Annals of Hematology (2019) 98:2303–2310
https://doi.org/10.1007/s00277-019-03764-1

ORIGINAL ARTICLE

Genetic characterization of pediatric primary hemophagocytic lymphohistiocytosis in China: a single-center study

Liping Zhang[1] · Zhigang Li[2] · Wei Liu[3] · Honghao Ma[1] · Tianyou Wang[1] · Rui Zhang[1]

Received: 4 March 2019 / Accepted: 20 July 2019 / Published online: 6 August 2019

Abstract

To study the genetic characteristics of primary hemophagocytic lymphohistiocytosis (pHLH) in China, we investigated the genetic data and clinical features of Chinese HLH patients. We retrospectively reviewed the genetic and clinical data of patients with HLH from November 2015 to June 2018. As a result, 26 patients were diagnosed with pHLH. The median age at diagnosis was 2.8 years (range 0.1–13.7 years). The probable overall survival at 12 and 24 months was 87.6% and 62.6%, respectively. Mutations in *PRF1* (38.4%) and *UNC13D* (26.9%) were the most common genetic abnormalities. Furthermore, we identified 19 novel mutations that had not been previously reported and were predicted to likely be pathogenic. In addition to HLH-associated genes, there were 27 other genes identified. Genotype-phenotype analysis showed that patients with disruptive mutations were significantly younger at diagnosis than those with other mutation types (2.9 years vs. 6.4 years, $P = 0.036$). Familial HLH patients were more prone to central nervous system involvement and seizures compared with other patients (83.3% vs. 37.5%, $P = 0.019$; 55.6% vs. 12.5%, $P = 0.04$, respectively). In summary, numerous new mutations in HLH-related genes and other genes were identified in Chinese children with pHLH. Significantly, disruptive mutation types were more likely to be found in younger patients, and familial HLH patients tended to exhibit central nervous system involvement and seizures.

Keywords Primary hemophagocytic lymphohistiocytosis · Genotype-phenotype · Children · Genetics · Clinical features · China

Introduction

Hemophagocytic lymphohistiocytosis (HLH), or hemophagocytic syndrome, is a rare and fatal disorder characterized by continuous activation of monocytes/macrophages, cytotoxic T cells, and natural killer (NK) cells. These overactivated cells can secrete a multitude of cytokines, resulting in uncontrolled hypercytokinemia and injury [1, 2]. There are two different types of HLH: primary HLH (pHLH), also known as inherited HLH, and secondary or acquired HLH. Primary HLH includes familial HLH (FHL), primary immunodeficiency-associated HLH, and EBV-driven HLH. FHL can be further divided into FHL2, FHL3, FHL4, and FHL5 according to the different causative genetic defects, such as *PRF1*, *UNC13D*, *STX11*, and *STXBP2*, respectively. However, no gene has yet been associated with FHL1 [3]. Mutations in the *RAB27A*, *LYST*, and *AP3B1* genes, found in Griscelli syndrome type II, Chediak-Higashi syndrome, and Hermansky-Pudlak syndrome type II, respectively, impair intracellular granule trafficking, resulting in both pigmentary abnormalities and defective cytotoxicity. In addition, EBV-driven

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00277-019-03764-1) contains supplementary material, which is available to authorized users.

✉ Tianyou Wang
wangtianyou@bch.com.cn

Rui Zhang
ruizh1973@126.com

[1] Department of Hematology and Oncology, Beijing Children's Hospital, Capital Medical University, Nanlishi Road No. 56, Xicheng District, Beijing 100045, People's Republic of China

[2] Hematology and Oncology Laboratory, Beijing Pediatric Research Institute, National Center for Children's Health, Beijing Key Laboratory of Pediatric Hematology Oncology, Key Laboratory of Major Diseases in Children, Ministry of Education, National Key Discipline of Pediatrics, Beijing Children's Hospital Affiliated to Capital Medical University, Beijing 100045, China

[3] Department of Hematology, Children's Hospital of Zhengzhou City, Zhengzhou 450053, China

pHLH can be found in patients with *SH2D1A*, *BIRC4*, *ITK*, *CD27*, and *MAGT1* mutations [3].

