Clinical Case Report

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Type 2 familial hemophagocytic lymphohistiocytosis in half brothers

A case report

Chunxia Liu, MD[a,*], Ming Li, MD[a], Xiaomei Wu, BS[b], Xiaojian Yao, BS[a], Li Zhao, PhD[a,*]

Abstract

Rationale: We describe a novel case of half-brothers suffering from type 2 familial hemophagocytic lymphohistiocytosis (FHL).

Patient concerns: A 15-year-old Chinese child was admitted to the hematology department. PRF1 gene coding revealed that he was c.282C>A/p.N94K heterozygous and had a c.1349C>T/p.T450M heterozygous mutation. One year later, his younger halfbrother suffered from the same disease. PRF1 gene coding revealed that the younger brother was c.282C>A/p.T450M heterozygous with a c.1349C>T/p.T450M heterozygous mutation. His mother and grandfather were confirmed to have c.1349C>T/p.T450M heterozygous mutations in exon 3.

Diagnoses: Half-brothers were diagnosed for type 2 familial hemophagocytic lymphohistiocytosis

Interventions: To our knowledge, this is a possible FHL and the children's mother may be a pathogenic gene carrier.

Outcomes: After being treated with the HLH-04 schedule, the symptoms of half-brothers were all improved.

Lessons subsections: Therefore, once FHL is diagnosed, HSCT needs to be done early, even if no perfect match is found.

Abbreviations: ALT = alanine aminotransferase, AST = aspartate aminotransferase, CSF = cerebrospinal fluid, EBV = Epstein–Barr virus, FHL = familial hemophagocytic lymphohistiocytosis, HLH = hemophagocytic lymphohistiocytosis, HSCT = hematopoietic stem-cell transplantation, NK = natural killer, WBC = white blood cell.

Keywords: familial hemophagocytic lymphohistiocytosis, hematopoietic stem-cell transplantation, *PRF1* gene

1. Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a group of syndromes characterized by infiltration of multiple organs and reduction of hemocyte caused by the excessive generation of inflammatory cytokines. It is an immune disorder in essence which caused by the uncontrolled activation of T lymphocytes and macrophages. The characteristic clinical feature is persistent fever, pancytopenia, hepatosplenomegaly, and hemophagocytic phenomena detected in the bone marrow, liver, spleen, and lymph tissue. Primary HLH includes familial hemophagocytic lymphohistiocytosis (FHL) and primary immunodeficiency syndrome. There is generally a positive family history or a defined genetic cause for the FHL. Natural killer (NK) cell and cytotoxic T lymphocyte function are decreased in cases of FHL, which are thought to be caused by genetic defects in the perforin/granzyme-mediated cytotoxic pathway. The onset of HLH symptoms is the uncontrolled immune activation which leads to extreme inflammation.[1–3]

Mutations in PRF1 occur in 15% to 50% of patients with familial HLH.[4] PRF1 is located at 10q21-22 and has 3 exons, with all coding sequences in exons 2 and 3. It has been clearly documented that PRF1 mutations cause decreased or absent perforin protein expression on the surface of cytotoxic cells. This condition prohibits cytotoxic and NK cells from destroying their target cells, which in turn leads to increased cytokine production and macrophage activation, causing the symptoms of HLH.[7] Five different forms of familial HLH have been described based on defects in different genetic material and genes, including chromosome arm 9q mutations (FHL1), PRF1 (FHL2), UNC13D (MUNC13-4) (FHL3), STX11 (FHL4), and STXBP2 (MUNC18-2) (FHL5).[5–10] Therefore, the purpose of this study was to identify a type 2 FHL in half brothers.

2. Case presentation

A 15-year-old boy who had been experiencing weakness, fever (temperature of >38.5°C), seizures, and pancytopenia for 4 months was admitted to the Department of Hematology, in May 2016. Bloodwork revealed decreased hemoglobin levels of 83 g/L (normal range, 110–140 g/L), a thrombocytopenia of 21×10^9/L (normal range, 100–300×10^9/L), leukopenia of 0.41×10^9/L (normal range, 4.0–10×10^9/L) with 26.8% neutrophils and 58.5% lymphocytes, and 14% atypical lymphocytes. He also had

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CL and ML contributed equally to this work.

Ethical Committee of the First Affiliated Hospital, Lanzhou University, approved the commencement of the study.

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[a] Department of Hematology, The First Affiliated Hospital, Lanzhou University, Lanzhou, [b] Department of Nursing, The Rehabilitation Center Hospital, Pingliang, Gansu, China.

[*] Correspondence: Chunxia Liu, Li Zhao, Department of Hematology, The First Affiliated Hospital, Lanzhou University, 1 Donggangxilu Street, Lanzhou 730000, Gansu, China (e-mails: chxliu@lzu.edu.cn, xq19910311@126.com).

