Journal of Pediatric Genetics 2 (2013) 163–169
DOI 10.3233/PGE-13062
IOS Press

Case Report

Inherited 5p deletion syndrome due to paternal balanced translocation: Phenotypic heterogeneity due to duplication of 8q and 12p

Pankaj Sharma[a], Neerja Gupta[a], Madhumita R. Chowdhury[a], Savita Sapra[a], Rashmi Shukla[a], Meena Lall[b] and Madhulika Kabra[a,*]

[a]*Division of Genetics, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi, India*
[b]*Center of Medical Genetics, Sir Ganga Ram Hospital, Rajender Nagar, New Delhi, India*

Received 25 June 2013
Revised 10 December 2013
Accepted 10 December 2013

Abstract. 5p deletion syndrome or Cri du Chat syndrome is a autosomal deletion syndrome, caused by the de novo deletion of chromosome 5p in the majority of the cases. Clinical features include developmental delay, microcephaly, subtle facial dysmorphism and high-pitched cry. With the advent of newer techniques such as multiplex ligation-dependent probe amplification, rapid diagnosis is possible and chromosomal microarray helps in accurate delineation of the breakpoints. In this study, we characterized probands from two Indian families who had duplication of another chromosome in addition to deletion of 5p region. In the first family, two females of 3 and 5 yr of age had deletion of 5p15.33p15.2 (14.7 Mb) and duplication of 8q24.21q24.3 (15.4 Mb). Proband in the second family was a 2-year-old female and had deletion of 5p15.33p14.3 (22.55 Mb) along with duplication of 12p13.33p13.31 (7.7 Mb). In both the families, father was balanced translocation carrier of the chromosomes involved. Patients in family 1 had overwhelming features of 5p deletion while patient in family 2, besides having features of 5p deletion, showed many features of 12p duplications. Prenatal diagnosis was possible in both the families. To the best of our knowledge, this is the first detailed molecular cytogenetic analysis and prenatal diagnosis report of 5p deletion syndrome from India.

Keywords: Cri du Chat syndrome, 5p deletion, developmental delay, multiplex ligation-dependent probe amplification, chromosomal microarray, prenatal diagnosis

1. Introduction

5p deletion syndrome also known as Cri du Chat syndrome or cat's cry syndrome is a rare condition resulting from a variable sized (5–40 Mb) deletion of the short arm of chromosome 5. This syndrome was first described by Lejeune et al. [1] in 1963. It is estimated to occur with an incidence of 1 in 15,000 to 1 in 50,000 live births [2,3]. High-pitched cat like cry probably caused by an abnormal larynx or epiglottis, is one of the most characteristic features that is usually considered diagnostic for this syndrome. Other features include microcephaly, hypertelorism, epicanthic folds, micrognathia, broad nasal bridge, low set ears, down turned corners of mouth and severe psychomotor retardation. There is a lot of phenotypic variability depending upon the size and type of

*Corresponding author: Madhulika Kabra, Division of Genetics, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi, 110029, India. Tel.: +91 11 26594585; Fax: +91 11 26588641; E-mail: madhulikakabra@hotmail.com.

cytogenetic abnormality. Deletion of 5p15.3 region is responsible for the typical cat like cry whereas involvement of 5p15.2 region accounts for the other phenotypic features [4,5]. We report here two families where index cases were found to have a variable deletion of the short arm of chromosome 5 and duplication of another chromosomal region. In both the cases unbalanced chromosome complement were derived from balanced paternal chromosomes. Phenotypic differences in probands of the two families are explained due to the effect of duplication of other chromosomes. Clinical diagnosis was confirmed by a combination of newer genomic profiling techniques, Multiplex Ligation-dependent Probe Amplification (MLPA) and Chromosomal Microarray (CMA), which helped in identifying genomic changes, providing appropriate genetic counseling and prenatal diagnosis.

