

A Fatal Case of Infantile Malignant Osteopetrosis Complicated by Pulmonary Arterial Hypertension after Hematopoietic Stem Cell Transplantation

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Infantile malignant osteopetrosis (IMO) is a rare and fatal autosomal recessive condition characterized by a generalized increased in bone density. Hematopoietic stem cell transplantation (HSCT) is the only effective and rational therapy with achieving long-term disease-free survival. However, complications with HSCT for IMO remain unclear. Here we describe a male infant with IMO, carrying two novel mutations in the T-cell immune regulator 1 (*TCIRG1*) gene. The *TCIRG1* gene encodes the $\alpha 3$ subunit of vacuolar H⁺-ATPase that plays an essential role in the resorptive function of osteoclasts. Direct sequencing of all 20 exons of the *TCIRG1* gene revealed a single nucleotide change in exon 11 (c1305 G > T), which causes the substitution of Asp (GAT) for Glu (GAG) at position 435, and a two-nucleotide deletion in exon 16 (c1952-1953 del CA), causing a frame-shift mutation. However, the functional consequence of each mutation remains to be determined. Allogeneic HSCT was performed in the patient at the age of nine months. Donor engraftment was achieved, and abnormal bone metabolism and extramedullary hematopoiesis were corrected. Graft-versus-host disease was mild (grade I). However, the patient died of complication of pulmonary arterial hypertension at seven months after the HSCT. Postmortem examination revealed prominent vascular wall thickening of the pulmonary artery and macrophage infiltration to alveoli. It should be noted that a patient with IMO has a risk for pulmonary arterial hypertension, and the evaluation of pulmonary arterial flow should be included in the assessment of each patient with IMO even after HSCT.

Keywords: graft versus host disease; hematopoietic stem cell transplantation; infantile malignant osteopetrosis; pulmonary arterial hypertension; T-cell immune regulator 1

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Introduction

Osteopetrosis is a group of rare, heritable disorders of the skeleton characterized by increased bone density on X-ray radiographs. Osteopetrosis is caused by the failure of osteoclast development or function (Askmyr et al. 2008). The overall incidence of osteopetrosis is about 1 : 20,000. The autosomal dominant form of osteopetrosis is usually asymptomatic, or diagnosed incidentally in late childhood. However, autosomal recessive form, also termed infantile malignant osteopetrosis (IMO), is usually diagnosed soon after birth with severe symptoms, including pancytopenia caused by bone marrow failure, hepatosplenomegaly, or macrocephaly with early closing of the fontanel. Mutations

in at least 10 genes have been identified as causative in humans, accounting for more than 70% of all IMO cases (Askmyr et al. 2008). We had reported one of these mutations previously (Michigami et al. 2002).

Here, we report a fatal case of IMO with novel mutations in the T-cell immune regulator 1 (*TCIRG1*) gene and fatal complication, pulmonary arterial hypertension (PAH), after hematopoietic stem cell transplantation (HSCT).

Case Presentation

A 4-month-old boy was referred to us for the evaluation of hepatosplenomegaly. He was the first baby of healthy unrelated Japanese parents, and his perinatal period and family history were unremarkable.

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The physical examination at the time of admission revealed failure to thrive: his body weight was 5,320 g (mean -3.7 s.d.), and body height was 56.6 cm (mean -2.4 s.d.). His fontanel was 2×2 cm in size and was mildly bulged. Chest auscultation revealed that the lung and heart sounds were clear. Palpation of the abdomen disclosed evident hepatosplenomegaly; the liver was enlarged 6 cm below the right costal margin, and the spleen was enlarged 5 cm below the left costal margin. Fundoscopic examination disclosed the normal optic nerve.

