When Cri du chat syndrome meets Edwards syndrome

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Abstract. It has been well established that the 5p deletion causes Cri du chat syndrome, typically characterized by a cat-like cry, and that duplication of 18q causes Edwards syndrome; the two are rare genetic abnormalities that separately lead to physical and mental impairments. However, the severity of the clinicopathological characteristics that arise when these two aberrations occur in one patient is unknown. Here, the first case in our knowledge of a single patient (a two-year-old female) with 5p partial monosomy and 18q partial trisomy is described. In the present study, chromosome microarray analysis was performed, which identified the imbalance of chromosomes 5 and 18 in the patient. The chromosome aberrations were further confirmed by fluorescence *in situ* hybridization. By comparing the phenotypes of combined case with those of the individual syndromes, severe clinical phenotypes of the 5p (5p15.33-p13.3) deletion were confirmed, however, the net effect of the duplication of 18q22.3-q23 was not determined, as this duplication only appeared to have a weak effect on the patient's phenotypes. The correlation between these chromosomal aberrations and their clinical features has implications for the identification of critical regions of 5p and 18q, particularly for the functional mapping of chromosome 18.

Introduction

Cri du chat syndrome (CdCS), which stems from a partial deletion of the short arm of chromosome 5 (5p-), is a rare genetic disorder occuring in ~1/15,000 to 1/50,000 live births (1), though it is more commonly identified in patients with mental retardation (~1/350) (1). As suggested by the name, CdCS is typically characterized by the occurrence of a high-pitched, cat-like cry, though this is lacking in certain cases (2). Therefore, the phenotype of CdCS may be divided into two categories, one for the typical cat-like cry and another for the other clinical traits. In principle, these two distinct phenotypes imply the existence of more than one critical region in 5p (3); differences in the sizes of deletions spanning these regions are likely responsible for some of the variability of CdCS.

While ~90% of cases are attributed to a sporadic or *de novo* deletion with random occurrence, a few arise from the malsegregation of a balanced translocation in the parental karyotype (4). Under this condition, the 5p monosomy tends to be accompanied by a partial trisomy of the genome. *A priori*, these patients may exhibit more severe phenotypes than those with only monosomy of 5p. In the case presented here, a trisomic portion was identified at chromosome 18.

Trisomy 18, or Edwards syndrome, is the second most common autosomal trisomy syndrome following trisomy 21 (Downs syndrome), and it results from full, mosaic, or partial trisomy of 18q. This syndrome has a very high mortality rate due to heart abnormalities, kidney malformations and other internal organ disorders. While full trisomy of 18q is the most prevalent form (5-8), partial trisomy of the terminal region of 18q is a very rare form that may present nonspecific abnormalities, including intrauterine growth restriction, microcephaly, a prominent forehead, hypertelorism, a short neck, mental retardation, seizure, laryngomalacia, atrial stenosis and club foot (9-12).

The deletion of 5p and partial 18q trisomy are rare occurances. The purpose of current study was to report an example of this unique combination in a female patient with an unbalanced translocation, giving rise to 5p deletion and 18q duplication, in addition to determining whether this case may assist in confirming critical regions of 5p that have previously been reported to cause typical CdCS, and whether partial trisomy of 18q influences the clinical characteristics, even though the CdCS phenotype prevails. To address these issues, the karyotype and phenotype of the patient and her parents were assessed using G-banding techniques, chromosome microarray analysis (CMA), and fluorescence *in situ* hybridization (FISH). The regions involved in or the extent of the 5p deletion and 18q duplication are good candidates for mapping the phenotypic disruptions associated with CdCS and Edwards syndrome. Therefore, determining which region has the genetic disorder in this case is of great clinical and etiological value.

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Materials and methods

Patient data. The two-year-old female patient was born at 39 weeks. She presented with laryngomalacia, atrial septal defect, a downturned mouth, transverse flexion creases and hypotonia, all of which are typical phenotypes of CdCS. In addition, the patient displayed psychomotor retardation, developmental delay and a long face, phenotypes which become evident with developing CdCS, however, she also had almond-shaped eyes and a bulbous nose (Fig. 1). Parental karyotyping detected an apparent balanced translocation (46, XY, t(5;18)(p13;q22)) in the father and no evident chromosomal abnormalities in the mother. Samples and pictures from the patient and her family were obtained following informed consent. This study was approved by the ethics committee of The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China.

