Cri-du-Chat Syndrome Cytogenetically Cryptic Recombination Aneusomy of Chromosome 5: Implications in Recurrence Risk Estimation

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Key Words

Genetic counseling · Monosomy 5p · Trisomy 5q

Abstract

Cri-du-chat syndrome is caused by haploinsufficiency of the genes on the distal part of the short arm of chromosome 5, and characteristic features include microcephaly, developmental delays, and a distinctive high-pitched mewing cry. Most cri-du-chat syndrome cases result from a sporadic de novo deletion that is associated with a low recurrence risk. On rare occasions, however, cri-du-chat syndrome with 5p monosomy can be accompanied by 5q trisomy. This combination is virtually always associated with parental large pericentric inversions. Among previously reported cri-du-chat syndrome cases with 5p monosomy accompanied by 5q trisomy, the aneusomy of chromosome 5 in all but one case was cytogenetically visible using G-banding. When an accompanying 5q trisomy is detected, a significant recurrence risk is expected. We here report on a patient with cri-du-chat syndrome phenotype who initially exhibited a normal karyotype on G-banding but in whom molecular analysis using multiplex ligation-dependent probe amplification and array comparative genomic hybridization revealed a 5p deletion accompanied by a 5q duplication. Parental chromosomal

testing led to the identification of a very large pericentric inversion, of which breakpoints resided at the terminal regions of 5p15.31 and 5q35.1. This information was vital for counseling the family regarding the significantly high recurrence risk.

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Cri-du-chat syndrome is caused by haploinsufficiency of the genes on the distal part of the short arm of chromosome 5. Characteristic features include microcephaly, developmental delays, and a distinctive high-pitched mewing cry, from which the syndrome derives its name [Neibuhr, 1978]. A clinical diagnosis can be confirmed using a fluorescence in situ hybridization (FISH) study with probes to detect the deletion of the so-called cri-du-chat critical interval [Zhang et al., 2005].

Most cri-du-chat syndrome cases result from a sporadic de novo deletion that is associated with a low recurrence risk. However, about 10–15% of all cases are caused by the unequal segregation of a parental balanced translocation where the 5p monosomy is accompanied by a trisomy of the terminal segment of the other chromosomal arm [Neibuhr, 1978]. For detecting the involvement of chro-

mosomes other than chromosome 5, chromosome painting with a chromosome 5 library may be helpful as an adjunct study to FISH investigations using probes for the so-called cri-du-chat critical interval [Schrock et al., 1996]. When a trisomy of another chromosome is indeed identified, parental chromosomal studies are warranted to evaluate the risk of recurrence. If either parent has a balanced translocation, the recurrence risk will be about 5–15%, depending on the parental origin.

On rare occasions, cri-du-chat syndrome with 5p monosomy can be accompanied by 5q trisomy. This combination is virtually always associated with parental large pericentric inversions that lead to the formation of an inversion loop, promoting the production of double segmental aneusomy or meiotic recombination aneusomy in the gametes [de Perdigão et al., 1989]. Among previously reported cri-du-chat syndrome cases with meiotic recombination, the aneusomy of chromosome 5 in all [Faed et al., 1972; Neibuhr, 1978; Beemer et al., 1984; Miyazaki et al., 1985; Schroeder et al., 1986; Sonoda et al., 1989; Ono et al., 1993; Levy et al., 2002] but one case [Akalin et al., 2006] was cytogenetically visible using G-banding. When an accompanying 5q trisomy is detected, a significant recurrence risk is expected [Anton et al., 2005]. It is important to be aware that neither a whole-chromosome painting nor a targeted 5p FISH study in the patient will detect the presence of 5q trisomy because the trisomic material cannot be differentiated from other parts of chromosome 5.

