CASE REPORT

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Cri-du-chat syndrome diagnosed in a 21-year-old woman by means of comparative genomic hybridization

Síndrome de cri du chat diagnosticado en mujer de 21 años por hibridación genómica comparativa

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| Abstract |

The cri-du-chat syndrome is caused by a deletion on the short arm of chromosome number 5. The size of genetic material loss varies from the 5p15.2 region only to the whole arm. Prevalence rates range between 1:15000 and 1:50000 live births. Diagnosis is suspected on infants with a high-pitched (cat-like) cry, facial dysmorfism, hypotonia and delayed psychomotor development. In adults, phenotypic findings are less specific. It is confirmed through high-resolution G-banding karyotype, fluorescent *in situ* hybridization or microarray-based comparative genomic hybridization (a-CGH).

The following is the case report of a 21-year-old female patient with severe mental retardation and trichotillomania, who does not control sphincters and does not bathe or eat by herself. Her communication is based only on sounds and dysmorphic facies. The G-band karyotype reported is 46, XX. a-CGH shows 18.583Mb interstitial microdeletion in 5p15.33p14.3, including the cri-du-chat critical region. In children or adults with unexplained mental retardation and normal karyotype results (like this case), an a-CGH should be performed to make an etiological diagnosis, establish the prognosis, order additional medical tests and specific treatments, and offer appropriate genetic counseling.

Keywords: Cri-du-chat Syndrome; 5p Deletion Syndrome; Comparative Genomic Hybridization; Mental Retardation (MeSH).

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| Resumen |

El síndrome de cri du chat o del maullido de gato es causado por una deleción en el brazo corto del cromosoma 5; el tamaño de la pérdida de material genético varía desde solo la región 5p15.2 hasta el brazo entero. La prevalencia va desde 1 por 15 000 habitantes hasta 1 por 50 000 habitantes. Su diagnóstico se puede confirmar con cariotipo con bandas G de alta resolución, hibridación fluorescente *in situ* o hibridación genómica comparativa por microarreglos (HGCm); este se sospecha en infantes con un llanto similar al maullido de un gato, fascies dismórficas, hipotonía y retardo del desarrollo psicomotor; sin embargo, en los adultos afectados los hallazgos fenotípicos son menos específicos.

Se presenta el caso de una mujer de 21 años con retardo mental severo y tricotilomanía, que no controla esfínteres y no se baña ni come sola; solo emite ruidos y tiene facies dismórficas. El cariotipo de bandas G es reportado 46, XX y la HGCm muestra microdeleción de 18.583Mb en 5p15.33p14.3, incluyendo región crítica de cri du chat. En pacientes de este tipo se debe realizar HGCm para hacer un diagnóstico etiológico, establecer un pronóstico, ordenar pruebas médicas adicionales y tratamientos específicos y realizar la adecuada asesoría genética.

Palabras clave: Síndrome del maullido del gato; Síndrome de deleción del brazo corto del cromosoma 5; hibridación genómica comparativa; Discapacidad intelectual (DeCS).

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Introduction

The cri-du-chat syndrome (CdCS) or cat meowing is a disease caused by a deletion in the short arm of chromosome 5. The size of the genetic material loss varies and ranges between the whole arm and the 5p15.2 region only. This syndrome affects from 1 in 15 000 to 1 in 50 000 inhabitants (1,2).

A CdCS diagnosis is suspected in infants with acute crying, similar to a cat's meow; size and weight less than two standard deviations;

hypotonia; psychomotor development delay, and particular facies characterized by rounded face, hypertelorism, broad nasal bridge, epicantic folds, micrognathia and ears with low implantation (3). The disease is confirmed through cytogenetic techniques such as karyotype by light microscopy with high-resolution G-bands. Hybridization fluorescence in situ (HFIS) should be performed as the first molecular cytogenetic test, with a specific probe that includes the 5p15.2 (4,5) region, for those who have a suggestive phenotype and unaltered karyotype.

In order to find the etiology in patients with unexplained mental retardation and nonspecific phenotypic findings —for instance, adult patients with CdCS characteristics—, comparative genomic hybridization by microarrays (a-CGH) has been suggested, which is a different molecular cytogenetic test. This test examines the entire genome and can find numerical and structural chromosomal alterations similar to those diagnosed by the karyotype, while detecting losses or excesses of genetic material with a higher resolution level than that diagnosed by conventional cytogenetic techniques (6).

The objective of this report is to present the case of a 21-year-old patient with severe mental retardation of unexplained origin and a karyotype that did not show any cytogenetic alteration. The a-CGH for this patient reported a microdeletion in 5p15.33 p14.3, which served as the basis for a CdCS diagnosis.