2. Case report

2.1. Family 1

Two female sibs aged 5 and 3 yr with global developmental delay were referred for genetic evaluation. They were born to a non-consanguineous couple. Both were born full term by normal vaginal delivery at home and had delayed cry. Anthropometric details at birth were not available for both sibs. There was no history of seizures, hearing impairment, visual problems, hyperactivity, aggressiveness or other behavioral abnormality in either sib. Examination of sibs showed the presence of microcephaly (head circumference, II-2:42 cm; II-4:41.5 cm, both <3rd centile), short stature (height, II-2:90 cm; II-4:80 cm both <3rd centile) and failure to thrive (weight, II-2:11 kg; II-4:6.8 kg both <3rd centile) as per World Health Organization standards. Both had high-pitched cry along with hypertelorism, synophrys, broad nasal ridge, full lips, short philtrum, wide mouth and hirsutism (Fig. 1A). Elder sib's magnetic resonance imaging of brain, abdominal ultrasound and thyroid profile were normal while younger sib's magnetic resonance imaging of brain was suggestive of hypoxic ischemic sequelae and abdominal ultrasound showed absence of right kidney. They were found to have profound intellectual disability on Vineland Social Maturity Scale. G-banded karyotype at 450 bands resolution was normal for both sibs. MLPA assay for subtelomeric region using SALSA MLPA kit P070-B2

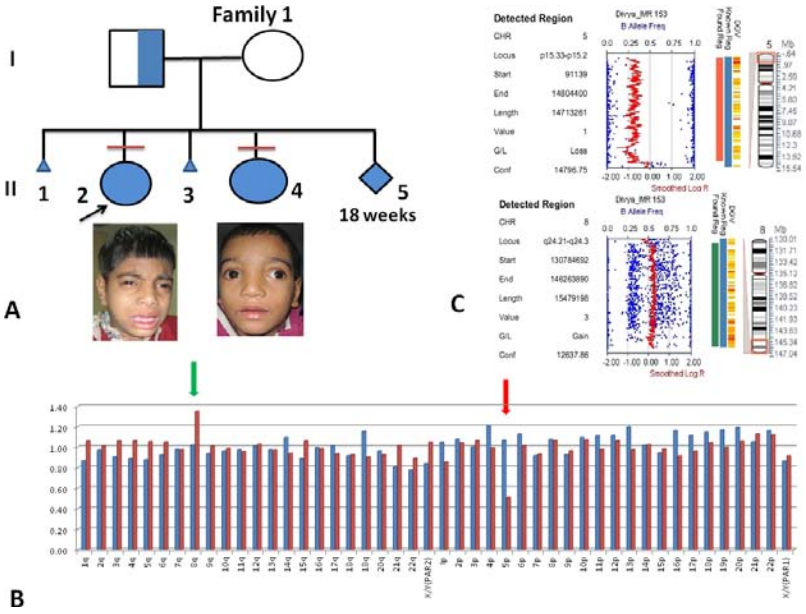

Fig. 1. (A) Pedigree and facial characteristics of affected children in family 1. Both sibs had facial dysmorphism in the form of hypertelorism, synophrys, broad nasal ridge, full lips, short philtrum, wide mouth and hirsutism. (B) Detection of subtelomeric anomalies by using multiplex ligation-dependent probe amplification kit P036-E1. The red arrow showing the copy number loss of 5p subtelomeric region and green arrow showing the copy number gain of 8q subtelomeric region. (C) Single nucleotide polymorphism array analysis illustrating 14.7 Mb loss of 5p (upper panel) and 15.4 Mb gain of 8q region (lower panel).