Conventional cytogenetic analysis. A 5 ml blood sample was collected from the patient and each of her parents. Lymphocytes were cultured from each sample and used for cytogenetic analysis according to the standard blood cytogenetic protocol (13). Further routine cytogenetic analysis was performed using G-banding at 550 band resolution (trypsin: Amresco, Solon, OH, USA; giemsa stain: Sigma-Aldrich, St. Louis, MO, USA).

CMA. CMA was performed using the Affymetrix cyto HD Array (Affymetrix, Santa Clara, CA, USA). DNA was amplified, labeled and hybridized to the CytoScan HD array platform according to the manufacturer's instructions. The array is specifically designed for cytogenetics research and offers >2 million markers across the genome, including single nucleotide polymorphism probes and probes to detect copy number variations (Cyto-arrays). CEL files obtained by scanning CytoScan arrays were analyzed with the Chromosome Analysis Suite software (Affymetrix), employing genome annotation data (version GRCH37, hg19). Only data achieving the manufacturer's quality cut-off levels were included in further analyses. Primarily, gains and losses that affected a minimum of 50 markers within a 100 kbp length were accepted.

FISH. The FISH test for confirming the der(5)t(5:18) of the derivative chromosome was conducted using the Vysis Cri-du-Chat region probe (Vysis, Downers Groove, IL, USA) and a combination of the CEP18 probes and 18q subtelomere-specific probes (Vysis) as described in the manufacturer's protocol, using the standard FISH protocol (14).

Results

CMA. The array detected two genomic anomalies in the patient's genome: a 32 Mb deletion at 5p15.33-p13.3 (chr 5: 1-32, 137, 848) (Fig. 2A) and a 6.6 Mb duplication at 18q22.3q23 (chr 18: 71, 368, 578-78, 014, 123) (Fig. 2B). The deletion encompasses the complete terminal region of chromosome 5 covering band 5p15.2 and band 5p15.3, which are well established CdCS critical regions. In contrast, a review of the literature shows the duplication, which resides in the terminal region of 18q, is not known to be associated with abnormal genotypes, appearing in phenotypically normal males (15).

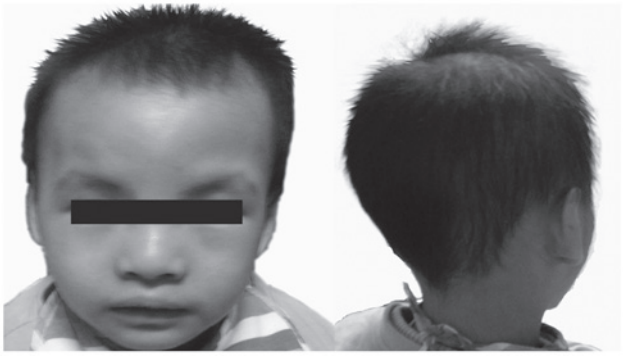

Figure 1. Clinical features of the female patient. She presented with laryngomalacia, a downturned mouth, almond-shaped eyes and a bulbous nose.

FISH analysis. FISH analysis of the proband and her parents confirmed the rearrangements and revealed that the partial monosomy of 5p and the partial trisomy of 18q were the result of a reciprocal unbalanced malsegregation derived from her father (Fig. 2C and D).

Comparative clinical features. The clinical phenotypes of the current patient, who carries these two rare genomic imbalances, were compared with the phenotypes of patients carrying only one of these genomic imbalances. The results are summarized in Tables I and II. While mental retardation or developmental delay was consistently observed in all patients with partial 5p monosomy, the present patient also presented a number of physical features that are not frequently observed in other patients, including hypotonia and a bulbous nose. Microcephaly was observed in certain patients and is a common phenotype of CdCS; however, it was not observed in the present case. In addition, the current study determined that the patient presented features that have not previously been described in other patients, such as transverse flexion creases, a downturned mouth, a long face and almond-shaped eyes. The more severe features observed in this patient may stem from the deletion at 5p15.33-p13.3, which is larger than deletions found in other patients. They may also be caused by the presence of the 18q duplication. However, patients with partial chromosome 18 trisomy alone did not exhibit the abnormalities observed in the current case, although mental retardation or developmental delay was a common feature in those patients. This suggests that in the present case, the 18q duplication may have a weaker effect on the phenotype than the larger 5p deletion.