The peripheral blood count showed mild anemia with thrombocytopenia and leukocytosis; hemoglobin 10.0 g/dL, red blood cell count of $3.98 \times 10^6/\mu\text{L}$, white blood cell count of $20,300/\mu\text{L}$, platelet count of $1.32 \times 10^5/\mu\text{L}$. Blood chemistry disclosed increased alkaline phosphatase levels (7,495 U/L: normal 150-450), hypophosphatemia (2.5 mg/dL: normal 4.0-6.0), increased intact parathyroid hormone levels (98 pg/ml: normal 15-75) and elevated $1,25(\text{OH})_2$ vitamin D levels (192 pg/mL: normal 10-55), while serum calcium level was normal (9.3 mg/dL: normal 8.5-10.5). Severe hypogammaglobulinemia (immunoglobulin G 35 mg/dL: normal 195-795 mg/dL) with a normal level of serum immunoglobulin M (35 mg/dL: normal 12-210 mg/dL) was observed. The levels of biomarkers associated with bone metabolism were also increased: bone alkaline phosphatase $787 \mu\text{g/L}$ (normal 50-150). The radiographs taken upon admission revealed characteristic signs of osteopetrosis, including diffuse sclerosis, “bone-in-bone appearance” and the loss of corticomedullary differentiation (Fig. 1A). Furthermore, nuclear imaging using $^{111}\text{Indium}$ revealed increased extramedullary hematopoiesis in the liver and spleen (Fig. 1B) (Cheow et al. 2001). A diagnosis

of IMO was made based on these findings.

After obtaining the informed consent by his parents, mutational analysis was performed by direct sequencing of all 20 exons of the *TCIRG1* gene that encodes the $\alpha 3$ subunit of the vacuolar proton-ATPase, as previously reported (Michigami et al. 2002). *TCIRG1* is important for the resorptive function of osteoclasts. We thus identified the two distinct mutations in the protein-coding region (Fig. 2):

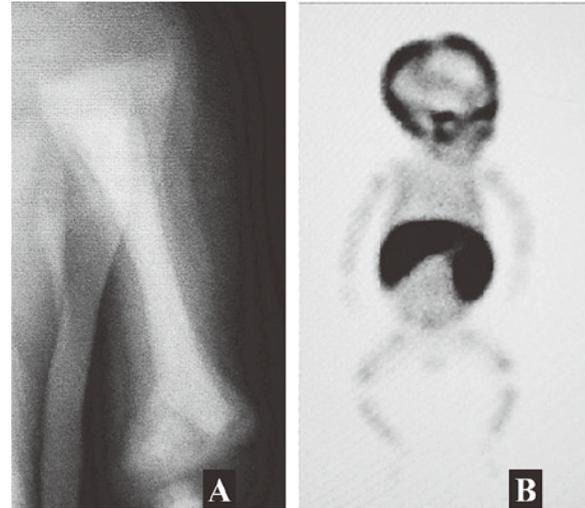


Fig. 1. Imaging studies on admission.

A: Bone X-ray of the humerus.

Diffuse sclerosis, “bone-in-bone” appearance and the loss of corticomedullary differentiation were demonstrated.

B: Bone marrow scintigraphy using $^{111}\text{Indium}$.

At 48 hours after the injection of isotope, extramedullary hematopoiesis was confirmed in the liver and spleen.

