Case Report

Challenges to allogeneic hematopoietic stem cell transplantation in a patient with GATA2 deficiency and persistent Epstein-Barr virus infection

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The germline *Guanine-adenine-thymine-adenine 2* (*GATA2*) heterozygous mutations (GATA2 deficiency) are identified as a complex immunodeficiency, which entails susceptibility to non-tuberculous mycobacterium (NTM), fungus, or Epstein-Barr virus (EBV) infection, and hematological disorders including myelodysplastic syndrome (MDS)/acute myelogenous leukemia, accompanied by characteristic sighs such as lymphedema or deafness. A 21-year-old woman with persistent EBV infection was diagnosed with GATA2 deficiency based on the onset of MDS and her family history. The patient underwent bone marrow transplantation from an unrelated donor. She developed steroid-resistant graft-versus-host disease (GVHD), leading to an NTM infection, the deterioration of lymphedema, and life-threatening gut bleeding caused by not only GVHD but also thrombotic microangiopathy. Although allogeneic hematopoietic stem cell transplantation is a potential therapeutic option in patients with GATA2 deficiency, GATA2 haploinsufficiency in non-hematopoietic cells may affect the post-transplant complications. Therefore, the establishment of treatment guidelines on donor and graft selection, the intensity of conditioning regimens, GVHD prophylaxis, and prevention of post-transplantation infections based on the history of infection and degree of organ damage is necessary for patients with GATA2 deficiency.
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Key Points

The germline Guanine-adenine-thymine-adenine 2 (*GATA2*) heterozygous mutations predispose to immunodeficiency and hematological disorders, such as myelodysplastic syndrome or acute myelogenous leukemia. Allogeneic hematopoietic stem cell transplantation is a useful therapeutic option, but many challenging issues remain for physicians.

Introduction

In 2011, a genetic abnormality in *guanine-adenine-thymine-adenine 2* (*GATA2*) was identified in four syndromes: the MonoMac syndrome, Emberger syndrome, DCML syndrome, and familial myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML). These syndromes have overlapping symptoms and signs and are caused by heterozygous GATA2 deficiency associated with heterozygous mutations in *GATA2*.[1] Allogeneic hematopoietic stem cell transplantation (HSCT) is an effective treatment to alleviate immunodeficiency and abnormal hematological findings,[2-5] though it remains challenging.[5-8]

Here, we describe the clinical course of HSCT in a 21-year-old woman with GATA2 deficiency persistently infected with Epstein-Barr virus (EBV), as well as discuss the challenges faced during treatment.