Discussion

In the patient, a large (32 Mb) terminal deletion of 5p was detected, covering all the CdCS critical regions that had been reported to date (p15.3, p15.2, p15.1, p14 and p13). The deletion of p15.3 is the direct cause of typical CdCS (16), while deletion of p15.2 is another cause of CdCS that does not cause the characteristic catlike cry (2). The loss of p15.1, p14 and p13 has been reported in individual families with inter-

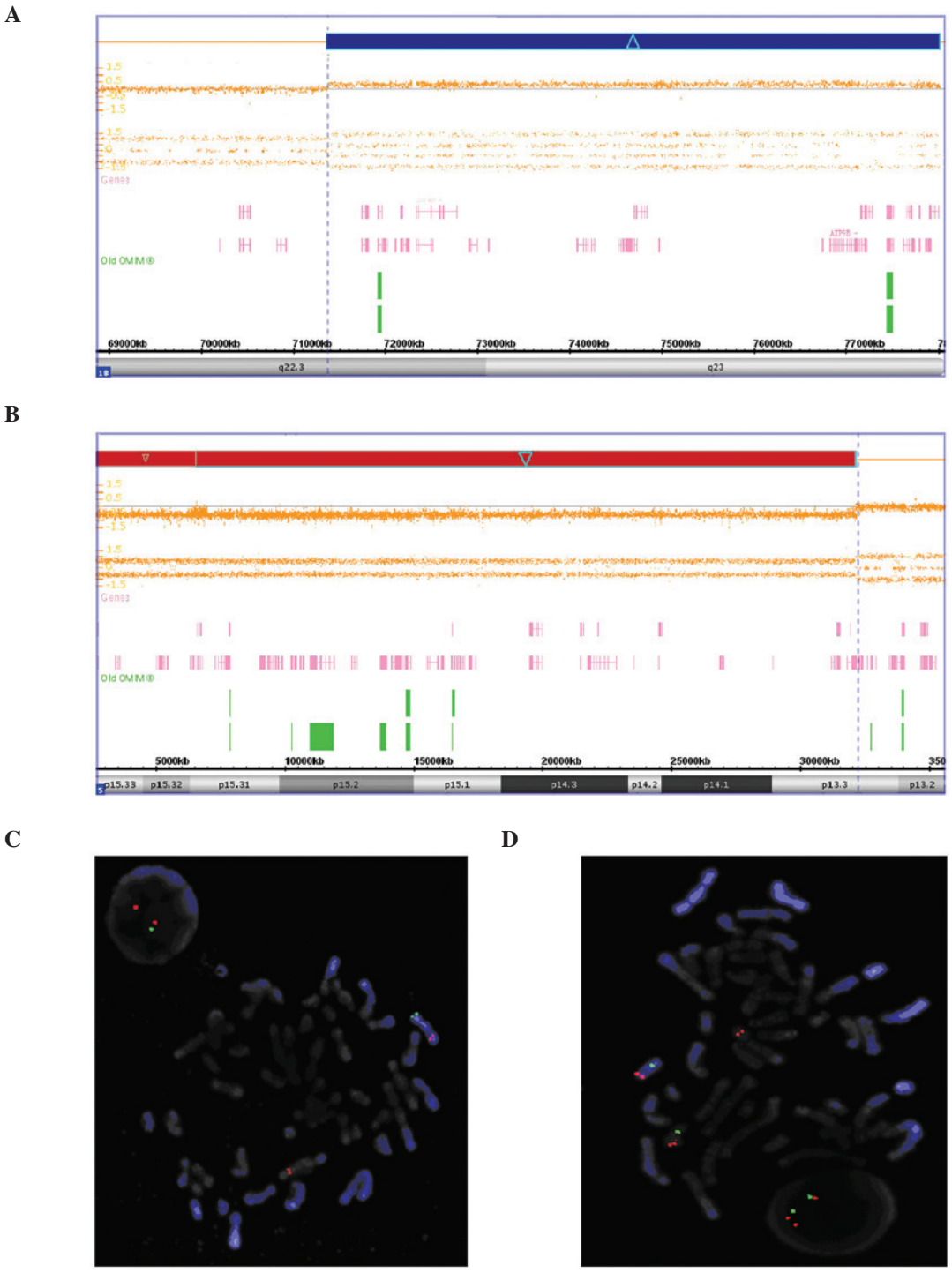

Figure 2. A deletion of 5p and a duplication of 18q analyzed using Chromosome Analysis Suite software. The array detected two genomic anomalies in the patient's genome, (A) a 32 Mb deletion at 5p15.33-p13.3 (chr 5: 1-32, 137,848) and (B) a 6.6 Mb duplication at 18q22.3q23 (chr 18: 71, 368,578-78,014,123). FISH results confirmed the deletion of Chr5 and the duplication of Chr18. Using the Vysis Cri-du-chat region probe, FISH analysis of metaphase cells taken from the proband showed one copy of chromosome 5 containing one red and one green signal, whereas its counterpart only contained one red signal, suggesting it is the derivative chromosome 5 or der (5). (C) Interphase cells showed two red signals and one green signal. Further FISH analysis for chromosome 18 confirmed balanced translocation of karyotype, with metaphase cells showing a red signal (18q telomeric probe)on one copy of chromosome 18 and a red signal on the other copy of chromosome 18 compared with both one green(CEP18 alpha satellite probe)and one red signal on the derivative chromosome 5 or der (5). (D) Interphase cells showed two green signals and three red signals. The labeled chromosomes 5, der (5), and 18 in the metaphase FISH pictures are identified by DAPI-banding using an ASI FishVision workstation (Applied Imaging). Magnification, x1,000. FISH, fluorescence *in situ* hybridization.