was carried out as per manufacturer's instructions to look for the alterations in subtelomeric regions of chromosomes. Amplification products were separated on ABI Prism-3130 Genetic Analyser (Applied Biosystem, Foster city, CA) and resulting data was analyzed using Genemapper software and Microsoft office excel spreadsheet. It revealed copy number loss of chromosome 5p and copy number gain of chromosome 8q subtelomeric region (Fig. 1B). These results were further confirmed by another SALSA-MLPA kit P036-E1 for subtelomeric region. To further delineate the breakpoints CMA analysis using Illumina Human Cyto-12 chip, which contains 300,000 probes across the genome for detecting copy number gains and losses was carried out. It revealed 14.7 Mb copy number loss of 5p15.33-p15.2 region and 15.4 Mb copy number gain of 8q24.21-q24.3 region (Fig. 1C) [arr5p15.33p15.2(91,139-14,804,400)×1 pat, 8q24.21 q24.3(130,784,692-146,263,890)×3 pat]. Based on the above findings a possibility of one of the parents being a balanced translocation carrier was considered. Hence, parental fluorescence in situ hybridization (FISH) study was carried out using Vysis ToTelVision Abbott Molecular probes of chromosome 5p and 8q. Metaphase FISH analysis revealed that father had a balanced translocation involving chromosome 5p and chromosome 8q telomeric regions (46,XY.ish t(5;8)(pter;qter) (Fig. 2A). Metaphase FISH for proband [46,XX.ish der(5)t(5;8)(pter;qter)pat] showed consistent findings with MLPA and CMA (Fig. 2B). Subsequently, 1 yr later prenatal diagnosis was offered in the next pregnancy at 18 wk of gestation after genetic counseling. Metaphase FISH analysis on cultured amniocytes was

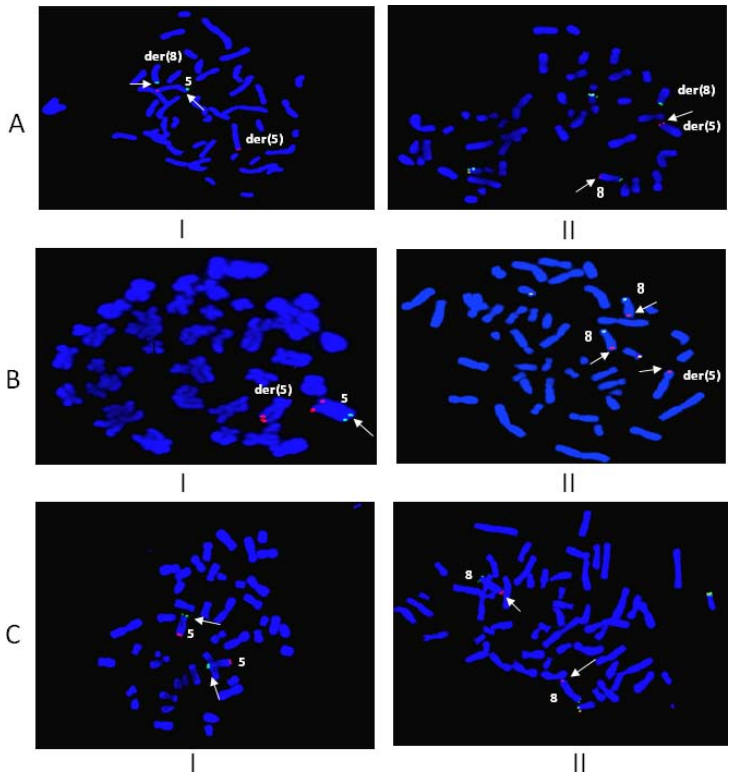

Fig. 2. Fluorescence in situ hybridization (FISH) results in family 1. Two Vysis TelVysion probe sets were used. Panel I mix contains telomeric probes for chromosome 5p (green color) and 5q (orange color) while Panel II mix contains telomeric probes for chromosome 8p (green color), 8q (orange color) and 17p (green and orange color). Fluorescent signals of only 5p and 8q are highlighted with an arrow. (A) Paternal metaphase representing reciprocal translocation of 5p and 8q telomeric regions. AI: Showing only one signal on chromosome 5p (green) while other translocated on 8q. AII showing one signal on chromosome 8q (red) while other translocated to 5p. (B) Metaphase FISH in patient 1. BI showing only one green signal of 5p representing monosomy of 5p while BII showing trisomy of chromosome 8q telomeric region, two orange signals of 8q on normal chromosome 8 and one on chromosome 5. (C) Metaphase FISH on amniotic fluid culture. CI showing the presence of two green signal on 5p representing normal chromosome 5 and CII showing two orange signals on chromosome 8 representing normal chromosome 8 in the fetus.

performed using telomeric probes for chromosome 5p and 8q. It showed two signals for telomeric region of chromosome 5p and chromosome 8q respectively (Fig. 2C), suggesting the absence of translocation between chromosome 5p and 8q in the fetus. MLPA using subtelomeric SALSA kit P070-B2 was also normal.

