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CASE REPORT

Chronic myeloproliferative neoplasm in adulthood in CBL syndrome harboring a splice-site *CBL* variant alongside a novel constitutional *CSF3R* variant

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Abstract

Casitas B-cell lineage (CBL) syndrome is a rare RASopathy known to predispose to CBL-mutated juvenile myelomonocytic leukemia (JMML) in childhood. Adulthood acute myeloid leukemia arising out of a genetic aberrancies consistent with prior CBL-mutated JMML has been twice previously described, but chronic myeloproliferative neoplasia has not. We present a case of progressive myeloproliferative neoplasm in adulthood in the context of CBL syndrome alongside a novel CSF3R variant. We also review pathogenic splice-site mutations in CBL-mutated JMML.

KEYWORDS
cancer genetics, chromosome 11q, infant leukemia, molecular pathogenesis, myeloproliferative disorder

Casitas B-cell lineage (CBL) syndrome is a rare autosomal dominant multisystem RASopathy with variable expression of a Noonan's-like phenotype featuring ectodermal, neurodevelopmental, immune, and cardiac defects. There is a predisposition toward juvenile myelomonocytic leukemia (JMML) with a peculiar tendency toward spontaneous hematologic remission. Reports of myeloid neoplasia in CBL syndrome in adulthood are limited to two cases of acute myeloid leukemia (AML). We now report the first case of chronic myeloid neoplasia in adulthood in CBL syndrome and describe its clinicopathologic features.

The female proband was born to non-consanguineous parents of Lebanese heritage. In infancy, she exhibited marked hypotonia, organomegaly, and failure to thrive. In childhood, she exhibited delayed developmental milestones and underwent an atrial septal defect repair.

At age of 21 years, during investigation for iron deficiency relating to menorrhagia, she was incidentally found to have moderate splenomegaly. Full blood examination showed a microcytic anemia with mild thrombocytopenia and normal white cell count and differential. Splenic length was 22 cm by ultrasonography. The initial bone marrow biopsy demonstrated a hypercellular marrow with left-shifted myeloid hyperplasia, megakaryocyte hyperplasia, and grade 2–3/4 reticulin fibrosis. Comprehensive metabolic investigations (listed in Table 2) identified no abnormalities and fluorescent in-situ hybridisation alongside chromosomal microarray demonstrated no genomic imbalances (specifically excluding *PDGFRA*, *PDGFRB*, *FGFR1*, *BCR*, and *ABL1* fusions), precluding a unifying diagnosis. She subsequently developed Hashimoto's thyroiditis

