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CASE REPORT

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# Juvenile myelomonocytic leukemia in CBL syndrome associated with germline splice-site mutations: Two case reports and a literature review

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**Abstract**

The clinical and laboratory criteria for hemophagocytic lymphohistiocytosis should be taken into account during the juvenile myelomonocytic leukemia diagnosis, specifically in CBL syndrome, to reveal the presence of primary rather than secondary associated hemophagocytosis.

**KEYWORDS**

CBL syndrome, hemophagocytic lymphohistiocytosis, juvenile myelomonocytic leukemia, splicing mutations

## 1 | INTRODUCTION

Casitas B-lineage lymphoma (CBL) syndrome is caused by heterozygous germline mutations in the *CBL* gene and is a rare and heterogeneous genetic disease characterized by musculoskeletal anomalies, dysmorphic features, congenital heart defects, and an increased risk of developing juvenile myelomonocytic leukemia (JMML). Clinical outcomes for JMML associated with CBL syndrome vary from spontaneous disease regression to an aggressive course requiring hematopoietic stem cell transplantation. Here, we report two pediatric patients with CBL syndrome who developed JMML. One patient debuted a rare episode of hemophagocytic lymphohistiocytosis, which was assumed to have developed in the context of JMML. We propose that the clinical and laboratory criteria for hemophagocytic lymphohistiocytosis should be considered during the JMML diagnosis to reveal the presence of primary rather than secondary associated hemophagocytosis.

We also emphasize the heterogeneity of the CBL syndrome spectrum with a review of the reported clinical and genetic characteristics of pediatric cases with *CBL* germline mutations.

CBL syndrome is caused by germline heterozygous mutations in the *CBL* gene. The syndrome clinically overlaps with the phenotypic features of Noonan syndrome, (developmental delay, congenital heart defects, and craniofacial anomalies) and shares an increased risk of developing juvenile myelomonocytic leukemia (JMML).[1-3] Patients with JMML present with a variable clinical spectrum, including fever, lymphadenopathy, skin rash, cough, hepatomegaly, pallor, and bleeding.[4] Management and treatment can vary from a "wait and watch" strategy to allogeneic hematopoietic stem cell transplantation (HSCT).[5] HSCT is indicated for patients with JMML and somatic mutations in *PTPN11*, *KRAS,* some *NRAS* mutations, and germline *NF1* mutations.[6] However, a watchful approach is recommended for patients with Noonan syndrome, CBL syndrome, and certain patients with somatic *NRAS* mutations, depending on the severity of the disease, given that spontaneous resolution of JMML is usually observed.[7-9] Here, we report two pediatric cases of CBL syndrome and JMML with different clinical outcomes, including the presence of hemophagocytic lymphohistiocytosis episodes in one case.

## 2 | CASES REPORTS

Patient 1 was the only child of healthy nonconsanguineous parents who was delivered early (at 34 weeks of gestation) due to maternal preeclampsia. At 2 months of age, the patient was hospitalized for persistent leukocytosis ($20.0 \times 10^9$ /L). The peripheral blood count showed 9% monocytes ($2.26 \times 10^9$ /L), 5% metamyelocytes, 2% myelocytes, and 1% promyelocytes. The bone marrow aspirate showed 5% blasts with mature monocytosis, 12% dysplasia, and no excess of blasts, all of which are compatible with JMML. The karyotype was 46, XY, and the genetic screening for *PTPN11*, *NRAS*, *KRAS*, and *CBL* genes revealed a novel heterozygous *CBL* splice-site mutation: c.1096-12_1096del. Sequencing of the patient's fibroblast DNA demonstrated a germline origin (Figure 1A), and parental DNA sequencing confirmed a de novo event. The presence of novel splice products was confirmed by RT-PCR (Figure 1B,C). At 4 months of age, the patient's physical examination detected somewhat redundant neck skin, slightly separated nipples, a distended abdomen, and an umbilical hernia. Abdominal ultrasound showed splenomegaly, and the echocardiogram revealed congenital pulmonic stenosis. One year after the diagnosis, the patient was growing adequately with normal neurologic development and hematologic stability.