2.2. Family 2

A 3rd gravida woman was referred at 18 wk of gestation for genetic counseling and prenatal diagnosis for having a 2-year-old female child with developmental delay. This child was born at term by a normal vaginal delivery with a birth weight of 2.5 kg. There was history of birth asphyxia. She had severe developmental and speech delay. She had generalized tonic clonic convulsions for which she was put on anti epileptic therapy. There was no deafness, visual problems, hyperactivity or abnormal behavior. Examination showed presence of microcephaly (head circumference 42.5 cm, <3rd centile), height 80.2 cm at 3rd centile and weight 8.5 kg, <3rd centile, as per World Health Organization standards. She had hypertelorism, downslanting palpebral fissures, epicanthic folds, periorbital puffiness, arched eyebrows, long palpebral fissures, short nose, low set ears, long philtrum, thin lips, down turned corners of the mouth and mild retrognathia (Fig. 3A). She also had feeble cry, single palmar crease, hypertonia and a

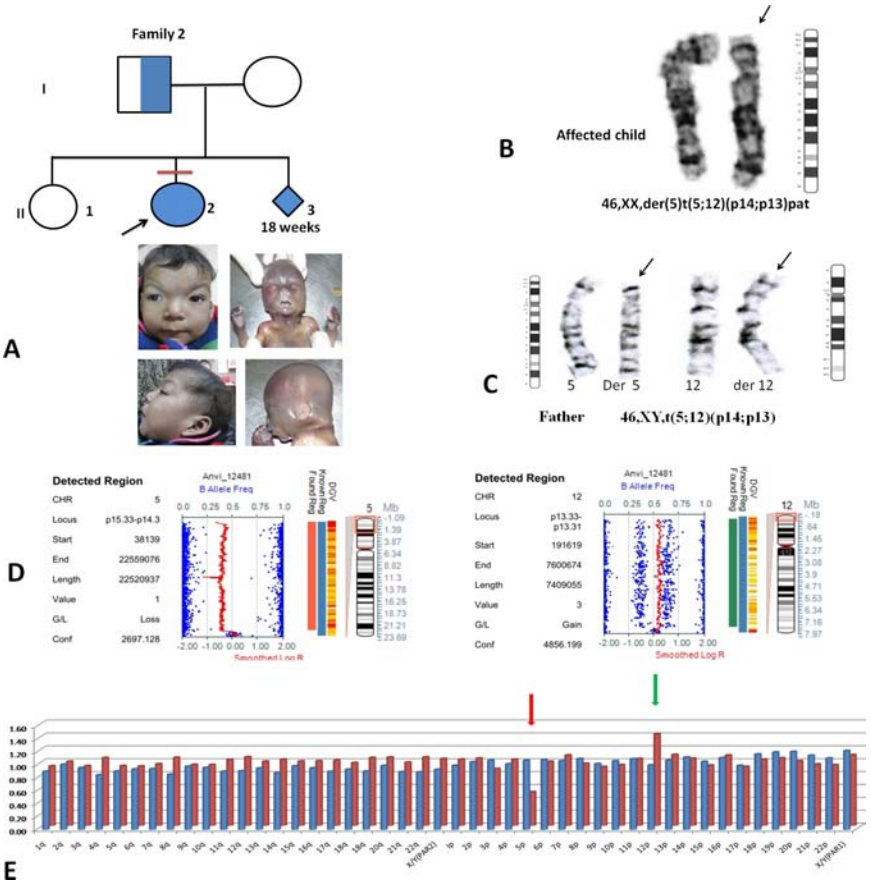

Fig. 3. (A) Pedigree and facial characteristics of the affected child and fetus in family 2. Note the similarities in facial phenotype in the form of hypertelorism, low set ears, short nose, long philtrum in the proband and the fetus. (B) Partial karyotype of the proband showing copy number loss on 5p. (C) Partial karyotype of the father showing balanced translocation for chromosome 5p and 12p regions. (D) Single nucleotide polymorphism array analysis illustrating 22.5 Mb copy number loss of 5p (left panel) and 7.4 Mb copy number gain of 12p region (right panel) (E) multiplex ligation-dependent probe amplification analysis using kit P070-B2 of the fetus showing copy number loss of chromosome 5p (red arrow) and gain of chromosome 12p (green arrow) subtelomeric regions.

