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ARTICLE

Narrowing the Duane syndrome critical region at chromosome 8q13 down to 40 kb

G Calabrese[1], L Telvi[2], F Capodiferro[1], E Morizio[1], A Pizzuti[3], L Stuppia[1,4], R Bordoni[3], A Ion[2], D Fantasia[1], R Mingarelli[5] and G Palka[1]

[1]*Dipartimento di Scienze Biomediche/Sez. Genetica Medica, Università di Chieti, Italy;* [2]*Laboratoire de Cytogénétique, Hôpital Saint Vincent De Paul, Paris, France;* [3]*Istituto di Clinical Neurologica, Università di Milano;* [4]*Istituto di Citomorfologia Umana Normale e Patologica del CNR, Chieti;* [5]*Mendel-CSS IRCSS, Rome, Italy*

Duane syndrome (MIM 126800) is an autosomal dominant disorder characterised by primary strabismus and other ocular anomalies, associated with variable deficiency of binocular sight. We have recently identified a <3cM smallest region of deletion overlap (SRO) by comparing interstitial deletions at band 8q13 in two patients (one described by Vincent *et al*, 1994, and the other by Calabrese *et al*, 1998). Here we report on another patient with Duane syndrome carrying a reciprocal translation t(6;8)(q26;q13). FISH and PCR analyses using a YAC contig spanning the SRO narrowed the Duane region to a <1cM interval between markers SHGC37325 and WI4901. In addition, the identification and mapping of two PAC clones flanking the translocation breakpoint, allowed us to further narrow the critical region to about 40 kb. As part of these mapping studies, we have also refined the map position of *AMYB*, a putative candidate gene, to 8q13, centromeric to Duane locus. *AMYB* is expressed in brain cortex and genital crests and has been previously mapped to 8q22. *European Journal of Human Genetics* (2000) **8**, 319–324.

Keywords: Duane syndrome; 8q13; physical mapping

Introduction

Duane syndrome (MIM 126800)[1] is an autosomal dominant syndrome with unknown etiology. It is responsible for a primary form of strabismus, accompanied by bilateral globe retraction and narrowing of the palpebral fissure, leading to deficiencies of binocular sight of variable severity.[2] The pathogenesis of this syndrome is unknown but, in some patients a muscular–neuronal origin has been proven.[3] Although mostly reported as an isolated disorder, in a few patients Duane syndrome was associated with deafness, renal defects, muscular and skeletal anomalies, and genetic heterogeneity has even been described.[4] Furthermore, chromosome anomalies have been observed in some Duane patients and an 8q12–13 contiguous gene syndrome including Duane syndrome has been proposed.[5] Recently, a comparison of boundaries in two patients with 8q13 deletions[5,6] allowed us to narrow down the Duane syndrome smallest region of overlap (SRO) to a <3 cM interval between D8S533 and D8S1767.[6]

In this study we report a Duane syndrome patient carrying a constitutional translocation involving region 8q13. Molecular analyses have allowed the refinement of the Duane locus, and the reassignment of the map position of the *AMYB* gene, proposed as a candidate gene due its expression in the hindbrain, neural retina and urogenital ridge.[7]

Correspondence: Giandomenico Palka MD, Via B. Buozzi 93–65121 Pescara, Italy. Tel: +39 0871 3554 136; Fax: +39 0871 3554 135; E-mail: gdpalka@unich.it
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Materials and methods

Patient

A 31-year-old man was referred for cytogenetic analysis because of infertility. On clinical evaluation he presented with hypoplastic external genitalia and glandular hypospadias. Additional clinical features included strabismus with amblyopia and narrowing of palpebral fissures which led to a diagnosis of Duane syndrome. Routine laboratory investigations, echocardiography, renal ecography and audiometry

were normal. Histological and endocrinological examinations revealed dysgenetic gonads.

Cytogenetic and FISH analyses

Chromosome studies were carried out on PHA-stimulated peripheral blood lymphocytes using high resolution GTG and RBG banding techniques.

