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LETTER TO EDITOR

DNA Ligase IV Deficiency Identified by Chance Following Vaccine-Derived Rubella Virus Infection

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Abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMT	Bone marrow transplantation
CDR3	Complementarity-determining region 3
GVHD	Graft-versus-host disease
HSCT	Hematopoietic stem cell transplantation
iVDRV	Immunodeficiency-related vaccine-derived rubella virus
PID	Primary immunodeficiency disease
KRECs	Kappa-deleting recombination excision circles
PBMCs	Peripheral blood mononuclear cells
SCID	Severe combined immunodeficiency
TRECs	T cell receptor excision circles
VDRV	Vaccine-derived rubella virus
VZV	Varicella-zoster virus

To the Editor,

Persistent vaccine-derived rubella virus (VDRV) infection is infrequently observed in immunocompetent individuals [1, 2]; however, most cases of VDRV are noted in patients with primary immunodeficiency diseases (PIDs) [3]. Latter cases are called immunodeficiency-related VDRV (iVDRV), which is associated with cutaneous and occasionally visceral granuloma formation. Intriguingly, iVDRV is frequently found in patients with DNA repair disorders, including ataxia telangiectasia, Nijmegen breakage syndrome, DNA ligase IV deficiency, and Artemis deficiency [4].

Here, we describe a Japanese patient with DNA ligase IV deficiency identified by chance following iVDRV, in whom successful bone marrow transplantation (BMT) with immune reconstitution led to the eradication of the rubella virus.

A 13-month-old Japanese girl presented with erythema on her cheeks and subsequent erythematous papules on her entire skin. She was born to nonconsanguineous Japanese parents at 39 gestational weeks. Her birth weight was 2040 g (− 2.84 SD), the height 44 cm (− 2.6 SD), and the head circumference 32 cm (− 0.97 SD). No recurrent or severe infection was noted after discharge. Her family history was negative for any hematologic or infectious diseases. Physical examination revealed mild hepatosplenomegaly and a bird-like facial

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appearance with microcephaly (Fig. 1a). She received bacille Calmette-Guerin (BCG) vaccine at 5 months of age and other live-attenuated vaccines, including for measles, rubella, mumps, and varicella-zoster virus (VZV) at 12 months of age. One month later, she developed maculopapular erythematous flat-topped symmetric eruption on the entire body (Fig. 1a). The patient was found to have severe hypogammaglobulinemia and mild leukopenia at 14 months of age (Table S1). Furthermore, she showed defects in IgG antibody production against the viral antigens of vaccines previously administered including VZV, mumps, measles, and rubella viruses. The patient was suspected of the underlying PIDs, and skin rash suggested a vaccine-derived virus infection. At 16 months of age, we screened for the causative virus by RT-PCR and PCR using throat swab, blood, and urine samples. RT-PCR for measles, mumps, and enterovirus and PCR for VZV were negative; however, RT-PCR for rubella virus was positive. The virus was compatible with the Takahashi vaccine strain 10 months after administration (Fig. S1). At 19 months of age, a skin biopsy was performed, revealing lymphohistiocytic infiltration characterized by scattered small granulomatous nodules in the dermis (Fig. 1b). Immunohistochemical analysis showed dermal infiltration of

