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BRIEF REPORT
A Novel Mutation in a Family With DNA Ligase IV Deficiency Syndrome

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DNA ligase IV deficiency syndrome (LIG4 syndrome) is a rare autosomal recessive disorder characterized by microcephaly, growth retardation, low birth weight, dysmorphic facial findings, immunodeficiency, pancytopenia, and radiosensitivity due to impaired repair of DNA double-strand breaks by non-homologous end-joining. Herein, we report two siblings with LIG4 syndrome with a novel mutation. One of the siblings, who had normocellular marrow, had autologous reconstitution after initial non-myeloablative conditioning and underwent successful second hematopoietic stem cell transplantation after conditioning with busulfan, cyclophosphamide, and anti-thymocyte globulin. Our findings indicate that transplantation with myeloablative conditioning can be used successfully in LIG4 syndrome patients. Pediatr Blood Cancer 2009;53: 482–484. © 2009 Wiley-Liss, Inc.

Key words: bone marrow failure; DNA repair; stem cell transplantation

INTRODUCTION

DNA ligase IV deficiency syndrome (LIG4 syndrome) is a rare autosomal recessive disorder caused by hypomorphic mutations in the DNA ligase IV gene (*LIG4*) [1]. Ligase IV protein is required for the repair of DNA double-strand breaks (DSBs) in mammalian cells via non-homologous end-joining (NHEJ), along with the Ku proteins, Artemis, DNA-dependent protein kinase, XRCC4, and Cernunnos/XLF [2–8].

Ligase IV-deficient patients are characterized by microcephaly, growth retardation, developmental delay, low birth weight, dysmorphic facial findings, immunodeficiency, pancytopenia, and pronounced clinical and cellular radiosensitivity, consistent with a defect in DSB repair [1]. LIG4 syndrome has some overlapping characteristics with Nijmegen breakage syndrome (NBS), ataxia telangiectasia (AT), and Fanconi anemia (FA). AT and NBS patients display clinical radiosensitivity, immunodeficiency, and elevated cancer incidence [9].

All known mutations of LIG4 gene in humans are hypomorphic and result in residual LIG4 activity, avoiding lethality during embryogenesis [1,10]. Herein, we report two siblings with a novel DNA ligase IV mutation, one of whom underwent hematopoietic stem cell transplantation (HSCT) as a treatment for LIG4 syndrome associated pancytopenia.

CASE REPORTS

Case 1

A 10-year-old female was referred to our center for pancytopenia evaluation. The onset of symptoms was at 4 years of age. History revealed a birth weight of 2,400 g following ovulation induction and term gestation. There was a first-degree consanguinity between parents. A maternal uncle had colon cancer. Recurrent upper respiratory tract and otitis media infections were striking. Developmental steps were appropriate for age. Physical examination revealed body weight, height, and head circumference (49.7 cm) were below the third percentile, low anterior hairline, a prominent nasal bridge, and bilateral epicanthi (Fig. 1). The initial hemogram findings at presentation to our center 2 years ago were as follows: hemoglobin 9.4 g/dl (11.5–15.5), WBC 3.3×10^9/L (4.5–13.5), MCV 98 fl (77–95), platelet 33×10^9/L (140–440), absolute neutrophil count (ANC) 1.3×10^9/L (1.5–8). Serum immunoglobulin (Ig) G was 696 mg/dl (608–1,572), IgM 25 mg/dl (43–207), IgA 103 mg/dl (33–236) at 8 years of age. Pancytopenia deepened progressively in the following years necessitating multiple erythrocyte and thrombocyte transfusions. The dysmorphic findings accompanying pancytopenia suggested a possible diagnosis of FA; however, DEB and mitomycin-C-induced chromosomal breakage analyses were all negative, although spontaneous breakages were reported to be increased. Bone marrow examination revealed normocellularity, inconsistent with FA. The common founder mutation in NBS, 657del5, was not found upon DNA analysis of the *NBS1*/*NBN* gene. HSCT was performed from the child's 6/6 HLA-matched father after fludarabine (35 mg/m^2/day for 5 days), cyclophosphamide (10 mg/kg/day for 4 days), and antithymocyte globulin (ATG) conditioning based on a putative diagnosis of DEB-negative FA. CD34 selection was performed on the bone marrow-derived graft. Cyclosporin A (CsA) (1 mg/kg/dose, q12hr, intravenous) was initiated on day −1 for graft-versus-host disease (GVHD) prophylaxis. Supportive regimen included fluconazole and acyclovir prophylaxis, gut sterilization with oral ciprofloxacin, G-CSF (5 μg/kg, starting at day +8) and 400 mg/kg weekly intravenous Ig while hospitalized, and every 2–4 weeks after. Autologous reconstitution developed with 100% host chimerism by the +23rd day of HSCT and CsA was stopped. A second HSCT of peripheral blood stem cells from her father, without CD34 selection, was performed after busulfan (1 mg/kg/dose, q12hr, for 4 days), cyclophosphamide (50 mg/kg/day for 3 days), and ATG conditioning. For GVHD prophylaxis methotrexate (5 mg/m^2/dose) on days +1, +3, +6 and CsA (1.5 mg/kg/dose, q12hr) were initiated. Neutrophil and platelet engraftment was achieved by +12th day of HSCT and she was discharged on +26th day and CsA was ceased after tapering by the end of the first year of transplantation.