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hypertriglyceridemia (fasting triglyceride level, 4.17 mmol/L; normal range, 0.8–1.8 mmol/L) and hypofibrinogenemia (fibrinogen level, 0.54 g/L; normal range, 2.0–4.0 g/L). His serum aspartate aminotransferase (AST) was 63 U/L (normal range, 1–49 U/L), serum alanine aminotransferase (ALT) was 57 U/L (normal range, 1–49 U/L), total protein was 42.0 g/L (normal range, 60–82 g/L), albumin was 29.2 g/L (normal range, 32–55 g/L), immunoglobulin was 12.8 g/L (normal range, 20–38 g/L), lactate dehydrogenase was 330.2 U/L (normal range, 125–240 U/L), and ferritin was 738.0 ng/mL (normal range, 30–400 ng/mL). His immunoglobulin levels were as follows: IgG 3.5 g/L, IgA 0.27 g/L, IgM 0.17 g/L, complement C3 0.89 g/L, and C4 0.24 g/L. Cerebrospinal fluid analysis showed the following: sugar qualitative 40–60 mg, red blood cells 6×10^6/L, white blood cells (WBCs) 28×10^6/L, 20% neutrophils, and 80% lymphocytes. Cerebrospinal fluid (CSF) biochemistry and brain effusion cytology were normal. CSF cytology showed no tumor cells and there were a few phagocytic cells, lymphocytes, and erythrocytes. Soluble CD25 was 27,600 μ/mL (normal reference value, 400–2700 pg/mL). Epstein–Barr virus (EBV) testing showed E+03 copies/mL (normal reference value <5.0E+02 copies/mL). Physical examination revealed that the patient's spleen was 15 cm below the left costal margin in the mid-clavicular line, with a soft and sharp margin and the liver was 7 cm below the right costal margin in the mid-clavicular line. Laboratory tests, including Wright staining of bone marrow smears, revealed hemophagocytosis in the bone marrow. Head-enhanced magnetic resonance imaging showed an abnormal signal in the bilateral caudate head of the pons. Whole body computed tomography scan showed multiple enlarged lymph nodes including cervical, peritoneal and mesenteric, mediastinal, left axillary, and inguinal. Histopathology of a splenic biopsy showed splenic sinus dilatation and congestion with scattered medium-sized splenic sinus cells with an irregular karyotype. Splenic puncture immunohistochemistry showed MPO(0), KI-67 (10%), CD7(2+), CD5(0), CD3(2+), CD20(0), CD23(0), PAX-5 (0), TDT(0), bcl-2(0), perforin (focal 1+), lysozyme (2+), CD56 (0), and CD30(0). The immunophenotype of bone marrow showed the following: CD3+ (right), CD4 part +, CD8 few +, CD20-, CD19-, KI-67+ < 5%, BCL-2-, CD138-, CD10-, and CyclinaD1-.

The *PRF1* gene coding sequence showed both c.282C>A/p.N94K heterozygous and c.1349C>T/p.T450M heterozygous mutations (Fig. 1) . Therefore, based on the aforementioned findings, the patient was clinically diagnosed with HLH. He was treated with methylprednisolone combined with cyclosporine and he had no fever, his blood corpuscle increased, and he became EBV negative. He was then treated with a gamma globulin infusion and VP-16 chemotherapy, and his triglyceride and fibrinogen levels decreased. He was treated with cyclosporine and prednisone and discharged from the hospital when his condition was stable (Table 1).

One year later, the patient's younger brother developed similar symptoms, excluding seizures. He was 12 years old. His hemoglobin level was 109 g/L (normal range, 110–140 g/L), platelet count was 80×10^9/L (normal range, 100–300 $\times 10^9$/L),

