European Journal of Human Genetics (1998) 6, 187–193

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ORIGINAL PAPER

Detection of an insertion deletion of region 8q13-q21.2 in a patient with Duane syndrome: implications for mapping and cloning a Duane gene

G Calabrese[1], L Stuppia[1], E Morizio[1], P Guanciali Franchi[1], F Pompetti[1], R Mingarelli[2,3], T Marsilio[4], M Rocchi[5], PE Gallenga[4], G Palka[1] and B Dallapiccola[2,3]

[1]*Cattedra di Genetica and*[4]*Cattedra di Oftalmologia, Università degli Studi 'G D'Annunzio', Chieti*
[2]*Mendel-CSS IRCSS, Rome*
[3]*Cattedra di Genetica Medica, Università Tor Vergata, Rome*
[5]*Istituto di Genetica, Università di Bari, Italy*

Duane syndrome (MIM126800) is an autosomal dominant disease responsible for 1% of all strabismus cases and has been related to a 8q12–13 contiguous gene syndrome. We report on an insertion of chromosome region 8q13–q21.2 on to band 6q25 in a patient presenting with Duane syndrome, mental retardation, and other dysmorphisms. FISH analysis using chromosome 8 radiation hybrid LIA2L indicated a concurrent deletion within the 8q rearranged region. These results were corroborated by STR-PCR analysis and FISH using YAC contig WC8.8 disclosed a deletion in 8q13. Comparison of the two known patients with Duane syndrome associated with deletion of 8q identifies a small region of overlap (SRO) of < 3 cM extending from D8S533 and D8S1767 in which a Duane syndrome locus is assigned. In addition YAC analysis in our patient showed that 8q rearrangement was rather complex since 8q deletion and insertion occurred in two distinct segments separated by a region which maintained its location on 8q.

Keywords: chromosome 8; deletion; Duane syndrome; mapping; complex rearrangement

Introduction

Duane syndrome is a primary form of strabismus consisting of an ocular motility defect with absent or severely limited abduction and variable limitation of adduction, associated with bilateral globe retraction and narrowing palpebral fissure.[1] This autosomal dominant (MIM 126800) disorder is responsible for approximately 1% of all strabismus cases. Duane syndrome has been clinically subclassified by Huber in three forms which cause deficiency of binocular sight due to altered ocular motility, eventually associated with nistagmus and other ocular anomalies. The pathogenesis of this syndrome is unknown but, in some patients at least, a muscular/neuronal origin has been proved.[2–3]

Correspondence: Giandomenico Palka, Via B Buozzi 93-65121 Pescara, Italy. Tel: +39 871 355 4137; Fax: +39 871 355 4135; E-mail: gdpalka@unich.it.
Received 11 July 1997; revised 16 September 1997; accepted 17 November 1997

Although Duane syndrome is considered a clinically distinct condition, genetic heterogeneity has been suspected. In fact a few atypical cases of Duane syndrome have been described in association with deafness, renal defects, muscular, and skeletal anomalies[4–7] and chromosome imbalances.[7–10] In particular a contiguous gene syndrome consisting of Duane syndrome, branchio-oto-renal syndrome (BOR), hydrocephalus and trapeze aplasia was found in a patient with 8q12.2–q21.2 deletion.[7] We report on an insertion deletion of chromosome 8q13–q21.2 in a patient presenting with Duane syndrome type I, severe mental retardation, and minor limbs abnormalities. Molecular studies were carried out in order to analyse and map this Duane syndrome containing region.

Materials and Methods

Patient

A 7-year-old girl, the first child of unrelated parents, was referred for cytogenetic analysis on the basis of severe mental retardation and dysmorphisms. Family history was unremarkable. At birth her length was 48 cm and weight 3250 g. At 7 years of age, she was 119 cm in height (5th centile) and weighted 25 kg (65th centile). On clinical evaluation she presented with microcephaly, bilateral deficiency of ocular abduction, impairment of adduction, bilateral globe retraction and narrowing of interpalpebral fissure in adduction (Duane syndrome type I according to Huber's classification)[2] prominent nasal root, anteverted nares, and micrognathia (Figure 1). Ears were normally implanted and no preauricular pit, tag and pinna malformation were observed. The patient also had short neck, brachydactyly and left club foot. Mental retardation was severe. Routine laboratory investigations, skull X-ray, echocardiography, renal echography, and audiometry were normal. An MRI revealed enlargement of lateral ventricle cornua.

