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Leaky Artemis Deficiency and EBV-Related Lymphoproliferative Disease: A Novel Case and Review of the Literature

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ABSTRACT

Introduction: Artemis (*DCLRE1C*) deficiency causes radiosensitive severe combined immunodeficiency (SCID), although hypomorphic cases can manifest later-onset immunodeficiency, autoimmunity, or lymphoproliferation. We report a 45-year-old man with humoral immunodeficiency, opportunistic infections, and recurrent EBV-positive diffuse large B-cell lymphoma (DLBCL).

Methods: Genetic analysis was performed to identify mutations in the *DCLRE1C* gene. Functional studies, including γH2AX assays to assess DNA damage repair and measurement of Type I interferon responses, were conducted to evaluate the impact of the variant. A literature review was performed to contextualize the findings.

Results: Biallelic p.Leu70del frameshift mutation in *DCLRE1C* was identified, leading to significantly decreased mutant protein expression. Functional testing confirmed impaired DNA damage repair, via γH2AX measurement, and elevated Type I interferon responses, indicating cytosolic DNA damage accumulation. A literature review highlighted EBV-positive lymphomas in leaky Artemis deficiency with high mortality rate.

Conclusion: Our report adds hypomorphic *DCLRE1C* deficiency as an inborn error of immunity that predisposes to EBV-associated lymphoproliferative disease.

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

1 | Introduction

Loss of Artemis function, through biallelic mutations in *DCLRE1C*, causes severe combined immunodeficiency (SCID) with heightened sensitivity to ionizing radiation and alkylating chemotherapy. Artemis possesses single-strand-specific 5′-to-3′ exonuclease activity, as well as endonuclease activity on 5′ and 3′ overhangs and hairpins, and regulates the cell cycle response to DNA damage. In conjunction with RAG1 and RAG2 proteins (which introduce DNA double-strand breaks); proteins of the nonhomologous end-joining (NHEJ) pathway (Artemis, Ku70, Ku80, and DNA-dependent protein kinase); and ligation proteins (DNA ligase IV, XRCC4, and Cernunnos-XLF), V(D)J recombination can proceed in B and T lymphocytes [1]. Without functional Artemis, this process is impaired, arresting lymphocyte development. Moreover, Artemis mediates the repair of double-strand DNA breaks resulting from the NHEJ, homologous recombination repair, and alternative forms of end-joining pathways [2]. Consequently, Artemis deficiency typically presents in childhood as $T^-B^-NK^+$ immunodeficiency with increased susceptibility to infections and heightened sensitivity to ionizing radiation. Artemis deficiency can be treated with hematopoietic stem cell transplantation in infancy, although growth delay, endocrinologic deficiencies, and dental abnormalities may subsequently develop if affected patients are exposed to alkylating agents as part of their conditioning for transplant [3].

In addition to the classical SCID phenotype, Artemis deficiency can also manifest as combined immunodeficiency (CID) outside of infancy/early childhood, Omenn syndrome, autoimmunity, and/or lymphoproliferation in patients with hypomorphic alleles [4, 5]. Here, we report an adult patient with leaky Artemis deficiency due to a homozygous hypomorphic variant in *DCLRE1C* with recurrent EBV-associated lymphomas, expanding the clinical phenotype of this disorder, while highlighting its predisposition to EBV disease.

2 | Methods and Results

The patient, first evaluated at age 35 for chronic sinusitis, was found to have hypogammaglobulinemia, diagnosed as common variable immunodeficiency (CVID), and initiated on IgG replacement therapy.

At 41, a second assessment was sought because of chronic giardiasis. Detailed medical history revealed recurrent upper respiratory tract infections; diffuse large B-cell lymphoma (DLBCL) of the upper gingiva at age 18 (no EBV studies done at that time; no residual tissue samples available for testing), treated with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP); EBER-positive DLBCL at age 27 involving the abdominal cavity, small bowel, and appendix, treated with rituximab-CHOP (R-CHOP); *Pneumocystis jirovecii* pneumonia (PJP) at age 27; thrush due to *Candida albicans* at age 29; recurrent onychomycosis due to *Trichophyton rubrum* at age 31; oral herpes due to HSV-1 at age 32; and viral conjunctivitis at age 36. The patient was of Tunisian origin and pedigree analysis revealed parental consanguinity. Immunologic investigations at 35 revealed T lymphopenia, near-absence of B cells, and normal levels of NK cells (Table 1). Although Artemis-SCID may be prone to maternal engraftment [10], evaluating the T cells for maternal origin was not possible, although this seemed unlikely given the patient's age. At age 39, he developed verruca vulgaris. Further investigations were then hampered because of the pandemic.

