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<th><strong>Allele</strong></th>
<th>Chairy</th>
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<tr>
<td><strong>Coordinate</strong></td>
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<td><strong>Base Change (assembly)</strong></td>
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<tr>
<td><strong>Gene</strong></td>
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<tr>
<td><strong>Gene Name</strong></td>
<td>DNA cross-link repair 1C</td>
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<tr>
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<td>Beutler Lab</td>
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</tr>
<tr>
<td><strong>Authors</strong></td>
<td>Xue Zhong, Jin Huk Choi, and Bruce Beutler</td>
</tr>
<tr>
<td><strong>Illustrators</strong></td>
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**Phenotypic Description**

Figure 1. *Chairy* mice exhibit increased body weights compared to wild-type littermates. Scaled weights are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (·) and standard deviation (±) are indicated.
Figure 2. **Chairy mice exhibit decreased frequencies of peripheral T cells.** Flow cytometric analysis of peripheral blood was utilized to determine T cell frequency. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean () and standard deviation () are indicated.
Figure 3. *Chairy* mice exhibit decreased frequencies of peripheral CD4+ T cells. Flow cytometric analysis of peripheral blood was utilized to determine T cell frequency. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean () and standard deviation () are indicated.
Figure 4. **Chairy** mice exhibit decreased frequencies of peripheral naive CD8 T cells in CD8 T cells. Flow cytometric analysis of peripheral blood was utilized to determine T cell frequency. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean () and standard deviation () are indicated.
Figure 5. *Chairy* mice exhibit increased frequencies of peripheral central memory CD8 T cells in CD8 T cells. Flow cytometric analysis of peripheral blood was utilized to determine T cell frequency. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean () and standard deviation () are indicated.
Figure 6. *Chairy* mice exhibit increased frequencies of peripheral effector memory CD4 T cells in CD4 T cells. Flow cytometric analysis of peripheral blood was utilized to determine T cell frequency. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (\(\mu\)) and standard deviation (\(\sigma\)) are indicated.
Figure 7. *Chairy* mice exhibit increased frequencies of peripheral effector memory CD8 T cells in CD8 T cells. Flow cytometric analysis of peripheral blood was utilized to determine T cell frequency. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean () and standard deviation () are indicated.
Figure 8. *Chairy* mice exhibit increased expression of CD44 on peripheral T cells. Flow cytometric analysis of peripheral blood was utilized to determine CD44 MFI. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean () and standard deviation () are indicated.
Figure 9. *Chairy* mice exhibit increased expression of CD44 on peripheral CD4+ T cells. Flow cytometric analysis of peripheral blood was utilized to determine CD44 MFI. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean () and standard deviation () are indicated.

The *Chairy* phenotype was identified among G3 mice of the pedigree R6113, some of which showed increased body weights compared to wild-type littermates (Figure 1). The *Chairy* mice showed reduced frequencies of T cells (Figure 2), CD4+ T cells (Figure 3), and naïve CD8 T cells in CD8 T cells (Figure 4) with concomitant increased frequencies of central memory CD8 T cells in CD8 T cells (Figure 5), effector memory CD4 T cells in CD4 T cells (Figure 6), and effector memory CD8 T cells in CD8 T cells (Figure 7), all in the peripheral blood. Expression of CD44 was increased on peripheral blood T cells (Figure 8) and CD4+ T cells (Figure 9).
Nature of Mutation

Figure 10. Linkage mapping of the increased effector memory CD8 T cell frequency using an additive model of inheritance. Manhattan plot shows -log10 P values (Y-axis) plotted against the chromosome positions of 58 mutations (X-axis) identified in the G1 male of pedigree R6113. Normalized phenotype data are shown for single locus linkage analysis without consideration of G2 dam identity. Horizontal pink and red lines represent thresholds of P = 0.05, and the threshold for P = 0.05 after applying Bonferroni correction, respectively.

Whole exome HiSeq sequencing of the G1 grandsire identified 58 mutations. All of the above anomalies were linked by continuous variable mapping to a mutation in Dclre1c: a T to C transition at base pair 3,452,863 (v38) on chromosome 2, or base pair 28,802 in the GenBank genomic region NC_000068. The strongest association was found with an additive model of inheritance to the normalized peripheral blood effector memory CD8 T cell frequency, wherein one variant homozygote and six heterozygous mice departed phenotypically from 11 homozygous reference mice with a P value of 0.000131 (Figure 10). A substantial semidominant effect was observed in most of the assays.

