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CASE REPORT

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An uninformative NIPT as an early indicator of cri-du-chat due to a chromosomal 5;18 translocation—An atypical presentation of a rare cytogenetic phenomenon

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Key Clinical Message

We present a patient with cri-du-chat syndrome secondary to a rare cytogenetic mechanism. Our patient was the product of a dichorionic diamniotic twin pregnancy initially flagged with soft markers on ultrasound and uninformative single-nucleotide polymorphism (SNP)-based noninvasive prenatal testing (NIPT) for chromosome 18. Subsequent NIPT using proprietary-targeted amplification methodology returned low risk for chromosomal aneuploidies 13, 18, and 21. Due to postnatal clinical findings, a clinical microarray and chromosomal karyotype confirmed cri-du-chat syndrome due to a de novo psu dic(5;18) (p15.2, p11.32). In this report we focus on these cytogenetic changes and discuss some of the current guidelines for prenatal microarray indications.

KEYWORDS

chromosomal rearrangement, cri du chat, genetics, microarray, NIPT

1 | INTRODUCTION

Cri-du-chat syndrome (CdCS) is a chromosomal abnormality characterized by a deletion of the short arm of chromosome 5 and a unique cat-like cry.[1] Other clinical features include microcephaly, distinctive facial features, intellectual disability, and psychomotor delay.[2] The incidence of CdCS is estimated to be 1 in 20,000–50,000 live births.[3,4] About 80% of cases are thought to be due to de novo deletions.[5] Other mechanisms include inheritance of unbalanced parental translocation and rare cytogenetic abnormalities such as ring chromosomes, de novo translocations, and mosaicism.[3]

In this report, we describe an individual with a de novo psu dic(5;18) (p15.2, p11.32). To the best of our knowledge, this is the first time a patient with CdCS has been reported secondary to a (5;18) imbalanced translocation. This case highlights the importance of early cytogenetic testing in the diagnosis of CdCS.

2 | CASE REPORT

Our patient was twin B of a diamniotic dichorionic twin pregnancy flagged prenatally with echogenic bowel and ventriculomegaly in twin A, and a normal ultrasound in

DS and MD contributed equally to this paper (Joint first authorship).

twin B. Follow-up level III obstetric ultrasound was normal. A single-nucleotide polymorphism (SNP)-based noninvasive prenatal testing (NIPT) was offered initially which was inconclusive for chromosome 18. Invasive testing with subsequent clinical microarray was not indicated based on soft markers alone per the Canadian College for Medical Genetics guidelines (CCMG).[6] Similarly, American College of Obstetricians and Gynecologists (ACOG) guidelines would also not support clinical microarray in this situation as the soft marker does not constitute a "major structural abnormality".[7] Neither body provides guidance on inconclusive NIPT and thus the patient was offered the option between a repeat NIPT or diagnostic testing. The patient opted for repeat NIPT. This was done through a secondary provider that utilizes proprietary-targeted amplification and returned low risk for trisomy 13, 18, 21 for both twins. Later in the pregnancy, oligohydramnios was flagged in both babies with intrauterine growth restriction in twin B. At 36+4 weeks, twin A measured at the 25th percentile and twin B at the 4th percentile. The twins were delivered at 37+0 weeks.

At birth, twin B exhibited a high-pitched cry, micrognathia, low-set posteriorly rotated ears, bilateral displaced nipples, and overlapping second toes. Birth weight, head circumference, and length were all below the tenth percentile. Echocardiogram revealed a small patent foramen ovale and a small restrictive anterior ventricular septal defect. Based on these clinical findings, a karyotype and microarray were performed. This revealed three copy number variants (CNV) consisting of a gain of uncertain significance, a loss of uncertain significance and a pathogenic terminal loss of approximately 12.5 Mb of chromosome region 5p15.33p15.2, consistent with CdCS.

Further cytogenetic analysis via karyotype revealed a pseudodicentric chromosome originating from a rearrangement between one chromosome 5 at breakpoint p15.2 and one chromosome 18 at breakpoint p11.32. Parental chromosome studies and targeted microarray analysis revealed that this copy number change was de novo in origin.

