Familial hemophagocytic lymphohistiocytosis type 5 in a Chinese Tibetan patient caused by a novel compound heterozygous mutation in STXBP2

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Abstract
Rationale: Familial hemophagocytic lymphohistiocytosis (FHL) is a fatal autosomal recessive immunodeficiency disease whose rapid and accurate diagnosis is paramount for appropriate treatment. Mutations in STXBP2 gene have been associated with FHL type 5 (FHL-5). Here, we report the first Tibetan Chinese patient diagnosed with FHL-5 caused by a novel compound heterozygous mutation in STXBP2.

Patient concerns: A 9-year-old girl who presented with recurrent fever, splenomegaly, pancytopenia, hypofibrinogenemia, and conspicuous bone marrow hemophagocytosis was diagnosed with haemophagocytic lymphohistiocytosis (HLH).

Diagnosis: FHL mutation analysis of the patient and her parents revealed that she presented compound heterozygosity for STXBP2: a novel missense mutation c.663G>C (p.Glu221Asp) and the known pathogenic splice-site mutation c.1247-1G>C (p.Val417LeufsX126). Bioinformatics analyses predicted that the new mutation was pathogenic and the FHL-5 diagnosis was confirmed.

Interventions: Upon diagnosis, HLH-2004-directed chemotherapy was instituted, but there was a relapse. Allogeneic hematopoietic stem cell transplantation (HSCT) was performed.

Outcomes: After transplantation, the patient presented implantation dysfunction, chronic graft-versus-host disease, and 5 episodes of pancreatitis. A follow-up after 5 years revealed that the patient had died of pancreatitis.

Lessons: This finding expands the spectrum of FHL-5-related mutations in Chinese patients and indicates a clear genotype-phenotype correlation of FHL-5 in China.

Abbreviations: ACMG = American College of Medical Genetics and Genomics, CTL = cytotoxic T lymphocyte, FHL-5 = familial hemophagocytic lymphohistiocytosis type 5, HLH = hemophagocytic lymphohistiocytosis, HSCT = hematopoietic stem cell transplantation, NGS = next generation sequencing.

Keywords: diagnosis, familial hemophagocytic lymphohistiocytosis type 5 (FHL-5), mutation, STXBP2

1. Introduction
Familial hemophagocytic lymphohistiocytosis (FHL) is a genetically heterogeneous autosomal recessive immune disorder characterized by poor clearance of target cells and perpetual state of immune activation.[1] The mutated genes in FHL lead to the deficiency of T lymphocyte and natural killer (NK) cell degranulation by inhibiting cytotoxic granule components or impairing their fusion machinery.[2] It is estimated that the incidence of FHL is approximately 0.12 to 0.15 per 100,000 children per year and FHL-5, which is caused by mutations of STXBP2, only accounts for 10% of all FHL cases.[2,3] Here we present a 9-year-old Tibetan Chinese patient with a novel compound heterozygous mutation in STXBP2 who developed FHL-5 and died even after allogeneic hematopoietic stem cell transplantation (HSCT).

2. Case report
A 9-year-old girl was admitted to our hospital in 2013 with recurrent fever, splenomegaly, and pancytopenia that had lasted more than 1 month. She was a Chinese Tibetan girl in previous good health and had been living in Tibet, China since her birth.
The patient was pallid and presented ecchymosis and edema of the lower extremities. The spleen was palpated 4 cm below the left costal margin. The patient’s parents had never presented similar symptoms and she had no siblings. Complete blood count revealed that hemoglobin was 58 g/L, absolute reticulocyte count was 70 × 10^9/L, white blood cells were 2.0 × 10^9/L, absolute neutrophils were 0.22 × 10^9/L, and platelets were 20 × 10^9/L. Liver function indicated hypoproteinemia (albumin was 27.7 g/L). Serum ferritin was significantly elevated (1204.3 ng/ml) and coagulation screening tests suggested hypo fibrinogenemia (145 ng/dl). Total plasma triglycerides and cerebrospinal fluid examination were normal. Bone marrow aspiration showed conspicuous hemophagocytosis and no malignant cells (Fig. 1). Though NK cell activity and sCD25 were not detected due to laboratory limitations, the diagnosis of hemophagocytic lymphohistiocytosis (HLH) was still made according to the diagnostic guideline criteria.[6]

To elucidate the underlying etiology of HLH, series examinations were performed. Serological investigations for the presence of Epstein-Bar virus, rubella, cytomegalovirus (CMV), herpes simplex virus, hepatitis B, hepatitis C, human immunodeficiency virus (HIV), salmonella, and mycoplasma were all negative. There was no positive result in the autoimmune antibody tests. No mass was found on CT scans of head, chest, and abdomen. Finally, a genetic investigation was carried out by next-generation sequencing (NGS). The results revealed that the patient was a compound heterozygous in the STXBP2 gene. Based on the visualization models for this novel mutation (Fig. 2), we hypothesized that the substitution of the conserved amino acid residue might affect protein folding, and/or reduce protein stability, causing its rapid degradation and/or loss of function.