At 45, he presented with persistent diarrhea, new ascites from nodular regenerative hyperplasia, and progressive malnutrition. Small bowel biopsies revealed marked intraepithelial lymphocytosis with villous blunting (Figure 1A). Microbial stains were negative for pathogens. Immunohistochemistry showed a lymphocytic infiltrate within the epithelium and lamina propria, composed almost exclusively of T lymphocytes. These findings prompted the initiation of oral budesonide, with a resolution of diarrhea over the next ∼4 weeks. Sirolimus was initiated after the third week of budesonide as a steroid-sparing strategy. Approximately 10 days later, he developed abdominal fullness with EBV viremia (21,380 copies/mL). Imaging identified new lesions in the small bowel, mesenteric lymph nodes, hepatic subcapsular region, peritoneum, liver, and abdominal wall (Figure 1B), histologically confirmed as EBV-positive DLBCL. Rituximab with gemcitabine, dexamethasone, and cisplatin (R-GDP) was initiated, but this was followed by intraabdominal sepsis, likely from extensive lymphomatous gastrointestinal wall involvement. The patient succumbed to the disease.

The constellation of hypogammaglobulinemia of unclear onset, recurrent extra-nodal DLBCL (two of which were EBV-positive), PJP, numerous viral infections, and superficial fungal disease, led to reclassification of the diagnosis as CID. Genetic investigations by whole-exome sequencing (WES) revealed a homozygous c.207-209delGTT (p.Leu70del) variant in *DCLRE1C*, confirmed by Sanger sequencing (Figure 1C). This variant has an allele frequency of 2.23e−5 in gnomAD, with no homozygotes. Western blot analysis of peripheral blood mononuclear cells showed faint expression of a shortened isoform of Artemis, suggesting significantly decreased *DCLRE1C* expression (Figure 1D).

Functional assays of DNA repair capacity were performed: Etoposide treatment of the patient's cells revealed increased γH2AX by flow cytometry, reflecting the accumulation of DNA double-strand breaks (Figure 1E). Because defective DNA repair produces cytosolic DNA accumulation leading to excessive Type I IFN responses [6], we also assessed this pathway and found increased expression of IFN-α, interferon-stimulated genes (ISGs) (Figure 1F), and phosphorylated-STAT1 levels (Figure 1G). These findings confirm that the c.207-209delGTT (p.Leu70del) variant results in loss of DNA repair function. Thus, the patient's leaky CID phenotype is due to autosomal recessive hypomorphic Artemis deficiency.

To determine if EBV-associated lymphoproliferative disease (EBV-LPD) is associated with leaky Artemis deficiency, we conducted a literature review, identifying seven additional cases (Table 2) [4, 7–9].

3 | Discussion

Complete loss of Artemis function results in SCID, typically with life-threatening opportunistic infections, chronic diarrhea, and

TABLE 1 | Immunological parameters across different age groups.

Age (years)	30	35		37		40.5		43		44.5		45.83	
Cell type [reference range]													
Neutrophils [1600–7700 cells/μL]	4330	3100		5050		3730		2890		6120		2590	
Monocytes [80–880 cells/μL]	830	600		750		670		640		820		430	
Lymphocytes [800–4400 cells/μL]	330	400		340		280		260		330		130	
$CD3^+$ T cells [661–2224 cells/μL; %]	ND	293	82	317	78	257	85	176	71	219	85	107	74
$CD4^+$ T cells [356–1573 cells/μL; %]	ND	105	30	122	30	103	34	47	19	55	21	25	17
$CD8^+$ T cells [113–804 cells/μL; %]	ND	185	52	193	47	153	51	130	52	156	65	84	58
$CD19^+$ B cells [43–396 cells/μL; %]	ND	ND		2	1	3	1	0	0	ND		0	0
NK cells [5–659 cells/μL; %]	ND	ND		84	20	39	13	71	29	ND		36	25
IgG [7.00–15.00 g/L]	2.96[a]	10.20		9.22		10.23		11.27		ND		6.66	
IgA [0.80–4.00 g/L]	0.07	< 0.07		0.07		< 0.10		< 0.1		ND		< 0.10	
IgM [0.50–3.00 g/L]	0.22	0.19		0.34		0.36		< 0.2		ND		0.21	
IgE [0–100 IU/mL]	5	ND		< 5		< 5		ND		ND		ND	

Note: Reference ranges are provided in brackets.Abbreviation: ND, not determined.