Case report

21-year-old female patient born to non-consanguineous parents. The patient's mother got pregnant at age 23, and had adequate prenatal care with two obstetrical ultrasounds that did not detect any abnormality. The patient was delivered via cesarean section due to post-term pregnancy and acute fetal distress. The newborn was hospitalized for 10 days due to poor suction.

The patient has poor psychomotor development, and has not received any type of education due to learning and aggression problems. In addition, by the time of the assessment, she was unable to speak —she only produces noises—. Sphincters are not controlled, she does not bathe or eat by herself and presents with trichotillomania. Auditory potentials show mild bilateral hypoacusis and no abnormalities on the echocardiogram. A karyotype with G-bands with a resolution level of 550 bands (as reported by the laboratory) was performed at age 1, showing the chromosomal formula 46, XX.

Physical examination showed the following special findings: dysmorphic fasciitis due to bitemporal depression, right epicanthic fold, large nose with broad nasal bridge, microretrognathia, persistently large open mouth, macroglossia, short subnasal philtrum, and single transverse palmar crease (Figure 1). a-CGH and nuclear magnetic resonance were requested, but were not performed because the patient offered resistance during the procedure.

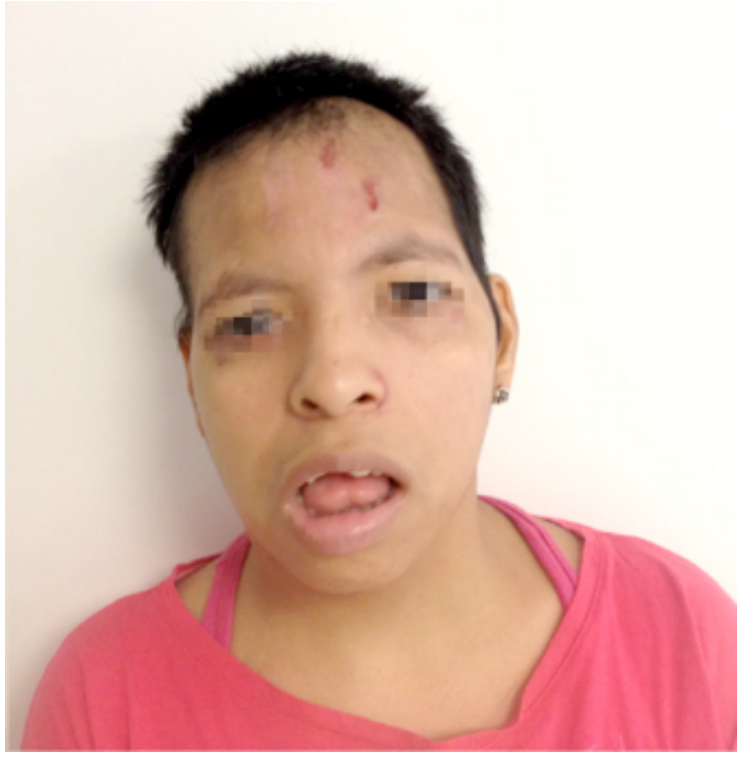

Figure 1. Patient with cri-du-chat syndrome.
Source: Own elaboration based on the data obtained in the study.

The genetic test was performed by extracting 10mL of peripheral blood in two tubes, one with sodium-heparin and another with ethylenediaminetetraacetic acid, which were subsequently sent to the Medical Genetics Laboratory of the Baylor College of Medicine. DNA was extracted using an automated nucleic acid extraction instrument (PerkinElmer Chemagen Technologie GmbH), which allows obtaining genetic material. A high-resolution chromosomal microarray analysis (HR-CMA) was performed using 180 000 oligos covering the whole genome at an average resolution of 30Kb.

Sample and control DNA were differentially labeled with fluorescence and were hybridized with the oligos. The a-CGH matrices used to analyze chromosome 5p were built by Agilent Technologies, which are based on the platform designed by the laboratory (BMGL V8.1.1). During the DNA study, the "Vysis Cri-du-Chat-Region Probe-

LSI D5S23" probe was labeled with green fluorochrome, and the "Vysis LSI EGR1" was used for control and labeled with orange fluorochrome. Results were analyzed using quantitative imaging methods and analytical software to identify the DNA sequences studied, as well as the loss (deletion), gain (duplication) or normality in the copies based on the presence or absence of signals for the D5S23 and EGR1 probes.

The patient's a-CGH result showed abnormal interstitial microdeletion of 18 583MB in the short arm of chromosome 5, 5p15.33p14.3, which includes the cri-du-chat critical region. Figure 2 shows a red area that identifies DNA in deletion and includes the CdCS critical region. The black spots represent chromosomes without an excess or deficit of genetic material.