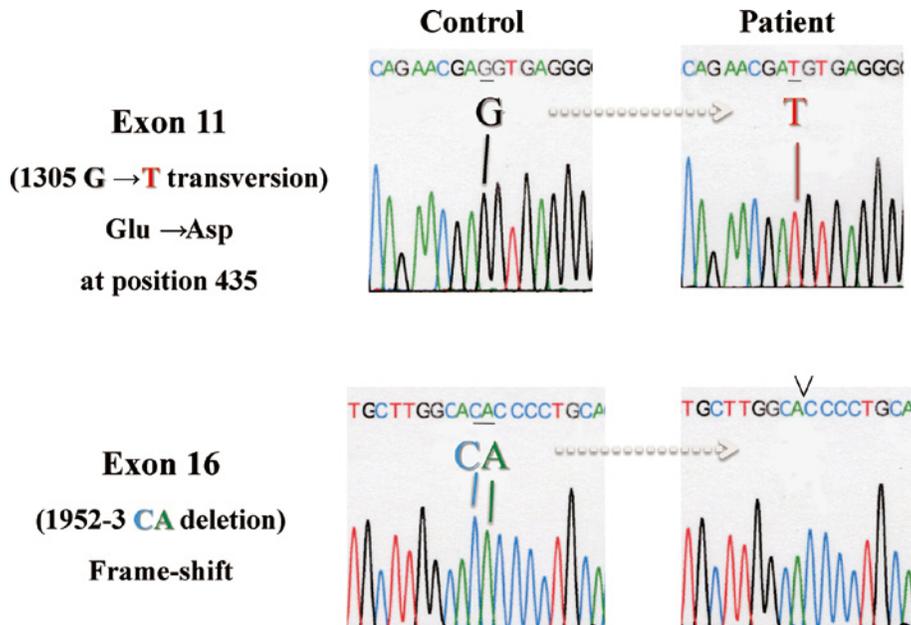


Fig. 2. Sequence analysis of the *TCIRG1* gene.

A single nucleotide change is present in exon 11 (c1305 G > T), which causes an amino acid substitution: Glu (GAG) to Asp (GAT). A two-nucleotide deletion (c1952-1953 del CA) is present in exon 16 of other allele, which causes the frame-shift mutation. Shown are the nucleotide sequences of the cloned DNA fragments, each carrying the novel mutation.

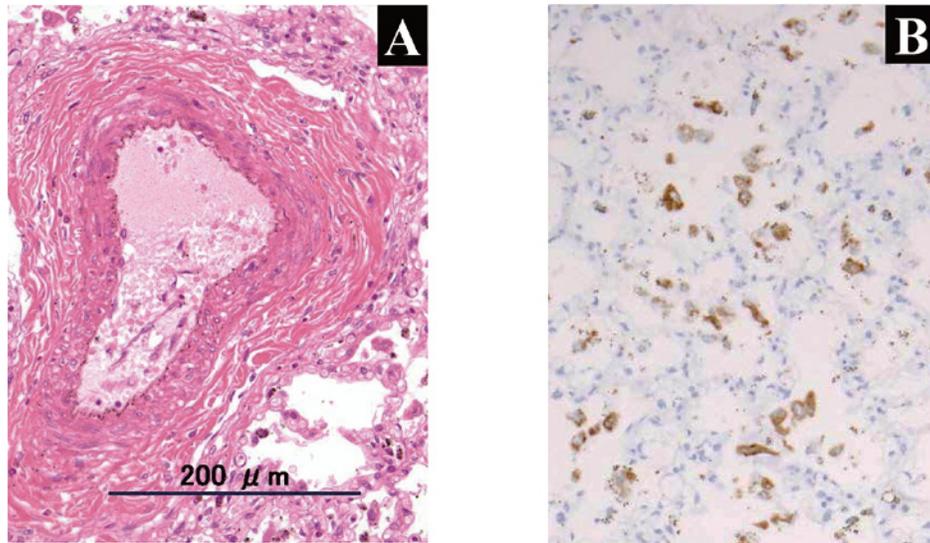


Fig. 3. Postmortem examination of the pulmonary artery.

A: Light microscopic findings of pulmonary artery.

Thickening of tunica media of pulmonary arterial wall was demonstrable (Hematoxylin and eosin staining, $\times 400$). A bar represents 200 μm .

B: Immunostaining of the pulmonary artery using anti-CD68 antibody.

Infiltration of macrophages reacting with anti-CD68 antibody (brown colored) into alveoli was seen ($\times 400$).

a single nucleotide change (c1305 G > T) in exon 11 and a two-nucleotide deletion (c1952-1953 del CA) in exon 16. The G > T transversion causes the substitution of Asp (GAT) for Glu (GAG) at position 435, and the two-nucleotide deletion causes a frame shift and premature termination. Thus, the patient is considered a compound heterozygote, although the functional consequence of each mutation remains to be determined. These mutations have not previously been identified (Frattoni et al. 2000; Kasow et al. 2004). The presence of mutations in *TCIRG1* gene also supports the diagnosis of IMO.