Significant improvements in pHLH patient survival have been achieved in the last two decades, due to the improved diagnostic criteria and treatment protocols (HLH-94 or HLH-2004) established by the International Histiocyte Society [1].

To date, the correlation between genetic characteristics and clinical features in pediatric pHLH has not been fully investigated. There had been no large cohort study in Chinese pediatric pHLH. In the present study, we conducted a large cohort analysis of genetic and immunological findings in children with pHLH, to clarify the genetic characteristics of Chinese pediatric pHLH.

Patients and methods

Patients

Patients with HLH underwent genetic and immunological tests (including the determination of natural killer (NK) cell cytolysis activity and CD107a on NK cells and cytotoxic lymphocytes (CTL)) at the Hematology Oncology Center of Beijing Children's Hospital from November 2015 to June 2018. The patients came from nonconsanguineous families. Clinical data including demographic characteristics, family history, and treatment outcomes were collected. The patients were treated according to either the HLH-94 or HLH-04 protocol. Written informed consent was obtained from the parents, and the study was approved by the Beijing Children's Hospital Ethical Committee.

Molecular studies

In all HLH patients, nucleotide sequencing of a panel of 726 immunodeficiency-associated genes (supplementary Table 1) was performed on the Illumina HiSeq 2000 platform (Illumina, USA). For mutations in pHLH-related genes, including *PRF1*, *UNC13D*, *STX11*, *STXBP2*, *LYST*, *AP3B1*, *RAB27A*, *SH2D1A*, *BIRC4*, *CD27*, *ITK*, and *MAGT1*, we verified the inherited genes by Sanger sequencing. The pathogenicity of single-nucleotide polymorphisms was predicted with SIFT databases and Polyphen2 [4, 5]. To verify whether a pathogenic variant has been previously reported, information was obtained from the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php) or the Clinvar database (https://www.ncbi.nlm.nih.gov/clinvar/).

Functional analysis of T cells and NK cells

The functions of NK cells and CTLs were assessed with flow cytometry (FACSCalibur flow cytometer, BD Biosciences, USA) in pHLH patients. The analyses included the determination of NK cell activity, CD107a degranulation assays, and expression analysis of related proteins such as perforin, granzyme, signaling lymphocytic activation molecule associated protein (SAP), and X-linked inhibitor of apoptosis protein (XIAP) [6].

Definition

The clinical diagnosis of HLH is based on the HLH-2004 diagnostic criteria. It includes fever, splenomegaly, cytopenias affecting ≥ 2 lineages, hypertriglyceridemia, and/or hypofibrinogenemia, hemophagocytosis (bone marrow, spleen, or lymph nodes), low or absent NK cell activity, ferritin ≥ 500 μg/L, and sCD25 (sIL2Rα) ≥ 2400 U/mL. These criteria (five of eight must be met) were utilized for the diagnosis of HLH. Primary HLH was defined as the presence of HLH-related genes and/or a family history in patients in the absence of secondary causes of HLH, such as infectious, malignant, or autoimmune triggers [1, 2, 6–8].

Central nervous system (CNS) involvement was defined as abnormal neuroradiological imaging and/or cerebrospinal fluid and/or the presence of neurological symptoms [9–11]. The abnormal brain imaging results included hemorrhage, high–signal intensity lesions, leptomeningeal enhancement, enlargement of ventricles, severe atrophy, or calcifications in magnetic resonance imaging. The neurological symptoms included coma, seizures, hemiplegia, ataxia, somnolence, tetraparesis, or meningeal irritation.

Overall survival (OS) was defined from the date of diagnosis through the date of death for any reason. The last follow-up was carried out in June 2018.