At a 13-month clinical follow-up, twin B was approximately 4 months behind her twin sibling developmentally. She was able to coo but had no single word use. She was able to roll and sit without support since the age of 10 months. With physiotherapy, she could stand while supported. She could feed herself and had pincer grasp ability at 10 months. She had issues with chewing for which she is receiving aid from occupational therapy.

3 | METHODS

3.1 | NIPT

Both NIPTs utilized third-party private testing. Initial NIPT used a SNP-based sequencing method and proprietary algorithms. Subsequent NIPT used proprietary-targeted amplification technology and analysis algorithm.

3.2 | DNA Extraction, QF-PCR, and Microarray Analysis

Quantitative fluorescence polymerase chain reaction (QF-PCR) and genomic microarray analysis on isolated DNA from peripheral blood cells was performed at the Cytogenetics Lab of Victoria Hospital, London Health Sciences Centre.

Using the Magna Pure Compact Nucleic Acid Isolation Kit I, (Roche Diagnostics GmbH) genomic DNA was extracted from peripheral blood cells. To detect both maternal contamination and autosomal aneuploidies, quantitative fluorescence polymerase chain reaction (QF-PCR) was conducted using an Aneufast QF-PCR kit (Genomed AG, Switzerland.[8] A SeqStudio Genetic Analyzer (Thermo Fisher Scientific) was used to separate the resulting DNA samples via electrophoresis. GeneMapper Software ver. 6.0 (Thermo Fisher Scientific) was subsequently used for allele analysis and investigation of specific markers.

Copy number variations (CNV) were identified via Infinium CytoSNP-850K v1.2 BeadChip array (Illumina, Inc.). Loss of heterozygosity (LOH) was identified through the same means. The resulting data were analyzed using BlueFuse Multi version 4.5(32178) (Illumina, Inc.). The following databases were used as references during analysis: CAGDB, ClinGen, ClinVar, DECIPHER, DCV, DGV, gnomAD, OMIM, UCSC.

All work was conducted at the Cytogenetics Lab within Victoria Hospital, London Health Sciences Centre.

3.3 | Chromosomal karyotype analysis

Trypsin-banded metaphase chromosomes were used for karyotyping following a method that utilized 550 band resolutions from peripheral blood lymphocyte cultures. From these, 10 metaphase spreads were used for analysis. Karyotypes were defined as per the International System for Human Cytogenetic Nomenclature (ISCN 2016).

3.4 | Fluorescence in situ hybridization (FISH)

Fluorescence in situ hybridization (FISH) was conducted using a BAC clone RP11-983C5 probe. This corresponded to chromosome 5 at nucleotide position chr5:13267979–13485824 (GRCh37/hg19 assembly). Standard FISH pre-treatment, hybridization, and fluorescence microscopy was then conducted for the sample as

per the manufacturer's specifications. The results were reported as per the ISCN 2016.

Additionally, to assess for a balanced chromosomal rearrangement of chromosome 5, parental chromosome analysis and FISH analysis were done as well.

4 | RESULTS

Initial NIPT using SNP based methodology was done and stated that there was an "atypical finding outside the scope of the test. A repeat specimen not indicated". Subsequently, NIPT was done using proprietary-targeted amplification methodology and returned results of a female fetus with low risk (less than 1/10,000) for Trisomy 13, 18, and 21.

Postnatally, karyotype and microarray testing were completed on peripheral blood (Figures 1–3). QF-PCR suggested no abnormalities in chromosomes 13, 18, 21, and X. Subsequent karyotype returned 45, XX, psu dic (5;18) (p15.2, p11.32), and microarray returned three CNVs. The first CNV is a pathogenic terminal loss of 12.5 Mb of chromosome region 5p15.33p15.2, consistent with Cri du Chat syndrome. The second CNV is a gain of 1 Mb of chromosome region 5p15.2, classified as uncertain significance, as it contains an OMIM gene that has not been associated with any known disorders, *LINC01194* (617097). The third CNV is a terminal loss of 520 kb of chromosome region 18p11.32, classified as uncertain significance with the loss of three OMIM genes that have not been associated with any known disorders—*THOC1* (606930), *COLEC12* (607621), and *USP14* (607274).

