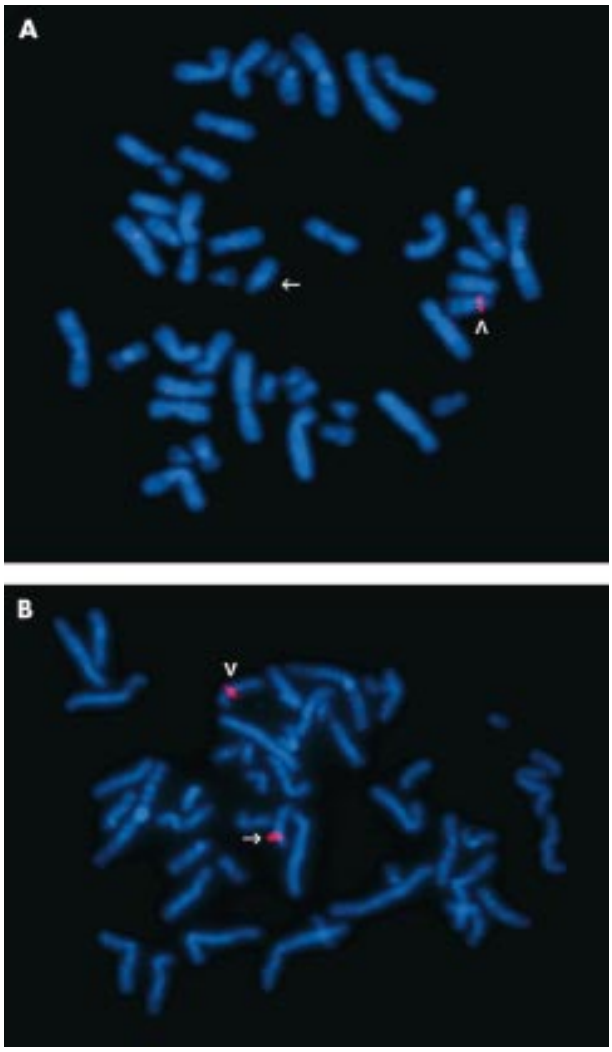

Figure 3 Fluorescence in situ hybridisation using digoxigenin labelled DOP-PCR products of (A) BAC RP11-26M6 (only one signal on normal chromosome 14), (B) BAC RP11-1007I4 (one signal on normal chromosome 14 and one signal on der(14)). The normal chromosome 14 is indicated by an arrowhead and the der(14) is indicated by an arrow.

Psychomotor retardation and microcephaly are common characteristics in both our case and the reported cases with larger 14q deletions.[1] Autistic-like behaviour and visual problems, such as cortical blindness, have also been reported in most of them. In order to identify potential candidate genes, we were especially interested in genes that may play a role in neurodevelopment. Recently, Breedveld *et al*[7] identified mutations in the gene encoding the thyroid transcription factor 1 protein leading to benign hereditary chorea (BHC). Knockout studies showed that this gene is involved in the differentiation of the striatum in the developing brain.[8] In one of their BHC families, two subjects in two subsequent generations could be identified as carriers of a 1.2 Mb microdeletion harbouring five transcripts (*MBIP*, *TITF1*, *NKX2.8*, *PAX9*, and *SLC25A21*; table 1, fig 4). Since patients with this microdeletion, which represents the telomeric segment of the deletion described here, do not show microcephaly, it is possible to conclude that the putative locus for this form of microcephaly maps more proximally. In this region, genes expressed in the developing brain such as genes from the KIAA library are especially promising candidates. However, variable expressivity of genes within the 1.2 Mb microdeletion[7] cannot formally be excluded as a cause of microcephaly.

In conclusion, we have reported a patient with an interstitial 14q deletion of less than 3.5 Mb and generated a contiguous transcription map of this region. In addition, we were able to identify candidate genes for the features found in our patient that warrant further functional investigations.

ACKNOWLEDGEMENTS

We thank the family for all their help in this study. This research was supported by grant 9522 from the Oesterreichischen Nationalbank to EP.