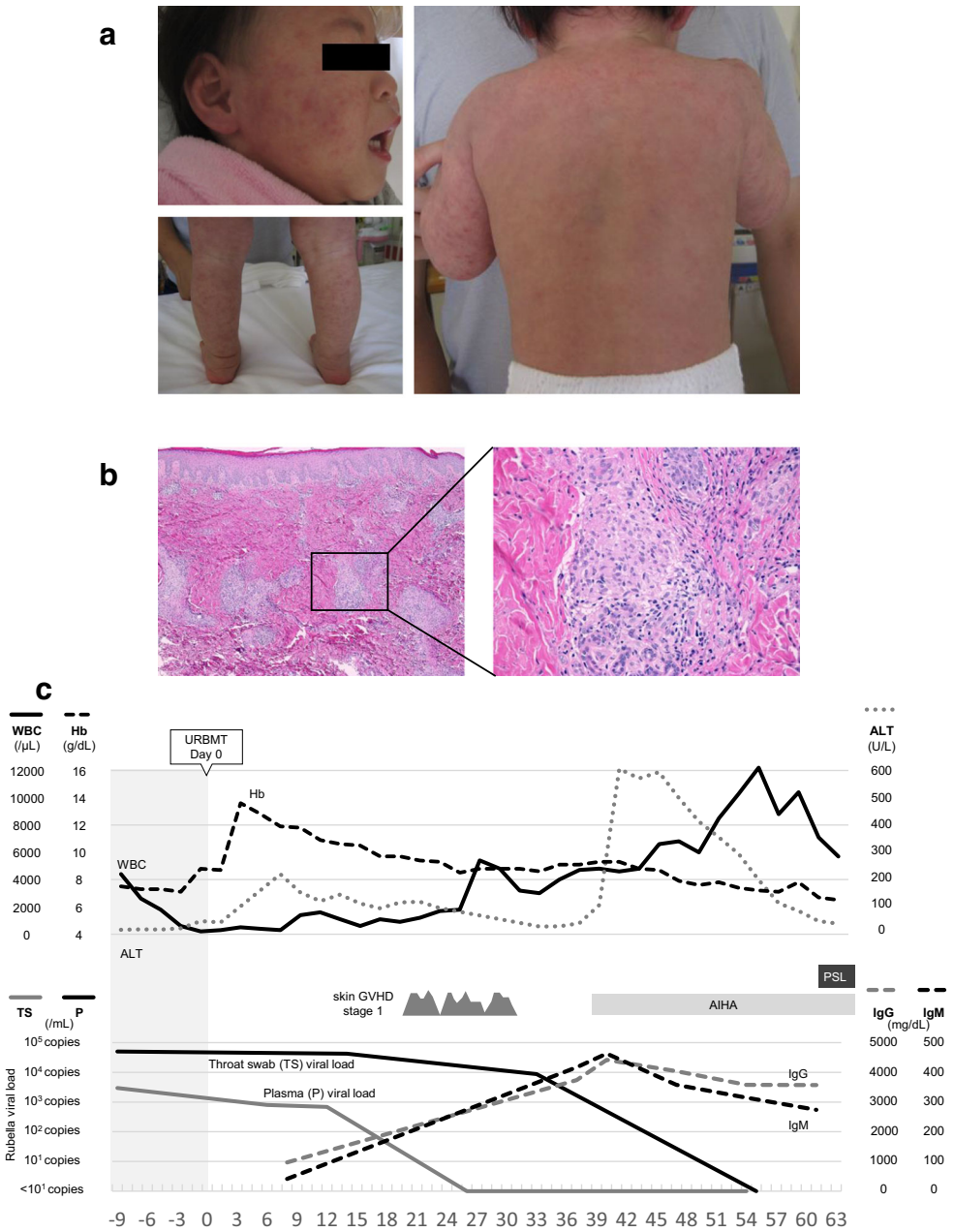

Fig. 1 Characteristic skin lesion and clinical course of the patient. **a** Skin erythema was mainly detected on the lower leg, cheek, and back. A "bird-like" facial appearance was exhibited. **b** Histopathological findings of the skin biopsy show lymphohistiocytic infiltration characterized by scattered small granulomatous nodules in the dermis Hematoxylin-eosin staining. **c** Clinical course of the patient. URBMT, unrelated bone marrow transplantation; GVHD, graft-versus-host disease; AIHA, autoimmune hemolytic anemia; PSL, prednisolone

$CD68^+$ macrophages and $CD163^+$ M2 macrophages (Fig. S2). Rubella antigen was not detected in immunohistochemical staining; however, low copy number of rubella virus RNA was detected using quantitative RT-PCR (data not shown). This discrepancy of quantitative RT-PCR analysis and immunohistochemistry might be attributed to the contamination of blood in the tissue or relatively low sensitivity of immunohistochemistry due to technical difficulties of handling antibody. Flow cytometric analysis revealed a reduced number of T cells, no B cells, and absolute dominance of NK cells (Table S1). The T cells were relatively dominated by γδ T cells, and reduced $CD4^+$ T cells were skewed to the memory phenotype. The levels of T cell receptor excision circles (TRECs) and kappa-deleting recombination excision circles (KRECs) were below the detection limit. The diversity of both TCR and IgH repertoires in the patient were lower than those in an age-matched healthy individual (Fig. S3). These immunological findings suggested that she had late-diagnosed $T^{low}B^-NK^+$ SCID. Combined with a bird-like facial appearance and microcephaly, the patient was suspected to have radiosensitive SCID. Genetic analysis demonstrated compound heterozygous mutations consisting of a missense and an early truncating mutation (c. G827A, p. G276D and c. 233_236delAGAG, p. R79Wfs*15) in the *LIG 4* gene and a missense mutation close to the ATP-binding domain p.R278, suggesting impaired catalytic capacity and another early truncating mutation. Relatively mild cases of DNA ligase IV deficiency caused by the combination of compound heterozygous missense and nonsense mutations have been previously reported [5]. Therefore, she was diagnosed with DNA ligase IV deficiency associated with iVDRV, although rubella antigens were not detected in the skin lesion.