5 | DISCUSSION

This pregnancy was flagged for soft markers and a failed NIPT for chromosome 18. Based on soft markers alone, microarray testing is typically not offered as per both SOGC-CCMG and ACOG guidelines.[6,7] As previously mentioned, neither body provides guidance on uninformative NIPT results. Thus, clinicians in this situation out of an abundance of caution offered multiple options including both a repeat NIPT and diagnostic testing. Although not supported by guidelines if microarray has been conducted in this case for investigation of soft markers and the inconclusive NIPT result, the CdCS diagnosis in twin B might have been identified prenatally.

In terms of this initial failure of NIPT, it may be due to testing methodology. The initial NIPT used an SNP-based screening tool. In the case of a terminal deletion, an SNP-based test would report a copy number variation for the missing terminal SNPs yet report a normal result for the unaffected inline SNPs. This may explain the inconclusive report, as it would not be possible for an SNP-based test to differentiate if this CNV was the result of a terminal deletion or homozygosity.

Postnatally, QF-PCR and clinical microarray were conducted due to newborn exam clinical findings. However, the QF-PCR report was normal for chromosome 18. At LHSC, D18S976 is the most terminal QF-PCR marker on

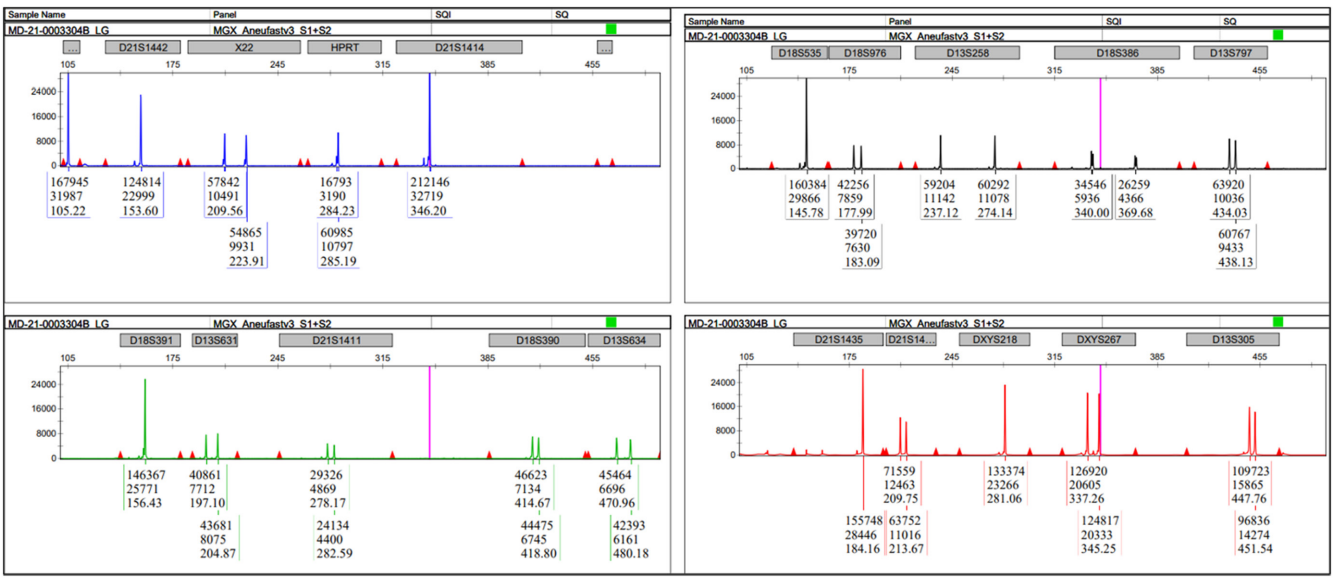

FIGURE 1 QF-PCR analysis of peripheral blood. Informative STR markers on all autosomal chromosomes demonstrate a normal 1:1 marker ratio. The presence of AMELX and the absence of AMELY and SRY is consistent with female gender. The most distal marker on 18p (D18S976) shows two normal peaks, confirming the breakpoint is more distal to the marker.