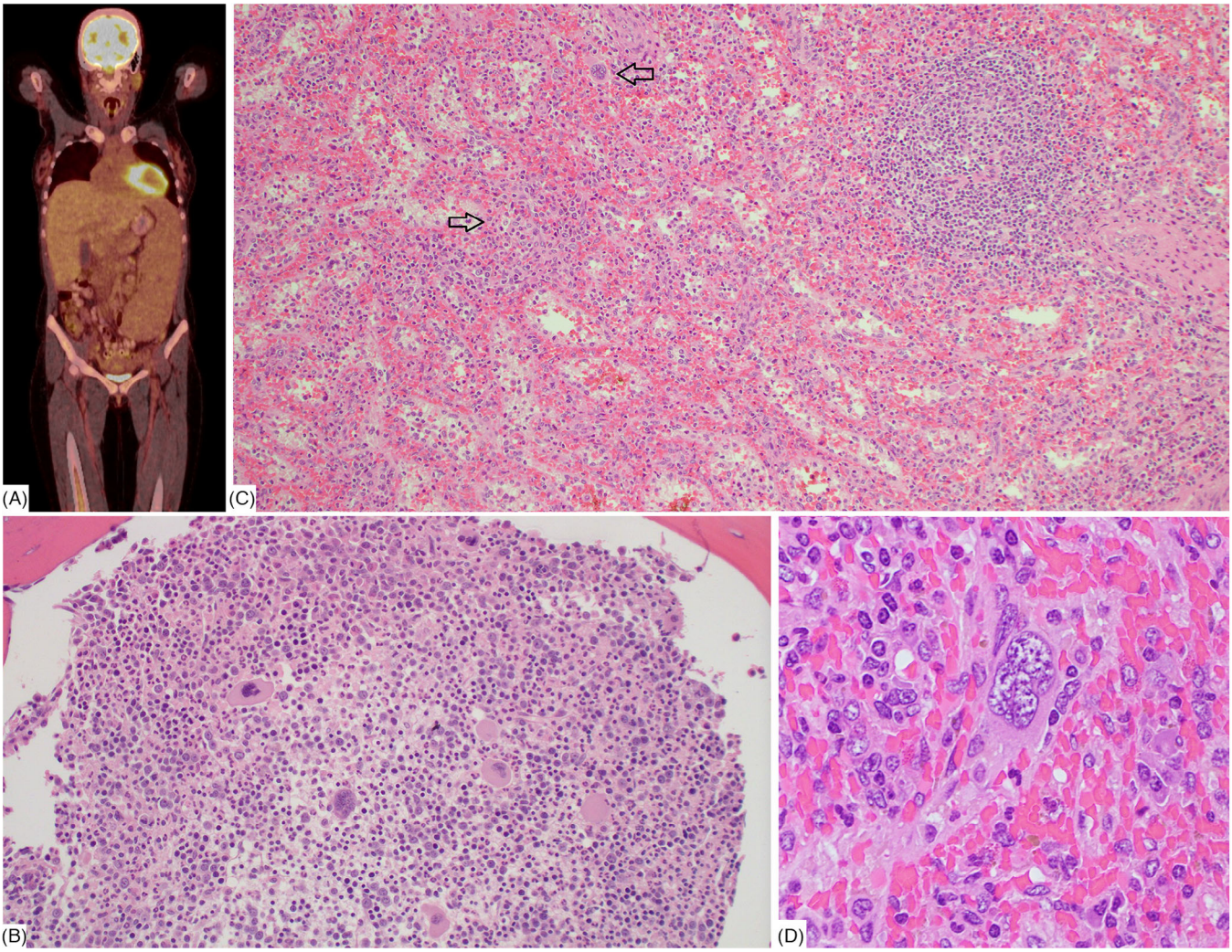

FIGURE 1 (A) Positron emission tomography (PET) scan demonstrating massive splenomegaly. (B) Bone marrow trephine—markedly hypercellular with panmyelosis. (C) Spleen biopsy (×20 magnification) marked expansion of splenic red pulp with congestion of splenic sinusoids. There is widespread morphological evidence of extramedullary hemopoiesis with megakaryocytes (left arrow), myeloid, and erythroid precursors (right arrow) identified. The cells are seen in the cords and in sinusoids. (D) Spleen biopsy (×100 magnification) extramedullary megakaryocyte.

with anti-thyroid peroxidase antibodies and commenced thyroxine supplementation.

Approximately 7 years after initial presentation, the patient presented acutely with a severe symptomatic cold antibody-induced hemolytic anemia with a hemoglobin nadir of 38 g/L. Longitudinal review of cell counts to date demonstrated a normal platelet and white cell count and including normal eosinophil, monocyte counts, not exceeding 0.5 × 10^9/L. Investigations demonstrated positive Mycoplasma pneumoniae serology. A broad autoimmune screen identified SS-B autoantibodies without a clinical correlate. A prolonged prothrombin time and mild reductions in factors VII, X, and XI were detected.

Clinical examination revealed massive splenomegaly, extending to the left iliac crest. A positron emission tomography scan (Figure 1A) revealed increased bone marrow fluorodeoxyglucose avidity and hepatosplenomegaly. Bone marrow biopsy during the hemolytic episode showed reactive erythroid dysplasia, marked hypercellularity (Figure 1B) with erythroid, and megakaryocytic hyperplasia alongside persistent increased reticulin staining.

The proband required transfusion of 34 units of packed red cells over 4 weeks and received rituximab with an eventual remission of hemolysis. A clinical decision was made to proceed to splenectomy which had been planned in the context of the patient's earlier refractory hemolysis, likely aggravated by massive splenomegaly.

The excised spleen measured 370 × 170 × 60 mm and weighed 2500 g. Splenic histology demonstrated florid extramedullary hematopoesis and is further described in Figure 1C,D. Flow cytometry detected a monocytic population accounting for 15% of splenic cells. Targeted next-generation sequencing on peripheral blood identified a *CBL* NM_005188.3: c.1096-1G>C, p.(?) splice variant, and a *CSF3R* NM_156039.3: c.1785_1786del p.(Tyr596Serfs*99) variant both at 50% variant allele frequency (VAF), consistent with an interim designation of myeloproliferative neoplasm, not otherwise specified (MPN-NOS) given the exclusion

of other compatible diagnostic entities per the 2022 WHO classification [1].

