

# Hematopoietic stem cell transplantation in two sisters with bone marrow failure associated with *POLE* gene variants

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## Abstract

*POLE* codes for DNA polymerase epsilon (Pol  $\epsilon$ ) and variants of Pol  $\epsilon$  catalytic subunit 1 can be pathogenic. We recently reported a novel *POLE* gene variant (p.[D1131fs];[T1891del]) which leads to bone marrow failure in two Japanese sisters. Here, we describe the successful course of hematopoietic stem cell transplantation of these two sisters. Both cases were born with congenital anemia and remained transfusion dependent. Their bone marrow showed gradually proceeding trilineage dysplasia. They both underwent HCT from an unrelated bone marrow donor and successfully achieved engraftment. Ten and eight years have passed respectively, and they are doing well without transfusion.

**Title:** Hematopoietic stem cell transplantation in two sisters with bone marrow failure associated with *POLE* gene variants

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Abbreviations key:

DNA	Deoxyribonucleic acid
Pol $\epsilon$	Polymerase epsilon
MDS	Myelodysplastic syndrome
HCT	Hematopoietic stem cell transplantation
RBCs	Red blood cells
WBCs	White blood cells
MCV	Mean corpuscular volume
IBD	Inflammatory bowel disease
HLA	Human leukocyte antigen
MAC	Myeloablative conditioning
GVHD	Graft-versus-host disease

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POLE codes for DNA polymerase epsilon (Pol  $\epsilon$ ) and variants of Pol  $\epsilon$  catalytic subunit 1 can be pathogenic. We recently reported a novel POLE gene variant (p.[D1131fs];[T1891del]) which leads to bone marrow failure in two Japanese sisters. Here, we describe the successful course of hematopoietic stem cell transplantation of these two sisters. Both cases were born with congenital anemia and remained transfusion dependent. Their bone marrow showed gradually proceeding trilineage dysplasia. They both underwent HCT from an unrelated bone marrow donor and successfully achieved engraftment. Ten and eight years have passed respectively, and they are doing well without transfusion.

## Main Text

### Introduction

DNA polymerase epsilon (Pol  $\epsilon$ ), which is composed of four subunits, is a core component of DNA replication. Pathogenic variants in *POLE* encoding Pol  $\epsilon$  catalytic subunit 1 (the largest subunit) is known to cause FILS and IMAGE1 syndrome, two syndromes with similar symptoms comprised of multiple malformations and organ dysfunctions, including facial dysmorphism, congenital adrenal hypoplasia, and immunodeficiency.<sup>1,2</sup> Recently, we reported a novel compound heterozygous variant in *POLE* (p.[D1131fs];[T1891del]) to be the cause of congenital anemia in two Japanese sisters.<sup>3</sup> Both sisters developed multilineage dysplasia compatible with myelodysplastic syndrome (MDS) and remained transfusion dependent. They both proceeded to hematopoietic stem cell transplantation (HCT). Here, we report the successful HCT outcomes of these two sisters.

### Cases

Case 1 is a 10-year-old girl who was born to non-consanguineous healthy Japanese parents. As we previously reported, she had severe macrocytic anemia without reticulocytopenia (red blood cells [RBCs]  $0.95 \times 10^{12}/L$ , hemoglobin level 43 g/L, mean corpuscular volume [MCV] 123 fL, reticulocytes  $158 \times 10^9/L$ , white blood cells [WBCs]  $2.3 \times 10^9/L$ , and platelets  $396 \times 10^9/L$ ) from birth. Anemia did not improve with oral iron supplements or erythropoietin administration, and she had to undergo recurring RBC transfusions. The first bone marrow examination was performed when she was 6 months old, showing normal cellularity and marked dysplasia only in the erythroid lineage. G-banding karyotyping of the bone marrow aspirate showed a normal female karyotype (46, XX). She also developed chronic bloody diarrhea at the age of 2 months. Inflammatory bowel disease (IBD), unclassified type, was diagnosed based on endoscopic and pathological findings at the age of 8 months. She became corticosteroid dependent and required intravenous hyperalimentation to treat malnutrition. A follow-up bone marrow examination at the age of 17 months revealed hypercellularity and dysplasia of trilineage cells without excess blasts. However, bone marrow examination before HCT showed multilineage dysplasia and 7.5% blastic cells, reminiscent of progression to MDS with excess blast-1. As she remained transfusion dependent, HCT was performed at the age of 1 year and 10 months from an unrelated human leukocyte antigen (HLA)-matched donor (8/8 allele matched). She received myeloablative