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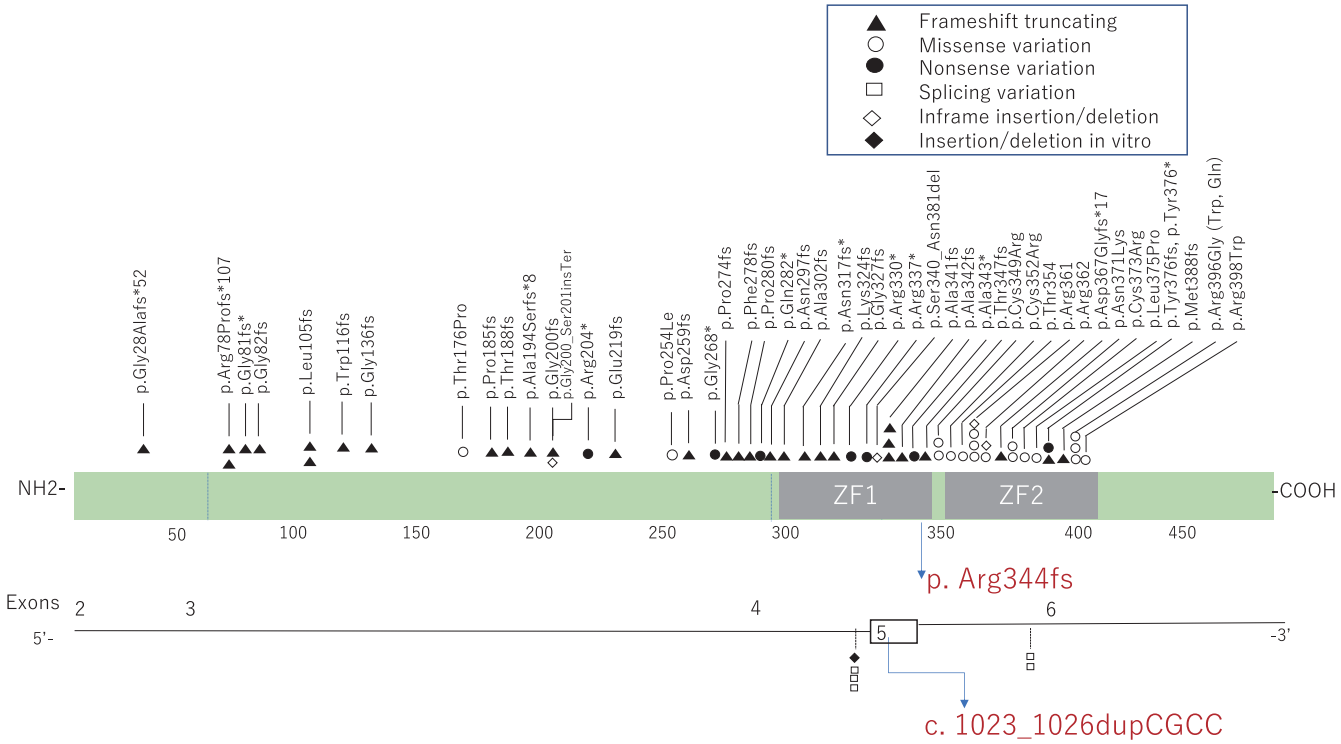
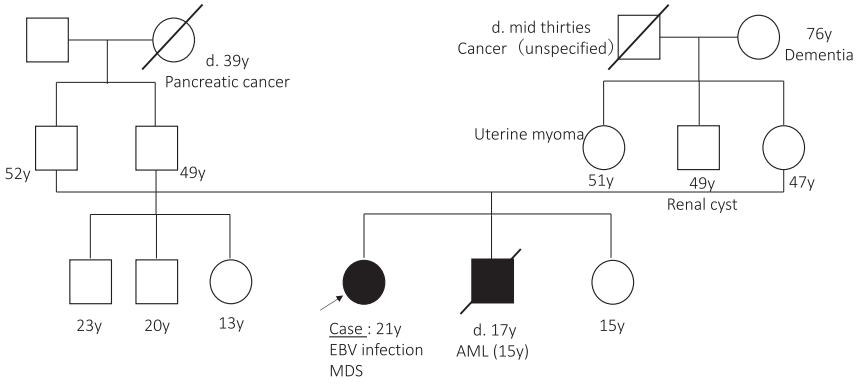


Figure 1. Results of germline *GATA2* gene analysis of the case, and previously reported mutations. The results were constructed with reference to the article by Yoshida et al[13].

Figure 2. Family tree of the case



Case Report

The patient was a 21-year-old female referred to our hospital for treatment. She was diagnosed with GATA2 deficiency concomitant with persistent EBV infection and MDS (See supplemental data for details).[8] A novel heterozygous *GATA2* mutation (c.1023_1026 dupCGCC, p.Arg344fs) was revealed in the patient (**Figure 1**), and her family history revealed AML in her younger brother (15-year-old), with no such findings in other relatives (**Figure 2**).

The patient required HSCT to treat her persistent EBV infection and MDS. During donor coordination at the Japan Marrow Donor Program (JMDP), she was treated with prednisolone (PSL) and cyclosporine A (CsA). As the MDS in this case was not accompanied by a karyotype abnormality, reduced-intensity conditioning was used as the conditioning regimen. However, because of the persistent EBV infection, the rapid destruction of infected cells by the transplantation regimen may induce uncontrolled hypercytokinemia.[9] Therefore, the patient was pretreated with low-dose cytarabine (20 mg/m^2) and etoposide (30 mg/m^2), along with anti-thymocyte globulin (ATG) was administered at the rate of 1.25 mg/kg/day from transplantation days -8 and -7 to prevent the T cell from causing hypercytokinemia. Subsequently, 30 mg/m^2 fludarabine (FLU) (from days -7 to -2), 70 mg/m^2 melphalan (MEL) (on days -3 and -2), 100 mg/m^2 etoposide (on days