stitial deletions (2,4), varying to different extents in different individuals. In the current case, the clinical phenotypes of 5p aberration conformed for the most part to the features of CdCS, confirming the critical regions reported in prior studies.

The region of chromosome 5p13.3 that was deleted in this patient includes 90 well-characterized genes, in addition to a number of predicted genes postulated to reside within this region. These genes are commonly deleted in patients with 5p-deletion syndrome (17), and are associated with the

Table I. Phenotypic comparison of patients with pure partial chromosome 5 monosomy.

	Decipher ID					Typical Cri du Chat Syndrome	Present case
Category	258252	276535	272684	270177	268106		
Size (Mb)	11.10 Mb	10.88 Mb	22.09 MB	8.64 Mb	14.25 Mb	12.52 Mb	32 Mb
Region	95302-11199684	95243-10972789	151737-22246071	22179-8659713	3209639-17455995	10001-12533304	1-32137848
Inheritance	Unknown	Unknown	Unknown	*de novo*	*de novo*	-	Parental
Weak high-pitched voice	-	-	-	-	-	+	+
Mental impairment	+	+	+	+	+	+	+
Transverse flexion creases	-	-	-	-	-	-	+
Downturned mouth	-	-	-	-	-	-	+
Hypotonia	-	+	-	-	-	-	+
Long face	-	-	-	-	-	-	+
Almond-shaped eyes	-	-	-	-	-	-	+
Bulbous nose	-	-	-	-	+	-	+
Microcephaly	-	+	-	-	+	+	-

common clinical features, including the characteristic facial features. The clinical abnormalities found by physical examination of the patient in the present study are primarily the result of the deletion of these critical 5p regions.