FISH and fibre-FISH analyses were performed according to Calabrese *et al*[6] and Fidlerova *et al*,[8] respectively. Biotinylated and FITC-labelled painting probes for chromosomes 6 and 8 (Cambio-Bouty, Italy), biotinylated 6q25-specific band probe (Li-Star FISH, Italy), YAC clones from contig WC8.8 (Généthon, France/Whitehead Institute, MIT, USA), PAC clones from RPCI-5 library (IGeR, Italy) were used. Probes were labelled by nick-translation prior to hybridisation.

Sequence tagged sites (STS) analysis

Fourteen STSs mapping within the Duane SRO at 8q13[6] and retrieved from CEPH/Généthon database[9] were tested by PCR on YAC clones to confirm integrity and on the PAC library for screening. Computer-aided search for sequences from genes and STS-ESTs mapped in the 8q13 region was performed using software available at the Whitehead Institute and Genome DataBase websites.[10,11]

Chromosome microdissection

Microdissection was carried out according to Zhang protocols[12] using a Nikon Eclipse inverted microscope and Nikon micromanipulator. Briefly, PCR using degenerated oligos[13] was performed on 40 copies of chromosomes der(6) and der(8) by a three-step amplification procedure. PCR products were then tested for content of STS markers from the SRO.

Results

Cytogenetic and YAC-based FISH characterisation of the 8q breakpoint

GTG banding in the patient revealed a reciprocal translocation t(6;8)(q26;q13) (Figure 1a) which was confirmed by FISH experiments with chromosome 6 and 8 specific paintings.

In order to characterise chromosome 8 breakpoint, six YAC clones mapping within the SRO previously identified (759A7, 953G3, 761H11, 897B11, 820E6 and 910F5)[6] were chosen. FISH analysis revealed that the four most centromeric clones 759A7, 953G3, 761H11, and 897B11 maintained the normal position at 8q13 on der(8). Clone 820E6 spanned the translocation 8q breakpoint as a large signal on normal chromosome 8, and one signal each on der(8) and der(6) (Figure 1b). Clone 910F5 showed a signal on der(6) in addition to that on the normal chromosome 8. To further refine the SRO, ten more YAC clones flanking 820E6 were chosen from the contig (Figure 2). Six clones, proximal to 820E6 (816D4 to 809G3) and sharing marker AFM238xc3, showed a normal location on der(8). The other four clones (925D9, 750C10, 883F2 and 743G1) were partially overlapping with clone 820E6 more centromeric markers WI4901 to D8S1775. Three of these clones (925D9, 750C10, and 883F2) spanned the translocation breakpoint. In particular, clone 925D9, in addition to a signal on normal chromosome 8, produced a large signal on der(8) and a tiny signal on der(6), while clones 750C10 and 883F2 displayed on der(6) a signal larger than the one on der(8). The remaining clone 743G1 did not span the translocation.

STS analysis

STS content of YACs[10] used for FISH was confirmed by PCR analysis. Microdissected der(6) and der(8) products were also analysed by PCR using six STS markers (WI7816, WI6151, AFM238xc3, WI4901, D8S1767, D8S1775) from the critical region. STS analysis showed that chromosome 8 DNA microdissected from der(8) was positive for markers WI7816 to AFM238xc3 (located proximally within the SRO), while DNA from der(6) was positive for STSs WI4901 to D8S1775 (located distally within the SRO) (data not shown). Marker AFM238xc3 was used to screen a PAC library but gave no positive clones, while WI4901 identified clone 1172j16. While these studies were in progress, a new STS marker, SHGC37325, has been placed between AFM238xc3 and WI4901 by radiation hybrid mapping.[11] By PCR analysis we found SHGC37325 in YAC clone 925D9. Because this is the most proximal clone spanning the 8q breakpoint, we proceeded with PAC library screening using this STS and identified a new clone, 832m7. FISH experiments with the two PAC clones showed that clone 832m7 maps on der(8) and 1172j16 on der(6) (Figure 3a). To understand the physical distance between the two clones 1172j16 and 832m7, we performed fibre-FISH experiments that revealed a 40kb gap between the two clones (Figure 3b).