The mutation corresponds to residue 1,259 in the mRNA sequences NM_146114 within exon 14 of 14 total exons.

```
1243 GAGGAAGACGATGATC T
CTTTGATGACCCCTCTA
386  -E--E--D--D--D--L--F--D--
     D--P--L--
```
The mutated nucleotide is indicated in red. The mutation results in a leucine to proline substitution at position 391 (L391P) in the Artemis protein, and is strongly predicted by Polyphen-2 to cause loss of function (score = 1.000).

Protein Prediction

Dclre1c encodes Artemis, a member of the metallo--lactamase protein superfamily (1). The metallo--lactamase proteins have two conserved domains: a metallo--lactamase domain (amino acids 10-193 in Artemis, SMART) and a -CASP (metallo--lactamase-associated CPSF ARTEMIS SNM1 P SO2) domain (amino acids 239-345 in Artemis, SMART) [Figure 24; (2); reviewed in (3)]. Together, the metallo-lactamase and -CASP domains are designated as the SNM1 domain [reviewed in (4)]. The SNM1 domain comprises the “catalytic core” of Artemis and contains nuclease activity as well as regulates protein-protein interactions including that of Artemis with the Cul4a-DDB1 ubiquitin complex [(5-7); reviewed in (4)].

The metallo--lactamase domain is a four-layered -sandwich with two mixed -sheets flanked by -helices (1;8;9). The metallo--lactamase domain has five highly conserved sequence motifs that function in metal coordination, substrate binding, and enzymatic activities (8;9). Motif 1 contains an aspartate at the end of two -strands of the first -sheet (8;10). Motif 2 contains a HxHxDH sequence (amino acids 33-38 in Artemis); the first two His residues in the motif (His33 and His35) are proposed to participate in metal ion coordination (10). The Asp amino acid in the motif (Asp37) is proposed to function in the hydrolysis reactions; the role of the third His (His38) in the motif is unknown (10). Asp37, His33, His35, His38, His115, and His319 coordinate the two active site metals, while Asp17, Asp136 and/or Asp165 are proposed to form salt bridges to the HxHxDH motif as well as H38 and H33 to stabilize the HxHxDH motif for optimum metal ion interaction (10). Motifs 3 and 5 are comprised of single histidines that are proposed to coordinate metal ions and the binding of negatively charged substrates (10). Motif 4 is a single aspartate that is proposed to participate in hydrolysis reactions (8;9). Asp165 or His319 are proposed to represent motif 5 (2;5). The -CASP domain is between motif 4 and motif 5 and has an / fold with a five-stranded -sheet surrounded on both sides with -helices (11). Amino acid 341 determines nucleic acid specificity (i.e., a His at 341 is found in RNA-specific enzymes, while a Val at 341 is found in DNA-specific enzymes (2); Val341 is essential for the interaction of Artemis with DNA (5).
The C-terminal region (CTR; amino acids 346-705) is dispensable for Artemis-mediated hairpin opening during V(D)J recombination (7) and is also required for DNA repair (12;13). Trp489, Phe492, and Ph493 within the CTR are essential for the formation of the Artemis-Ligase IV/XRCC4 complex (14). Ma et al. proposed an inhibitory role for the CTR, which would regulate Artemis function in the absence of DNA double-strand breaks (DSBs) (7). Several sites within the CTR of Artemis are phosphorylated by the phosphatidylinositol-3-OH kinase-like (PIK) kinases DNA-dependent protein kinase catalytic subunit (DNA-PK<sub>CS</sub>; see the record for clover), ataxia telangiectasia mutated (ATM), and ATM- and Rad3-related (ATR) in response to DNA damage or cellular stress (7;15-18). Three basal (Ser503, Ser516, and Ser645) and 11 DNA-PKcs–mediated phosphorylation sites have been located in the CTR (6;7;19). Ser645 is also phosphorylated by ATM in response to ionizing radiation (IR) (15;17). Mutation of Ser645 to alanine (Ser645Ala) did not affect survival of the mutant cells after exposure to IR, indicating that phosphorylation at Ser645 is not functionally critical (7). Artemis phosphorylation is not required for Artemis-dependent DSB repair and V(D)J recombination (see “Background” section for more information about V(D)J recombination) (6).