Patient 2 was the second child of healthy nonconsanguineous parents who was born at 40 weeks of gestation. At 5 months of age, the patient was referred to our hospital with suspected liver failure. He had a history of hypotonia, feeding difficulties, and two previously "sepsis-like" episodes, the most recent associated with brief transient cytopenia, increased acute phase reactants, and hepatosplenomegaly. Preliminary studies for hyperinflammatory syndromes and immunodeficiencies were inconclusive, despite an in vitro natural killer activity of 0%. An exome sequence analysis of bone marrow DNA revealed the heterozygous *CBL* splice-site mutation c.1228-2A > G. The germline and de novo

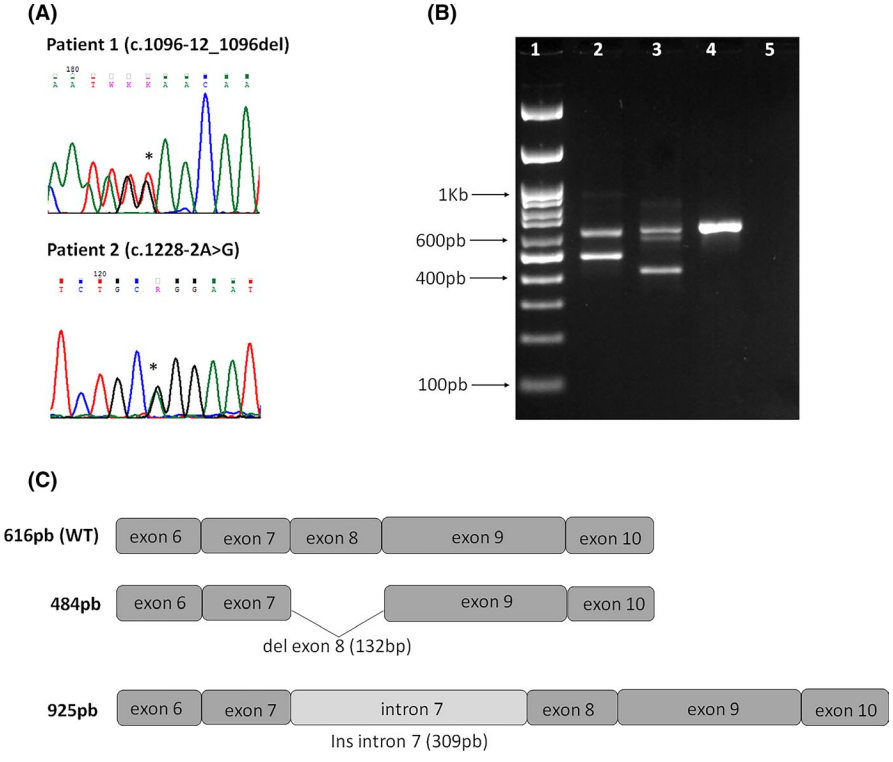


**FIGURE 1** Characterization of *CBL* splice-site variant. A, Electropherograms from fibroblast DNA samples from patient 1 (c.1096-12_1096del) and patient 2 (c.1228-2A>G). Mutations are indicated by an asterisk (*). B, RT-PCR agarose gel. Lane 1:100-bp DNA ladder; lane 2: patient 1 c.1019-12_1096del; lane 3: patient 2 c.1228-2A>G; lane 4: control sample (wild type); lane 5: blank. C, Schematic representation of transcript products as a consequence of the novel splice-site mutation c.1096-12_1096del. RT-PCR, real-time reverse transcription polymerase chain reaction; del, deletion; ins, insertion