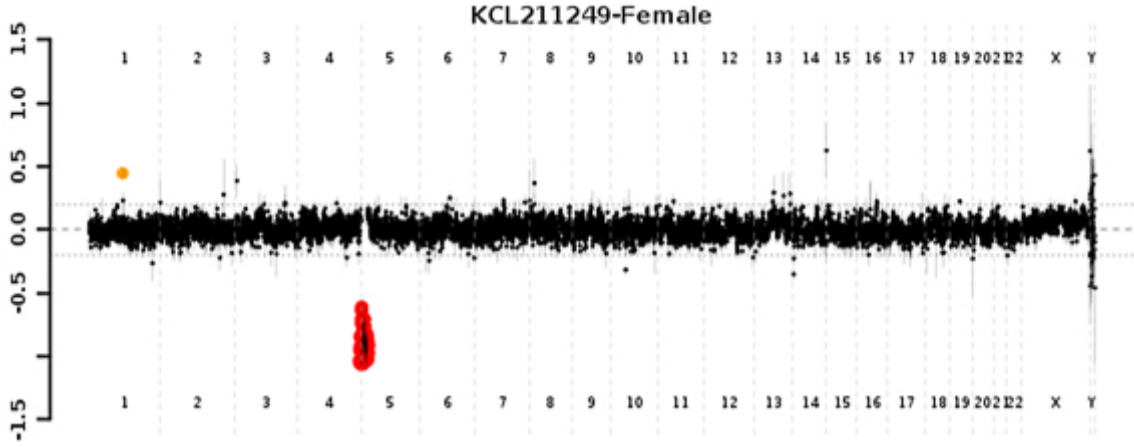

Figure 2. Graphical representation of the result for comparative genomic hybridization by microarray.
Source: Own elaboration based on the data obtained in the study.

A diagnosis of cri-du-chat syndrome by microdeletion, OMIM # 123450, was established. Since the patient's mother had a tubal ligation and the father had no reproductive expectation, no tests were performed to establish if any of them were carriers of a balanced translocation involving the chromosomal region under study. Both parents signed an informed consent to take photographs and use the medical history data.

Discussion

Lejeune *et al.* (7) first described CdCS in 1963. They gave the syndrome this name due to the characteristic cry of affected infants, which resembles a meowing kitten. The prevalence is very low, although it is estimated at 1 for every 350 individuals with mental retardation (1,2,8). This syndrome is caused by a deletion of the short arm of chromosome 5, which can involve total loss of the material on the short arm, or affect only the 5p15.2 region (9).

In CdCS, the loss of genetic material in a specific locus of the short arm of chromosome 5 has been related to particular phenotypic traits in affected patients. Likewise, a critical region located at 5p15.2, 1.7MB in size and strongly related to mental retardation, language delay, typical crying, microcephaly and dysmorphic fascicles has been described (8,10). Deletion in that region implies the loss of the CTNND2 genes, which encode the δ-catenin protein, and of MARCH6, which encodes the protein of the same name (membrane-associated RING-CH6 finger protein). The first is involved in cell motility, expressed in the early stages of neuronal development, especially in cellular migration processes, which is related to the characteristic mental retardation. The second is an E3 ubiquitin-ligase located in the membranes of the endoplasmic reticulum, which has been associated with typical acute crying, language problems and facial features in CdCS (11).

In addition, other genes with 5p loci and clinical significance in CdCS have been reported. SEMA5A (locus 5p15.31) encodes the semaphorin 5A protein associated with mental retardation caused by the reduction in the number of neurons to be differentiated, and to the migration from neurogenesis zones, which generates changes in the organization of the periventricular cortex layers and base ganglia (9). SLC6A3 (locus 5p15.33) encodes a dopamine transporter and is linked to attention deficit hyperactivity disorder and autistic features. CDH18, CDH12, CDH10 and CDH9 (locus 5p14.1 to 5p14.3) encode critical cadherins in cell adhesion during synapse formation and axonal growth. Finally, TERT (locus 5p15.33) produces reverse transcriptase associated with accelerated shortening of telomeres and premature aging (11,12).

In 2005, Zhang *et al.* (13) published a phenotype/genotype correlation study using microarray-based comparative genomic hybridization. They described three contiguous regions on the short arm of chromosome 5 called MR (referring to mental retardation): region I with locus 5p15. 31, size 1.2MB, related to moderate to severe mental retardation; region II, proximal to region I, size 8.9MB, associated to less severe mental retardation, and region III, proximal to region II, 13.8MB and without a consistent phenotype. Mental retardation increases progressively with the joint loss of the described regions, and the critical region would include MRI. In addition, the same authors specifically associated crying to the 5p15.31 region, language delay to the 5p15.32-15.33 region, and dysmorphic features to the 5p15.2-15.31 region (8,11,13). The loss of genetic material in this patient was located in the 5p15.33 p14.3 region, which includes all fragments of genetic material related to the pathological phenotype in CdCS. This loss accounts for severe mental retardation, microcephaly, and marked language deficit.