The patient underwent HSCT from 2 locus-mismatched family members at the age of 9 months. The preparative regimens consisted of busulfan 4 mg/kg/day (day-8, -7, -6, and -5), cyclophosphamide 50 mg/kg/day (day-3 and -2), and anti-thymocyte globulin 2 mg/kg/day (day-4, -3, and -2). FK506 (tacrolimus) 0.02 mg/kg/day (day-1) and methotrexate were administered to prevent graft versus host disease (GVHD). Nucleated cells (1.1×10^{10} cells), including 3.7×10^7 CD34-positive cells, were injected. The patient reached an absolute neutrophil count of $> 500/\mu\text{L}$ at 11 days after HSCT and reached a platelet count of $> 20,000/\mu\text{L}$ at 91 days after HSCT. Restriction fragment length polymorphism confirmed that $> 95\%$ of the circulating peripheral blood cells in the engrafted patient were composed of donor cells (Kapelushnik et al. 2001; Jaing et al. 2006).

Following successful engraftment, the radiographic findings, bone marrow scintigraphic findings and biomarkers associated with bone metabolism had improved (Cheow et al. 2001). Hypercalcemia was not observed. Grade I GVHD was developed, but no treatment was necessary.

However, 6 months after HSCT, the patient developed cyanosis. The echocardiogram demonstrated right atrial and ventricular enlargement, paradoxical movement of the ventricular septum, and tricuspid regurgitation. These findings indicated that the patient suffered from pulmonary hypertension. Despite intensive care, the patient died of cardiac failure at the age of 16 months. Postmortem examination disclosed hypertrophy of the right ventricle, vascular wall thickening of the pulmonary artery, and macrophage infiltration to alveoli (Fig. 3A, B).

Discussion

IMO is fatal if left untreated, as more than 70% of affected children die within the first decade of life due to bone marrow failure (Askmyr et al. 2008). Several kinds of treatment, including high doses of active vitamin D and long-term therapy with interferon- γ , have been reported to be effective, but with limitation. HSCT seems to be the most and only effective and rational therapy, achieving long-term disease-free survival in more than 70% of those with matched sibling donors (Driessen et al. 2003; Steward et al. 2004).

HSCT can improve intrinsic osteoclast abnormalities in this disorder, especially caused by the *TCIRG1* gene mutation and reduce bone mineral density associated with normalization of bone remodeling (Kasow et al. 2004; Askmyr et al. 2008). However, many complications have been reported in the patients with IMO after HSCT.

Our patient is worthy of special mention with regard to the development of PAH after HSCT. The occurrence of PAH in IMO has been occasionally reported, but the reasons for this association have remained obscure. A previ-

ous report of a patient with *TCIRG1* mutations who developed severe PAH in the absence of HSCT suggests that PAH in patients with IMO does not simply reflect a drug-related adverse event in HSCT. Furthermore, the fact that several other *TCIRG1*-mutated patients did not present with PAH argues against the hypothesis that the association of IMO and PAH may represent a novel and specific disease entity (Kasow et al. 2004; Steward et al. 2004). It has been hypothesized that granulocyte-macrophage colony stimulating factor (GM-CSF) may play a key role for IMO (Orchard et al. 1992).

In agreement with this hypothesis, postmortem examination in the present case revealed that macrophages infiltrated to the alveoli around the thickened vascular wall of the pulmonary artery, while there has been no previous report regarding the pathological findings of PAH in IMO. We, therefore, postulate that cytokine(s) such as GM-CSF play some role in the development of PAH in IMO caused by *TCIRG1* gene mutations. First, both mutations in the *TCIRG1* gene in IMO may cause dysregulation in the development of immune cells in the bone marrow. Second, dysregulated immune cells produce aberrant cytokines including GM-CSF. Third, elevated levels of GM-CSF promote the proliferation of macrophages and increase the levels in circulation. Finally, increased circulating levels of macrophages infiltrate the alveoli, which results in PAH (Vergadi et al. 2011).

In summary, we report a rare case of IMO with novel mutations in the *TCIRG1* gene. The patient died of PAH at 16 months of age despite successful HSCT. The postmortem examination has suggested that macrophages play some role in the development of PAH in IMO. It should be noted that a patient with IMO has a greater risk for PAH. Thus, the evaluation of pulmonary arterial flow should be included in the assessment of each patient with IMO even after HSCT to permit the early diagnosis of PAH and to attempt treatment.

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Conflict of Interest

The authors declare no conflict of interest.

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