small café au lait spot on the lower abdomen. G-banded karyotype analysis revealed paternally derived derivative chromosome 5 in the proband, 46,XX,der(5)t (5;12)(p14;p13)pat (Fig. 3B). Maternal karyotype was normal while paternal karyotype revealed balanced translocation for chromosomes 5p and 12p regions, 46,XY,t(5;12)(p14;p13) (Fig. 3C). CMA analysis using Illumina Human Cyto-12 chip detected 22.55 Mb copy number loss of 5p and 7.7 Mb copy number gain of 12p [arr5p15.33p14.3(38,139-22,559,076)x1pat, 12p13.33p13.31(191,619-7,600,674)x3pat] (Fig. 3D). Fetal high-resolution ultrasound in this pregnancy revealed presence of mild polyhydramnios, single umbilical artery, agenesis of corpus callosum, hypoplastic cerebellum and teardrop shaped lateral ventricles with mildly short long bones. MLPA analysis on cell lysates from amniotic fluid using SALSA kit P070-B2 showed copy number loss of chromosome 5p and copy number gain of chromosome 12p subtelomeric region (Fig. 3E). Cultured amniocytes confirmed the presence of unbalanced karyotype 46,XX,der(5)t(5;12)(p14;p13)pat. Parents chose to terminate the pregnancy after genetic counseling. Fetal autopsy confirmed the ultrasound findings and the fetus had similar facial dysmorphism as the previous affected child (Fig. 3A).

3. Discussion

Cri du Chat syndrome is associated with a variable phenotype depending upon the size of the deletion. Critical region for this syndrome has been localized to 5p15.2-15.3 [4,6]. A large number of repetitive sequences, which may account for its instability, flanks this region. Almost 90% of the patients have de novo deletions and in approximately 10% of the cases, deletions are due to meiotic malsegregation of a balanced chromosomal rearrangement in parents, paternal carriers being more common [7]. In both of our families, father was a carrier of a balanced translocation involving the common chromosome 5. Adjacent-1 segregation during meiosis may have led to the segmental monosomy of Cri du Chat region and segmental trisomy of the other chromosomal region involved. Although isolated 5p deletion syndrome is a well-defined entity, patients with unbalanced translocations can have a mixed phenotype due to partial trisomy of the other chromosome.

To the best of our knowledge, there are no reports for inherited 5p deletion syndrome along with involvement of chromosome 8q. There are reports on partial trisomy of 8q due to rearrangements with other chromosomes [8,9]; reports on isolated submicroscopic duplications of 8q24.21-q24.3 are rare [10,11]. Similarly, there is variability in the severity of phenotype for 12p duplication. Partial trisomy of 12p13.33 region is now considered to manifest with facial features overlapping with Pallister-Killian syndrome [12]. In family 1, both the affected sibs had overwhelming features of 5p deletion making the clinical diagnosis relatively easier. Proband in family 2, besides having few features of 5p deletion such as cat cry during the initial months of life and feeble cry later on, showed many features of 12p duplication such as broad forehead, prominent cheeks, short nose with anteverted nostrils, long philtrum and hence a mixed phenotype. Deletion of distal 5p14 region does not have any phenotypic consequences [7]. Phenotypic manifestations in Cri du Chat syndrome are mainly due to the deletion from 5p15.1 towards telomere region encompassing the critical region. [7]. As reported in previous studies, the phenotypic manifestations in our case may also be due to the deletion from 5p15.1. Table 1 shows phenotypic comparison of these patients with the reported clinical features of 5p deletion, partial 8q trisomy and partial 12p trisomy.

Diagnosis in family 1 required detailed molecular cytogenetic analysis using a combination of techniques such as MLPA, CMA and FISH to delineate the breakpoints and to know the origin of deletion. In family 2, unbalanced karyotype on routine cytogenetics showed terminal deletion with breakpoint in distal 5p14. CMA was helpful in precise delineation of breakpoints making phenotype array correlation easier.