Statistics

SPSS version 20.0 (IBM, Armonk, USA) was used for all statistical analyses of the data. The *t* test was used to determine the differences between two numerical variables obeying normal distribution between two groups. Pearson's chi-square test was used to determine whether there was a difference in qualitative variables between groups. OS was estimated by Kaplan-Meier survival analysis and compared with the log-rank test. The results were considered to be statistically significant at $P < 0.05$.

Results

General information of the pHLH patients

Twenty-six patients with HLH were diagnosed with pHLH, as shown in Table 1. In our cohort, there were 14 boys and 12 girls, with a male:female ratio of 1.17: 1. The median age at diagnosis was 2.8 years (range, 0.1–13.7 years). Ten patients

had affected siblings. Collectively, twenty children survived with or without active disease; six patients died due to disease progression or complications, such as infection. The probability of overall survival (OS) at 12 and 24 months after pHLH diagnosis was 87.6% and 62.6%, respectively (Fig. 1).

Mutations in HLH-related genes

Gene mutations in HLH-related genes were detected in 26 children (Table 1). Generally, mutations in *PRF1* (10/26, 38.4%), *UNC13D* (seven out of 26, 26.9%), and *XIAP* (four out of 26, 15.3%) were common in the pHLH patients. In the present study, mutations were not found in the *STX11*, *AP3B1*, *RAB27A*, *CD27*, or *MAGT1* genes (Fig. 2, Table 1). Compound heterozygous pathogenic mutations were found in 18 patients (*PRF1* in 10, *UNC13D* in 6, *LYST* in 1, and *ITK* in 1). Hemizygous pathogenic mutations in *SH2D1A* and *BIRC4* were detected in 6 patients and were inherited from the patients' mothers, as indicated by Sanger sequencing.

Altogether, there were 37 mutation sites with possible pathogenicity predicted by SIFT and Polyphen2 software. Among these mutation sites, 13 and 12 mutations were located in *PRF1* and *UNC13D*, respectively. Further analysis indicated 19 mutations that likely lead to pathogenic consequences but had not been reported elsewhere (see Table 1, asterisk). This result indicated the existence of 19/37 likely pathogenic mutations that had not been previously reported. Additionally, 18 mutations that had been previously reported as pathogenic were found in our study, as shown in Table 1 and supplementary Table 2 (including the listed reference articles). Notably, the aforementioned 19 new gene mutations were not only possible pathogenic sites predicted by SIFT and Polyphen2 software but were also clinically relevant and deleterious.

Among the 19 novel mutations, five mutations, in *PRF1* (c.1450G>A, P1 and c.218C>T, P5), *ITK* (c.85C>T and c.243+1G>A, P20), and *STXBP2* (c.497C>T, P18), were proven to be associated with a family history, as affected siblings who were characterized by HLH manifestation died after birth without genetic testing and timely diagnosis and treatment. Fourteen mutations were detected in patients with compound heterozygous mutations or hemizygous mutations in the HLH-related genes. These sites involved were located in *PRF1* (c.65 delC, c.1535T>G, c.394G>A), *UNC13D* (c.1978-1979insATTACCG, c.3049G>A, c.596+5G>A, c.2077C>T, m.284C>A), *LYST* (c.377del), *BIRC4* (c.589C>T, c.1253-1256delAGAA, c.813delT, exon 4, and exon 5 deletion), and *SH2D1A* (c.3G>A). These molecular defects were associated with decreased CD107a expression or NK cell activity or corresponding protein defects, as shown in Table 1.