**TABLE 1** CBL germline mutations

| | CBL mutation | | | | | | | Clinical characteristics | | |
|---|---|---|---|---|---|---|---|---|---|---|
| No.cases | Mutation | Germ | Inheritance | Age Dx (y.o)[a] | JMML | HSCT | Follow-up (mo)[a] | Status | Phenotypic features | Ref |
| 1 | p. Tyr235* | NA | Familial | 0.6 | - | - | NA | Alive | neurologic hypotonia; craniofacial and skeletal anomalies; pulmonary valve anomalies | 10 |
| 1 | c.1096-1G>C | Het | NA | 0.9 | + | + | 30 | Dead | developmental delay; café-au-lait spots, heart disease, cerebral hypoxia | 2 |
| 2 | c.1096-1G>T | NA | De novo | 1 - 8 | - | - | 24-120 | Alive | feeding difficulties.; developmental delay; neurologic hypotonia; craniofacial and skeletal anomalies; pulmonary valve stenosis; dark skin, muscular hypotonia, abnormal brain myelination | 10, 11 |
| 1 | c.1096-1delGG | Het | De novo | 2.1 | + | + | 26 | Alive | NA | 12, 9 |
| 1 | c.1096-4 1096-1delAAAG | Het | NA | 0.2 | + | NA | 4.8 | Dead | feeding difficulties; developmental delay; neurologic hypotonia; craniofacial and skeletal anomalies; pulmonary valve stenosis | 7 |
| 1 | c.1096-12_1096del | Het | De novo | 0.16 | + | - | 7 | Alive | **craniofacial and skeletal anomalies; pulmonary valve stenosis; hepatomegaly, splenomegaly; skin lesions** | Present study |
| 5[b] | p. Gln367Pro | Het | De novo | 0.5-9 | - | - | 3.6-174 | Alive | feeding difficulties; developmental delay; neurologic hypotonia; craniofacial and skeletal anomalies; heart disease; café-au-lait spots; ovarian teratoma; embryonal rhabdomyosarcoma | 11, 21-23 |
| 1 | p. Glu369_ Tyr371del | Het | De novo | 3.2 | - | - | NA | Alive | feeding difficulties; developmental delay; neurologic hypotonia; craniofacial and skeletal anomalies; pulmonary valve stenosis; dark skin; ophthalmological disease | 10 |
| 2 | p. Tyr371Asn | Het | NA | 1.3-3 | + | - | 8.4-120 | Alive | craniofacial and skeletal anomalies; JXG; heart disease; ophthalmological disease; moyamoya disease | 2, 13 |
| 18[c] | p. Tyr371His | Het | De novo | 0.6-10 | + | + | 9.6-199.2 | Alive | feeding difficulties; developmental delay; craniofacial and skeletal anomalies; café-au-lait spots; heart disease; JXG; vasculitis; splenomegaly | 1, 2, 10-12, 21, 22, 24, 25 |
| 5[d] | p. Tyr371Cys | Het | Familial | 0.6-1.6 | + | - | 828 (7.5y) | Alive | developmental delay; café-au-lait spots; craniofacial anomalies; ophthalmological disease; heart disease; splenomegaly; thyroid cancer | 2, 26 |
| 1 | p. Leu380Pro | Het | NA | 0.65 | + | + | 21.6 | Dead | developmental delay; JXG | 2 |
| 1 | p. Cys381Gly | Het | De novo | 2.9 | - | - | 443.6 | Alive | HLH; auto-immune manifestations | 12 |

(Continues)