A PAC library screening was also performed using ESTs WI7886 and WI7816, located at the 5' and 3' UTR, respectively of the *AMYB* gene.[10,11] *AMYB* has been proposed as a putative candidate gene for Duane syndrome. A positive PAC clone was obtained, 889el5, and mapped by FISH to chromosome 8q13 and on der(8) (Figure 3c).

Discussion

We have previously proposed a SRO for Duane syndrome included between markers D8S533 and D8S1767 which harbours a 3cM region located between the loci for Freidreich ataxia with vitamin E deficiency and the Branchio-Oto-Renal syndrome gene *EYA1* at 8q13.[6]

In the present study we have described a patient with gonadal dysgenesis and Duane syndrome heterozygous for a translocation involving 8q13. Cytogenetic evidence for a reciprocal translocation t(6;8)(q26;q13) was confirmed by FISH analysis using a panel of 16 YAC clones from a 8q13 contig. In addition, *in situ* hybridisation localised the 8q breakpoint in a region between markers AFM238xc3 and WI4901. The location of the translocation breakpoint on 8q

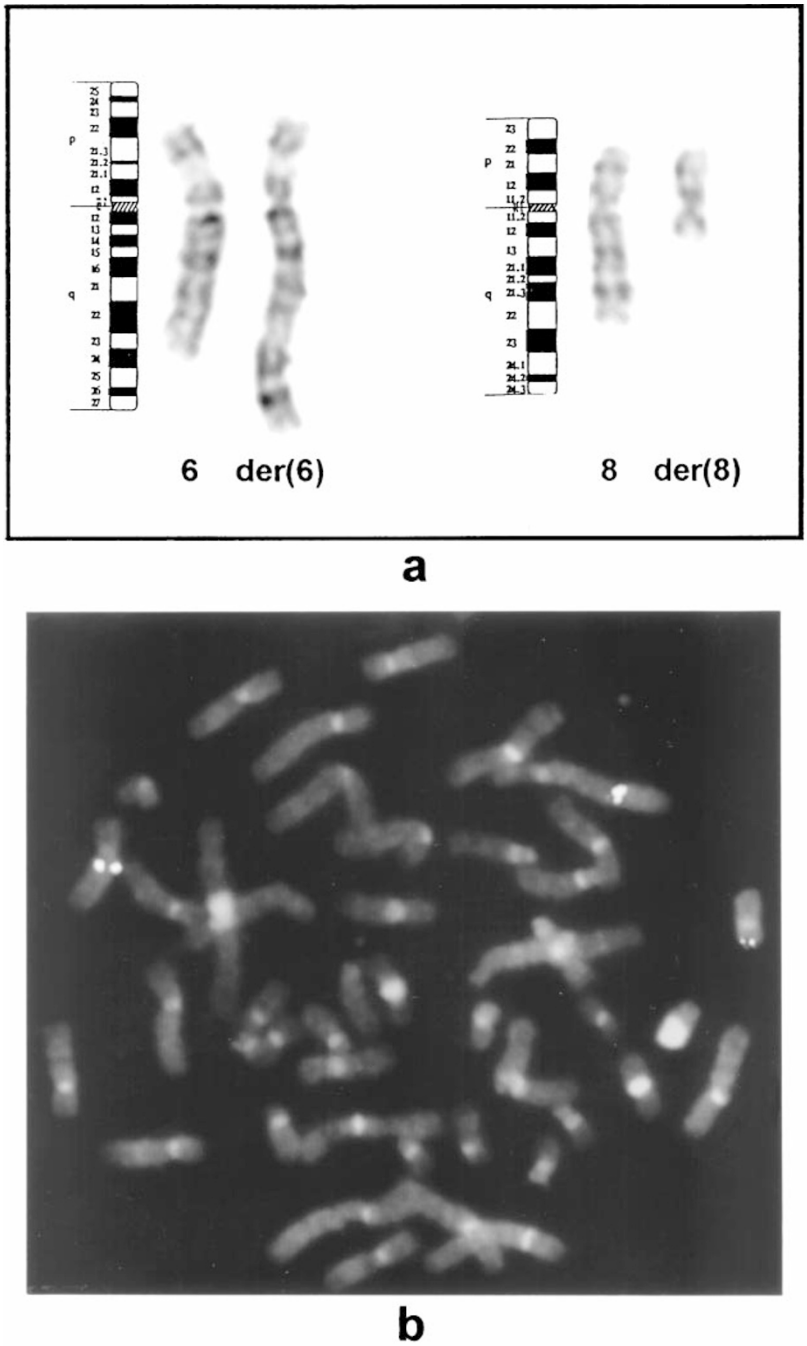

Figure 1 **a** Partial karyotype of Duane patient showing a t(6;8)(q26;q13); **b** FISH analysis with YAC clone 820E6 shows a large signal on normal chromosome 8, one signal on der(8) and one signal on der(6) as for bridging the 8q13 breakpoint.

was also confirmed by STS content analysis of microdissected DNA from der(6) and der(8). To refine the SRO map, we have isolated two PAC clones positive for markers WI4901 and SHGC37325, respectively. SHGC37325 maps distal to AFM238xc3 and proximal to WI4901.[11] By FISH analysis these clones were found to border the 8q13 breakpoint. Thus, the breakpoint maps between the two clones in a segment estimated to be approximately 40 kb, as measured fibre-FISH (although the involvement of PAC ends flanking this region cannot be excluded).

At present no gene has been assigned to this Duane syndrome gene interval.[11] Based on the possible neuromuscular origin of Duane syndrome and the gonadal dysgenesis present in the patient, an electronic search for genes at the