[a]Earliest available measurement, was obtained before initiation of Ig replacement therapy.

failure to thrive in infancy. Hypomorphic variants are associated with leaky onset and increased susceptibility to infections and may also manifest with autoimmunity (e.g., cytopenia), dysregulated inflammation (e.g., inflammatory bowel disease), Omenn's syndrome (erythroderma, elevated serum IgE levels, eosinophilia), and/or lymphoproliferative disease. In the current case, the patient's clinical course suggested a leaky SCID syndrome. WES revealed a homozygous frameshift mutation c.207-209delGTT (p.Leu70del) in *DCLRE1C* with a faint expression of an abnormal isoform by Western blot (using different commercial mono- or polyclonal antibodies), consistent with diminished protein expression. Using previously established methods to directly measure DNA double-strand breaks (by γH2AX) or their consequence (Type I IFN response from accumulation of DNA-repair associated cytosolic DNA), we further show that the patient's cells have impaired capacity for DNA-damage repair, as well as basally excessive Type I IFN response (which has been previously reported in Artemis-deficient patients [6, 11]. These findings confirm that the identified *DCLRE1C* variants are deleterious, causing hypomorphic Artemis deficiency in this patient. The long survival of this patient, which is the longest identifiable among published cases in the literature, is notable.

The striking clinical feature is the recurrence of EBV-positive DLBCL. Given the protracted time period, we were unable to determine if the patient's first episode was related to EBV, although the latter two were; it is unclear if these episodes represent relapse or de novo recurrence. A growing number of IEIs are recognized to predispose to EBV-related disease, presenting as severe infectious mononucleosis or hemophagocytic lymphohistiocytosis (HLH) or as a lymphoproliferative disorder. Artemis deficiency is not classically considered among IEIs predisposing to EBV-associated disease, most likely because of its severity with other manifestations in infancy. However, our case and the accompanying literature review identify that hypomorphic *DCLRE1C* deficiency is associated with an increased risk of EBV-driven lymphoma. Moreover, its development in leaky Artemis deficiency appears to confer a poor prognosis, with a high mortality rate.

The mechanisms by which loss of Artemis function regulates EBV-mediated transformation require further elucidation. In addition to T lymphocytopenia with reduced cell-mediated immunity onto EBV-infected cells, a B-cell-intrinsic process may be implicated involving EBV's encoded latent membrane protein 1 (LMP1), an oncogene essential for B-cell transformation. LMP1 compromises the host's capacity for DNA repair, increasing cellular sensitivity to DNA-damaging agents [12], compounding the defect in Artemis deficiency. Genotoxic stress normally prompts p53 to induce cell cycle arrest and apoptosis [13], but