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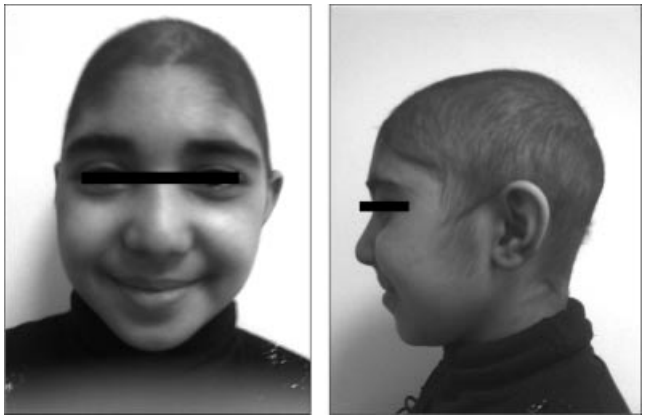

Fig. 1. Case 1 at age of 10 showing prominent microcephaly and low anterior hair line.

Case 2

The other sibling was a 6-year-old male who presented to our center at $3\frac{1}{2}$ years of age with widespread ecchymoses. Personal history revealed a birth weight of 1,000 g following a spontaneous 30 weeks of gestation and recurrent upper respiratory and urinary tract infections. In addition to microcephaly (head circumference 45 cm), his body weight and height were also below the third percentile. Besides inguinal hernia, a prominent nasal bridge and bilateral epicanthi were the positive physical findings. Developmental steps were appropriate for age. Hemogram at $3\frac{1}{2}$ years of age was as follows: hemoglobin 14.1 g/dl (11.5–13.5), WBC 3.2×10^9/L (5.5–15.5), MCV 90 fl (75–87), platelet 86×10^9/L (140–440), and ANC 0.9×10^9/L (1.5–8.5). DEB and mitomycin-C-induced chromosomal analyses were all within normal limits, whereas spontaneous breakages were found to be increased like his sister. Bone marrow examination revealed normocellularity. The 657del5 mutation for NBS was not found upon DNA analysis. Serum IgG was 490 mg/dl (608–1,572), IgM 43 mg/dl (43–207), IgA 101 mg/dl (33–236) at 6 years of age. Karyotype from bone marrow sample revealed 46,XY. Serum vitamin B12, folate, and alpha-fetoprotein (AFP) levels were within normal limits in both siblings.

DISCUSSION

Facial dysmorphology, pancytopenia, growth retardation, low birth weight, absence of DEB sensitivity, increased spontaneous chromosomal instability, normal serum AFP, recurrent infections, low IgG levels, and negative for the NBS founder mutation excluded the possible diagnoses of FA, NBS, and AT. LIG4 syndrome was considered likely. Mutational analyses of the family were performed and revealed a novel sequence variant in the *LIG4* gene. A homozygous trinucleotide deletion, 1762delAAG, was detected in the brother (case 2), resulting in the deletion of a lysine at amino acid position 588 of the DNA ligase IV protein (588delK). Both parents were confirmed to be heterozygous for the 1762delAAG deletion. The sister's (case 1) pretransplantation DNA was available; however, PCR of her DNA was unsuccessful and the deletion could not be demonstrated in her.

The 1762delAAG, 588delK variant is a new *LIG4* sequence variant that has not been reported before. The lysine residue at position 588 is the last amino acid of a ''conserved peptide'' of unknown function that is highly conserved amongst DNA ligase proteins [11]. Using SIFT algorithm, lysine is present at this position in human, mouse, rat, chicken, *Drosophila*, and yeast DNA ligase IV homologs. The only substitution allowed at this amino acid position is arginine, which is found in budding yeast. Thus, while the 588delK variant does not alter the reading frame of the DNA ligase IV protein, it is highly likely that this amino acid deletion constitutes a mutation, which has deleterious effects. Indeed, Western blot analysis of cells from case 2 showed dramatically reduced levels of DNA ligase IV protein, indicating that the 588delKvariant destabilizes the ligase IV protein (Fig. 2).