To define the underlying genetic basis, trio whole exome sequencing was performed on buccal samples. This confirmed the previously identified *CBL* and *CSF3R* variants in the proband at 43% and 42%, respectively. These were absent in parental samples; both were de novo germline variants. A diagnosis of CBL syndrome of de novo origin was made.

The *CBL* gene located on the long arm of chromosome 11, encodes an intracellular adaptor protein involved in surface membrane receptor signal transduction and receptor recycling through its E3 ubiquitin ligase activity. *CBL* variants in CBL syndrome were first demonstrated in the exon 8 ring finger domain, resulting in an amino acid change in the Y371 phosphorylation site conferring constitutive RAS–MAPK activity. Copy number neutral loss of heterozygosity (CN-LOH) of 11q has been identified in those who develop JMML [2]. Persistent 11q CN-LOH may remain following spontaneous or treatment-related hematologic remission (Niemeyer et al.), signifying acquired clonal hematopoesis.

The *CBL:c.1096-1G>C* variant is located at the non-coding intron 7/exon 8 splice-site boundary. The 50% VAF indicates retained heterozygosity, consistent with the microarray result. Intron 7 splice-site variants were first identified in a case series [4], including two JMML cases with retained heterozygosity in hematopoetic tissue, thus suggesting at the time that 11q CN-LOH is not a requisite step in leukemogenesis. The first functional assessments of splice-site variants at the intron 7–exon 8 donor/acceptor splice-site boundary demonstrated aberrant splicing with alternative transcripts featuring absent and truncated exon 8 segments [4], confirming this is a pathogenic variant by ACMG criteria (PM2, PS3, PS2).

To date, there have been eight published reports of JMML arising with *CBL* variants in the intron 7–exon 8 splice-site boundary (Table 1). Five had confirmed constitutional origin, one had a somatic CBL variant and two have unverified origins. Of the reported cases, only four had confirmed germline status, and additional genetic abnormalities were only identified in cases with negative or unverified germline status. Therefore, 5/5 of reported germline cases exhibited de novo, intronic pathogenic CBL variants with retained heterozygosity in hematopoetic tissue, similar to the proband described. Of note, only childhood phenotypic information is available for these five individuals. Furthermore, assessment for concomitant single nucleotide variants had been limited to the canonical RASopathy genes (at most *PTPN11*, *SOS1*, *RAF1*, *NRAS*, *BRAF*, *MEK1*, *MEK2*, *CBL*, *NF1 SHOC2*) and only one case included chromosomal microarray, potentially leaving structural rearrangements unidentified. The largest cohort of CBL-mutated JMML to date has recently been described, where 33 patients underwent a 26-gene targeted panel and microarray. Only one secondary somatic CBL variant was identified with the panel used [8].

Our patient's *CSF3R* 2-base pair deletion which results in a frameshift has not been previously reported and is "likely pathogenic" by ACMG criteria (PM2, PM4, PS2). It is expected to produce a tyrosine to serine change at amino acid residue 596 with further disruption in the terminal 241 amino acids, a region spanning the transmembrane and cytoplasmic domains. Variation in this region leads to markedly altered receptor degradation and therefore increased signaling in myeloid neoplasia such as atypical chronic myeloid leukemia and are a monogenic cause of chronic neutrophilic leukemia [9]. Constitutional *CSF3R* variants have also recently been identified as candidate germline risk alleles for myeloid neoplasms [10].

Additive constitutional mutations have previously been described in CBL syndrome; crosstalk between *CBL* and *SH2B3* constitutional variants were thought to produce a phenotype with immune dysregulation and associated coagulopathy that co-segregates with affected kindreds [11]. The *CBL* gene products c-CBL, b-CBL, and CBL act as a Lyn-Src adaptor to mediate intracellular CSF3R signaling [12]. The concurrence of *CBL* and *CSF3R* variants with a shared signaling pathway, and the conspicuous absence of 11q CN-LOH leads us to strongly consider if these are co-operative mutations culminating in a heretofore undescribed myeloproliferative neoplasm in adulthood. This suggests that potential "second hits" may be unrecognized in JMML with retained 11q heterozygosity that have not undergone exome or targeted sequencing for co-mutation.

