Interstitial 22q11 microdeletion excluding the ADU breakpoint in a patient with DiGeorge syndrome

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DiGeorge (DGS) and velocardiofacial (VCFS) syndromes are frequently associated with microdeletions within the 22q11 region. The phenotypic spectrum is broad and microdeletions have been found in asymptomatic adults and children with quite subtle manifestations. The severity of the phenotype does not correlate with the extent of the deletion. The 22q11 region contains moderately repetitive sequences (1-4). The cosmid sc11.1 detects two loci within 22q11, sc11.1A being the proximal locus and sc11.1B the distal one. Nearly all patients identified with a 22q11 deletion have a large deletion encompassing the 2 Mb defined by sc11.1, regardless of their phenotype (5). The distal boundaries of the deletion are variable, and cosmid loci distal to sc11.1B are not consistently included in the deleted region (6). The proximal breakpoint seems to be more constant. Morrow et al. (4) reached similar conclusions by using highly polymorphic markers. The region of interest has been narrowed (3) and the critical region is limited distally by the breakpoint of an unbalanced translocation resulting in a visible deletion in a DGS patient. This region contains the proximal sc11.1 locus and the breakpoint of a balanced translocation (ADU breakpoint) present in a DGS patient and her mildly symptomatic mother (7). Recently a single patient with a smaller deletion than the interval defined by the two sc11.1 loci has been reported (8). This small deletion includes sc11.1A and the entire length of the critical region previously defined.

Several candidate genes have been mapped to the commonly deleted region. Two of these genes map to the critical region distal to the ADU breakpoint (2,9-11). Recently, an open reading frame disrupted by the rearrangement and another transcript in the vicinity of the ADU breakpoint were reported (12). This disrupted putative gene called DGCR3 appears to be the best candidate for the major manifestations of the DGS and VCFS identified thus far. However, no point mutations in any of these genes have been found in non-deleted patients.

We would like to report the case of a child with DGS whose deletion is smaller than the commonly deleted region and excludes a large portion of the critical region. This girl (patient G) was born at 35 weeks gestation after an uncomplicated pregnancy. At 15 days of age, truncus arteriosus was diagnosed. On examination, she had slight facial dysmorphism, consistent with the features commonly reported in 22q11 deleted patients (round face, small ears, small mouth and short, poorly defined philtrum). However, the typical prominent root of the nose was lacking. The congenital heart defect was surgically repaired and the thymus was recorded as normally sized. Serum calcium was normal. After surgical repair, the clinical course was unremarkable and, at 20 months of age the psychomotor development was in the normal range.

Fluorescence in situ hybridization analysis was performed according to previously described techniques (13). Cosmid probes were labelled by nick-translation with biotin 11d-UDP, according to BRL protocol. The hybridization signal was

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Figure 2. Schematic representation of the order of cosmid loci mapped to 22q11 region. The commonly deleted region is represented with the position of the DGCR. Patient G had a deletion smaller than the commonly deleted region. To investigate more precisely the proximal boundary of this deletion, we performed FISH with cosmids from the 3F4 contig region (9). Only loci 58C and 141F were deleted. Therefore, we have localized the proximal deletion breakpoint to between 141F and 3F4. The distal breakpoint was localized between Cos 40 and Cos 17. Distances between markers and breakpoints are not to scale.

Figure 3. FISH on metaphases of patient G with cosmids from 3F4 contig. (a) With cosmid 141F, there is one single hybridisation signal indicating a monozygous status for this locus. (b) With cosmid 3F4, two different signals are present.

revealed by FITC labelled-avidin, amplified twice with additional layers of biotinylated goat anti-avidin and avidinFITC, and chromosomal DNA was counterstained by propidium iodide.

Hybridization with cosmid sc4.1 revealed a single signal in all metaphases, indicating that the corresponding locus was deleted. With cosmid sc11.1, there were two hybridisation signals but one was much weaker than the other, suggesting that only one locus probed by sc11.1 was deleted (Fig. 1A). This was confirmed by performing hybridization in interphase nuclei (Fig. 1B). In order to define more precisely the extent of the deletion, we performed hybridization with several cosmids corresponding to loci mapped to the commonly deleted region, to the critical region or distal to the commonly deleted region (Fig. 2). Cosmid loci sc4.1 and cos40 were deleted, indicating that it was sc11.1B which was deleted. The proximal boundary was located between two cosmid loci of the 3F4 contig (9) (Fig. 3A and B), distal to the ADU breakpoint.

To the best of our knowledge, this represents the first case of interstitial deletion, the proximal boundary of which is distal to the ADU breakpoint. These results do not fit the hypothesis of a single gene disrupted by ADU, unless a large
A final hypothesis requires a second mutational event proximal to the deletion. This event could be a point mutation in a major gene disrupted by ADU, possibly DGCR3, or a second very small deletion not detectable by the probes now available.

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