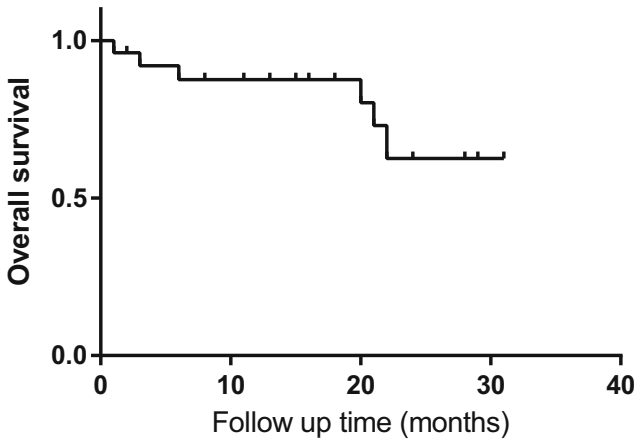

Fig. 1 Overall survival of 26 patients with primary hemophagocytic lymphohistiocytosis in China

Among the 18 mutations that were found in pHLH patients and were reported in other studies (shown in supplementary Table 2), the most common mutation was c.1349C>T in *PRF1*, appearing in the 6 patients with FHL2 (P1, P2, P4, P7, P8, and P9). Among these 18 mutations, nine mutations were detected in patients with a family history, and nine mutations were detected in patients with compound heterozygous mutations or hemizygous mutations in the HLH-related genes. These mutations were accompanied by impaired CD107a expression, decreased NK cell activity, or corresponding protein defects in the patients.

Other gene mutations detected in children with pHLH

Interestingly, 27 genes (affiliated with immunodeficiency-associated gene panels) that were not common HLH-related genes were detected in patients with pHLH. Notably, there were 4 genes in which mutations were found in more than 2 patients, including *TFR2*, *PMS2*, *DNMT3B*, and *SPTA1*, as shown in Table 2. The above gene-related pathways are mainly shown in Table 2. They included the cytokine signaling pathway, DNA double-strand break repair pathway, DNA methylation pathway, and the iron metabolism in placenta pathway. Specifically, compound heterozygous mutations in *SPTA1* were detected in P1. P14 exhibited a family history and presented heterozygous mutations in *UNC13D*. It is widely accepted that HLH is an autosomal recessive inheritance disorder. It is possible that intron mutations in *UNC13D* or synergistic pathogenicity in other gene mutations remain to be recovered in P14. Other unknown genes may be involved in the pathogenesis of HLH, which is currently being examined in patients.

Correlation between genotypes and clinical features

To determine whether the genotype was related to phenotype of pHLH patients, we further studied the clinical characteristics of our cohort of pHLH patients. In this study, compound heterozygous mutations and hemizygous mutations in *SH2D1A* and *BIRC4* were found in 18 and 6 patients,

Table 1 Clinical and genetic results for primary HLH patients with mutations in HLH-associated genes