The literature on the manifestations CBL syndrome in adulthood is sparse, the syndrome only having been recognised in 2010 [2]. The first described features of this syndrome are those that overlap with other RASopathies and the features of CBL-variant JMML. As further reports have emerged over the past decade, additional phenotypic manifestations such as coagulopathy, autoimmunity and immune dysfunction with predisposition to non-JMML myeloid neoplasia have become apparent, as collated in Table 2. In particular, the autoimmune features and MPN-NOS were only exhibited by the case proband upon reaching adulthood, illustrating the need for longitudinal case histories in CBL syndrome.

Reports to date of myeloid neoplasms in CBL syndrome in adulthood are incredibly rare, and do not clearly implicate causality of the constitutional *CBL* variant in adult leukemogenesis. The most extensive longitudinal family study to date of CBL syndrome describes two patients with features suspicious for adulthood myeloproliferative neoplasms [13]. The study proband received a retrospective diagnosis of JMML based on historical chart review and repeat bone marrow aspirate at age 36 demonstrating hypercellularity, a pathogenic CBL variant and 11q CN-LOH. However, this bone marrow aspirate occurred 33 years after cytotoxic treatment for "familial childhood chronic myeloid leukemia" and was also 30 years post-splenectomy, rendering attribution of cause difficult. Similarly, one kindred in the series is described as having moderate splenomegaly at age of 32 years; however, active toxoplasmosis infection was also evident on the splenectomy histology as an alternate explanation.

Adult-onset AML in CBL syndrome was first reported by Becker et al. [14]. This was demonstrated to arise from a post-JMML clonal hematopoetic state due to detectable 11q CN-LOH in the leukemia tissue. CBFC::MYH11 translocation was also identified as a genetic driver. The second case of AML in a CBL syndrome affected patient harboring a cryptic t(7;11) resulting in increased copy number of the KMT2A locus alongside a pathogenic U2AF1 SNV, again on a

TABLE 1 Cases with intron 7 splice-site variant features.

Hematologic clinical features	CBL variant	CBL allelic status by tissue	Additional genetic abnormality	Ref
JMML at 1 year of age with massive splenomegaly. Died at 3 years old of cerebral hypoxia.	De novo c.1096-1G>C	Germline analysis not reported Hematopoetic tissue: 11q CN-LOH (confirmatory method not described)	45, XY, −16	[2]
4-Year-old atypical hemolytic uremic syndrome.	De novo c.1096-1G>T	Germline status not assessed Hematopoetic tissue: 11q CN-LOH inferred by VAF	*CFH* variant on WES	[5]
JMML at 4 years of age eventual death with rhabdomyosarcoma.	c.1096-1G>C, no parental testing	Germline tissue: CBL wild type Hematopoetic tissue: heterozygous CBL confirmed by microsatellite analysis	Germline NF1 heterozygote with NF1 CN-LOH in tumoral tissue	[3]
Progressive splenomegaly in adulthood. Factors IX and X deficiency.	De novo c.1096-1G>C	Germline tissue heterozygous Hematopoetic tissue: heterozygous CBL inferred by buccal tissue WES VAF	Germline CSF3R heterozygote	Current patient
JMML with persisting splenomegaly at 11 years. Factors II, IX, X, and XII deficiency with bleeding diathesis.	De novo c.1096-1G>T	Germline status not assessed Hematopoetic tissue: heterozygous CBL	None described. Only assessed for PTPN11, SOS1, RAF1, NRAS, BRAF, MEK1, MEK2, and CBL	[4, 6]
JMML at 4 years with post-HSCT relapse.	De novo c.1096-1delGG	Germline tissue heterozygous Hematopoetic tissue: heterozygous CBL confirmed by microsatellite analysis	None described.	[3]
JMML at 2 months, stabilized without treatment.	De novo c.1096-12_1096del	Germline (skin fibroblast): heterozygous Hematopoetic tissue; heterozygous CBL inferred by VAF	None described. Only assessed for PTPN11, NRAS, KRAS, and CBL	[7]
Organomegaly with JMML.	c.1096-4 1096-1 delAAAG, no parental testing	Germline tissue (muscle): Heterozygous Hematopoetic tissue: heterozygous CBL	None described. Only assessed for PTPN11, SOS1, RAF1, RIT1, KRAS, and SHOC2	[4]
Splenomegaly and leukocytosis at birth.	c.1098-1 G>T	Germline tissue: heterozygous Hematopoetic tissue: heterozygous CBL	None described on co-assessment of ASXL1, BRAF, CBL, DNMT3A, ETV6, EZH2, FLT3, GATA2, JAK3, KRAS, MAP2K1, NF1, NRAS, PTPN11, RAC2, RAF1, RIT1, RRAS, RRAS2, RUNX1, SAMD9, SAMD9L, SETBP1, SH2B3, SOS1, and ZRSR2	[8]