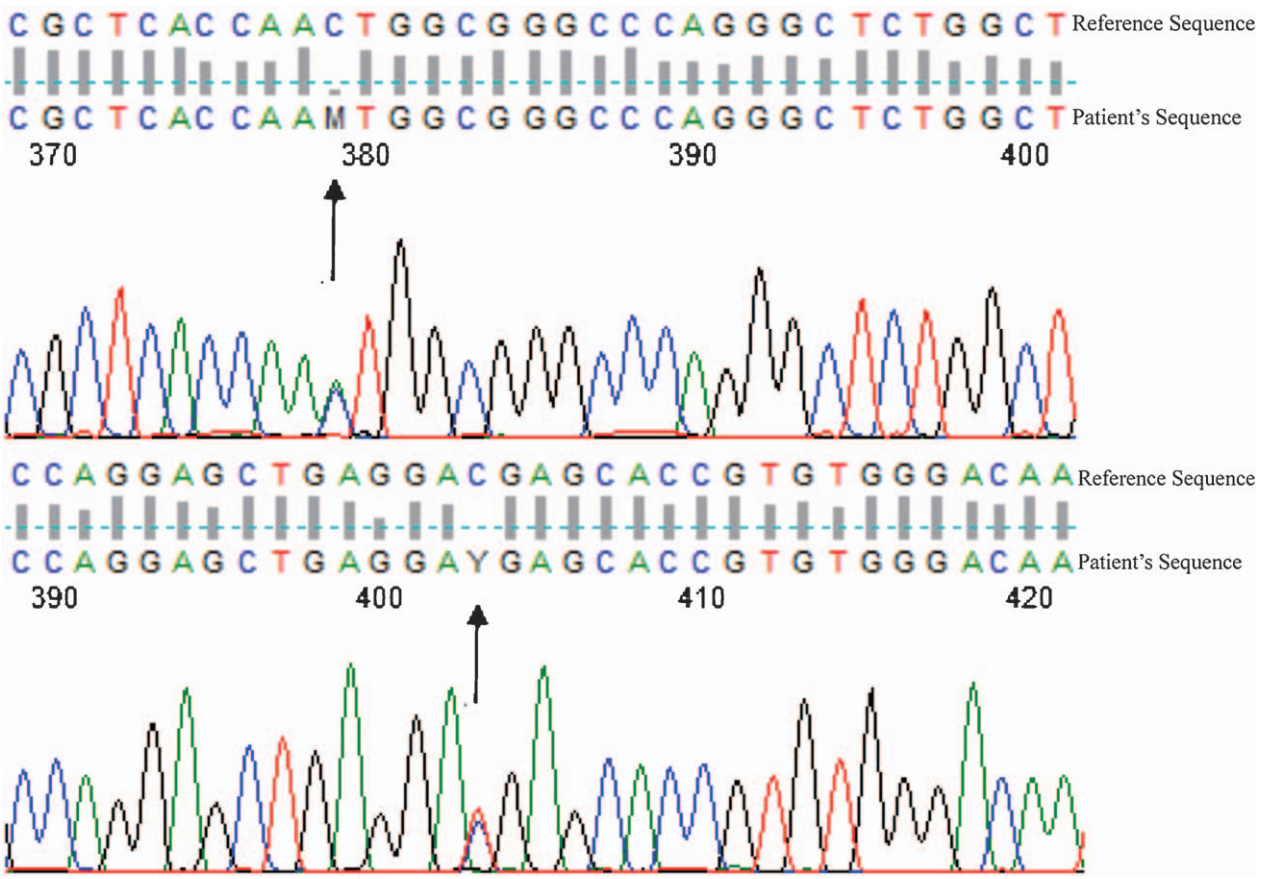

Figure 1. The *PRF1* genes at exon 2 and exon 3 were analyzed by polymerase chain reaction in patient. (A) Exon 2: c.282C>A (p.Asn94Lys) (heterozygosis). (B) Exon 3: c.1349C>T (p.Thr450Met) (heterozygosis)).

Table 1

Summary of laboratory data.

	Hemoglobin (110–140 g/L)	Platelet (100–300 x 10^9/L)	Leukocyte (4.0–10 x 10^9/L)	Triglyceride (0.8–1.8 mmol/L)	Fibrinogen (2.0–4.0 g/L)	AST (1–49 U/L)	ALT (1–49 U/L)	LDH (125–240 U/L)	Ferritin (30–400 ng/mL)	SCD25 (400–2700 pg/mL)	EBV (<5.0E+02 copies/mL)
Patient	83	21	0.41	4.17	0.54	63	57	330.2	738	27,600	E+03
Brother	109	80	5.27	2.4	1.42	58	48	342	897.9	>7500	1.16E+04

ALT = alanine aminotransferase, AST = aspartate aminotransferase, EBV = Epstein–Barr virus, LDH = lactate dehydrogenase.

and WBC count was 5.27×10^9/L (normal range, $4.0–10 \times 10^9$/L) with 26.8% neutrophils and 10.6% monocytes. He also presented with hypertriglyceridemia (fasting triglyceride level, 2.4 mmol/L; normal range, 0.8–1.8 mmol/L and hypofibrinogenemia (fibrinogen level, 1.42 g/L; normal range, 2.0–4.0 g/L). His AST level was 58 U/L (normal range, 1–49 U/L), ALT level was 48 U/L (normal range, 1–49 U/L), lactate dehydrogenase was 342 U/L (normal range, 125–240 U/L), and ferritin was 897.9 ng/mL (normal range, 30–400 ng/mL). Immunoglobulin levels were as follows: IgG 3.72 g/L, IgA 0.78 g/L, IgM 0.77 g/L, complement C3 1.25 g/L, and C4 0.23 g/L. CSF biochemistry was normal and brain effusion cytology showed few lymphocytes present. CSF cytology showed no tumor cells in the smears. Soluble CD25 was greater than 7500 U/mL (normal reference value, 400–2700 pg/mL). EBV testing showed 1.16E+04 copies/mL (normal reference value <5.0E+02 copies/mL). Bone marrow histopathology revealed that the proliferation of hematopoietic tissues was obviously active, the proportion of granulocytes was increased, and there were no abnormalities in erythrocytes or megakaryocytes. The tissue cells and the lymphocytes were easy to see and phagocytosis was occasionally seen. The *PRF1* gene coding sequence showed c.282C>A/p.T450M heterozygous and c.1349C>T/p.T450M heterozygous mutations (Fig. 2). The patient's mother and grandfather were confirmed to have a c.1349C>T/p.T450M heterozygous mutation in exon 3 (Figs. 3 and 4). The patient's sister and grandmother's *PRF1* gene had no mutations at exon 2 and exon 3 (Figs. 5 and 6). The patient was treated with the HLH-04 schedule. The treatment improved the general condition of the patient and resulted in a decreased spleen size and complete remission of HLH, which was demonstrated by a reduction in ferritin levels to within the normal range, and the recovery of blood cell counts and fibrinogen levels to within the normal range.