In both the families, prenatal diagnosis was possible. In family 1, prenatal ultrasound was normal and combination of MLPA and FISH was useful in providing prenatal diagnosis. In the second family, high-resolution ultrasound showed the presence of mild polyhydramnios, single umbilical artery, agenesis of corpus callosum, hypoplastic cerebellum and mildly short long bones. These features are reported in both 5p deletion and 12p duplication [13,14]. MLPA using SALSA kit P070-B2 and karyotype analysis from amniotic fluid showed the presence of unbalanced chromosomes in the fetus.

Phenotypic diversity in patients from both the families is explained due to the involvement of another chromosome in addition to common chromosome 5. The origin of the deleted and duplicated chromosomes was paternal in both of our cases. This is in concordance with the previous reports of prevalence of paternal

Table 1
Phenotypic comparison of patient 1, 2 and 3 with reported features of 5p− and 8q+ and 12p+

Clinical features	5p− [15]	8q+ [8,9]	Patient 1	Patient 2	5p−	12p+ [14,16]	Patient 3
Microcephaly	+	NA	+	+	+	−	+
Macrocephaly	−	NA	−	−	−	+	−
Round face	+	−	−	−	+	+	+
Fore head (prominent/ broad)	−	Prominent	−	−	−	+	Broad
Broad/depressed nasal bridge	Broad	NA	Broad	Broad	Broad	Depressed	Depressed
Flat nose with short septum	−	+	−	−	−	+	+
Palpebral fissure up	−	+	+	+	−	+	−
Palpebral fissure down	+	NA	−	−	+	−	+
Hypertelorism	+	+	+	+	+	+	+
Epicanthal folds	+	+	−	−	+	+	+
Strabismus divergent/ convergent	+	−	−	−	+	−	−
Short philtrum	+	−	+	−	+	−	−
Down turned corners of the mouth	+	−	+	−	+	−	+
Prominent cheeks	−	+	−	−	−	+	+
Low-set ears	+	+	−	−	+	+	+
Thin upper lip	−	−	−	−	−	+	+
Everted lower lip	−	−	−	−	−	+	−
Micrognathia	+	+	+	+	+	+	+
Typical cry	+	−	+	+	+	−	+
High arched palate	+	−	+		+	+	
Short neck	+	+	−	+	+	+	+
Wide spaced nipples	−	+	−	−	−	−	−
Pectus excavatum	−	+	−	−	−	−	−
Hypertrichosis	−	+	+	−	−	−	−
Single palmer crease	+	NA	−	−	+	−	+
Clinodactyly	+	+	+	+	+	+	−
Brachydactyly	−	+	−	−	−	−	−
Renal malformations	+	NA	+	− Unilateral agenesis	+	−	−
Cryptorchidism	+/−	+	−	−	+/−	+	−
Congenital heart defect	+/−	+	−	−	+/−	+	− (Patent foramen ovale)
Scoliosis	+	+	−	−	+	−	−
Coxa valga	+	+	−	−	−	NA	−
Hypotonia	+	+	−	−	+	+	−
Speech delay	+	NA	+	+	+	−	+
Seizures	+	−	−	−	+	+	+
Deafness	−	NA	−	−	−	+	−
Growth retardation	+	+	+	+	+	−	−
Developmental delay	+	+	+	+	+	+	+

NA = Not available.

carrier (90%) in Cri du Chat syndrome. This study highlights the importance of newer molecular cytogenetic techniques revealing cryptic subtelomeric abnormalities, which are not detectable by routine karyotyping as most subtelomeric region of chromosomes are stained G-band negative. MLPA was useful for detecting the submicroscopic imbalances in both families and providing a rapid prenatal diagnosis. When compared with CMA, MLPA can detect only up to 45 nucleic acid targets in the genome. Nevertheless, it is a high throughput, cost effective and rapid technique for the detection of submicroscopic copy number gains and losses in patients with idiopathic intellectual disability, which makes it very useful technique especially in middle and low-income

group countries. MLPA and CMA, while being high throughput, cannot detect balanced chromosomal rearrangements. FISH, though time consuming and labor intensive, is still crucial to uncover balanced chromosomal rearrangement.