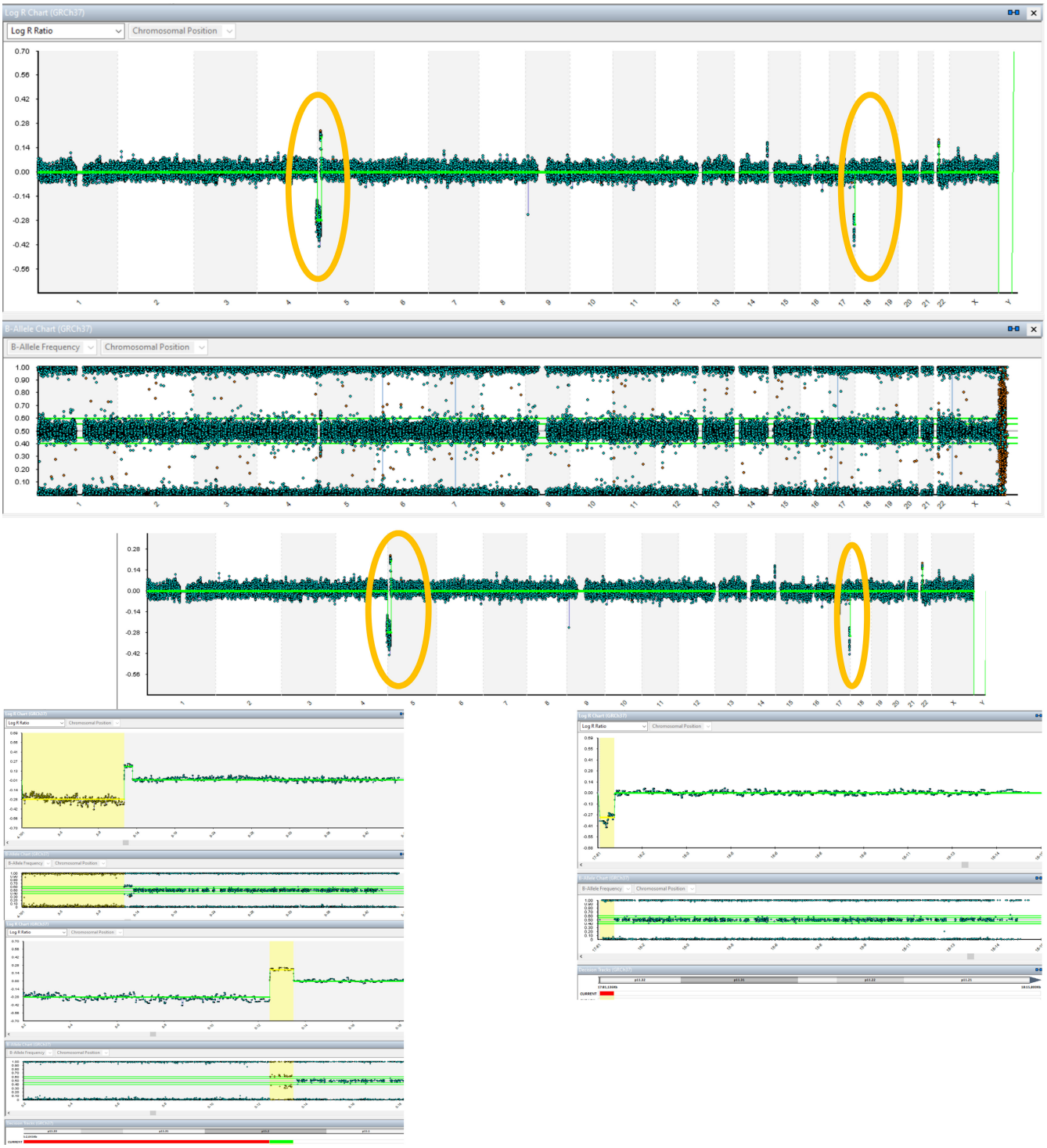

FIGURE 2 Microarray data show the terminal loss and interstitial gain on 5p as well as the terminal loss on 18p. Both LogR and BAF are consistent with the copy number changes.

chromosome 18. Results indicated two peaks for D18S976 suggesting a deletion distal to the marker. Thus, this deletion cannot be identified through QF-PCR alone and highlights the importance of other genetic testing modalities, such as microarray and FISH.