Figure 1 *Proband phenotypes.*

Cytogenetic Analysis and Fluorescence in situ *Hybridisation*

Chromosome studies were carried out on PHA-stimulated peripheral blood lymphocytes using GTG banding technique.[11]

FISH analysis was performed according to Calabrese *et al*.[12] Biotinylated and FITC-labelled chromosomes 6 and 8 painting probes (Cambio-Bouty, Milan, Italy), radiation hybrids LIA2L, HY95L and HY95A1 for region 8q11–q21,[13] and YAC clones from contig WC8.8 (Whitehead Inst. MIT, USA[14]) were used. DNA from hybrids was Alu-PCR amplified with BK33 primers.[15] Hybrid PCR products and YAC DNA were biotin and/or digoxigenin labelled by nick translation (Life Technologies, Gaithersburg MD, USA) prior to hybridisation.

Genotyping

Sixteen polymorphic sequence tagged sites (STS) mapping at 8q11.2–q21 were retrieved from the CEPH/Généthon and Whitehead Institute databases[16] and tested on DNA samples from the patient and her parents by PCR according to recommended protocols. PCR products were run on polyacrilamide or Metaphor XR (FMC, Rockland ME, USA) gels. A deletion in the patient was considered when three PCR experiments failed to amplify one parental allele.

Results

Cytogenetic and FISH Analyses

GTG banding in the proband revealed an insertion of segment 8q11.2□ q13 on to 6q25 (Figure 2), whilst the parents had normal karyotypes.

FISH experiments with chromosome 6 and 8 specific paintings confirmed chromosome 8q insertion on to chromosome 6q25 (Figure 3a). In order to refine the

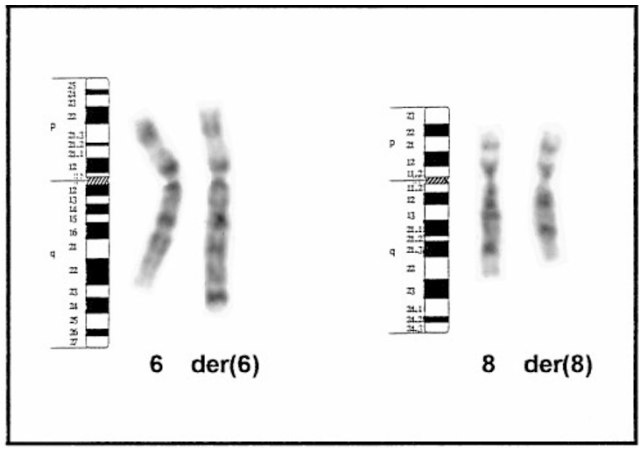


Figure 2 *Partial karyotype of Duane patient showing an insertion of segment 8q11.2–q13 on to chromosome 6q25.*