The patient was managed with prophylactic treatment of intravenous immunoglobulin, sulfamethoxazole/trimethoprim, itraconazole, acyclovir, isoniazid, and vitamin B6; however, she developed leukopenia and thrombocytopenia at 18 months of age. Bone marrow aspiration revealed normal morphology but reduced number of nuclear cells. She was administered granulocyte colony-stimulating factor. At 22 months of age, she underwent unrelated BMT from an 8/8 HLA-matched 43-year-old female donor followed by reduced intensity conditioning, with 62.7×10^7 nucleated cells and 82.2×10^5 $CD34^+$ cells per kg body weight. The conditioning regimen consisted of fludarabine (35 mg/m^2/day for 5 days, day 9~5), cyclophosphamide (5 mg/kg/day for 4 days, day 5~2), and rabbit anti-thymocyte globulin (1.25 mg/kg/day for 4 days, day 9~6). The graft-versus-host disease (GVHD) prophylaxis consisted of tacrolimus and short-term methotrexate. Neutrophil engraftment was achieved on day 12 (Fig. 1c). On day 20, she developed a maculopapular rash on the face. She was diagnosed with grade I acute GVHD (stage: skin 1, liver 0, gut 0). Corticosteroid ointment relieved the skin symptoms. On day 29, mixed chimerism of 90% donor cells was confirmed in peripheral blood using short tandem repeat-PCR technique. On day 37, blood examination revealed profound elevation of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) accompanied by dysgammaglobulinemia (Fig. 1c). Seroconversion was demonstrated on day 42, with positive rubella virus IgG and IgM being detected. The elevation of AST, ALT, and IgG resolved without any treatment. Flow cytometric analysis demonstrated an extraordinary increase in the number of $CD27^+$ memory B cells of total B cells (84.4%, normal value: 5.1% ± 3.5%). Reconstruction of B cell immunity may have caused the dysgammaglobulinemia and seroconversion of rubella antibody. Rubella virus was detected on day 9 in plasma and throat swab, but it became undetectable in plasma on day 26 and in throat swab on day 55 (Fig. 1c). Accordingly, the skin erythema disappeared. On day 61, the patient suddenly developed severe anemia with reticulocytosis. Both direct and indirect antiglobulin tests were positive. Haptoglobin level was normal. Notably, serum free light chain showed a skewed ratio of kappa/lambda 5.01. IgH rearrangement was positive for DH1-6/JH (data not shown). The patient's condition was suspected of being associated with immune reconstitution syndrome, in which kappa chain-skewed atypical plasma cell cytosis might lead to extravascular autoimmune hemolytic anemia. Oral prednisolone was administered for 210 days, and the hemolytic anemia was improved. On day 182, peripheral blood analyses showed full donor chimerism. Peripheral B cells increased to 367/μL, and immunoglobulin replacement therapy was stopped.

iVDRV is a unique complication in patients with PID, frequently comprising DNA repair disorders, including DNA ligase IV deficiency [4, 6]. M2 macrophage is associated with the inability to eradicate the virus, and that aberrant $CD8^+$ T cell responses are also thought to play a role [7]. RA27/3 was a rubella virus strain vaccine that was commonly administered in the USA [7, 8]. In one study, only half of the patients with PID and iVDRV were found to have positive rubella antigens in the skin [4]. Although rubella antigen was not detected in the skin of our patient, the histological finding of M2 macrophage accumulation might be indicative of iVDRV. Furthermore, our patient presented with the persistence of a generalized rash, whereas the previously reported cases were characterized by localized, discrete granuloma lesions predominantly on the extremities and face [4].

DNA ligase IV deficiency is characterized by $T^-B^-NK^+$ SCID and profound radiosensitivity, as well as a "bird-like" facial appearance, microcephaly, growth retardation, developmental delay, and progressive bone marrow failure [9, 10]. Our patient showed hypomorphic immunological phenotype with few T cells and B cells; however, these cells revealed skewed patterns of TCR and IgH repertoires, respectively. Forty-one patients with DNA ligase IV deficiency have been reported [5, 11]. Hematopoietic stem cell transplantation (HSCT) is the only curative treatment; however, no optimal conditioning regimen has been established because of unpredictable toxicity. In our case, we considerably reduced the intensity of the conditioning regimen compared with a

previous case [12]. These reduced doses may have been optimal for our patient because she achieved rapid engraftment with full donor chimerism and good immune reconstruction, as well as minimal adverse events. Notably, the patient presented with immune reconstitution syndrome following rubella virus eradication, consisting of hepatitis, kappa-restricted oligoclonal dysgammaglobulinemia, and hemolytic anemia. Although we reduced the dose of cytotoxic drugs in the conditioning regimen, the occurrence of malignancy, including lymphomas [5], should be carefully monitored.

In conclusion, we described the first Japanese case of iVDRV, which helped us to identify DNA ligase IV deficiency. The patient successfully underwent BMT with ultra-reduced-intensity conditioning, which completely eradicated the rubella virus infection followed by the immune reconstitution syndrome. Neonatal mass screening of TRECs and KRECs might facilitate early detection of SCID and B cell deficiency in patients and avoid inoculation of live-attenuated vaccines. In the near future, neonatal mass screening for PIDs would be available in Japan.

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Authors' Contributions KM contributed to data collection and writing of the manuscript. AH and TK performed the genetic analysis. YM performed the virological study. MH contributed to the pathological diagnosis. KM, AH, AN, MS, CN, KW, NM, MT, and KI treated the patient. SN and TM supervised the study. HK conceptualized the study and extensively edited and revised the manuscript.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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