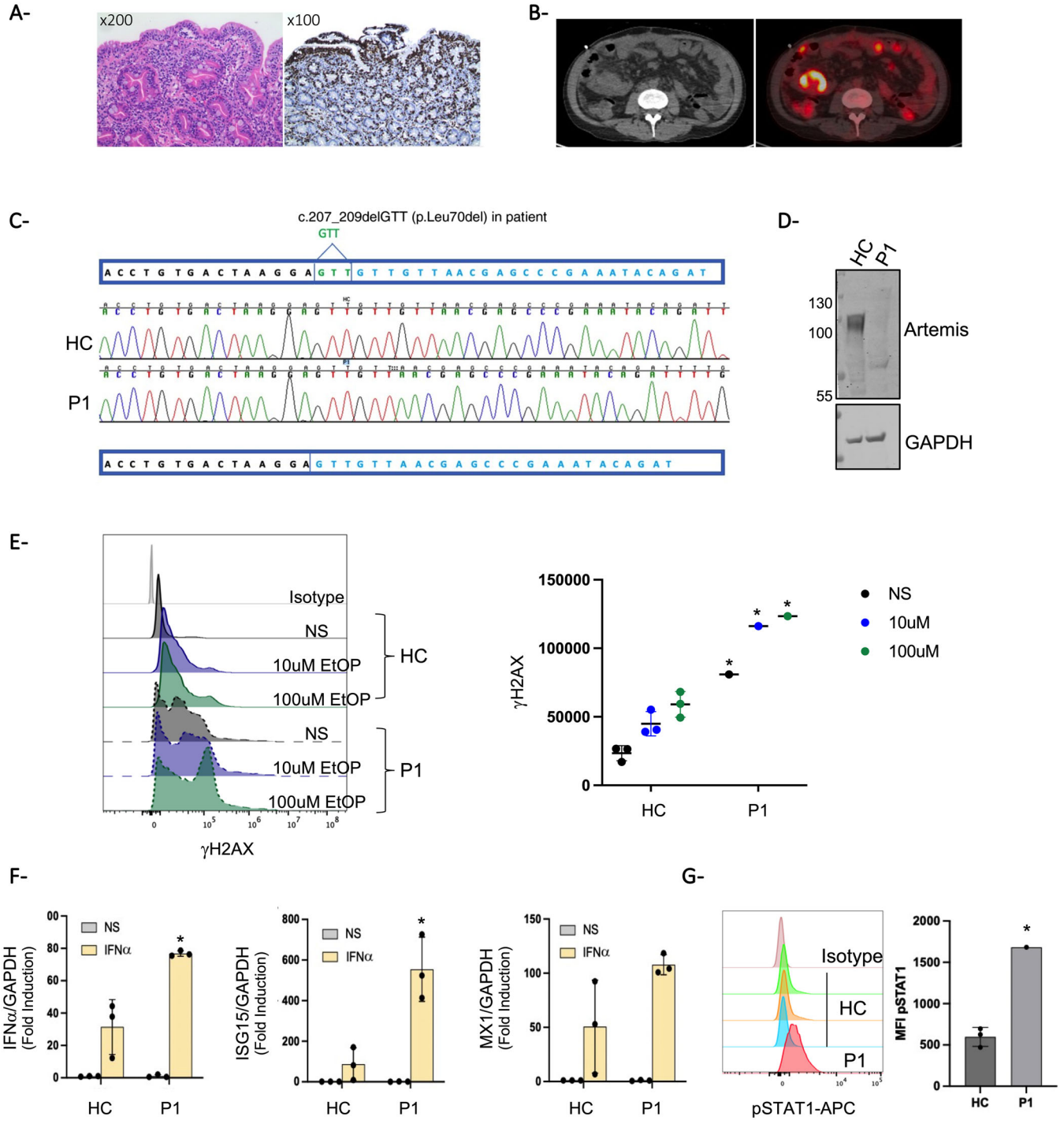

FIGURE 1 | A) Jejunal mucosa with H&E (left) and immunohistochemitry (right) showing marked intraepithelial $CD3^+$ lymphocytosis. (B) Positron emission tomography–computed tomography (PET-CT) scan demonstrating intense FDG-avid activity in the small bowel, including the jejunal loop. (C) Electropherogram demonstrating wild-type Artemis sequencing (healthy control, HC) and that of the homozygous patient (P1), along with the corresponding deletion. (D) Western blot analysis of Artemis in whole cell lysates from PBMCs of two healthy controls (HCs) and the patient (P1). GAPDH served as a loading control. (E) Histogram obtained by γH2AX flow cytometry analysis of PBMC treated with Etoposide (10 or 100 μM) for 1 h. (F) Expression of *IFN-α*, *ISG15*, and *MX1* as determined by qRT-PCR following 4 h of stimulation with IFN-α (50 ng/mL) in PBMCs from healthy control (HC) and patient (P1). The values represent the mean ± SEM fold change in expression relative to the housekeeping gene (*GAPDH*). (G) STAT1 phosphorylation in PBMCs from HC or P1, as determined by flow cytometry. MFI, mean fluorescence intensity. Data represent the means from triplicates. *$p < 0.05$ between HC and P1.

TABLE 2 | Literature review on EBV-associated lymphoproliferative disease (EBV-LPD) in leaky Artemis-deficient patients, identifying seven additional cases.