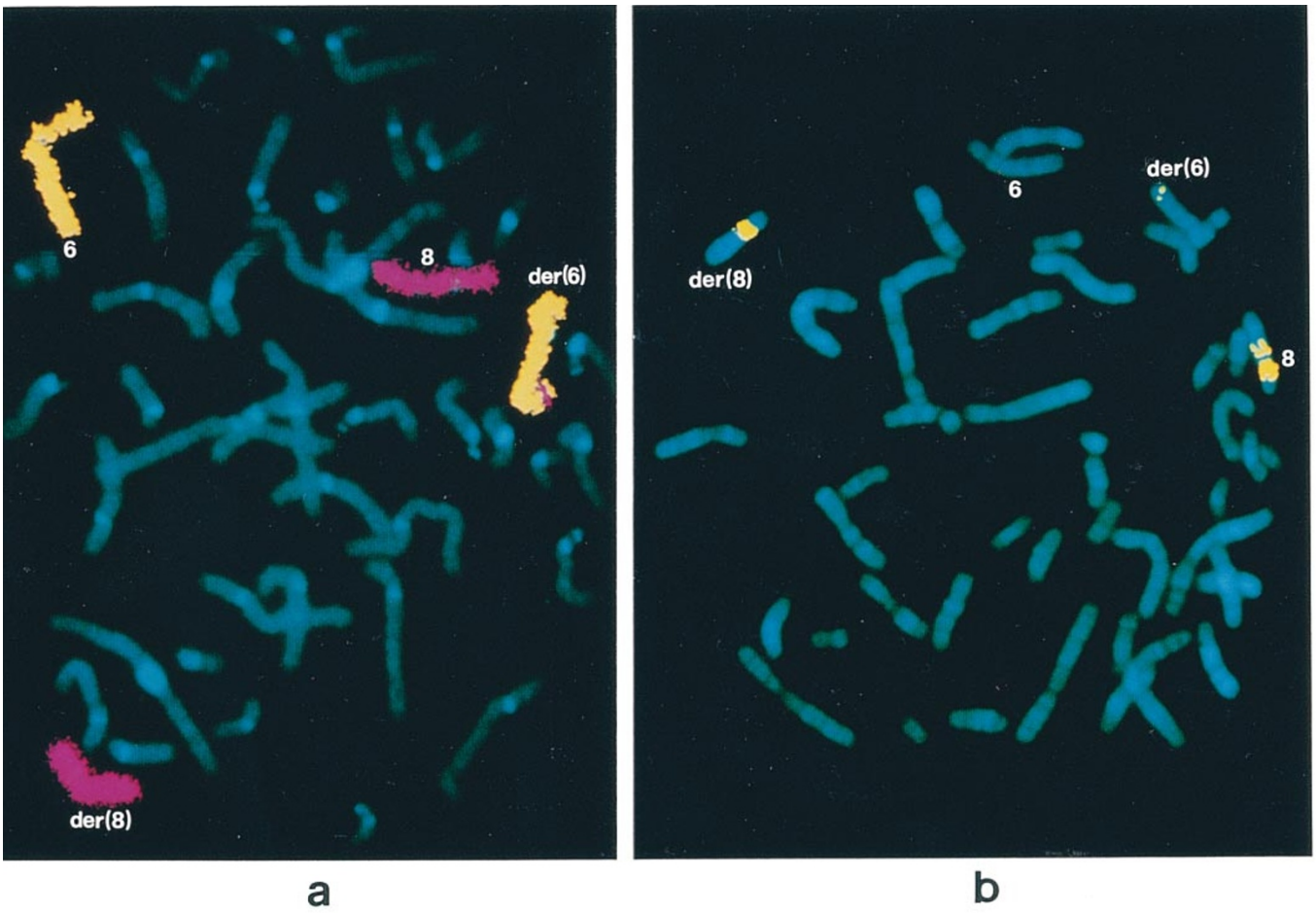


Figure 3 *(**a**) FISH with painting probes for chromosome 6 (yellow signal) and chromosome 8 (red signal) showing insertion of material of chromosome 8 at band 6q25; (**b**) FISH with radiation hybrid LIA2L. Chromosome 8q material on proximal q arm in der(8) and inserted in der(6) is fewer than that on normal chromosome 8 indicating that a deletion has occurred along with the insertion on to 6q25.*

chromosome 8 breakpoints, radiation hybrids LIA2L, HY95L and HY95A1 were tested (Figure 4 and Figure 5). Hybrids HY95L and HY95A1 showed an apparent normal painting of der(8), while LIA2L was translocated onto 6q25, a small signal being present onto der(6) (Figure 3b). These results relocated the 8q breakpoints at 8q13 and 8q21.2 and suggested a concurrent deletion within this 8q region.

Genotyping

Markers D8S601, D8S1763, D8S510, D8S1841, D8S1840, D8S533, D8S543, D8S279, and D8S286 were informative in this family (Figure 4). Analysis of the polymorphic loci in the proband showed paternal allele loss of markers D8S1841, D8S1840 and D8S533. These results were corroborated by STS analysis content of the three radiation hybrids. As expected, only the translocated and deleted LIA2L hybrid was found positive for the deleted markers between D8S1841 and D8S533.