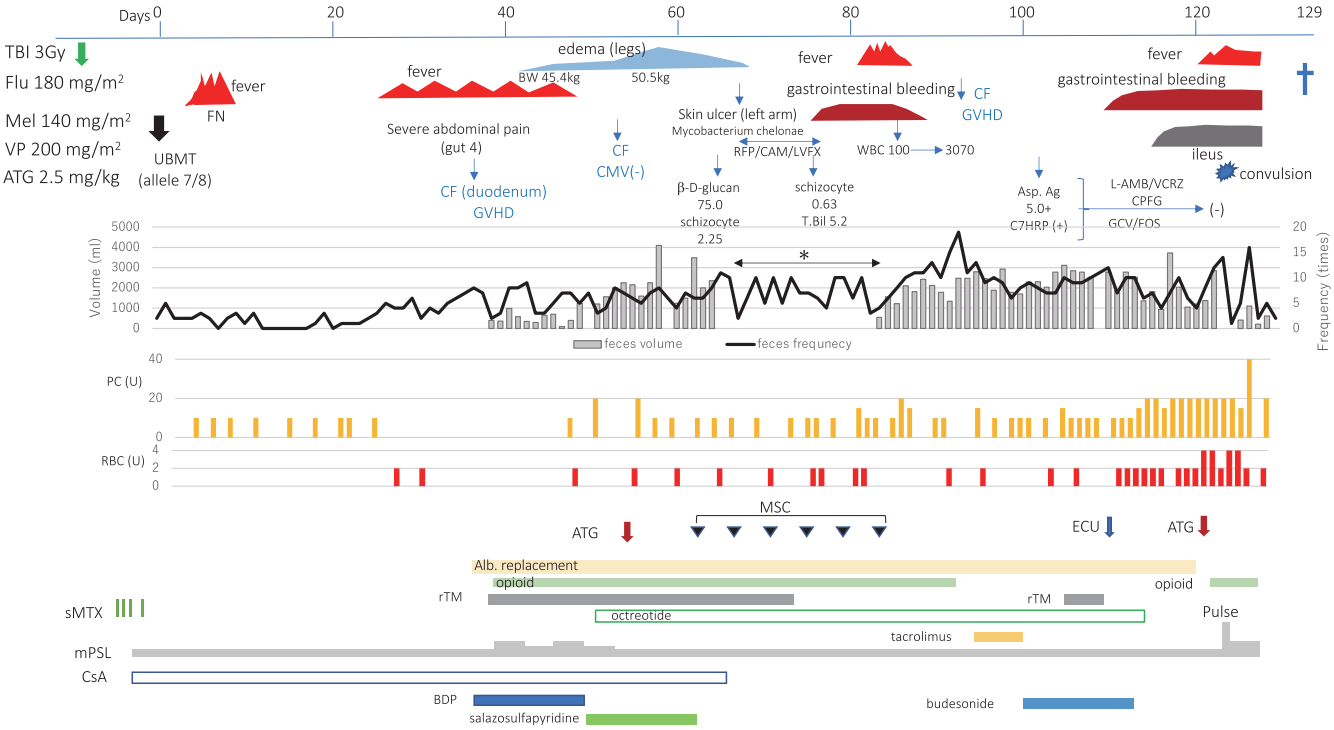


Figure 3. Clinical course after bone marrow transplantation. TBI, total body irradiation; Flu, fludarabine; Mel, melphalan; VP, etoposide; ATG, anti-thymocyte globulin; UBMT, unrelated bone marrow transplantation; FN, febrile neutropenia; CF, colon fiberscope; GVHD, graft-versus-host disease; CMV, cytomegalovirus; T. Bil, total bilirubin; Asp. Ag, Aspergillus antigen; C7HRP, cytomegalovirus pp65 antigens; L-AMB, liposomal amphotericin B; VCRZ, voriconazole; CPFG, caspofungin; GCV, ganciclovir; FOS, foscavir; MSC, mesenchymal stem cell; ECU, eculizumab; rTM, recombinant thrombopoietin; sMTX, short course methotrexate; mPSL, methyl-prednisolone; CsA, cyclosporine A; BDP, beclomethasone propionate ; *, Feces volume data was not available during this period.