References	Age (of LPD onset)/Sex	*DCLRE1C* mutation	Impact on Artemis protein	EBV LPD characteristics	Additional comments
[4]	9 months/M	Compound heterozygous with genomic deletion of exons 1–3	Loss of expression	Diagnosed on lymph node biopsy. Abnormal B cells in blood and CSF	The patient was deceased 5 days after diagnosis of LPD. On autopsy, lymphoma was found in lung, liver, striated muscle
	5 years/F (non-consanguineous sibling of above)	Seven-nucleotide deletion (T1384–A1390) in exon 14	p.D451fs*10	EBV viremia detected before BMT; B-cell lymphoma in the liver found 38 days after BMT	Deceased 12 days after diagnosis of LPD
[8]	7 years/F	Homozygous c.512C>G	p.P171R; hypomorphic	Hodgkin's lymphoma; successfully treated with chemotherapy	Cervical and mediastinal lymphadenopathy with granulomatous inflammation
		Homozygous c.1307|AGGAT GCT|1308ins	p.C436*; loss of expression		
[9]	22 years/M	Homozygous c.632G>T	p.G211V	DLBLC (non-GC-B type EBV$^+$) in the neck. Simultaneous, different, preauricular B-cell lymphoma (unclassifiable; with features intermediate between DLBLC and classical Hodgkin's lymphoma). The above was treated with R-CHOEP and radiation therapy with complete remission. DLBCL-like relapse in the gastroventricular mucosa, involving the pancreas and spleen; treated with isophosphamide, methotrexate, and etoposide, with complete remission	Underwent haploidentifical SCT from heterozygous father. The patient died on Day 167 post-SCT from infections (including *Pneumocystis jirovecii* pneumonia, HSV viremia, *Staphylococcus aureus* bacteremia, *Candida albicans* candidemia)
[7]	13 years/F	Homozygous c.1299_1306dup	p.C436*	Retroperitoneal large B-cell lymphoma	Received rituximab once, but abruptly deteriorated and died
	15 years/M (No known relation to the above patient)			EBV-related lymphoma in mediastinum and liver	Rapid deterioration with respiratory failure before chemotherapy
[10]	13.5 years/M	Compound heterozygous with			
		c.109 + 2T>C	Altered splicing	EBER-positive colon lymphoma (B cell, non-Hodgkin); treated with chemotherapy	Deceased on the day he received SCT (1.5 years after the diagnosis of colon lymphoma)
		c.1147C>T	p.R383X		
Roussel et al. (current report)	18 years/M	Homozygous c.207–209delGTT	p.Leu70del	Gingival DLBCL (unable to confirm EBV status) Abdominal wall and gastrointestinal EBV-DLBCL EBV viremia with abdominal wall, hepatic, and intestinal EBV-DLBCL	

Note: The table summarizes the age of onset, mutation impact, LPD characteristics, and outcomes.

LMP1 also induces the expression of ΔNp73α, an antagonist of p53 [14]. Consequently, impaired DNA repair, genotoxic stress, and EBV LMP1 lead to cell cycling proceeding without arrest, despite DNA damage, and loss of apoptosis. This context of EBV latent infection of DNA repair-deficient B-cells, with impaired T-cell-mediated immunity, may be permissive for recurrent lymphomagenesis. EBV-related lymphoproliferation may also occur in IEIs due to defects in T or B cells or impaired cytotoxicity by T or NK cells [11, 15]. T or NK cell-specific immunity to EBV could not be evaluated in our patient, although the WES data found no evidence of such IEIs. A deeper molecular understanding of this interplay may lend insight into the development of some EBV-associated B-cell lymphomas.

In conclusion, we report an older adult with hypomorphic Artemis deficiency and underline that this condition may confer susceptibility to EBV disease, specifically, B-cell lymphoma.

Author Contributions

Lucie Roussel: investigation, methodology, supervision, writing – review and editing. **Stéphane Bernier**: methodology. **Gertruda Evaristo**: investigation, methodology**. Anna Perez**: resources. **Sanabelle Zaabat**: resources. **Romina Pace**: resources. **Yichun Sun**: investigation. **Isabelle Angers**: Investigation. **John Storring**: resources. **Donald C. Vinh**: conceptualization, methodology, investigation, formal analysis, validation, resources, writing – original draft, writing – review and editing, project administration, funding acquisition.

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Ethics Statement

The study was approved by the McGill University Health Centre (MUHC) institutional review board–approved research protocol (GEN10-256).

Consent

Informed consent was received from all participants.

Conflicts of Interest

Donald C. Vinh has received funding support from the Jeffrey Modell Foundation, FRQS, and the Canadian Institutes of Health Research. He has served on advisory boards for Astra Zeneca, Merck Canada, Moderna, Takeda, and Xediton. He has a patent application pending (Electronic Filing System ID: 40101099) unrelated to this work. The other authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author, DCV, upon reasonable request.

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