Acknowledgement

We would like to thank Dr. Shivaram Shastri, Scientist, Division of Genetics, Department of Pediatrics, AIIMS for helpful discussion in manuscript preparation. This work was supported by the grant from Department of Biotechnology, India.

References

[1] Lejeune J, Lafourcade J, Berger R, Vialatte J, Boeswillwald M, Seringe P, et al. 3 cases of partial deletion of the short arm of a chromosome 5. C R Hebd Seances Acad Sci 1963;257: 3098–102 (in French).

[2] Higurashi M, Oda M, Iijima K, Iijima S, Takeshita T, Watanabe N, et al. Livebirth prevalence and follow-up of malformation syndromes in 27,472 newborns. Brain Dev 1990; 12(6):770–3.

[3] Niebuhr E. The Cri du Chat syndrome: epidemiology, cytogenetics, and clinical features. Hum Genet 1978;44(3):227–75.

[4] Overhauser J, Huang X, Gersh M, Wilson W, McMahon J, Bengtsson U, et al. Molecular and phenotypic mapping of the short arm of chromosome 5: sublocalization of the critical region for the cri-du-chat syndrome. Hum Mol Genet 1994;3(2): 247–52.

[5] Gersh M, Goodart SA, Pasztor LM, Harris DJ, Weiss L, Overhauser J. Evidence for a distinct region causing a cat-like cry in patients with 5p deletions. Am J Hum Genet 1995;56(6): 1404–10.

[6] Mainardi PC. Cri du Chat syndrome. Orphanet J Rare Dis 2006;5;1:33.

[7] Mainardi PC, Perfumo C, Calì A, Coucourde G, Pastore G, Cavani S, et al. Clinical and molecular characterization of 80 patients with 5p deletion: genotype-phenotype correlation. J Med Genet 2001;38(3):151–8.

[8] Puvabanditsin S, Garrow E, Rabi FA, Titapiwatanakun R, Kuniyoshi KM. Partial trisomy 8q and partial monosomy 18p: a case report. Ann Genet 2004;47(4):399–403.

[9] Schinzel A. Partial trisomy 8q in half-sisters with distinct dysmorphic patterns not similar to the trisomy 8 mosaicism syndrome. Hum Genet 1977;37(1):17–26.

[10] Concolino D, Iembo MA, Moricca MT, Rapsomaniki M, Marotta R, Galesi O, et al. A de novo 8q22.2-24.3 duplication in a patient with mild phenotype. Eur J Med Genet 2012; 55(1):67–70.

[11] Bonaglia MC, Giorda R, Tenconi R, Pessina M, Pramparo T, Borgatti R, et al. A 2.3 Mb duplication of chromosome 8q24.3 associated with severe mental retardation and epilepsy detected by standard karyotype. Eur J Hum Genet 2005; 13(5):586–91.

[12] Wilkens A, Liu H, Park K, Campbell LB, Jackson M, Kostanecka A, et al. Novel clinical manifestations in Pallister-Killian syndrome: comprehensive evaluation of 59 affected individuals and review of previously reported cases. Am J Med Genet A 2012;158A(12):3002–17.

[13] Teoh XH, Tan TY, Chow KK, Lee IW. Prenatal diagnosis of cri-du-chat syndrome: importance of ultrasonographical markers. Singapore Med J 2009;50(5):e181–4.

[14] Izumi K, Conlin LK, Berrodin D, Fincher C, Wilkens A, Haldeman-Englert C, et al. Duplication 12p and Pallister-Killian syndrome: a case report and review of the literature toward defining a Pallister-Killian syndrome minimal critical region. Am J Med Genet A 2012;158A(12):3033–45.

[15] Mainardi PC, Pastore G, Castronovo C, Godi M, Guala A, Tamiazzo S, et al. The natural history of Cri du Chat Syndrome. A report from the Italian Register. Eur J Med Genet 2006; 49(5):363–83.

[16] Kondo I, Hamaguchi H, Haneda T. Trisomy 12p syndrome: de novo occurrence of mosaic trisomy 12p in a mentally retarded boy. Hum Genet 1979;46(2):135–40.