conditioning (MAC) composed of busulfan (1.2 mg/kg) four times daily on days -9 to -6 (total dose, 19.2 mg/kg) and cyclophosphamide (50 mg/kg) once daily from days -5 to -2 (total dose, 200 mg/kg). The infused bone marrow cells contained  $3.0 \times 10^6$ /kg of CD34-positive cells. Graft-versus-host disease (GVHD) prophylaxis was performed with tacrolimus and short-term methotrexate (15 mg/m<sup>2</sup> on day +1 and 10 mg/m<sup>2</sup> on days +3, +6, and +11). Engraftment was achieved on day +22 with full donor chimerism. She developed grade II acute skin GVHD on day +94 which resolved immediately after initiating corticosteroid treatment. However, she developed grade III gastrointestinal GVHD after discontinuing corticosteroid on day +140. The effect of reinitiated corticosteroid was insufficient, and infliximab was added. Her bloody stool and diarrhea gradually resolved, and corticosteroid was stopped approximately 1 year and 4 months after developing gastrointestinal GVHD. Infliximab was discontinued 3 years after discontinuing corticosteroid. Approximately ten years have passed since HCT, and she is doing well without active complications or transfusion demand and all her immunosuppressants have been discontinued. However, due to prolonged tube feeding associated with inflammation of the gastrointestinal tract, she is unable to ingest orally. After HCT, a novel compound heterozygous variant in *POLE*(p.[D1131fs];[T1891del]) was found by genetic analysis using her dermal fibroblasts.

Case 2 is a 3-year-old girl, a younger sister of Case 1. She also had severe macrocytic anemia without reticulocytopenia (RBCs  $1.71 \times 10^{12}$ /L, hemoglobin level 54 g/L, MCV 117 fL, reticulocytes  $30 \times 10^9$ /L, WBCs  $12.8 \times 10^9$ /L, and platelets  $306 \times 10^9$ /L) from birth and became transfusion-dependent. Genetic analysis of her peripheral blood cells revealed the same variant of *POLE* as in Case 1. Bone marrow findings at 3 months of age were similar to those in Case 1, with dysplasia only in the erythroid without hypoplasia. Dysplasia of all three lineages was seen at 13 months of age (G-banding karyotyping showed a normal female karyotype (46, XX), with 8% blastic cells. These findings indicated MDS with excess blast-1. However, blastic cells decreased to 4.2% at 16 months of age. Unlike Case 1 she did not develop IBD. She remained transfusion dependent and there were fluctuations in the proportion of blasts. HCT was performed at the age of 1 year and 5 months from an unrelated HLA one allele mis-matched donor. She received conditioning composed of fludarabine (25 mg/m<sup>2</sup>) on days -8 to -4 (total dose, 125 mg/m<sup>2</sup>), melphalan (90 mg/m<sup>2</sup>) once daily from days -4 to -3 (total dose, 180 mg/m<sup>2</sup>), anti-thymocyte globulin (2.5 mg/kg) once daily from days -7 to -6 (total dose, 5.0 mg/kg) and total body irradiation of 3 Gray on day 0. The infused bone marrow cells contained  $6.3 \times 10^6$ /kg of CD34-positive cells. GVHD prophylaxis was the same as in Case 1. Engraftment was achieved on day +16 with full donor chimerism. She developed grade III acute gastrointestinal GVHD on day +24 and was treated with immunosuppressive agents including corticosteroid, mycophenolate mofetil, and additional methotrexate. However, her symptoms were refractory and required addition of mesenchymal stem cell therapy. Meanwhile, pathological findings of her gastrointestinal biopsy revealed intestinal thrombotic microangiopathy and recombinant human soluble thrombomodulin was also administered. Her gastrointestinal symptoms gradually resolved, and each treatment was able to be tapered off without recurrence. Two years have passed since HCT, and she is doing well without active complications or transfusion demand and all her immunosuppressants have been discontinued.

## Discussion

Recently, we described a novel human Pol  $\epsilon$  defect associated with hematopoietic failure.<sup>3</sup> The patients in our current report are the same siblings reported there and thus may represent the first HCT report of Pol  $\epsilon$  defect associated hematopoietic failure. Both cases presented with trilineage dysplasia with fluctuations in the proportion of blasts in bone marrow, which meets the diagnostic criteria of MDS-EB-1.<sup>4</sup> Along with persistent transfusion dependency, HCT was performed according to protocol for MDS patients.<sup>5,6</sup> MAC was selected in Case 1 because her bone marrow showed hypercellularity and, without precedent, MAC was considered ideal to obtain definite engraftment. As expected, Case 1 achieved stable full donor chimerism. Based on this experience, we decided to reduce the conditioning intensity of Case 2 to avoid cardiac toxicity<sup>7</sup> and late complications, including impaired fertility.<sup>8</sup> HCT certainly resolved their bone marrow failure, and they became transfusion independent. In Case 1, IBD also resolved after HCT. The association of IBD and *POLE* gene variant is uncertain; however, reconstitution of the immune system may be beneficial, as in other monogenic forms of IBD.<sup>9</sup> On the other hand, as with variants in the exonuclease domain of the *POLE*,<sup>10</sup>

there may be outcome factors other than bone marrow failure in these cases, and continued careful follow-up is essential.

### Author contributions

KF, NI, IT, H. Shimizu, A. Ishiguro, KA, KM, and A. Iguchi were the attending physicians. TU and TD supported laboratory examinations. KF and A. Iguchi wrote the manuscript. YG, H. Sakaguchi, and DT supervised the case and helped write the manuscript. All authors revised and approved the manuscript.

### Conflict of interests

The authors declare no conflicts of interest.

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