Pt	Age/Gd	Gene mutation sites	Family history	NK cells activity (%)	Degranulation (NK %/CTLs)	Protein (NK cells/CTLs)	Treatment response	Outcome
P1	13.7/F	*PRF1*: c.1349C>T (het, mis, pathogenic) c.1450G>A* (het, mis, pathogenic)	Yes	14.1↓	Not available	Not available	NR	Died
P2	0.2/M	*PRF1*: c.1349C>T (het, mis, pathogenic) c.853_855del (het, del, pathogenic)	Yes	Not available	10.3/2.1↓	Perforin 17↓/below limit↓	Relapse	Alive
P3	4/F	*PRF1*: c.133G>A (het, mis, pathogenic) c.116C>A (het, mis, pathogenic)	No	10.9↓	4.3↓/4.6↓	Normal	HSCT, CR	Alive
P4	4.2/F	*PRF1*: c.1349C>T (het, mis, pathogenic) c.65 delC* (het, del, pathogenic)	No	9.3↓	11.5/7.4	Perforin 14↓/below limit↓	HSCT, CR	Alive
P5	3/F	*PRF1*: c.1349G>A (het, mis, pathogenic) c.218C>T* (het, mis, pathogenic)	Yes	12.1↓	Not available	Not available	HSCT, CR	Alive
P6	0.4/F	*PRF1*: c.673C>T (het, mis, pathogenic) c.1535T>G* (het, mis, pathogenic)	No	Not available	8.5↓/2.2↓	Not available	HSCT, CR	Alive
P7	4.9/F	*PRF1*: c.1349C>T (het, mis, pathogenic) c.853_855del (het, del, pathogenic)	Yes	15.5	17.0/2.5↓	Perforin 41↓/9	HSCT, CR	Alive
P8	0.8/F	*PRF1*: c.1349C>T (het, mis, pathogenic) c.1090_1091delCT (het, del, pathogenic)	Yes	12.4↓	Not available	Not available	HSCT, CR	Alive
P9	11.3/M	*PRF1*: c.1349C>T (het, mis, pathogenic) c.503G>A (het, mis, pathogenic)	No	13.2↓	5.7↓/3.7	Perforin 13↓/8	NR	Alive
P10	6/F	*PRF1*: c.133G>A (het, mis, pathogenic) c.394G>A* (het, mis, pathogenic)	No	14.3↓	14.6/2.6↓	Perforin 71↓/16	Relapse	Alive
P11	0.1/M	*UNC13D*: c.2448-13G>A (het, spli, pathogenic) c.118-308C>T (het, spli, pathogenic)	Yes	13.5↓	1.6↓/1.3↓	Normal	HSCT,	Alive
P12	0.8/F	*UNC13D*: c.3049G>A (het, mis, pathogenic) c.1978-1979insATTACCG* (het, ins, pathogenic)	No	13.4↓	2.5↓/1.6↓	Normal	Relapse	Alive
P13	6.2/M	*UNC13D*: c.2831-13G>A (het, spli, pathogenic) c.640C>T (het, non, pathogenic)	Yes	Not available	Not available	Not available	Relapse	Alive
P14	1/F	*UNC13D*: c.766C>T (het, non, pathogenic)	Yes	14.1↓	2.6↓/1.4↓	XIAP 1↓/45↓	HSCT, CR	Alive
P15	9/F	*UNC13D*: c.2588G>A (het, mis, pathogenic) m.284C>A (het, untran, pathogenic)	No	14.8↓	9.0↓/4.3	Perforin 68↓/17 Granzyme B 68↓/64	HSCT	Died
P16	7/F	*UNC13D*: c.3049G>A* (het, mis, pathogenic) c.596+5G>A* (het, spli, pathogenic)	No	21.5	2.3↓/1.3↓	Normal	HSCT	Alive
P17	0.3/M	*UNC13D*: c.2077C>T* (het, mis, pathogenic) c.1596+1G>C (het, spli, pathogenic)	No	14.9↓	8.7↓/4.7	Not available	HSCT, CR	Alive
P18	12/F/	*STXBP2*: c.497C>T* (het, mis, pathogenic)	Yes	11.6↓	7.8↓/1.0↓	Not available	Relapse	Died
P19	7/M	*LYST*: c.244C>T (het, mis, pathogenic) c.377del* (het, del, pathogenic)	No	15.5	0.6↓/1.1↓	Normal	HSCT	Alive
P20	9/F	*ITK*: c.85C>T* (het, mis, pathogenic) c.243+1G>A* (het, spli, pathogenic)	Yes	15.8	25.4/1.9↓	Normal	Relapse	Alive
P21	2.7/M	*SH2D1A*: c.3G>A*(hemi, mis, pathogenic)	No	11.9↓	12.0/1.4↓	XIAP 47↓/38↓	NR	Died
P22	2.2/M	*SH2D1A*: c.163C>T (hemi, non, pathogenic)	No	13.9↓	16.1/2.2↓	SAP 46/36↓	HSCT	Alive
P23	0.3/M	*BIRC4*: c.813delT* (hemi, del, pathogenic)	No	14.1↓	7.1↓/2.7↓	XIAP 46↓/32↓	HSCT	Alive
P24	0.7/M	*BIRC4*: c.589C>T* (hemi, non, pathogenic)	No	12.6↓	2.6↓/1.9↓	Normal	HSCT	Died