The clinical microarray testing revealed three CNVs located terminally on the short arms of chromosome 5 and 18, which were large in size and contained OMIM genes. LogR values of a small region between the chromosome 5 duplication and deletion indicated a normal region with

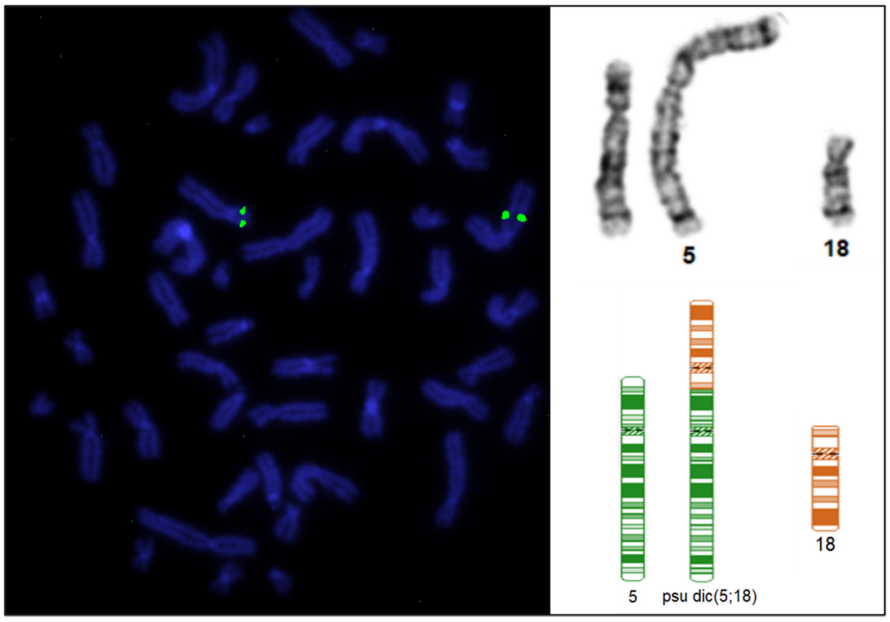

FIGURE 3 FISH and GTG banding analysis show a 45, XX, psu dic(5;18) (p15.2;p11.32) karyotype.

two copies. This pattern is suggestive of an inverted duplication with terminal deletion. Inverted duplications with a terminal deletion are complex chromosomal rearrangements with only two examples in the 5p region reported in literature previously.[9,10] In both cases, the duplicated chromosome portion size was larger than the deleted chromosome portion size.[9,10] To the best of our knowledge, this is the first report of a patient in which the deleted region of chromosome 5p is larger than the duplicated region. Furthermore, in a B-allele frequency analysis, normal samples generate three values of 1, 0.5, and 0. The chromosomal duplication of 5p was noted again through the generation of four values, which is expected from a gain region. Similarly, the deletions in chromosomes 5 and 18 were noted due to generation of only two values, indicating a loss of heterozygosity.

Lastly, FISH analysis was conducted using the RP11 probe to confirm the microarray results. The signal intensity was observed to be higher on the derivative chromosome, indicating that it also contains the duplication. As there was only one signal on the derivative chromosome, but significantly brighter than the other chromosome 5, it signifies a tandem duplication on the derivative chromosome.

In terms of mechanism, the inverted duplication and deletions likely arose from a symmetric U-type recombination between homologous chromosomes, followed by breakage of the dicentric chromosome distal to the fusion site in anaphase.[11] To prevent loss of coding DNA, broken chromosome ends stabilize by telomere healing or telomere capture.[11] In this instance, the distal 18p material attached to the distal end of 5p, suggesting that following the terminal 5p inv-dup-del event, the chromosome was stabilized via telomere capture of distal 18p. Segmental duplications are highly homologous DNA regions with an upper limit of 400 kB in length that occur in multiple areas in the genome and are thought to be locations fruitful for recombination.[12] A high proportion of these regions are found in the sub telomeric region of chromosome 5 and in moderate proportion in chromosome 18 when compared to the rest of the genome.[13]

