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Next generation sequencing revealed DNA ligase IV deficiency in a “developmentally normal” patient with massive brain Epstein–Barr virus-positive diffuse large B-cell lymphoma

DNA ligase IV deficient patients usually present with microcephaly, low birth weight, growth and/or mental retardation, dysmorphic facial findings, variable immunodeficiency, pancytopenia, and radiosensitivity due to impaired repair of DNA double-strand breaks by non-homologous end-joining [1,2]. Non-Hodgkin’s lymphoma is known to be the most frequent malignancy associated with immunodeficiency. Diffuse large B-cell lymphoma (DLBCL) is considered to be the most common type frequently associated with Epstein–Barr virus (EBV) and having a predilection for extranodal sites [3]. We report a case of the female patient with DNA ligase IV deficiency syndrome, who had no clinical features of that syndrome (Fig. 1A) and developed EBV-associated DLBCL with the right lung involvement and a massive brain tumor lesion at the age of 2 years old. Next generation sequencing revealed c.2736 + 3delC, c.8 C>T (p.A3V) and c.26C>T (p.T9I) in LIG4 gene and additional polymorphisms in ATM, NOD2 and NLRP3 genes.

A healthy female was born at term (weight 3050 g., height 50 cm, circumference of the head 34 cm) from a non-consanguineous marriage, and was vaccinated BCG without any clinical complications. She had a history of primary EBV infection at the age of 11 months with persisted EBV in blood proven by PCR. She had stomatitis with ulceration and perforation of the hard palate. Six months later she developed encephalitis with facial and oculomotor nerve paresis, generalized convulsions, hemiplegia of the right side of the body. PCR of cerebrospinal fluid was positive for EBV. Brain CT revealed multiple foci of calcification (Fig. 1B,C,D). The patient received short courses of antiviral treatment as well as intravenous immunoglobulin and dexamethasone with a short-term clinical response.

At the age of 2 years and 3 months, she had another episode of active EBV infection with fever and cough followed by a rapid deterioration of the neurological status. Chest CT scan showed foci in the right lung (Fig. 2A,B,C). Brain CT revealed negative dynamics with tumor masses in the white matter in the left brain stem with moderate contrast enhancement and decreased density and differentiation of gray and white matter. Lung and brain biopsy was performed, and the diagnosis

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**Fig. 1.** Patient at the age of 2 years and 10 months without dysmorphic facial findings and microcephaly, (A) written informed consent was obtained from parents for publishing photo and clinical data Brain CT scan of the patient at the age of 1 year and 7 months reveals multiple focal calcification in the right hemisphere of the brain (B), in the left (C), in both (D), probably as a manifestation of viral encephalitis.
Abnormalities in the DNA reparation machinery gave the reasons to assume DNA recombination defects (RAG1, RAG2, Artemis, DNA PKcs and others) [1]. Next generation sequencing was performed, and it revealed c.2736 + 3delC, c.8 C>T (p.A3V) and c.26C>T (p.T9I) in LIG4 gene. A small deletion and p.A3V (PolyPhen-2 software gave a slightly higher the score of damaging 0.38, substitution was predicted to be benign) were inherited from her mother and the point mutation (p.T9I) (PolyPhen-2, Score of damaging = 0.52, the mutation was predicted to be possible damaging) was identified in her father. The presence of two missense substitutions inherited from both parents with additional deletion from mother was the reason for establishing the genetic diagnosis for the patient. Additional polymorphisms in ATM gene c.146C>G, p.549C, NOD2 gene c.2653G>C, p.A885P and in NLRP3 gene c.410G>A, p.R137H were found.

She was treated with NHL-BFM-based chemotherapy and received 4 courses of high-dose methotrexate (5 g/m²) and a course of high-dose cytarabine (12 g/m²) combined with dexamethasone. She received rituximab as well, both intravenously (six doses) and intrathecally (two doses). During the chemotherapy, the patient received antiviral treatment (acyclovir, ganciclovir and foscarnet). A partial response was achieved, but a rapid brain tumor growth occurred after the fifth course of chemotherapy, while EBV DNA by PCR remained positive both in blood and cerebrospinal fluid (Fig. 2G,H,I). She died seven months after DLBCL was diagnosed due to lymphoma.
progression and severe infectious complications. An autopsy was not performed.

Confirmation carriers of mutations in the parents gave a possibility to carry out prenatal diagnosis in this family. At the twelfth week of pregnancy, chorionic villi were taken from the mother, and prenatal diagnosis was performed. Only the paternal mutation p.T9I was detected to carry out prenatal diagnosis in this family. At the twelfth week of pregnancy, chorionic villi were taken from the mother, and prenatal diagnosis was performed. Only the paternal mutation p.T9I was detected.

Twenty-eight cases of identified mutations in LIG4 gene have been previously reported [5–10]. They were divided into seven distinct categories: 1) leukemia; 2) DNA ligase IV deficiency syndrome; 3) Dubowits syndrome; 4) Omenn syndrome; 5) radiosensitive severe combined immunodeficiency [5]; 6) Seckel syndrome with AML/MDS [8]; 7) slowly-progressing SCID [10]. Patients with LIG4 gene had increased incidence of hematological malignancies [1,2,4–9]. Additionally, 8 out of 28 had hematological malignancies, two of them EBV-associated or negative lymphomas [6,7]. Twenty-seven out of 28 had facial abnormalities [4–8]. The main differences of our case included lack of classic clinical stigmata, the presence of B cells in the peripheral blood and an early age of lymphoma manifestation in comparison with other patients who were described. The only similar case of DNA ligase IV deficiency to be reported was a “developmentally normal” 14-year-old boy (with presumably normal growth) who exhibited severe radiosensitivity during treatment for leukemia [9].

We present a patient with a combination of clinical and immunologic data, which were not previously reported as DNA ligase IV deficiency. Next generation sequencing helped to identify a genetic basis of atypical severe combined immunodeficiency complicated with a massive brain and lung EBV-positive DLBCL and to reveal previously unreported phenotype due to mutations in LIG4 gene.

Conflict of Interest

The authors declare that they have no conflict of interest.

References


Svetlana O. Sharapova

Research department, Belarusian Research Center for Pediatric Oncology, Hematology and Immunology, Minsk region, Belarus

Corresponding author at: Research department, Immunology Laboratory, Belarusian Research Center for Pediatric Oncology, Hematology and Immunology, Minsk region, settlement of Borovliani 223053, Belarus.

E-mail address: sharapovasv@gmail.com.

Elizabeth Yenhui Chang

Department of Pathology and Laboratory Medicine All Children’s Hospital
Johns Hopkins Medicine, St. Petersburg, FL, USA

Irina E. Guryanova
Inna V. Proleskovskaya

Alina S. Fedorova
Elena A. Rutskaya

Olga V. Aleinikova

Research department, Belarusian Research Center for Pediatric Oncology, Hematology and Immunology, Minsk region, Belarus

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