Informed consent was obtained from the patient for publication of this case report and accompanying images.

3. Discussion

The FHL is an autosomal recessive genetic disease and the incidence rate is approximately 0.12/10,000 per year in

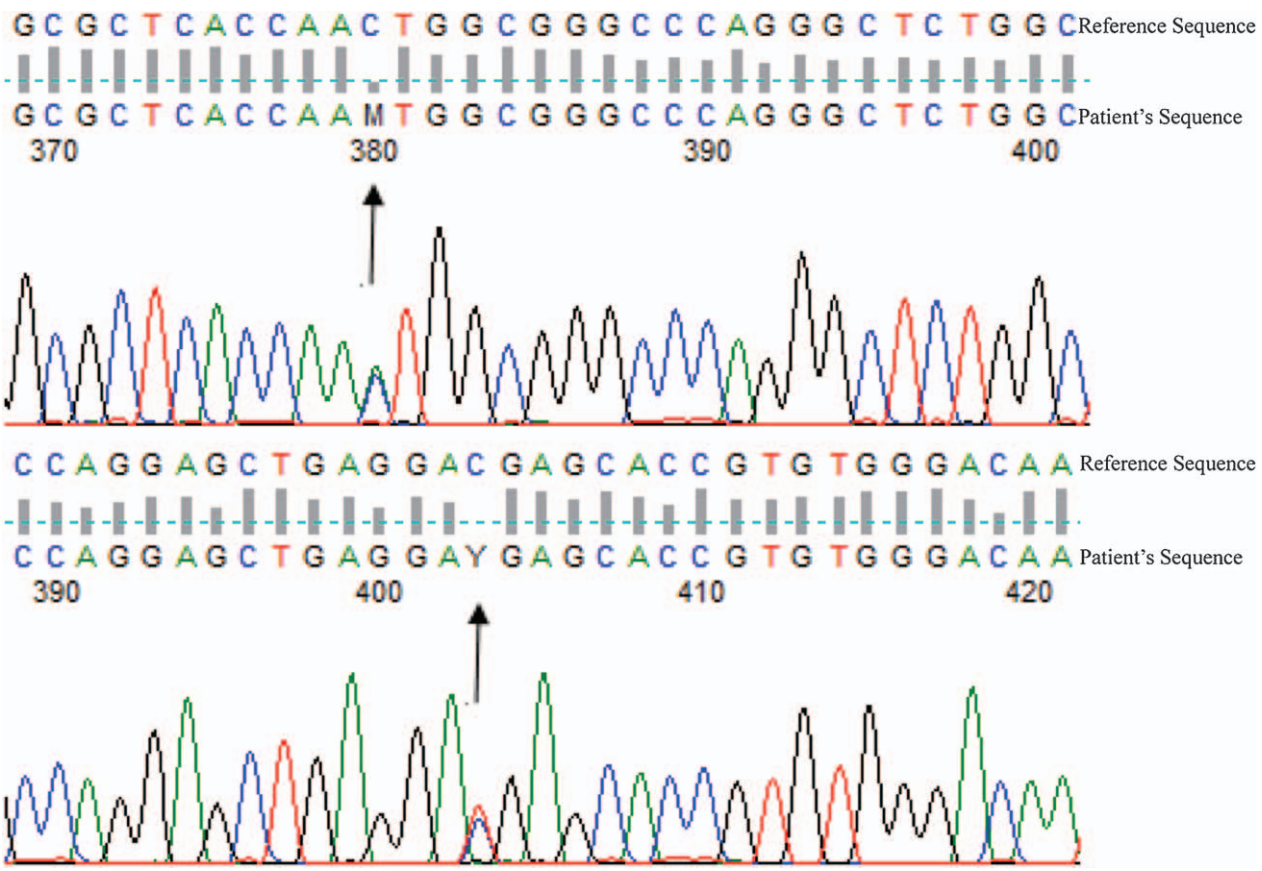

Figure 2. The *PRF1* genes at exon 2 and exon 3 were analyzed by polymerase chain reaction in patient's little brother. (A) Exon 2: c.282C>A (p.Thr450Met) (heterozygosis). (B) Exon 3: c.1349C>T (p.Thr450Met) (heterozygosis).