Abbreviations: CBL, casitas B-cell lineage; CN-LOH, copy number neutral loss of heterozygosity; HSCT, haematopoetic stem cell transplant; JMML, juvenile myelomonocytic leukemia; VAF, variant allele frequency; WES, whole exome sequencing.

TABLE 2 Clinical features of intron 7 variant casitas B-cell lineage (CBL) syndrome.

	Index proband	Reports
Neurodevelopmental	Hypotonia, failure to thrive requiring enteral feeds for first 3 months, developmental delay	Delayed myelination, microcephaly [2]
Ectodermal	Café-Au-Lait spots	Lentigines [2]
Cardiovascular	Atrial septal defect, supraventricular tachycardia[a]	Pulmonary stenosis, left ventricular hypertrophy with LVOTO, supraventricular tachycardia [2], Moya Moya, hypertensive cardiomyopathy
Coagulopathy	VII, X, and XI deficiency	II, IX, X, XII, von willebrand factor deficiency [6, 11, 14]
Autoimmunity	Hashimoto's thyroiditis[a]	ALPS-like syndrome [11]
Organomegaly	Progressive hepatosplenomegaly with monocytic infiltrate[a]. No metabolic abnormality on assessment of angiotensin-converting enzyme, glucocerebrosidase and chitotriosidase activity, leukocyte lysosomal, sphingolipid, glycosylation, and glycogen storage enzyme levels	Resolution of splenomegaly with remission of JMML [2]
Syndromic features	Short neck, telecanthus, bilateral ptosis	Noonan-like features [2]
Hematologic	MPN-NOS[a], normal HbF %	AML [14, 15], persistence of hemoglobin F [2]
Ophthalmologic	Nil to date	Optic atrophy [2]

Abbreviations: ALPS, Autoimmune lymphoproliferative syndrome; AML, acute myeloid leukemia; JMML, juvenile myelomonocytic leukemia; LVOTO, Left ventricular outflow tract obstruction, MPN-NOS, myeloproliferative neoplasm, not otherwise specified.
[a]Identified in adulthood.

background of clonal hematopoesis signified by 11q CN-LOH [15]. In contrast, we demonstrate that myeloproliferative neoplasia in CBL syndrome in adulthood may retain 11q heterozygosity.

CBL syndrome has manifestations that differ over the human lifetime, and hematological features that may vary with the genotype. This unique case constitutes the first description of concurrent pathogenic constitutional CSF3R and CBL variants and the first chronic-phase myeloid neoplasm with retained 11q heterozygosity in adulthood described in CBL syndrome.

AUTHOR CONTRIBUTIONS

George Mason collected and analyzed the data and wrote the paper. Rhian Aghajani collected the data and wrote the paper. Brieana Dance collected and analyzed the data. William Stevenson and Jad Othman contributed to data analysis. Naomi Mackinlay and Linda Goodwin followed up with the patient, collected the data, and provided their expertise.

CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in ClinVar at https://www.ncbi.nlm.nih.gov/clinvar/variation/45196/ and https://www.ncbi.nlm.nih.gov/clinvar/variation/844629/ (reference numbers VCV000045196.14 and VCV000844629.4).

ETHICS STATEMENT

Formal ethics approval is not required by the NSLHD HREC for case reports when they involve less than five patients.

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

N/A.

CLINICAL TRIAL REGISTRATION

The authors have confirmed clinical trial registration is not needed for this submission.

PATIENT CONSENT STATEMENT

The authors confirm that written informed consent was obtained from the patient for the publication of this case report in accordance with the Declaration of Helsinki.

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