Identification of YACS in the 8q Rearranged Region

Eighteen YAC clones were selected from the YAC contig WC8.8 of the CEPH Mega-YAC Library based on the above STS/hybrid results. These clones were tested by FISH in the proband and in two unrelated controls (Table 1). Apparently all clones, except 850G4, were not chimeric and disclosed a unique signal at 8q13–21.1 in the normal chromosome 8 in the patient. YAC clone 850G4 showed signals at 8q13 and 8q23 (Figure 5a). FISH analysis using the most centromeric clones 799C11, 814A6, 937E2 and 751A2 revealed signals in der(8), 937E2 and 751A2 disclosing a faint signal. Clones 942E7 to 820E6 disclosed signals in normal chromosome 8, and no signal in der(8), while

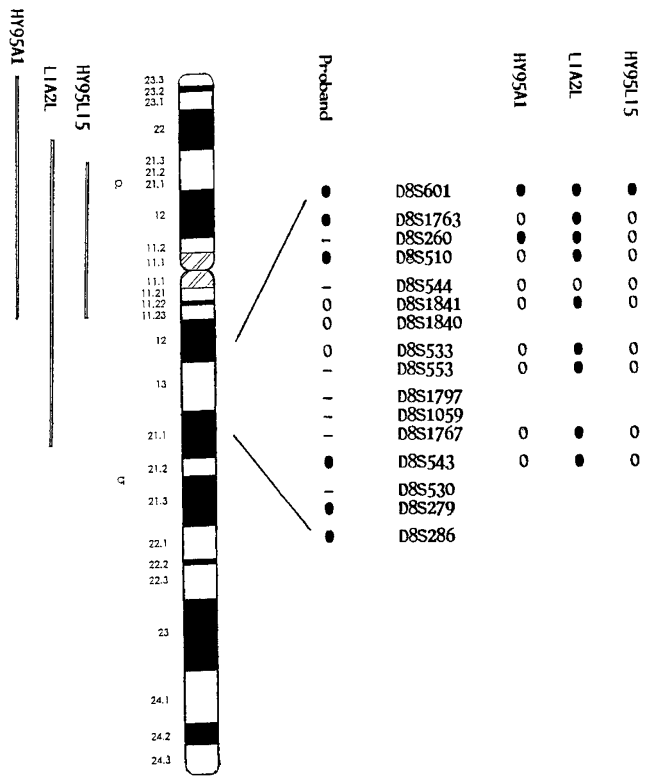


Figure 4 *Radiation hybrids and polymorphic markers used for chromosome 8 analysis. From left to right, hybrids chromosome coverage, chromosome 8 diagram, and STS markers tested in the proband and hybrids are reported. Empty circles indicate deleted markers. Uninformative STSs are shown with symbol (-).*

910F5 showed signals in der(8) and der(6). These signals were small compared to the very large signal found in normal chromosome 8. FISH using the most telomeric clones 876D10 to 944C9 displayed signals in normal chromosome 8 and der(6) (Table 1). Two-colour FISH experiments in interphase nuclei, combining clone 910F5 with the more proximal overlapping clones 937E2 or 751A2, showed two separate large signals relative to the YAC loci on the normal chromosome 8. In addition there were one small signal for clone 910F5 corresponding to the inserted region onto der(6), and two tiny signals, one for 910F5 and one for the more proximal clones 937E2 or 751A2, which were always joined (Figure 5b). YAC clones were also tested in the proband's parents, which showed chromosome 8 hybridisation patterns fitting well with published mapping results.

A summary of STSs and FISH results in reported in Table 1.