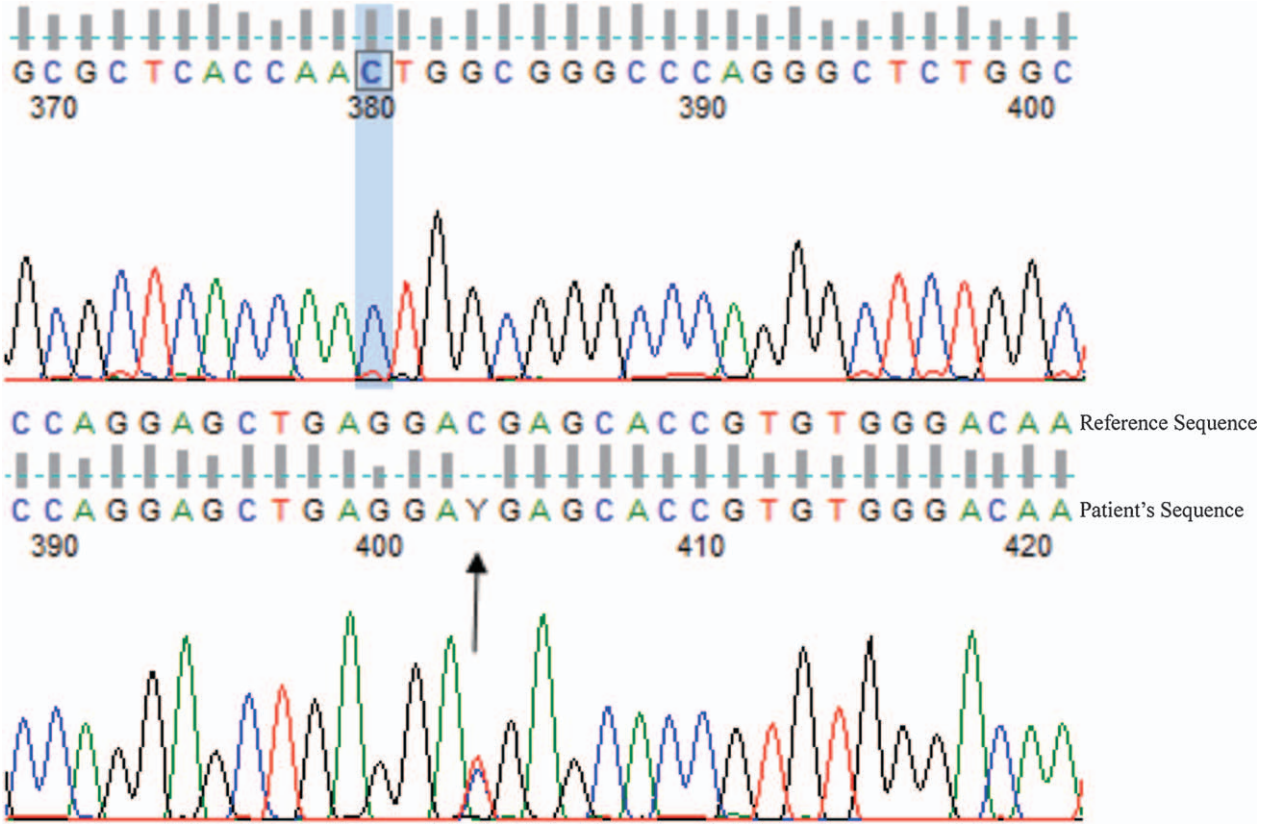

Figure 3. The *PRF1* genes at exon 2 and exon 3 were analyzed by polymerase chain reaction in patient's mother. (A) Exon 2: No variationc.282C>A (p.Asn94Lys). (B) Exon 3 c.1349C>T (p.Thr450Met) (heterozygosis).