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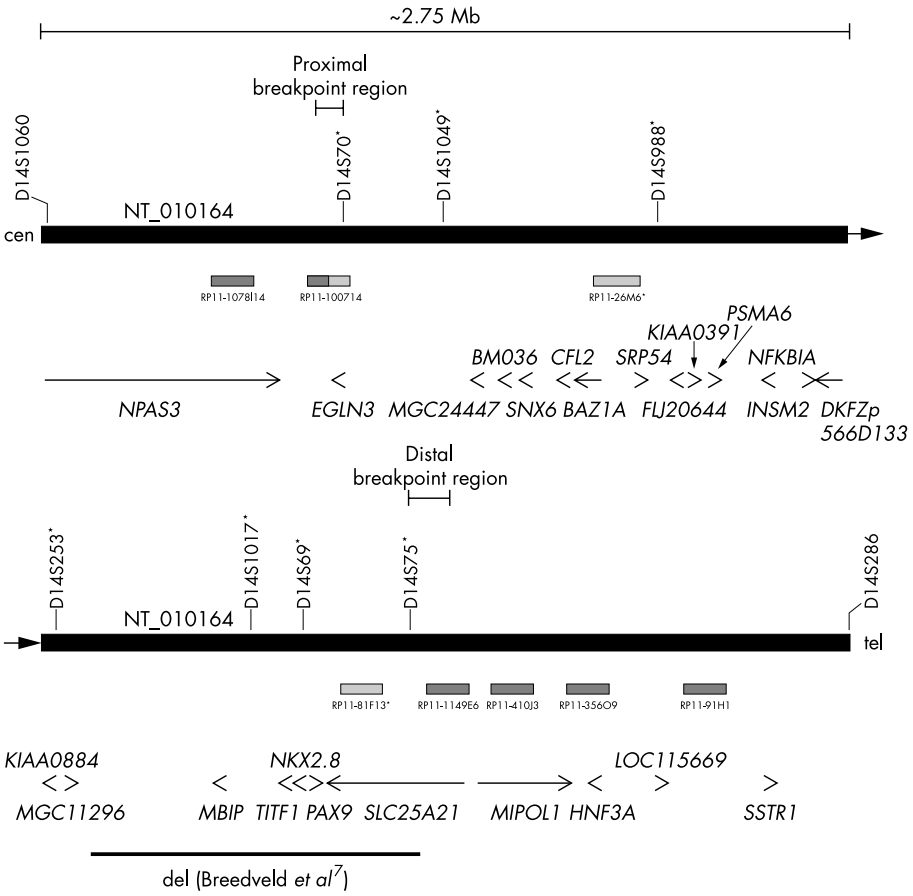

Figure 4 Transcription map of the genomic interval between markers D14S1060 and D14S286 on human chromosome 14q. The proximal and distal breakpoint region of the der(14) in our patient is shown. The deleted interval reported by Breedveld *et al*[7] is also shown. Genes are indicated by arrows or arrowheads. Markers and BAC clones deleted on the der(14) in our patient are indicated by an asterisk.

Table 1 Genes mapped to the interval between markers D14S1060 and D14S286

Gene/cDNA*†	Gene function	Locus ID‡	Accession No
NPAS3	Basic helix-loop-helix PAS protein	64067	NM_022123
EGLN3	Implicated in the regulation of growth, differentiation and apoptosis, in muscle and nerve cells	112399	NM_022073
MGC24447	Function unknown	171546	NM_138288
BM036	Uuncharacterised bone marrow protein	55837	NM_018453
SNX6	Interacts with members of the transforming growth factor-beta family of receptor serine-threonine kinases	58533	NM_021249
CFL2	Actin binding protein which is involved in the translocation of actin-cofilin complex from cytoplasm to nucleus	1073	NM_021914
BAZ1A	May act as a chromatin mediated transcriptional regulator	11177	NM_013448
SRP54	Signal recognition particle subunit	6729	NM_003136
FLJ20644	Function unknown	55012	NM_017917
KIAA0391	Function unknown	9692	NM_014672
PSMA6	Proteasome subunit	5687	XM_046642
NFKBIA	Inhibits NFkB activity by binding the rel domains of NF-kB components	4792	NM_020529
INSM2	Insulinoma associated protein	84684	NM_032594
DKFZp566D133	Function unknown	26134	XM_050005
KIAA0884	Function unknown	23000	XM_046660
MGC11296	Function unknown	84312	NM_032352
MBIP	Interacts with MUK/DLK/ZPK and inhibits its activity to induce JNK/SAPK activation	51562	NM_016586
TITF1	Involved in differentiation of the striatum and the developing brain	7080	NM_003317
NKX2.8	Transcription factor	26257	NM_014360
PAX9	Involved in development of stratified squamous epithelia as well as various organs and skeletal elements	5083	NM_006194
SLC25A21	Mitochondrial oxodicarboxylate carrier	89874	NM_030631
MIPOL1	Function unknown	145282	XM_085077
HNF3A	Transcriptional activator for liver specific transcripts	3169	NM_004496
LOC115669	Function unknown	115669	XM_056434
SSTR1	Somatostatin receptor	6751	NM_001049

*Genes are listed from centromere to telomere.
†A deletion in this patient lies between *EGLN3* and *SLC25A21*.
‡NCBI LocusLink (http://www.ncbi.nlm.nih.gov/LocusLink/).