Table 1 (continued)

Pt	Age/Gd	Gene mutation sites	Family history	NK cells activity (%)	Degranulation (NK %/CTLs)	Protein (NK cells/CTLs)	Treatment response	Outcome
P25	2.2/M	*BIRC4*: c.1253-1256 del AGAA* (hemi, del, pathogenic)	No	Not available	10.6/2.6↓	Not available	HSCT	Died
P26	1.5/M	*BIRC4*: Exonic deletion* (hemi, large del, pathogenic)	No	14.6↓	2.34↓/1.3↓	XIAP 54↓/49↓	Relapse	Alive

Pt, patient; *Gd*, gender; *F*, female; *M*, male; Gene mutation sites: het heterozygous, *hemi* hemizygous, *mis* missense, *del* deletion, *spli* splicing, *ins* insertion, *non* nonsense, *untra* untranslated region. *Protein*, XIAP X-linked inhibitor of apoptosis protein, *SAP* signaling lymphocytic activation molecule associated protein. *HSCT* hematopoietic stem cell transplantation, *NR* not remission, *CR* complete remission. (The normal values for functional analysis and proteins are as follows: NK cell activity (coculture of NK and target cells) ≥ 15.11%; CD107a degranulation assay: NK cells > 10% or CTLs ≥ 2.8. Perforin NK cells > 98% or CTLs ≥ 2%. Granzyme: NK cells ≥ 77% or CTLs ≥ 6%. XIAP: NK cells ≥ 59% or CTLs ≥ 61%. SAP: NK cells ≥ 26% or CTLs ≥ 43%. The specific experimental results and reference values were received and supported by the Aipuyi Medical Testing Center in Beijing

respectively. These 24 patients were divided into 2 groups: group 1 was composed of 16 patients with disruptive mutations, including indels, deletions, nonsense mutations, and splice errors [2]; the others were classified as group 2. The average age of group 1 was significantly younger than that of group 2 (2.9 years vs. 6.4 years, $F = 3.719$, $P = 0.036$). For example, P11 (belonging to group 1) exhibited two destructive mutations of *UNC13D* (c.2448-13G>A, c.118-308C>T), and the age at onset was also the earliest (15 days after birth).

All pHLH patients were divided into the FHL (including FHL2, FHL3, and FHL5, $n = 18$) and non-FHL groups ($n = 8$), as shown in Table 1. There was a significant difference in the incidence of CNS involvement between these two groups (83.3% vs. 37.5%, $\chi^2 = 5.462$, $P = 0.019$). Additionally, we found that the incidence of seizure was also different in these two groups. There were 10 (55.6%, 8, 2 with FHL2, and FHL3, respectively) and 1 (12.5%, 1 with *BIRC4*) patients who experienced seizures in the FHL and non-FHL groups, respectively ($\chi^2 = 4.206$, $P = 0.04$).

Discussion

This study revealed that mutations in the *PRF1* and *UNC13D* genes were predominant in Chinese children with pHLH, similar to the results from Italian, Turkish, and Korean groups [8, 12, 13]. In addition, this study discovered 19 novel mutations in HLH-associated genes, most of which were predicted to be deleterious to protein function. Further pedigree analysis and functional studies would verify the pathogenicity of these mutations. These studies would benefit from progress in the construction of cell and animal models of HLH [14] and from gene-editing technology, such as clustered regularly interspaced short palindromic repeats (CRISPR) technology [15]. Alternatively, these results indicate the need for the development of detection methods for these and other novel or nonhotspot mutations in future clinical studies in addition to hotspot mutations.