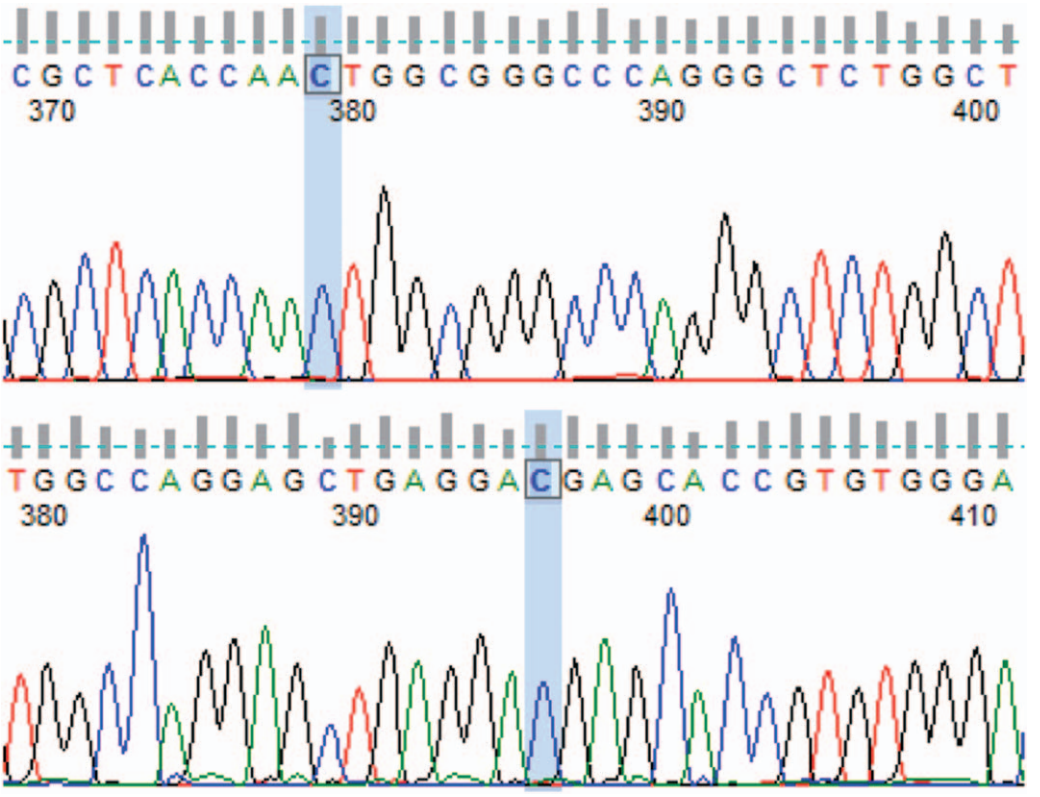

Figure 4. The *PRF1* genes at exon 2 and exon 3 were analyzed by polymerase chain reaction in patient's sister) Exon 2: No variationc.282C>A (p.Asn94Lys). (B) No variationc.1349C>T (p.Thr450Met).