This case demonstrates the importance of cytogenetic testing such as microarray and FISH analysis in studying complex chromosomal rearrangements. Although not supported by current ACOG or SOGC-CCMG guidelines, microarray testing following the inconclusive results from NIPT paired with soft marker ultrasound findings would have most likely yielded an earlier diagnosis. Both NIPT and QF-PCR were unable to identify these changes suggesting the importance of follow-up testing with microarray and FISH. Furthermore, from a cytogenetics perspective, this case represents the first reported CdCS patient with a (5;18) imbalanced translocation as well as the first reported 5p inverted duplication and deletion in which the deleted region is greater than the duplicated region.

AUTHOR CONTRIBUTIONS

Devanshi Shukla: Conceptualization; writing – original draft; writing – review and editing. **Matthew Dinunzio:** Conceptualization; writing – original draft; writing – review and editing. **Samantha Colaiacovo:** Data curation; writing – review and editing. **Anahita Mohseni Meybodi:** Data curation; methodology; writing – review and editing. **Maha Saleh:** Conceptualization; supervision; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

All authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available upon request from corresponding author.

ETHICS STATEMENT

The local IRB deemed the study exempt from review.

CONSENT

Written informed consent was obtained from the patient to publish this report in accordance with the journal's patient consent policy.

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REFERENCES

1. Lejeune J, Lafourcade J, Berger R, et al. 3 cases of partial deletion of the short arm of a 5 chromosome. *C R Hebd Seances Acad Sci*. 1963;18(257):3098-3102.
2. Espirito Santo LD, Moreira LM, Riegel M. Cri-Du-chat syndrome: clinical profile and chromosomal microarray analysis in six patients. *Biomed Res Int*. 2016;1:2016-2019.
3. Niebuhr E. The cri du chat syndrome: epidemiology, cytogenetics, and clinical features. *Hum Genet*. 1978;44:227-275.
4. Higurashi M, Oda M, Iijima K, et al. Livebirth prevalence and follow-up of malformation syndromes in 27,472 newborns. *Brain Dev*. 1990;12(6):770-773.
5. Cerruti MP. Cri du Chat syndrome. *Orphanet J Rare Dis*. 2006;1(1):1-9.
6. Armour CM, Dougan SD, Brock JA, et al. Practice guideline: joint CCMG-SOGC recommendations for the use of chromosomal microarray analysis for prenatal diagnosis and assessment of fetal loss in Canada. *J Med Genet*. 2018;55(4):215-221.
7. American College of Obstetricians and Gynecologists. Microarrays and next-generation sequencing technology: the use of advanced genetic diagnostic tools in obstetrics and gynecology. ACOG Committee opinion no. 682. American College of Obstetricians and Gynecologists. Obstet Gynecol. 2016;128:e262-8.
8. Mann K, Fox SP, Abbs SJ, et al. Development and implementation of a new rapid aneuploidy diagnostic service within the UK National Health Service and implications for the future of prenatal diagnosis. *The Lancet*. 2001;358(9287):1057-1061.
9. Wang JC, Coe BP, Lomax B, et al. Inverted duplication with terminal deletion of 5p and no cat-like cry. *Am J Med Genet A*. 2008;146(9):1173-1179.
10. Sreekantaiah C, Kronn D, Marinescu RC, Goldin B, Overhauser J. Characterization of a complex chromosomal rearrangement in a patient with a typical catlike cry and no other clinical findings of cri-du-chat syndrome. *Am J Med Genet*. 1999;86(3):264-268.
11. Zuffardi O, Bonaglia M, Ciccone R, Giorda R. Inverted duplications deletions: underdiagnosed rearrangements. *Clin Genet*. 2009;75(6):505-513.
12. Sharp AJ, Locke DP, McGrath SD, et al. Segmental duplications and copy-number variation in the human genome. *The American Journal of Human Genetics*. 2005;77((1)):78-88.
13. Zhang L, Lu HH, Chung WY, Yang J, Li WH. Patterns of segmental duplication in the human genome. *Mol Biol Evol*. 2005;22(1):135-141.

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