Discussion

Mapping a Locus for Duane Syndrome to 8q13

Vincent *et al*[7] found a dir ins(8) (q24.11q13.3q21.13) which harboured a 10 cM deletion encompassing the BOR syndrome related gene EYA1 in a patient with BOR syndrome associated with Duane syndrome, hydrocephalus, and trapeze aplasia.[7,17] In the present study we have described a patient with 8q deletion associated with a complex phenotype, including Duane syndrome, without any signs of BOR syndrome or other 8q related disorders.[7,17–19] Cytogenetic evidence of an insertion or region 8q13–21.2 on to chromosome 6q25 was confirmed by FISH analysis using whole chromosome painting probes, radiation hybrids and YAC clones. In addition *in situ* hybridisation disclosed a deletion within band 8q13. Microsatellite analysis indicated that the deletion affected the paternal chromosome similar to the patient reported by Vincent *et al*.[7] The deleted region boundaries were placed at D8S510 and D8S543. Thus, the chromosomal loss spanned D8S544 to D8S1767 in a region of about 6 cM according to published genetic distance.[16] Comparison of the two patients with del 8q associated with Duane syndrome pinpoints a small region of overlap (SRO) included between markers D8S533 and D8S1767. We have tentatively assigned a Duane syndrome locus to this region, which spans 3 cM and is located between the more proximal Friedreich ataxia with selective vitamin E deficiency locus[19] and the more distal BOR syndrome locus.[17,20]

Finally, the association of Duane syndrome and hydrocephalus is very rare in the literature, whilst hydrocephalus and mental retardation are quite aspecific features of chromosome imbalances.[21,22] Nevertheless, the concurrence of ventriculomegaly in the two patients with Duane syndrome and del 8q supports the hypothesis of a contiguous gene syndrome at 8q13.[7]

Rearrangement Characterisation

FISH analysis of the 8q region included between markers D8S1763 and D8S286 using YAC clones of the contig WC8.8 disclosed a rather complex rearrangement in the proband. The deleted region spanned from YAC clone 942E7 to 820E6, whilst the 8q region inserted on to chromosome 6q25 included the more distal clones 876D10 to 944C9 (Table 1). Clone 910F5, mapping between 820E6 and 876D10, disclosed signals on to 6q25 and der(8). The concurrence of 910F5 signals both on der(8) and der(6) suggests that the deletion