**TABLE 1** (Continued)

| | CBL mutation | | | | | | | Clinical characteristics | | |
|---|---|---|---|---|---|---|---|---|---|---|
| No.cases | Mutation | Germ | Inheritance | Age Dx (y.o)[a] | JMML | HSCT | Follow-up (mo)[a] | Status | Phenotypic features | Ref |
| 1 | p. Lys382Glu | Het | Familial | 18 | - | - | NA | Alive | craniofacial and skeletal anomalies; Arnold Chiari malformation | 21 |
| 3[e] | p. Cys384Arg | NA | NA | 1.4-2.2 | + | - | 19.2-99.6 | Dead | developmental delay; ophthalmological disease; heart disease; JXG | 2 |
| 1 | p. Asp390Tyr | Het | De novo | 3 | - | - | 144 | Alive | feeding difficulties; developmental delay; neurologic hypotonia; craniofacial and skeletal anomalies | 21 |
| 1 | p. Asp390Val | Het | De novo | 40 | - | - | NA | Alive | Acute myeloid leukemia, splenomegaly, hereditary spherocytosis | 21 |
| 1 | p. Cys396Arg | Het | NA | 0.1 | + | - | 217.2 | Alive | developmental delay; heart disease; ophthalmological disease; hearing loss | 2 |
| 1 | p. His398Arg | Het | Familial | 1.5 | + | - | NA | Alive | NA | 2 |
| 1 | p. Cys404Arg | Het | De novo | 1.1 | + | + | 70.8 | Alive | NA | 2 |
| | **CBL mutation** | | | | | | | **Clinical characteristics** | | |
| **No. cases** | **Mutation** | **Germ** | **Inheritance** | **Age Dx (y.o)[a]** | **JMML** | **HSCT** | **Follow-up (mo)[a]** | **Status** | **Phenotypic features** | **Ref.** |
| 1 | p. Trp408Arg | Het | De novo | 3.6 | + | - | 112.8 | Alive | developmental delay; intracranial germinoma; café-au-lait spots; JXG; ophthalmological disease | 2 |
| 5 | c.1228-2A>G | Het | De novo | 0.4-5 | + | + | 5-135 | Alive | developmental delay; craniofacial and skeletal anomalies; moyamoya disease; café-au-lait spots; autoimmune disease; muscular hypotonia; splenomegaly; self-revolving exanthema; HLH | 2, 12, 13, Present study |
| 1 | p. Phe418Ser | Het | Familial | 5.7 | - | - | 34 | Alive | neutrophilic dermatosis; splenomegaly; development delay | 12 |
| 1 | p. Phe418Leu | Het | Familial | 0.5 | + | - | 72 | Alive | NA | 12 |
| 1 | p. Arg420Gly | Het | De novo | 1.6 | - | - | 53 | Alive | moyamoya disease; splenomegaly; JXG | 12 |
| 1 | p. Arg420Gln | Het | Familial | 0.5 | - | - | 96 | Alive | neurologic hypotonia; craniofacial and skeletal anomalies; café-au-lait spots; dark skin; heart disease | 21 |

Abbreviations: Dx, diagnosis; Het, heterozygous; HLH, hemophagocytic lymphohistiocytosis; HSCT, hematopoietic stem cell transplantation; JMML, juvenile myelomonocytic leukemia; JXG, juvenile xanthogranuloma; mo, months; NA, not available; Ref, reference; y.o, years.

[a]Range of age at diagnosis and range of months of follow-up were informed for more than one case description.

[b]Bülow et al (2014) described a JMMLcase without somatic LOH. Hanson et al (2014), reported a case of ovarian teratoma, and the subject described by Ji et al (2019) presented embryonal rhabdomyosarcoma.

[c]Described has a de novo event in 10 subjects. Sixteen of 18 cases presented JMML, 15 of them received HSCT, and four of them died.

[d]Number of cases based on four childhood JMML, members of a 35 years follow-up family, described by Pathak et al (2015). One of these subjects died at 16 months of age without a specific JMML diagnosis.