We here report on a patient with cri-du-chat syndrome phenotype who exhibited a normal karyotype on G-banding but had an accompanying 5q trisomy to address the need to perform appropriate molecular investigations, such as a FISH analysis using a 5q subtelomere probe, multiplex ligation-dependent probe amplification (MLPA) or array comparative genomic hybridization (CGH), to search for the presence of cryptic 5q trisomy, so that families with cri-du-chat syndrome can be provided with appropriate genetic counseling.

Patient and Methods

The proband was delivered at 32 weeks of gestation by cesarean section (because of hydramnios) to a 31-year-old gravida 1, para 0 Japanese woman. The parents were nonconsanguineous. The Apgar scores were 4 at 1 min and 7 at 5 min. The infant's birth weight was 1228 g (below the 3rd percentile).

At birth, she was noted to have a cardiac murmur and was diagnosed as having a ventricular septal defect. She reportedly had a high-pitched mewing cry during infancy. She developed an upper airway obstruction accompanied by a flat larynx and underwent a tracheotomy at the age of 5 months. The ventricular septal

Fig. 1. Proband at 3 years. Note the broad nasal bridge, the prominent forehead, upslanting palpebral fissures, the bulbous nose, preauricular tags, and the smooth philtrum. In addition, the patient had a bifid uvula.

defect was repaired at the age of 3 years. She was able to walk at the age of 3 years and started to speak meaningfully, through a speech cannula, at the age of 3 years and 6 months.

At 3 years and 7 months, the patient was referred to us for evaluation because of multiple congenital anomalies and severe delays in psychomotor development. A physical examination revealed several dysmorphic features including a broad nasal bridge, a prominent forehead, a bulbous nose, preauricular tags, a bifid uvula, a smooth philtrum, and a short fifth finger (fig. 1).

After obtaining informed consent from the parents, the following investigations were performed. Her G-banded karyotype was reported as normal (fig. 2a). An MLPA analysis using 2 sets of subtelomeric probes (P036E and P070; MRC Holland) [Schouten et al., 2002; Rooms et al., 2006] revealed the deletion of the 5p subtelomere and a duplication of the 5q subtelomere with both kits.

A FISH analysis of the peripheral blood using TelVysion multicolor FISH probe panel No. 5 (Vysis) confirmed the deletion of the 5p subtelomere and the duplication of the 5q subtelomere (fig. 2b). Parental testing revealed that one of the parents had a pericentric inversion of the 5p and 5q subtelomeres (fig. 3). We concluded that meiotic crossing-over in the parent within the inverted segment of chromosome 5 gave rise to the unbalanced karyotype in the child. The parents did not wish to disclose the parental origin of the inversion.

An array CGH of the proband's genomic DNA using the Agilent Human Genome CGH 244A Oligo Microarray Kit (containing over 236,000 probes) revealed a loss in the copy number for the 5p subtelomeric region and a gain in the copy number for the 5q subtelomeric region (fig. 4). The size of the deletion was 9.1 Mb, extending from 75149 to 9094540 on 5p, whereas that of the duplication was 11 Mb, extending from 169407034 to 180629412 on 5q (UCSC hg18; NCBI Build 36).

Fig. 2. Results of cytogenetic studies in the proband. **a** GTG-banded chromosomes 5. Although the karyotype was initially reported normal, reevaluation after the results from molecular studies were known indicated a derivative chromosome der(5) inv(5)(p15.31q35.1)del(5)(p15.31)dup(5)(q35.1). **b** Fluorescence in situ hybridization analysis. Metaphase spreads revealed a deletion of the subtelomere of the short arm and a duplication of the subtelomere of the long arm in one of the chromosome 5 homologs when hybridized with a 5p subtelomere probe (green signal, arrowhead) and 5q centromere probes (red signals, arrows).

Fig. 3. Results of cytogenetic studies in the parent with cryptic pericentric inversion. **a** GTG-banded chromosomes 5 revealing a large pericentric inversion with subtle changes in the telomeric banding pattern. **b** Fluorescence in situ hybridization analysis. Metaphase spreads revealed an inversion of the subtelomere of the short arm and the subtelomere of the long arm in one of the chromosome 5 homologs when hybridized with a 5p subtelomere probe (green signals, arrowheads) and 5q centromere probes (red signals, arrows).