The diagnosis of LIG4 syndrome was established after HSCT in case 1. The initial conditioning regimen given for HSCT was non-myeloablative based on a putative diagnosis of DEB negative FA. We used non-myeloablative conditioning in order to decrease the risk of chromosomal breakage, since there exists increased risk of malignancy in FA patients with the use of potent chemotherapeutics. However, autologous reconstitution developed after the HSCT. The normocellular bone marrow of the patient was considered a progression of FA to myelodysplatic syndrome. Thus, a second HSCT was performed after myeloablative conditioning using peripheral blood-derived stem cells without CD34 selection, resulting in successful engraftment. The diagnosis of LIG4 syndrome was established after second HSCT, from the studies of the pretransplantation samples of the patient.

This is the second LIG4 syndrome case that has been successfully treated by HSCT, but utilizing a different conditioning

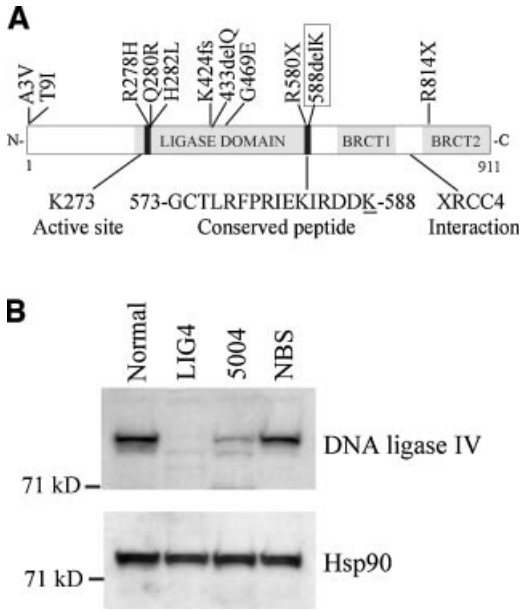

Fig. 2. The summary of LIG4 syndrome mutations including the current cases. **A**: Primary structure of DNA ligase IV protein and reported LIG4 syndrome mutations. **B**: Western blot analysis of DNA ligase IV protein expression in patient cells. Protein lysates were prepared from normal human primary fibroblast cells (normal), primary fibroblasts from a DNA ligase IV patient homozygous for the mutation R814X (LIG4), bone marrow mesenchymal cells from patient 5004, and primary fibroblasts from a NBS patient. Twenty micrograms of protein per lane was separated by SDS–PAGE and Western blotted. Immunoblots were probed with polyclonal antisera specific for DNA ligase IV, upper panel, or with polyclonal antibody specific for Hsp90 as a loading control.

regimen. Previously, Gruhn et al. [12] transplanted a LIG4 patient with pancytopenia and hypocellular bone marrow with cellular dysplasia. Since a diagnosis of LIG4 syndrome had been established, they conditioned with fludarabine (30 mg/m^2/day for 4 days), cyclophosphamide (10 mg/kg/day for 4 days), ATG (15 mg/kg for 4 days), resulting in successful engraftment of the unmanipulated bone marrow from an HLA-matched sibling. The successful result of HSCT with non-myeloablative conditioning in their case could be attributed to hypocellular bone marrow. However, in LIG4 syndrome patients with normocellular bone marrow, this type of conditioning may not be adequate, as in our case, and may require a more potent conditioning. Use of total body irradiation in these cases should be avoided because of the radiosensitive nature of the disease.

In contrast to these successful HSCT of LIG4 patients, several reports describe failed transplant of LIG4 patients with subsequent recipient mortality [10,13]. All of these LIG4 cases were characterized by severe combined immunodeficiency with hypocellular bone marrow. In an attempt to understand this poor prognosis, O'Driscoll and Jeggo [14] found that ligase IV-deficient cells are unexpectedly sensitive to CsA, either alone or in combination with busulfan or fludarabine, resulting in increased DSB that are inadequately repaired in the hypomorphic ligase IV background. In the successful transplant reported by Gruhn et al. [12], a CsA concentration at the lower end of the range (3 mg/kg/day) was used. In our study, we used a CsA dose of 2 and 3 mg/kg/day on the consecutive transplants of the patient, which were similarly low doses.

In conclusion, diagnosis of LIG4 syndrome should be considered in patients with cytopenia, dysmorphic facial findings, recurrent infections, and growth retardation whose AFP is normal and DEB test and nibrin mutation analyses are negative. Successful results can be achieved by HSCT after appropriate conditioning based on the LIG4 patient's bone marrow cellularity.

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