Accompanying the terminal deletion of 5p, a partial duplication of 18q was detected in this case. In a previous study, patients with partial trisomy of chromosome 18 displayed severe Edwards syndrome (18), though complete duplication of chromosome 18 is typically required for the pathogenesis of Edwards syndrome. Several authors have attempted to identify the critical regions responsible for phenotypes in full trisomy 18 by comparing the clinical features of patients with various chromosome 18q duplications. A number of candidate critical regions for Edwards syndrome have been proposed on the basis of a few individual reports, however no consensus has been reached regarding the location (11,19-22). The proposed critical regions associated with dysmorphic features include 18q11.2, 18q21.1q21.2, and 18q22.3qter; the 18q duplication (18q22.3q23) reported in the current study partially overlapped with one of these critical regions. Nevertheless, a phenotypically normal male patient was reported to have a large duplication of the terminal 17.4 Mb of chromosome 18, including 18q22.3q23 (15), which contradicts its proposed role as a critical region. Furthermore, certain authors have argued against the existence of a critical region due to the interaction of cis-acting genes from several parts of chromosome 18, which are necessary to produce the full trisomy 18 phenotype (18). Literature searches for direct evidence of an effect of 18q22.3q23 duplication suggests no associations, although certain dysmorphic phenotypes seemingly linked to this region indicate an association.

While these results suggest a *prima facie* conclusion that the region from 18q22.3 to q23 has no effect on dysmorphic phenotypes, it is still possible that the clinical features associated with duplication of this region are easily masked by more severe phenotypes from other chromosomal aberrations. In contrast, a recent study using high-resolution array-CGH in 29 patients with 18q deletions concluded that the 4.3 Mb region located within 18q22.3q23 is a critical region for the 'typical' 18q-phenotype (23). It is possible that the critical region defined in cases of deletion is also partly responsible for the phenotypes classically described for duplication of 18q. A previous study indicated the existence of concentration-sensitive genes within 18q22.3q23; to date, 29 well-annotated genes have been found in this region, including the microsomal cytochrome b5 gene (*CYB5A*; 613218) and the gene for phosphatase specific for the C-terminal domain (*CTD*) of RNA polymerase II subunit A (*CTDP1*, *604927). The *CTDP1* gene product is involved in the initiation of gene expression (24) and may have a role in linking transcription elongation with splicing (25). It remains difficult to predict the effect of an increased expression of these genes. A similar patient to that of the current study was identified in the database DECIPHER. This patient (256304) had a loss of 36 Mb at chr 5: 130, 931-36, 780, 974, and a gain of 37 Mb at chr 8: 40, 832, 757-77, 966, 288, arising from a balanced parental rearrangement, and presented with atrioventricular canal defect (https://decipher.sanger.ac.uk/patient/256304). It is notable that this larger duplication, which includes the segment duplicated in the present

Table II. Phenotypic comparison of patients with pure partial chromosome 18 trisomy.

Category	Decipher ID					Present case
	3793	255714	259905	266270	268116[a]	
Size (Mb)	14.83 Mb	18.53 Mb	5.93 Mb	77.92 Mb	7.37 Mb	6.6 Mb
Region	178428-15008636	14116-18540053	362803-6288431	83701-78001525	363264-7730691	71368578-78014123
Inheritance	*de novo*	Unknown	*de novo*	Unknown	Unknown	Parental balanced translocation
Weak high-pitched voice	-	-	-	-	-	+
Mental impairment	+	+	+	-	-	+
Transverse flexion creases		-	-	-		+
Downturned mouth	-	-	-	-	-	+
Hypotonia	+	-	-	+	-	+
Long face	-	-	-	-	-	+
Almond-shaped eyes	-	-	-	-	-	+
Bulbous nose	-	-	-	-	-	+
Microcephaly	-	-	-	-	-	-

[a]The phenotype of this patient is annotated as nothing found.

patient, coincides with less-severe abnormalities, although it is possible that the patient's phenotypes were not fully detailed. It is unclear what effect the 18q duplication has in the present patient's phenotype. We hesitate to conclude that there is no effect from the 18q22.3-q23 duplication; however, the current study has demonstrated that the net effect is weak. This may serve as a guide for identifying the critical regions in Edwards syndrome and for analyzing the functional areas on chromosome 18.

In the current study, the patient showed a variety of genetic manifestations that were markedly different from those typical of CdCS. We report the first clinicopathological characteristics of a patient with a combined chromosomal disorder of 5p partial monosomy and 18q partial trisomy. The phenotypes of 5p monosomy displayed in this patient agree with previous reports, and the 18q partial trisomy may have a weak effect, which is considerably dwarfed by the severity of CdCS. The correlation between those unique features determined by the karyotype identified with CMA and validated by FISH will shed light on the functional mapping of chromosome 18.

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