Results and Discussion

We report on a patient with cri-du-chat syndrome phenotype and a cytogenetically cryptic recombination aneuploidy of the terminal regions of the short and long arms of chromosome 5. G-banding chromosome testing was negative, but a subtelomeric MLPA analysis revealed a 5p deletion accompanied by a 5q duplication, and a subsequent array CGH confirmed these abnormalities. By reevaluation of the G-banded chromosomes, the aberration was indeed visible and compatible with the breakpoints in 5p15.31 and 5q35.1 indicated by array CGH. The findings in the proband prompted us to perform parental chromosomal testing, which led to the identification of a very large pericentric inversion with breakpoints resided at the terminal regions of 5p15.31 and 5q35.1. This information was vital for counseling the family regarding the significantly high recurrence risk, which contrasts the relatively low risk of recurrence associated with the more commonly observed pure terminal deletions.

Given that the inversion occurred in relatively telomeric regions, the recombination of the inverted chromosome 5 and its normal homolog is expected to occur

Fig. 4. Result of array CGH analysis and schematic diagram of the extent of the deletion. Array CGH analysis revealed the 9.1-Mb terminal deletion of 5p and the 11-Mb terminal duplication of 5q (gray areas). Filled boxes represent proposed candidate regions for cat-like cry and speech delay (see text).

in an almost regular fashion. When the inverted chromosome undergoes an odd number of recombination in both arms at loci proximal to the inversion breakpoint, the inversion will be resolved. When the inverted chromosome undergoes an even number, including zero, of recombination in both arms, the inversion will be retained. When the inverted chromosome undergoes an odd number of recombination in one arm but an even number in the other, unbalanced chromosomes will be generated. Given the relative smallness of the deleted or duplicated region, the chance of a fertilized egg derived from a gamete with such an unbalanced chromosome actually surviving may be relatively high; thus, the risk of the parent having another affected child is considerable.

The deletion interval on 5p revealed using array CGH contained the 2 intervals of the so-called 'cri-du-chat critical interval', as defined by a high-resolution mapping study [Zhang et al., 2005]: a 1.5-Mb interval between D5S2054 and D5S676 associated with a cat-like cry and a 3.2-Mb interval between D5S417 and D5S635 associated with a speech delay. Since the proband did have a characteristic high-pitched cry and speech delay, the phenotype was well correlated with the genotype. The duplicated region, 5q34–5qter, recapitulates the duplicated interval reported by Levy et al. [2002], who suggested that the characteristic features that can be ascribed to this re-

gion include a prominent forehead and a bulbous nose. Because the report by Levy et al. lacked a facial photograph, a direct comparison of these features is not possible. Nevertheless, these 2 features were present in the presently reported patient. However, we suspect that it would be difficult to appreciate the possible presence of 5q trisomy solely on a clinical basis.

The prospective screening of patients with multiple congenital anomalies and a characteristic highly pitched cry by MLPA or array CGH is warranted to estimate the incidence of this variant of cri-du-chat syndrome. Indeed, a previous patient with a mild developmental delay of a degree that was atypical for cri-du-chat syndrome and a questionable 'cat-like cry' was ascertained to have a recombination aneuploidy of the most subtelomeric regions of 5p and 5q by performing a FISH-based subtelomere screening of all the chromosomal arms [Bocian et al., 2005].

In conclusion, we reported on a patient with cri-du-chat syndrome phenotype who had a cytogenetically cryptic recombination aneuploidy of chromosome 5 resulting from a parental pericentric inversion. An MLPA analysis successfully unraveled the cryptic recombination aneuploidy, and an array CGH analysis was instrumental in precisely defining the aneuploidy. The principle illustrated in the present report may be applicable to apparent terminal deletions of other chromosomes as well.

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