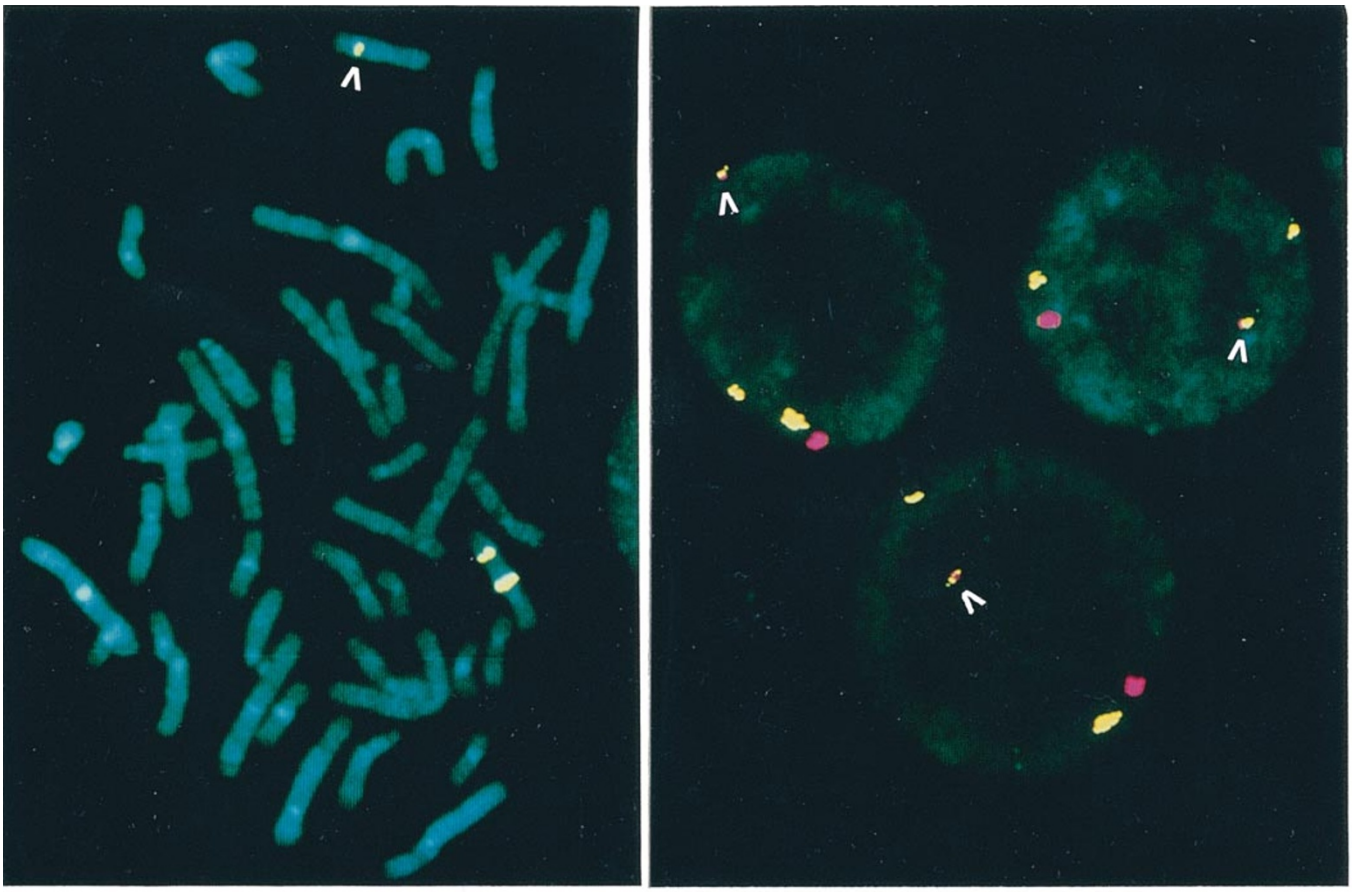


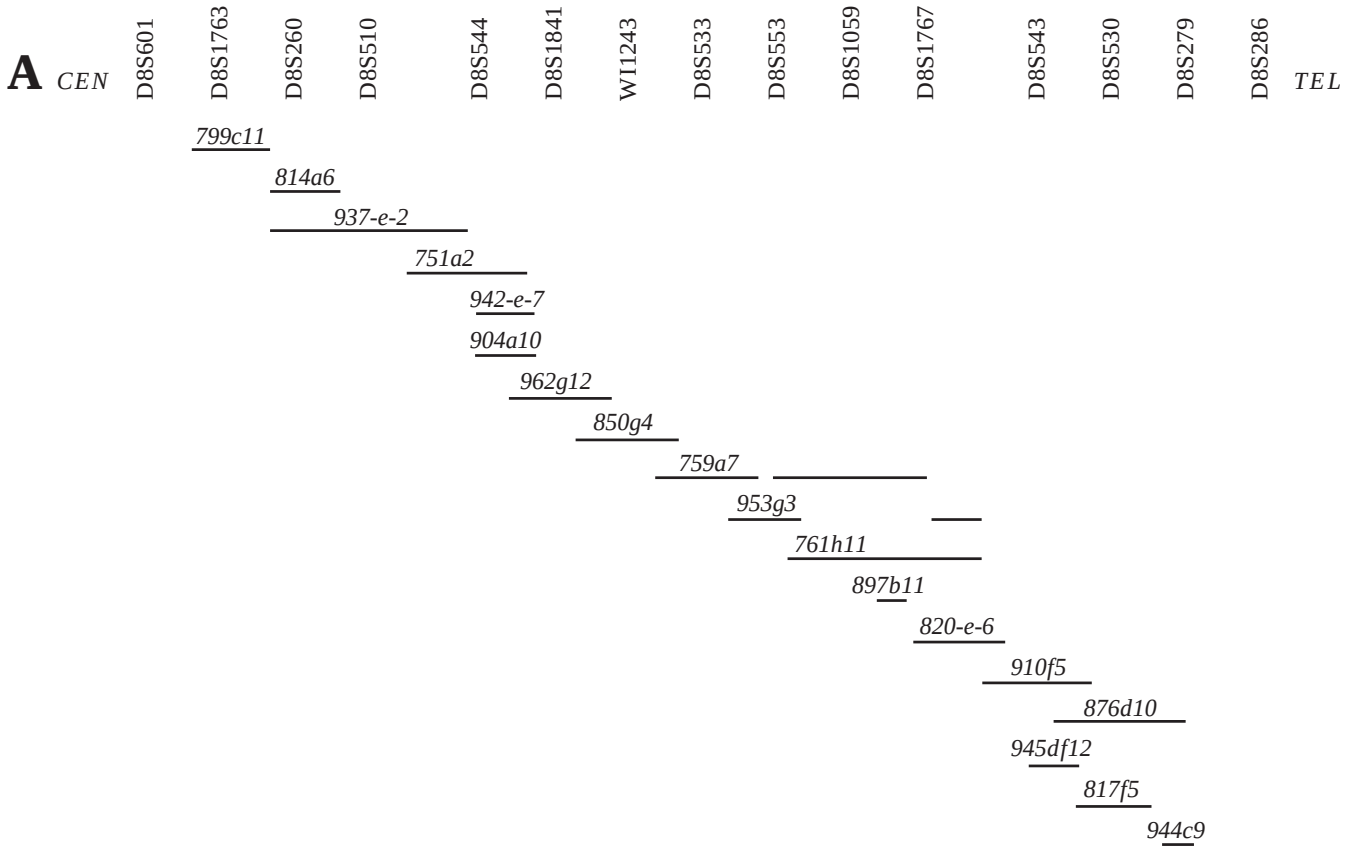
Figure 5 *(**a**) FISH analysis with YAC clone 850G4 showing signals on 8q13 and 8q23–24 on normal chromosome 8 and only the distal hybridization site on the der(8) (arrow) (**b**) two-color FISH analysis on interphase nuclei of the patient using clone 937E2 (red signals) and clone 910F5 (yellow signals). Hybridisation shows in each nucleus two large signals relative to the YAC loci on the normal chromosome 8, one smaller yellow signal corresponding to the 8q region inserted onto der(6), and two tiny signals, one red and one yellow, appearing joined (arrows).*