-3 and day -2), and total body irradiation (TBI, 3 Gy, on day -1) were administered. Transplantation was performed using 7/8 human leukocyte antigen (HLA) allele-matched unrelated bone marrow cells (3.1×10^8 cells/kg). As prophylaxis for graft-versus-host disease (GVHD), CsA, a short-course methotrexate, and methylprednisolone (mPSL) at 0.5 mg/kg/day were employed. Leukocyte engraftment occurred on day 20 after transplantation. A temperature of over 38℃ was observed after day 28, along with gut-related symptoms (abdominal pain and protein-losing diarrhea) (**Figure 3**). The pathologic findings obtained from gastrointestinal endoscopy performed on day 36 were consistent with GVHD. The abdominal pain was intolerable and required opioids. Acute GVHD was localized in the gut (stage 4, grade Ⅲ) at that time. The prophylactic dose of mPSL was increased (1 mg/kg), and beclomethasone propionate, a nonabsorbable steroid, was administered. However, the gut symptoms were steroid-resistant, and the frequency of diarrhea was approximately 10 times/day with a volume reaching 2 L/day. Edema of the lower legs was observed from approximately day 40, which gradually progressed to the thighs and lower abdomen, and patient's body weight increased by approximately 5 kg (10%). On day 48, since the total bilirubin concentration exceeded 2 mg/dL, an abdominal echocardiography was performed considering the possibility of sinusoidal obstructive syndrome/veno-occlusive disease; however, no evidence of hepatomegaly or paradoxical traffic of the portal vein was observed. Edema was resistant to albumin supplementation and was a possible exacerbation of lymphedema associated with GATA2 deficiency. Somatostatin analogue was also started to relieve her gastrointestinal symptoms.[10] Thus, for the gut GVHD, 1 mg/kg of ATG was administered on day 56, moreover human mesenchymal stem cells were administered twice-weekly starting on day 63. However, no improvement was observed following these treatments. Redness and swelling were observed on the skin of the left upper arm, partially complicated with skin ulcer. After the biopsy, *Mycobacterium chelonae* was isolated from a skin ulcer. On day 66 after HSCT, fragmented red blood cells increased by 2.25%, whereas anemia and thrombocytopenia deteriorated. By day 76, the patient experienced severe gut bleeding and required daily blood transfusion. As the patient's gut symptoms were predicted to be caused by GVHD or thrombotic microangiopathy (TMA), recombinant thrombomodulin was initiated,[11]

and CsA was discontinued after reduction. Hyperbilirubinemia with direct bilirubin predominance was also observed from 76 days after transplantation, indicating hepatic GVHD. Tacrolimus administration was initiated on day 90 but was discontinued because of the deterioration of TMA. Biopsy of the small intestinal mucosa on day 87 also revealed histology consistent with GVHD; however, we reviewed the histopathological findings for TMA complications. Although intravascular thrombi were not observed, pathological findings, such as fewer CD8-positive T cells and more CD68-positive macrophages infiltrating the mucosa, were suggestive of TMA (**Figure 4**). The frequency of diarrhea declined on day 124 but gut bleeding continued. Additionally, abdominal echocardiography showed dilatation of the intestine and filling of the intestinal contents, suggesting a complication of the ileus due to exacerbation of gastrointestinal symptoms caused by GVHD/TMA. The clinical symptoms did not improve with various treatments, and the patient died of multiple organ failure caused by massive gastrointestinal hemorrhage 127 days after transplantation.

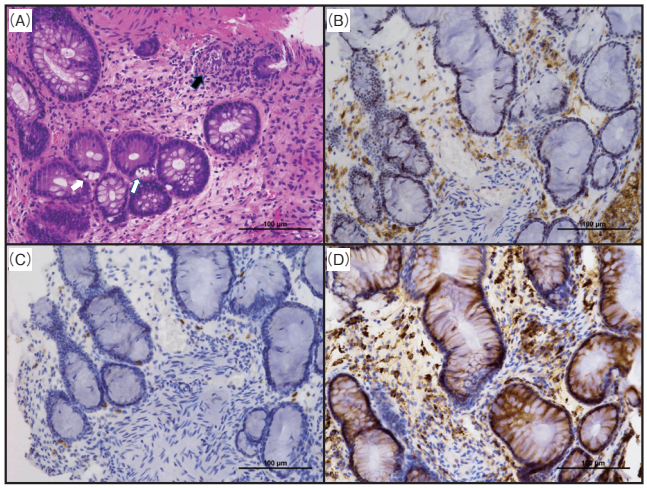


Figure 4. Pathological findings of sigmoid colon mucosa biopsy tissue on day 87. The hematoxylin and eosin stain specimen demonstrated atrophy and loss of the crypts (black arrow), and apoptosis of the epithelium at the base of the crypts (white arrows) (A). CD4-positive T cells (B) infiltrated the interstitial space significantly more than CD8-positive cells (C), and CD68-positive macrophages infiltrated remarkably (D) compared to T cells (B, C).