It was noteworthy that genetic data were acquired from only 726 immunodeficiency-associated genes in this study, which probably caused most of the information on the whole exome and genome to be overlooked. The small number of genes sampled was the primary reason that no mutations were found in other patients with presumed pHLH due to a positive refractory or relapse history. It has been illustrated that cis-regulatory variation modifies the penetrance of coding variants and that joint regulatory and coding variant effects are an important part of the genetic architecture of human traits and contribute to modified penetrance of disease-causing variants [16]. Therefore, whole-exome sequencing or whole-genome sequencing can play an important role in the diagnosis, treatment choice, and study of pathogenesis in primary HLH.

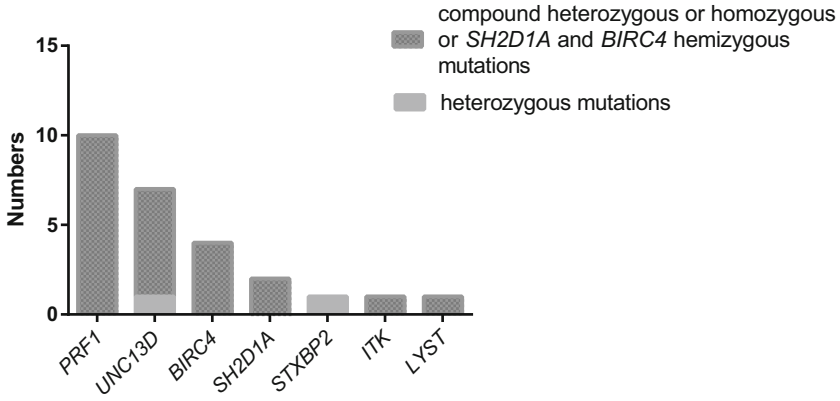

Fig. 2 Comparison of the HLH-causing gene mutations in pHLH patients. The results show that *UNC13D* ($n = 7$), *PRF1* ($n = 10$), and *BIRC4* ($n = 4$) were the primary gene mutations in 26 pHLH patients. Light gray bars represent heterozygous mutations in HLH-causing genes; dark gray bars represent compound heterozygous, homozygous, or hemizygous mutations in *SH2D1A* and *BIRC4*

It is believed that CNS involvement in HLH results from extension of the abnormal immune response in pHLH to and through the blood-brain barrier [17]. Imaging examination, for example, shows gadolinium enhancement supporting the presence of active inflammation in and around the brain [18]. We previously demonstrated that a higher percentage of children with FHL exhibit severe CNS syndrome than other HLH patients, and seizure, one of the most severe CNS symptoms, is associated with the worst prognosis [11, 19]. Thus, the observation made in this study that children with FHL are prone to seizure suggests that FHL patients with CNS syndrome, particularly seizures, should be treated with salvage chemotherapy or HSCT as soon as possible.

Mutations in the coding genes of proteins involved in the cytotoxic pathways of cytotoxic T or NK cells have been regarded as the main mechanism of pathogenesis in HLH [20]. In this study, four genes were found to be mutated in more than 2 patients. Interestingly, the most common pathway was DNA double-strand break repair or methylation pathway. It has been reported that defective double-strand break repair in mice with severe combined immunodeficiency was due to an abnormal repertoire of antibodies and T cell receptors during development [21, 22]. Thus, to a certain extent, mutations in genes functioning in DNA double-strand damage repair or regulation may be involved in the pathogenesis of primary HLH. It was noteworthy that compound heterozygous mutations in *SPTA1* (c.6789-7C>T, c.2805+7T>G) were found in patient 1. Mutations in the *SPTA1* gene result in a variety of hereditary red blood cell disorders, including hereditary elliptocytosis and spherocytosis [23]. This gene encodes scaffold proteins of the erythrocyte membrane skeleton, which are commonly responsible for hereditary elliptocytosis [24]. However, the relationship between these gene mutations and HLH warrants further investigation.