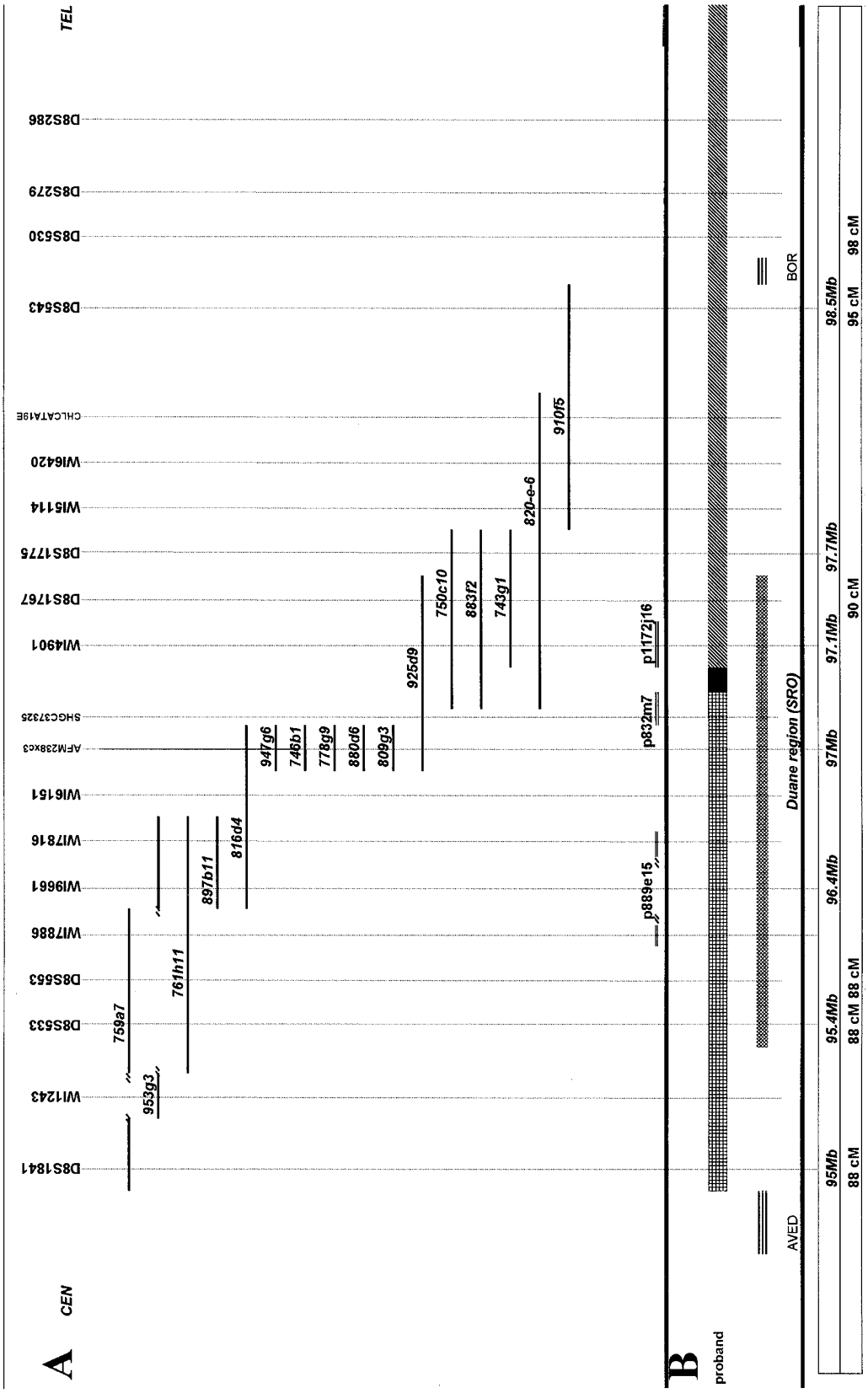

Figure 2 STS, YAC and PAC content map from the 8q13 region. **A** YAC contig and PAC clones within the Duane region. Markers not present in the clones are represented as gaps flanked by small double dashed lines. **B** The deletion in the patient here reported, and the Duane smallest region of deletion overlap (SRO) (Calabrese *et al*[6]) are shown. Gridded bars for regions normally located on der(8), and hatched bar for translocated segment on to der(6) are reported with respect to the contig map. Black box indicates the breakpoint region. Freidreich ataxia with vitamin E deficiency (AVED) and BOR loci are also represented. The physical and genetic distances between markers are as in the GDB chromosome 8 radiation hybrid map (GDB, USA), and WC8.8 contig map (Whitehead Inst, MIT, USA), respectively. Maps not to scale.