Discussion

Patients with GATA2 deficiency can be persistently infected with EBV.[12] When the EBV genome load is high before transplantation, pre-conditioning drugs can cause rapid destruction of EBV-infected cells, leading to uncontrolled hypercytokinemia and organ damage. Hence, reducing the amount of EBV DNA by chemotherapy before transplantation is crucial.[9] In our case, chemotherapy was not indicated based on the hematopoietic stem cell-level damage associated with GATA2 deficiency. Treatment-related deaths occured in cases of remission-induction chemotherapy in GATA2 deficiency, including in patients with AML.[2] In this case, cytopenia associated with MDS was relieved by PSL and CsA, which also reduced the EBV genome load decreased from 5.0×10^5 (at the time of admission) to 10^2 (before transplantation) copies/mL. Before administrating of FLU/MEL, a low-dose of chemotherapy followed by ATG were useful for preventing hypercytokinemia. Moreover, post-transplant EBV-DNA quantification was below the sensitivity of the PCR assay at any time, and EBER staining of the intestinal biopsy tissue showed no association of her gut symptoms with EB virus infection. Thus, HSCT safely and effectively eliminated the persistent EBV infection.

Based on the results of EWOG-MDS cohort study (n=508), overall survival and outcome were not influenced by the presence of germline *GATA2* mutation in children and adolescents with MDS.[2] However, in the present case, several transplantation-associated complications occurred, including non-tuberculous mycobacterium (NTM) infection, severe lymphedema extending from the lower leg to the lower body, and lethal gastrointestinal TMA-like complications, which may be characteristic of GATA2 deficiency patients. Hoffman et al. compared the outcomes of HSCT in two groups of patients who developed hematopoietic disorders, MDS or AML, with or without GATA2 deficiency. The 5-year rate of overall and disease-free survival in the GATA2-deficient group were 65% and 55%, showing no significant difference from the control group. However, event-free survival was 7±6% in the GATA2 deficiency group, showing a significant difference from that in the control group (28-33%). In addition, a higher frequency of thrombosis and neurological complications was observed in GATA2-deficient patients.[5] Bogaert et al. also reported a similar case of treatment-resistant chronic GVHD after transplantation that resulted in death due to myocardial infarction.[6] Because GATA2 is expressed in the vascular endothelium, haploinsufficiency of GATA2 protein may predispose the patient to thrombosis in case of possible endothelial damage due to GVHD or associated therapeutic agents.[5,6] Moreover, in the setting of HSCT for GATA2-deficient patients from HLA-matched siblings or unrelated donors, high frequency severe acute GVHD (26% of grade Ⅱ-Ⅳ) and

chronic GVHD（46%）were observed.[3] Thereafter, intensified GVHD prophylaxis, such as tacrolimus or post-transplant cyclophosphamide, achieved excellent treatment results.[4] These results suggest that adequate GVHD prophylaxis and therapy are important for HSCT in patients with GATA2 deficiency.

This patient was diagnosed with GATA2 deficiency because her younger brother had AML with monosomy 7. [8] Based on reports of the natural history of GATA2 deficiency [2,14,15], the symptoms are variable, and the same genetic abnormality can produce completely different symptoms and clinical courses. Her brother had no history of infection and symptoms associated with GATA2 deficiency. He also received HSCT, but encountered no serious complications, including TMA in the early post-transplant period.[8] Additionally, for transplantation between blood relatives, it is necessary to analyze *GATA2* in the donor. Patients transplanted from the unaffected donors of GATA2 deficiency can subsequently develop hematopoietic disorders and immunodeficiency associated with donor-derived GATA2 haploinsufficiency after transplantation, resulting in death [7,8].

Overall, although HSCT is an effective therapeutic modality for GATA2 deficiency, donor and graft selection, GVHD prophylaxis, and post-transplantation infection prevention should be cautiously considered based on the history of infection and degree of organ damage before transplantation. Therefore, establishing treatment guidelines that include the care of unaffected individuals is necessary.

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Authors' Contributions

N. S. designed the treatment plan of this patient and wrote the manuscript. M. O., A. T., R. O., and T. K. provided patient care. K. S. reviewed the manuscript.

Conflict of Interest disclosure

The authors declare that they have no conflict of interest.

Statement of Ethics

Written informed consent was obtained from the patients' parents for publication of the details of their medical records.

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