and the insertion occurred on to two distinct segments separated by a region included in YAC 910F5 which maintained its original location in 8q. Normal pattern of hybridisation using YACs in the parents indicated that the rearrangement in the patient was *de novo* and consisted of five breaks, including two each for deletion and insertion in 8q, and one in 6q25.

FISH analysis using clone 910F5 showed small signals both on der(8) and der(6) supporting a deletion also within this insert, which contains the distal limits of the deletion and the proximal breakpoint of the inserted region. This interpretation is in agreement with FISH experiments using clone 910F5 and the more proximal overlapping clones 937E2 or 751A2, showing very small signals on der(8). FISH analysis of interphase nuclei, in addition to signals on normal chromosome 8 and der(6), also showed two tiny signals corresponding to 910F5 and 937E2 or 751A2 (Figure 5b). Joining of these signals indicated sequence fusion following deletion. These data also placed the proximal deletion boundary within the overlapping clones 937E2 and 751A2.

In conclusion, it is known that complex chromosome rearrangements could hamper a genotype–phenotype correlation. However, the association of Duane syndrome and deletion 8q13 in the present patient and in another unrelated patient[7] supports the location of a gene for Duane syndrome at 8q13. The interval region around the putative 8q Duane gene has been narrowed down to a < 3 cM interval based on genetic-physical

Table 1 STS and YAC content map from the 8q region



Notes: (**A**) YAC contig of region 8q13–q21 (**B**) the deletion in the patient here reported, in Vincent's paper[7] and the putative Duane locus interval are shown: dark bars for regions normally located on der(8), dashed bars for translocated segments, and gridded bars for deleted segments are reported in respect of YAC clone sites and/or STSs. Fredreich ataxia with vitamin E deficiency (AVED) and BOR loci are also represented. The genetic distance between markers are as in the WC8.8 contig map (Whitehead Institute, MIT, USA).

map of region[23] included between D8S533 and D8S1767.

Acknowledgements

The authors wish to thank C Sala at DIBIT Centre, Milan, for providing the YAC clones. The financial support of Telethon-Italy (grant E420) is gratefully acknowledged.

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