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# The many faces of Artemis-deficient combined immunodeficiency — Two patients with DCLRE1C mutations and a systematic literature review of genotype–phenotype correlation

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**Abstract** Defective V(D)J recombination and DNA double-strand break (DSB) repair severely impair the development of T-lymphocytes and B-lymphocytes. Most patients manifest a severe combined immunodeficiency during infancy. We report 2 siblings with combined immunodeficiency (CID) and immunodysregulation caused by compound heterozygous Artemis mutations, including an exon 1–3 deletion generating a null allele, and a missense change (p.T71P). Skin fibroblasts demonstrated normal DSB repair by gamma-H2AX analysis, supporting the predicted hypomorphic nature of the p.T71P allele. In addition to these two patients, 12 patients with Artemis-deficient CID were previously reported. All had significant morbidities including recurrent infections, autoimmunity, EBV-associated lymphoma, and carcinoma despite having hypomorphic mutants with residual Artemis expression, V(D)J recombination or DSB repair capacity. Nine patients underwent stem cell transplant and six survived, while four patients who did not receive transplant died. The progressive nature of immunodeficiency and genomic instability accounts for poor survival, and early HSCT should be considered.


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## 1. Introduction

V(D)J recombination is an essential process for normal development of B- and T-lymphocytes [1]. Defects in V(D)J recombination classically manifest as T−B−NK+ severe combined immunodeficiency (SCID) [2]. V(D)J recombination is initiated by the creation of DNA double-strand breaks (DSBs), which are introduced by RAG1 and RAG2 proteins at recombination-specific sequences that flank V, D, and J gene units, and are repaired by the nonhomologous end joining (NHEJ) pathway [3]. Ku70, Ku80 and DNA-PKcs together constitute the DNA-dependent protein kinase (DNA-PK). DNA-PK promotes the endonucleolytic activity of Artemis, which is required for the opening of hairpin structures created upon RAG1/2-specific DNA cleavage. DNA ligase IV, X-ray cross-complementation group 4 (XRCC4) and Cernunnos–XLF are responsible for the final ligation step [4]. Defects in the NHEJ pathway confer radiosensitivity and genomic instability, leading to increased susceptibility to malignancy [5].

Artemis deficiency is the most common form of radiosensitive SCID [6,7]. Other molecular defects known to cause radiosensitive SCID in humans include DNA-PKcs [8], DNA ligase IV [9] and Cernunnos–XLF [10]. Residual recombination events permitted by hypomorphic mutants may allow the emergence of T-lymphocytes with restricted T-cell receptor repertoires. Expansion of these oligoclonal T-cells often leads to normal or elevated peripheral T-cell numbers, and when activated they cause tissue inflammation and damage [11,12]. Defects in T-lymphocyte differentiation also compromise normal development of T-regulatory cells, and the restricted number of mature thymocytes reduces the efficiency of thymic negative selection, permitting autoreactive T-cell clones to emerge [13]. Omenn syndrome, characterized by erythroderma, eosinophilia, enlarged lymphoid tissues and opportunistic infections is a well-recognized form of 'leaky' SCID and occurs most frequently in V(D)J recombination defects [14]. In addition, hypomorphic mutants in the genes involved in this process are now increasingly recognized as responsible for combined immunodeficiencies (CID) complicated by autoimmunity, granulomatous inflammation, lymphoproliferative disease and malignancies, and the age of presentation extends beyond infancy to childhood and even adulthood [15,16].

The wide phenotypic range of CID associated with RAG1 and RAG2 is well described [15–17]. A recent review of atypical SCID highlighted the frequent occurrence of immune dysregulation in patients with recombination defects, which were dominated by RAG1 and RAG2 deficiencies [16]. Genomic instability as a result of defective DSB repair distinguishes Artemis deficiency from RAG1 and RAG2 defects, giving rise to additional oncogenic potential and possibly worse prognosis. In this article, we report two siblings affected by Artemis-deficient CID, both with significant immunodysregulation. We then proceeded to a systematic literature review of all reports of Artemis-deficient CID. Our primary objective was to define the range of phenotypic features in hypomorphic Artemis deficiency. Our secondary objective was to evaluate any genotype–phenotype correlation which could potentially account for the 'leaky' disease in these patients.

## 2. Materials and methods

### 2.1. Patients

We analyzed the clinical and immunological features of 2 siblings with CID (P1.1 and P1.2) caused by deficiency in Artemis. Informed consent was obtained from the family according to the procedures established by the ethics committee of the institution.

### 2.2. Cell culture and DSB repair analysis

Primary skin fibroblasts from IBR.3 (control), patient P1.2 (IL640), F02/385 (Artemis-null), and 618BR (Artemis-deficient, leaky phenotype) were grown in minimal essential medium (MEM) as previously described [18]. 618BR was derived from a patient who became symptomatic of CID at the age of 27 years, and her fibroblasts displayed normal G1 phase DSB repair but a defect in G2 phase [18]. The DSB repair assay was performed by analyzing the rate of loss of phosphorylated histone H2A (γ-H2AX) foci in non-dividing, confluent cultures at G0 and G2 phase. Fibroblasts were plated at $1.5–2 \times 10^5$ cells on a coverslip in a Petri dish and left to grow at 37 °C. To assess DSB rejoining in G1 phase, fibroblasts were exposed to 3 Gy γ-rays when they reached confluency. γH2AX foci were enumerated at 0, 2 and 8 h after γ-irradiation. Cells were fixed with 3% paraformaldehyde, 2% sucrose phosphate-buffered saline (PBS) for 10 min at RT and permeabilized in 0.2% Triton X-100 in PBS for 2.5 min at RT, followed by primary antibody staining with anti-phospho-H2AX (Ser139) mouse monoclonal antibody (Upstate Technology) and secondary antibody staining with FITC-conjugated anti-mouse IgG (Sigma). Nuclei were counterstained with DAPI and γ-H2AX foci were quantified using fluorescent microscopy (Leica). To evaluate DSB repair in G2 phase, cells were plated 24 h before γ-irradiation, and were treated with aphidicolin immediately following irradiation to inhibit the progression of S-phase cells into G2 during the period of repair. γH2AX foci were examined 2 and 8 h later in G2 phase cells, which were identified using anti-CENP-F (Abcam). Two experiments were performed on separate days for confirmation, and 3 readings of γ-H2AX were obtained for each experiment.

### 2.3. Genetic diagnosis

Genetic analysis of *RAG1*, *RAG2* and *DCLRE1C* (Artemis) was performed on genomic DNA extracted from peripheral blood for both patients. Multiplex ligation-dependent probe amplification (MLPA) was also performed to detect a gross exon deletion in *DCLRE1C* gene.

### 2.4. Protein sequence analysis

To deduce the functional implication of amino acid substitutions, the degree of sequence conservation across species and members of β-CASP family of metallo-β-lactamases was analyzed. Amino acid sequences of Artemis orthologs and paralogs (human *DCLRE1A* [*SNM1A*], human *DCLRE1B* [*SNM1B*], and *SNM1* in yeast [*PSO2*, *Saccharomyces cerevisae*]) were obtained from Basic Local Alignment Search Tool (BLAST) [19]