The *Chairy* mutation results in a leucine to proline substitution at position 391 (L391P); Leu391 is within the CTR.

**Please see the record kiwis for more information about Dclre1c.**

**Putative Mechanism**

Artemis has several functions. Artemis is an endonuclease that functions in homologous recombination, nonhomologous end-joining (NHEJ), and class switch recombination (20-24). For a detailed explanation of Artemis functions in these processes, please see the kiwis record. Artemis regulates recovery from the G2 checkpoint in response to IR through regulation of cyclin B/Cdk1 activation by retaining Cdk1/cyclin B at the centrosome and inhibiting its nuclear import during prophase (15-18). Artemis is also involved in S phase checkpoint recovery in response to replication fork blocking lesions (25). At the S phase checkpoint recovery, Artemis interacts with SCFFbw<sup>7</sup> to mediate the degradation of cyclin E via the SCFFbw<sup>7</sup> E3 ligase complex (25). Artemis interacts with the tumor suppressor p27 during the G1 phase of the cell cycle and is required for the ubiquitination and degradation of p27 by the Cul4A-DDB1 complex, which is required cell cycle progression at the G1-S and G0 to S transitions (26). Artemis is required for normal proliferative control of multipotent mesenchymal stem/progenitor cells (MSCs), especially after exposure to cytostress stimuli (27). Dclre1c deficiency resulted in chromosomal damage as well as enhanced resistance and proliferative potential in primary MSCs after stress (27). Artemis is a negative regulator of p53 in response to oxidative stress in primary cells and cancer cell lines (28).
Mutations in \textit{DCLRE1C} are linked to severe combined (TBNK\(^+\)) immunodeficiency associated with increased radiosensitivity (RS-SCID; OMIM: \#602450), Athabascan SCID (SCID-A; OMIM:#602450), and Omenn syndrome (OS; OMIM: \#603554) \((1; 29; 30)\). Patients with RS-SCID exhibit defects in V(D)J recombination resulting in early maturation defects in B and T cells \((1)\). Patients have absence of complete V(H)-J(H) gene rearrangements and subsequent differentiation arrest of B cells at the pre-BCR checkpoint \((30)\). As a result, the patients display a complete absence of T- and B lymphocytes \((31)\). Some RS-SCID patients have a predisposition to B cell lymphoma \((32)\). Most patients with RS-SCID exhibit early lethality (at approximately 1 year of age) due to opportunistic infections. SCID-A is an autosomal recessive disorder in peoples of the Athabascan-speaking Native Americans \((1; 29)\). Similar to RS-SCID, patients present with an absence of both T and B cells due to defective coding joint and precise, but reduced signal joint formation during V(D)J recombination \((29)\). OS is an autosomal recessive condition in which patients present with symptoms of SCID as well as erythrodermia, hepatosplenomegaly, lymphadenopathy, and alopecia \((33)\). OS patients are classified as T\(^+\)B\(^+\)NK\(^+\) SCID \((33)\). Patients with OS often exhibit elevated or normal T cell counts that are activated and skewed toward a Th2 phenotype \((34; 35)\). V(D)J coding joints are normal in the T cells of OS patients. B cells in the OS patients are not detected. The eosinophilia and high IgE levels are the result of increased secretion of the Th2-type cytokines \((36)\). The other immunoglobins were reduced or not detectable in the serum of OS patients. NK cell functions and numbers were unaffected in patients with OS. Patients with OS exhibit lethality; bone marrow transplantation is often successful in treating patients with OS \((36)\). A \textit{DCLRE1C} truncation mutation, D451fsX10, results in loss of the C-terminus of Artemis and has been linked to partial immunodeficiency and aggressive EBV-associated lymphoma \((32)\). Patients have low levels of T and B cells, but exhibited lymphocytopenia and died of recurrent infections or lymphoma progression \((32)\).
A spontaneous Dclre1c mutant (37) and Artemis knockout (Dclre1c−/−) mice exhibit early T- and B-cell maturation arrest as well as increased sensitivity to IR (31; 38; 39). The number of CD11c+MHCII+ dendritic cells, CD3+NK1.1+ NK cells, CD11b+ monocytes and Gr1+ granulocytes were comparable to those in wild-type mice (37; 40). In the bone marrow of Dclre1c−/− mice, B cell development was blocked at the B220+/CD43+ progenitor stage resulting in loss of B220+ CD43 precursor and B220+ IgM+ immature B cell (12; 31; 37; 38). Dclre1c−/− mice also lacked peripheral B220+/IgM+ B cells and T cells (38-40). Thymocyte numbers were reduced by approximately 50-fold in the Dclre1c−/− mice compared to wild-type or heterozygous littermates. Thymocytes in the Dclre1c−/− mice were predominantly DN T cells, but low numbers of DP and SP thymocytes were observed in some Dclre1c−/− thymocytes indicating that some T cell development occurred (31; 37-39). Most thymocytes were arrested in the CD44+CD25− ("DN3") stage of development and few matured to the DP stage; the number of DN1 cells in the thymus were also reduced compared to wild-type levels (37; 40). The thymus in the Dclre1c−/− mice did not have a lymphocytic cortex and scattered lymphoid cells with abundant mitotic figures (40). T and B cell frequency were reduced in the lymph nodes and spleens of the Dclre1c−/− mice (40). B cell development was arrested at the early progenitor stage (B220+CD43+) in the lymph nodes and spleens of the Dclre1c−/− mice (40). In the lymph nodes and spleen of some Dclre1cN/N mice, SP T cells (mostly CD4+ CD8) were observed that also expressed surface CD3 and TCR/ (38). The spleens from the Dclre1c−/− mice had reduced fully developed lymphoid follicles; most lymphoid cells had larger nuclei with less dense chromatin and prominent nucleoli (40). The lymph nodes from the Dclre1c−/− mice were smaller than those in wild-type mice and were depleted of mature lymphocytes and lymphoid follicles (40). Both T and B cell proliferation were reduced in response to Con A and LPS, respectively in the Dclre1c−/− mice compared to wild-type mice (40).