[e] One of three subjects is from a familial case who receive HSCT, and the other two described cases did not die from JMML complications.

origin of the mutation were confirmed by DNA sequencing of the patient's fibroblasts (Figure 1A) and blood leucocyte samples from his parents. On admission, the patient presented with progressive cytopenia and massive splenomegaly (10.3 cm). He also developed progressive monocytosis (>1.0 × $10^3$ /μL). On suspicion of JMML, a new bone marrow aspiration was performed, but no dysplastic signs were observed. The patient's karyotype was 46, XY, no BCR/ABL was detected, and the blast percentage was 2.5%. Granulocyte-macrophage colony-stimulating factor hypersensitivity was positive. Another "sepsis-like" episode was observed, with persistent fever, progressive hepatobiliary dysfunction, and a hyperinflammatory status with hyperferritinemia, hypofibrinogenemia, and hypertriglyceridemia. The patient subsequently met the clinical and analytical criteria for the diagnosis of JMML and hemophagocytic lymphohistiocytosis (HLH). No correlation between HLH and the viral copy number of Epstein-Barr or cytomegalovirus was observed. Despite the recommended "wait and watch" approach for patients with CBL syndrome and due to the recurrent hemophagocytic episodes, the patient underwent matched unrelated HSCT treatment with dexamethasone and etoposide according to the HLH 2004 protocol. At the 6 month follow-up, the patient was stable, with complete donor chimerism and no signs of a hemophagocytic-like episode.

## 3 | DISCUSSION

A total of 26 germline *CBL* mutations have been reported in 59 cases of CBL syndrome (Table 1), most of which were missense mutations (77%). Eleven patients harbored splice-site mutations, located at intron 7 or 9, causing in-frame deletions of the RING finger domain responsible for the E3 ubiquitin ligase activity.[2,10-13] Nine of these patients developed JMML, and 78% (n = 7) required HSCT. All JMML cases with the c.1228-2A>G mutation underwent HSCT, in contrast with those with splice-site mutations on intron 7, 50% of whom underwent HSCT, suggesting that a splice-site mutation located at intron 7 could be associated with a favorable outcome (Table 1).

HLH is a life-threatening hyperinflammatory disease from a series of underlying conditions that trigger uncontrolled acute inflammation, including infections, a weakened depressed immune system, autoimmune diseases, autoinflammatory diseases, and malignancy, such as T-cell leukemia and B-cell lymphoma.[14,15] HLH has no pathognomonic clinical manifestation or specific laboratory finding, and the diagnosis is based on the presence of 5 of 8 clinical and laboratory parameters defined by the Histiocyte Society.[16,17] HLH has also been reported in rare childhood JMML cases related to juvenile xanthogranuloma and with lymphadenopathies such as Kikuchi's disease.[18-20] The HLH observed in patient 2 is rarely reported in CBL syndrome (Table 1), given that the only case reported by Strullu et al (2013) presented HLH syndrome but not JMML. Regarding the clinical outcome of our second patient, due to the corroborating diagnosis of JMML, it was assumed that his inflammatory outcome developed in the context of JMML, either as an initial clinical manifestation or as a secondary phenomenon. We therefore propose that the clinical and laboratory criteria for HLH should be considered during JMML diagnosis to reveal the presence of primary associated hemophagocytosis.

The genetic characterization of these two patients confirms the heterogeneity of the clinical features and disease outcomes in CBL syndrome and emphasizes the need for close clinical management to improve decision making, particularly in those patients who require HSCT.

### CONFLICT OF INTEREST

The authors declare no conflicts of interest.

### AUTHOR CONTRIBUTIONS

All authors: significantly contributed to the manuscript and reviewed and agreed with the content of the final version. In addition to writing the manuscript, Leila Cardoso and Adela Escudero López: were responsible for the genetic tests of one of the patient and data interpretation of both *CBL* mutations. Susana Riesco and María Isidoro-García: were responsible for the genetic diagnosis of one of the patients and participated in the review of the published germline mutations in *CBL*. Víctor Galán Gómez, María Dolores Corral Sánchez, and Antonio Pérez-Martínez: formed the medical team in charge of the patients' clinical management; and were involved in the clinical review of previously reported cases of CBL syndrome.

### ETHICAL APPROVAL

The ethics committee of La Paz University Hospital approved this study, and informed consent was obtained from the parents of the patients according to the Declaration of Helsinki.

### DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article.

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