3. Discussion

FHL-5 is associated with mutations in STXBP2 and was initially identified as a genetic subtype of FHL in 2009 by zur Stadt et al.[6] STXBP2, which has 19 exons, is located on 19p13 and encodes for the 593-amino acid STXBP2 protein.[7] The STXBP2 gene also results in an amino acid change (p. Glu221Asp), and 1 known pathogenic splice-site mutation (c.1247-1G>C)[8] were detected by NGS in a compound heterozygous state of the STXBP2 gene. Based on the visualization models for this novel mutation (Fig. 2), we hypothesize that the substitution of the conserved amino acid residue might affect protein folding, and/or reduce protein stability, causing its rapid degradation and finally resulting in decreased Munc18-2 expression. In our patient, one novel missense mutation, c.663G>C (p.Glu221Asp), and 1 known pathogenic splice-site mutation (c.1247-1G>C)[8] were performed in accordance with this assumption. Taken together, we infer that the novel missense mutation, along with the known pathogenic mutation, is responsible for the FHL-5 phenotype of this patient. Still, further in vitro biochemical studies are needed to confirm this hypothesis.

To date, FHL-5 has mainly been described in patients from the Middle East, of Turkish origin or European descent, who very often had consanguineous parents.[6,10] Only limited data have been reported from Asian countries. To the best of our knowledge, this is the first case report of FHL-5 from a non-consanguineous family of Tibetan Chinese ethnicity. Distinct mutations may be associated with various ethnic origins and
variable clinical presentations. Exon 15 splice-site mutations occur predominantly in German and Turkish patients and have not yet been reported in Asians. Patient age at diagnosis of FHL-5 with exon 15 splice-site mutations is significantly higher than that of patients with other mutations. Moreover, exon 15 FHL-5 shows a less severe NK cell and cytotoxic T lymphocyte (CTL) degranulation deficiency. The highest recorded onset age of an FHL-5 patient with exon 15 splice-site mutations is 19 years old. Our patient developed FHL-5 when she was 9 years old, thus qualifying as a late-onset FHL-5. Moreover, the levels of Munc18-2 and syntaxin-11 in patients with a homozygous missense mutation are markedly lower than those of patients with a homozygous splice-site mutation or with compound heterozygosity with a splice-site mutation. Therefore, we suppose this patient did not develop HLH until 9 years of age due to the compound heterozygosity of STXBP2, which includes the splice-site mutation in exon 15. Our case demonstrates that there is a clear genotype-phenotype correlation in FHL-5. Besides, in some late-onset FHL-5 patients, extracellular staining of CD107 and CTL cytotoxicity can reach the lower normal range. We therefore speculate that it is possible that the patient’s mother’s cytotoxicity and degranulation assays of NK cells and CTLs were in the lower normal range. Although the patient’s mother has no current HLH-related clinical symptoms, we consider she has a high risk of developing HLH in the future with trigger factors such as infection, autoimmune disease, and tumors. However, there is a possibility that she may never develop HLH, and it would be very valuable to follow her up.

4. Conclusion

Early genetic testing is not only a rapid and accurate approach for the diagnosis of FHL-5, but it can also help in the identification of carriers and high-risk relatives who could receive genetic counseling. However, the mutation features of FHL-5 in China remain unclear. Our results expand the Chinese spectrum of pathogenic mutations in STXBP2 and can definitely contribute to the elucidation of the genotype-phenotype correlation of FHL-5 in China.
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Author contributions

Conceptualization: Xia Guo.
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References

[1] Degar B. Familial hemophagocytic lymphohistiocytosis. Hematol Oncol Clin North Am 2015;29:903–13.
[2] Zhao XW, Gazendam RP, Drewniak A, et al. Defects in neutrophil granule mobilization and bactericidal activity in familial hemophagocytic lymphohistiocytosis type 5 (FHL-5) syndrome caused by STXBP2/Munc18-2 mutations. Blood 2013;122:109–11.
[3] Meeths M, Horne A, Sabel M, et al. Incidence and clinical presentation of primary hemophagocytic lymphohistiocytosis in Sweden. Pediatr Blood Cancer 2015;62:346–52.
[4] Henter JI, Horne A, Aricó M, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 2007;48:124–31.
[5] Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for molecular pathology. Genet Med 2015;17:405–24.
[6] zur Stadt U, Rohr J, Seifert W, et al. Familial hemophagocytic lymphohistiocytosis type 5 (FHL-5) is caused by mutations in Munc18-2 and impaired binding to syntaxin 11. Am J Hum Genet 2009;85:482–92.
[7] Lee KO, Yoo KH, et al. Prevalence of type 5 familial hemophagocytic lymphohistiocytosis in Korea and novel mutations in STXBP2. Clin Genet 2016;89:222–7.
[8] Pagel J, Beutel K, Lehmburg K, et al. Distinct mutations in STXBP2 are associated with variable clinical presentations in patients with familial hemophagocytic lymphohistiocytosis type 5 (FHL5). Blood 2012;119:6016–24.
[9] Spossett WA, Sanmillan ML, McCormick ME, et al. Hemophagocytic lymphohistiocytosis caused by dominant-negative mutations in STXBP2 that inhibit SNARE-mediated membrane fusion. Blood 2015;125:1566–77.
[10] Stepensky P, Bartram J, Barth TF, et al. Persistent defective membrane trafficking in epithelial cells of patients with familial hemophagocytic lymphohistiocytosis type 5 due to STXBP2/MUNC18-2 mutations. Pediatr Blood Cancer 2013;60:1215–22.

Figure 3. Sanger sequencing results in STXBP2 of our patient’s parents: her father was a heterozygote of c.663G>C and mother was a homozygote of c.1247-1G>C.