A targeted Dclre1c mutation (ArtP70/P70; Dclre1ctm1Jsek) in exon 14 resulted in a coding of a premature stop codon at amino acid 449 (Asp449X) and mimics a human DCLRE1C allele, Artemis-P70 (D451X) (12). The ArtemisP70 protein can interact with DNA-PKcs, and it retains exo- and endonuclease activities, but it is not phosphorylated (12). The number of thymocytes and splenocytes in the ArtP70/P70 mice were reduced compared to wild-type and heterozygous mice, but higher than those in Dclre1c knockout (Dclre1c−/−; Dclre1ctm1Fwa) mice (12). The ArtP70/P70 mice exhibited impaired V(D)J recombination, DSB repair, and increased chromosomal instability (12). T cell development was impaired at the DN3 stage, but some T cells did progress to the DP and SP stages (12). In the ArtP70/P70 mice, B cell development was defective at the transition from the pro-B to pre-B stage resulting in reduced percentage and number of pre-B cells than wild-type mice (12). The number of surface IgM-expressing cells in the bone marrow and peripheral lymphoid organs was reduced in the ArtP70/P70 mice (12). The ArtP70/P70 mice displayed a reduced frequency of both D to J and V to DJ rearrangements within the TCR- locus (12). A modest decrease in the levels of D_H to J_H rearrangements in the pro- and pre-B cells from ArtP70/P70 mice; significant levels of D_H−J_H rearrangements occurred (12). Mouse embryonic fibroblasts from the ArtP70/P70 mice exhibited an intermediate hypersensitivity to IR compared to Dclre1c−/− mice (12).
Conditional knockout of Dclre1c in mature B cells (Dclre1c<sup>tm2.1Jpdv</sup>) resulted in defective switching to certain isotypes (IgG3 and IgA) after B cell activation or after KLH immunization; CSR was not significantly affected (39).

The Chairy mouse phenocopies the previously characterized Dclre1c mutant and knockout models, indicating that the D165G mutation results in loss of Artemis function.

References