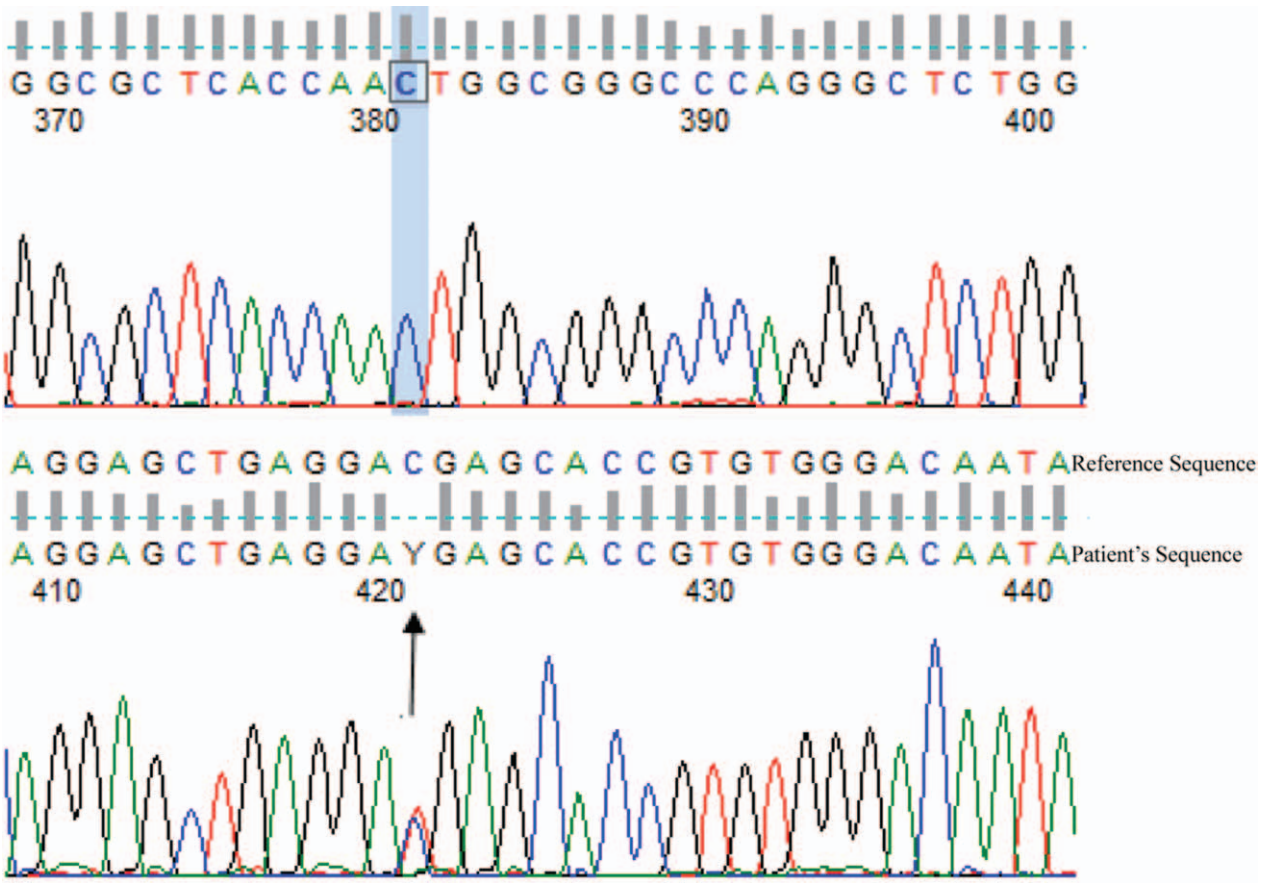

Figure 5. The *PRF1* genes at exon 2 and exon 3 were analyzed by polymerase chain reaction in patient's grandfather. (A) Exon 2: No variationc.282C>A (p. Asn94Lys). (B) Exon 3 c.1349C>T (p.Thr450Met) (heterozygosis).

newborns. The incidence rate is 0.342/10,000 in Asia and male and female incidence is approximately 1:1.[11] Most patients are infants and young children, but they can also be adolescents or adults. The oldest age of onset was reported to be 65 years.[4,12] In 1999, Stepp et al[13] first confirmed the relationship between PRF1 and FHL2. This mutation results in a cytokine storm, which was the pathogenesis of FHL2.[11,14] At present, there are approximately 70 mutations in PRF1, which occur in second and third of the exons' coding region. The most common locus at c.1122G>A can cause a 374 tryptophan codon to become a stop codon, which produces nonfunctional PRF proteins. This mutation had a higher incidence[1,15,16] in patients with HLH in Turkey. *PRF1* gene mutations vary in different countries, such as c.50delT, which are common in Americans. In Japan, c.1090-91delCT was the most common gene mutation site.[1,2] S168N and T450M have been reported in our country. Rs885821 and rs885822 are the most common SNP loci,[5] but there are no reports about the existence of gene polymorphism and susceptibility to HLH in PRF1.

There is no definite mechanism for the delay in the age of onset of FHL2, but it may be related to the absence or partial expression of perforin in vivo.[14] Gao et al[6] through whole genome sequencing analysis found that the *PCDH18* gene mutation may be an important mechanism for the second potential triggers of FHL2 in addition to the PRF1 mutation. The age of the patient older than 2 years, clinical manifestations, and laboratory findings are in line with the hemophagocytic syndrome (HPS) diagnostic criteria specified by the international organization of cell society of 2004 and DNA sequencing results. C.949G>A, c.1228C>T, or c.1349C>T mutations result in delated onset in patients with FHL. Ishii et al[7] reported patients who have the c.1349C>T mutation, while patients with c.282C>A/p.N94K mutations have not been reported at home or abroad. The patient was EBV positive, and EBV infection was not only a potential cause of acquired HLH but also closely related to various types of lymphoma. It can also be a trigger for primary HLH. Therefore, the patient was presumed to be FHL2 triggered by EBV infection.

The main clinical manifestation of the older brother, in this case, was immunodeficiency and epilepsy. However, the symptoms of the younger brother were mild, without immunodeficiency or epilepsy. The patient and his younger brother, mother, and grandfather all had c.1349C>T/p.T450M heterozygous mutations in exon 3. Therefore, this family conformed to FHL. However, the older brother showed a c.282C>A/p.T450M heterozygous mutation in exon 2, while the younger brother had a c.282C>A/p.N94K mutation. His mother and grandfather were normal. Because the 2 patients were half-brothers, their fathers did not have the *PRF1* gene. Why did they have the same mutation in exon 2 even though this mutation did not exist in their maternal line? Their mother may be a pathogenic gene carrier. Wang et al[8] reported 18 identified mutations in HLH-related genes and c.1349C>T/p.T450M was the one of mutations among them.