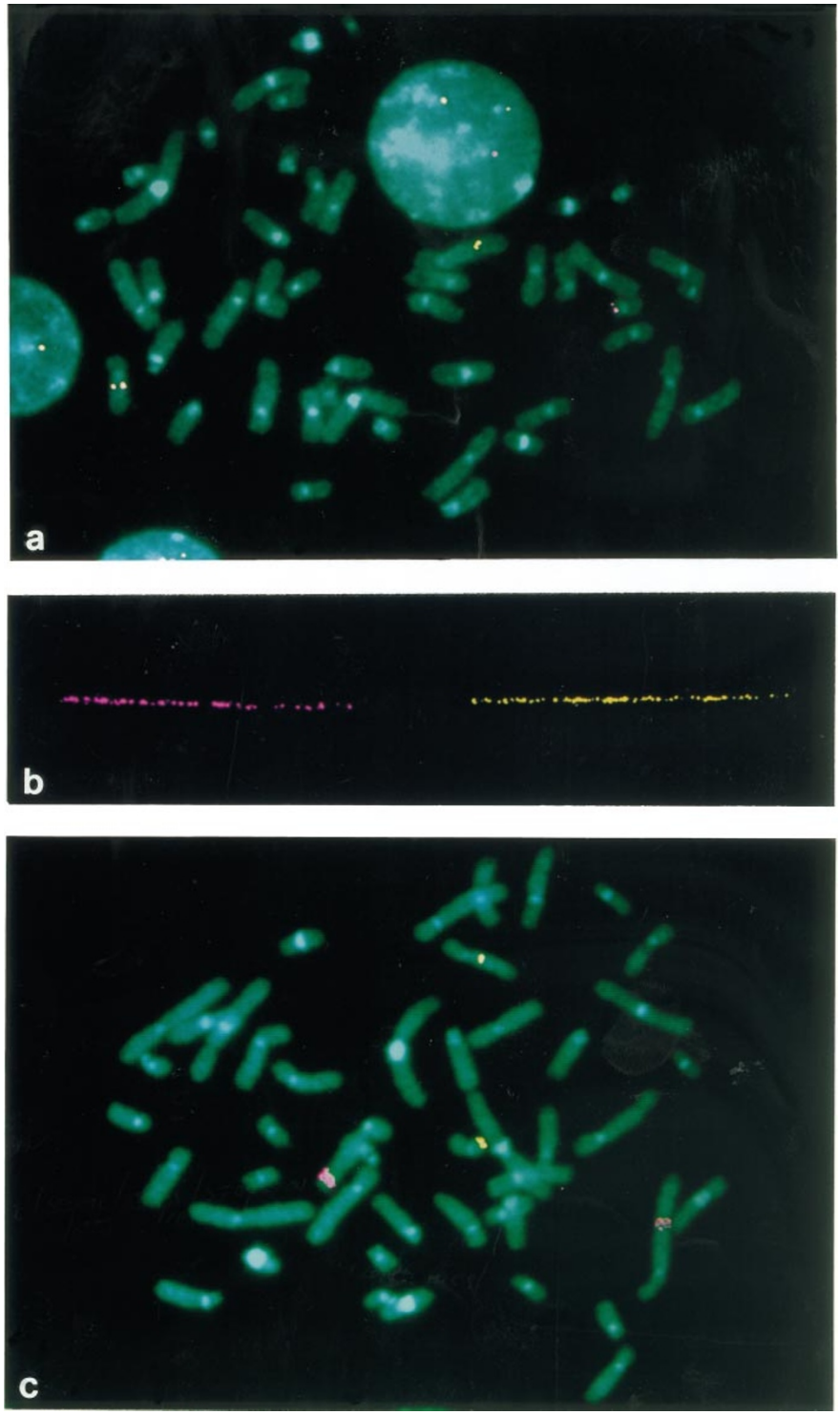

Figure 3 **a** Dual color FISH using PAC clones 832m7 and 1172j16 selected with markers SHGC37325 and WI4901, respectively. Clone 832m7 shows signals on der(8) (red), while clone 1172j16 shows signals on der(6) (yellow-green). On the normal chromosome 8, signals from the two probes result in an orange-yellow spot due to combined yellow-green and red fluorescences; **b** fibre-FISH showing PAC clones 832m7 (red array) and 1172j16 (yellow-green array) as separated by a 40 kb gap on the basis of the insert length of the two clones (120 kb each); **c** FISH with PAC clone 889e15 displaying yellow spots on normal chromosome 8 at band q13 and on der(8). A control 6q25 specific painting probe is also shown in red.

8q12–q21 region suggested *AMYB* as a putative candidate gene because of its expression in hindbrain, neural retina and genital crests.[7] Two ESTs, WI7886 and WI7816 containing 5' and 3'UTR AMYB sequences, mapped within the Duane SRO, suggested that the site for *AMYB* was more proximal, at 8q13, than previously reported 8q21–q22. This was demonstrated by FISH using PAC clone 889el5 positive with the two ESTs which showed signals on der(8) only. Therefore, our data map the *AMYB* locus at least 300 kb proximal to the 8q breakpoint in the patient and exclude *AMYB* as a Duane syndrome candidate gene.

In conclusion, the association of Duane syndrome with a 8q13 rearrangement in the patient described here and in two previously reported cases[5,6] strongly supports the location of a gene for Duane syndrome at 8q13. In this study the 8q Duane syndrome gene interval has been narrowed down to a 40 kb interval between markers SHGC37325 and WI4901. These results will greatly facilitate the identification of the causative gene.

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