Between 80% and 90% of the deletions that cause CdCS are de novo, about 10% to 15% are the result of unbalanced translocations of parental, and some particular cases are attributed to rare cytogenetic alterations (1). The risk of recurrence is minimal in cases in which the deletion is de novo; however, the possibility of gonadal mosaicism in one of the parents cannot be disregarded. The probability is higher and becomes more important in familial balanced translocation cases that include the 5p fragment. Cerruti *et al.* (14) show that the risk of progeny with unbalanced chromosome alteration ranges between 8.7% and 18.8%, and that it is similar in carriers of both sexes. In cases

such as the one reported here, a balanced translocation in parents that included the missing 5p fragment in the patient (5p15.33p14.3) should be ruled out through a metaphase HFIS using the specific probe.

CdCS patients may present with typical crying, short stature and weight, severe psychomotor development delay, hypotonia, particular facies (4,8), recurrent vomiting, respiratory difficulties, severe heart disease and jaundice, which can lead to death within the first year of life (15), although none of them was found in the treated patient. As patients grow, the face becomes long, thin and asymmetrical, the nose becomes more prominent, and hypertelorism, epicanto and micrognathia are attenuated. Mental retardation becomes evident in preadolescents and adolescents, who usually do not speak and present periods of aggressive behavior and self-mutilation (9,15); all these characteristics were observed in the treated patient. Over time, the phenotypic traits in these patients become less specific and clinical diagnosis is more difficult (16). A differential diagnosis in adult patients without changes in karyotype can be performed based on the Mowat-Wilson and Wolf-Hirschhorn syndromes, 1p36 monosomy, 17q21 microdeletion, among others.

Usually, the diagnosis is suspected when the classic phenotypic characteristics appear in children younger than one year. To confirm it, a karyotype with G-bands must be performed, which, depending on the resolution level, can detect the total or partial absence of genetic material in the short arm of chromosome 5 (17). However, the diagnostic capacity of the karyotype in structural chromosomal alterations, such as CdCS, varies depending on whether the chromosomes are observed in prometaphase or metaphase, the type of staining and the level of resolution obtained according to the observed bands. In metaphase karyotypes with Giemsa staining (G-bands) and resolution levels between 400 and 500 bands, alterations greater than 10MB can be detected. The diagnostic capacity improves when the chromosomes are observed in prometaphase, which lead to diagnose losses or excesses of genetic material greater than 3MB (18).

The karyotype of the treated patient was performed with metaphase chromosomes, had a resolution of 550 bands and did not report any numerical or structural alterations despite having a deletion of 18MB in 5p that should have been found in that study. This may have happened because chromosome analysis by cytogenetics is a dependent operator, which does not occur with a-CGH, and has limitations in the resolution level due to difficulties in the visualization of specific areas considering the characteristics of the staining (18). Hence, the level of bands reached on chromosome 5 may not be optimal for the diagnosis.

In cases where the karyotype does not show any alteration and the phenotypic characteristics are suggestive, HFIS must be performed using probes that detect losses of fragments of different sizes in the short arm of chromosome 5, including the 5p13 to p15 regions; the most used is the one that hybridizes with the CdCS 5p15.2 critical region (5). In the case reported here, the karyotype was not repeated and the HFIS was not used, since this was an adult patient with no specific phenotype.

A-CGH has been suggested to find the specific etiology in patients with mental retardation, autism and multiple congenital anomalies (6); this is a molecular test that detects losses or excess of genetic material smaller than those diagnosed with cytogenetic techniques by light microscopy. However, it is not sensitive to detect chromosomal rearrangements such as balanced translocations or specify insertion location (19). Diagnosis rates in patients with delayed psychomotor development and mental retardation increased from 3% with karyotype to 12.2% with a-CGH (6).

Comparative genomic hybridization is available in Colombia and is performed in two laboratories of Bogotá D.C. or in reference laboratories from Europe and the USA, as was the case with this patient. The importance of the test lies in obtaining a diagnosis and thus establishing prognosis and ordering additional medical tests and treatments specific to the syndrome, besides offering genetic and reproductive advice (4,14,19).

Conclusions

The case of a 21-year-old female patient was reported in this paper. The patient presented with severe mental retardation of unexplained origin, with unchanged karyotype, and a-CGH with microdeletion in 5p15.33 p14.3, which led to the CdCS diagnosis.

A-CGH should be performed in adult patients with unexplained mental retardation with karyotype and without alteration, in order to obtain an etiological diagnosis, establish prognosis and perform differentiated interventions and genetic counseling.

Authors' contributions

WS and JRC performed the clinical diagnosis and the analysis of the a-CGH result. All the authors participated in the literature review and document writing.

Conflict of interest

None stated by the authors.

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