Without treatment, FHL can be life-threatening. The median survival time for children who are not treated after diagnosis is

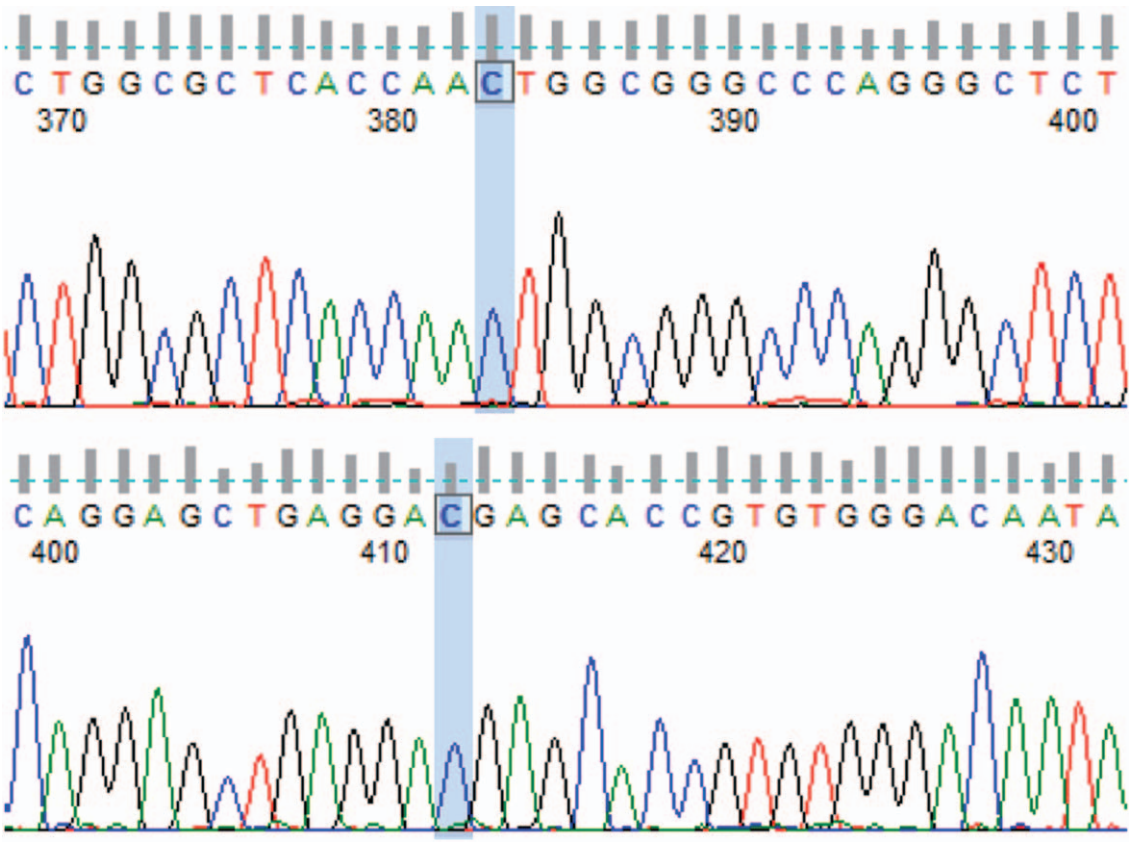

Figure 6. The *PRF1* genes at exon 2 and exon 3 were analyzed by polymerase chain reaction in patient's grandmother. (A) Exon 2: No variationc.282C>A (p. Asn94Lys). (B) Exon 3: No variationc.1349C>T (p.Thr450Met).

<2 months.[3] The treatment of this disease requires simultaneous treatment in 3 areas: treatment of infectious triggering factors, suppression of excessive immune responses, and correction of potential genetic defects. The elimination of triggers, suppression of immune response, and chemotherapy are necessary for the improvement of prognosis in HLH. There is no need to wait for DNA sequencing results to treat if FHL is suspected. The HLH-94 or HLH-04 programs are the most commonly used schema. The survival rates of patients with HLH were greatly improved. Although the guidelines are based on HLH studies in children, they are still widely used in adults. In addition, hematopoietic stem cell transplantation (HSCT) is a radical treatment for HLH. The International Association of Tissue Cells, in a large sample of clinical research, found that patients with FHL who underwent HSCT had a 50% 5-year survival rate, while children who did not undergo HSCT all died.[9] Patients with a positive family history, genetic diagnosis or serious illness, or those with continued active HLH should undergo continuous chemotherapy or HSCT[10] as early as possible. If there is no family history and no primary etiology associated with secondary HLH, the possibility of FHL should be considered after repeated treatment according to the HLH-2004 regimen. Patients with FHL tended to relapse more frequently before HSCT, especially at the end of the initial treatment. If symptoms of the central nervous system appear, intrathecal injection should be considered. HSCT should be performed as soon as possible after remission of disease. HSCT is the only way to maintain long-term disease-free survival in children with FHL. Therefore, once FHL is diagnosed, HSCT needs to be done early, even if no perfect match is found.

4. Author contributions

Conceptualization: Xiaojian Yao.
Data curation: Xiaomei Wu.
Investigation: Ming Li.
Writing – original draft: Chunxia Liu.
Writing – review & editing: Chunxia Liu, Li Zhao.

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