The cumulative effect of polygenic mutations in HLH-related and other genes on the pathogenesis of HLH and treatment response has not been thoroughly elucidated to date. It has been hypothesized that multiple mutations in the same or different pathways result in an increased individual risk of developing HLH [25, 26]. Therefore, HLH may be a threshold disease to some extent. When mutations in 2 or more related pathway genes exceed this threshold, HLH immunopathology may occur. This possibility suggests that there might be a coordinated effect of these mutated genes and the coding proteins in HLH pathogenesis. Alternatively, the existence of mutations in genes involved in the same or different pathways indicates the requirement for a specific chemotherapy regimen or targeted drugs, such as ruxolitinib, in the treatment of HLH [27–30]. A more precise or targeted treatment strategy may be helpful in the correction of HLH immunopathology.

In summary, the determination of gene mutations is not only a good indicator for predicting prognosis in pHLH but

Table 2 Other gene mutations detection and pathways involved in the pHLH patients

Gene	Number of patients ($n = 26$) (%)	Nucleotide change	Zygosity	Signal pathway
SPTA1	2 (8%)	c.6789-7C>T, c.2805+7T>G (P1) c.480C>G (P5)	Com Het	Cytokine signaling in immune system
PMS2	2 (8%)	c.706-5T>- (P1,14)	Het	DNA double-strand break repair
DNMT3B	2 (8%)	c.1603G>A (P14) c.1804G>A (P21)	Het Het	DNA methylation
TFR2	2 (8%)	c.224C>T (P21) c.590A>G (P14)	Het Het	Iron metabolism in placenta

P patient, *Het* heterozygous mutations, *Com* compound, heterozygous mutations, *SPTA1 spectrin alpha, erythrocytic 1*, *TFR2 transferrin receptor 2*, *PMS2 pms1 homolog 2*, *DNMT3B dna methyltransferase 3 beta*

is also an important factor when considering differential treatment. We hope that primary HLH can be rapidly diagnosed to facilitate the initiation of life-saving treatments and preparation for HSCT.

Acknowledgements We thank all of the patients and their families for their kind cooperation.

Authors' contributions Liping Zhang collected clinical the patient data, wrote the paper, and designed and performed the research. Wei Liu and Honghao Ma collected clinical patient data and analyzed the patients. Zhigang Li analyzed the patients, revised the paper, and designed and performed the research. Tianyou Wang and Rui Zhang analyzed the patients, revised the paper, designed and performed the research and contributed patient data. All authors read and approved the final manuscript.

Funding This study was funded by the National Science and Technology Key Projects (No. 2017ZX09304029004), the Beijing Municipal Science & Technology Commission (No. Z171100001017050), the National Natural Science Foundation of China (Nos. 81700186 and 81800189), the Beijing Municipal Administration of Hospitals' Youth Programme (QML20181205), the Scientific Research Common Program of Beijing Municipal Commission of Education (Nos. KM201710025019 and KM201910025011), the Talent Training Project-Fostering Fund of the National Natural Science Foundation of Beijing Children's Hospital, Capital Medical University (No. GPY201713), the Special Fund of the Pediatric Medical Coordinated Development Center of the Beijing Hospitals Authority (No. XTZD20180202), and the Guangdong Province Key Laboratory of Popular High-Performance Computers of Shenzhen University (SZU-GDPHPCL2017).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent and ethics approval Written informed consent was obtained from the parents, and the study was approved by the Beijing Children's Hospital Ethical Committee.

References

1. Henter JI, Horne A, Aricó M, Filipovich AH, Imashuku S, Ladisch S, McClain K, Webb D, Winiarski J, Janka G (2007) HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 48:124–131
2. Al-Samkari H, Berliner N (2018) Hemophagocytic lymphohistiocytosis. Annu Rev Pathol 13:27–49
3. Chandrakasan S, Filipovich AH (2013) Hemophagocytic lymphohistiocytosis: advances in pathophysiology, diagnosis, and treatment. J Pediatr 163:1253–1259
4. Ng PC, Henikoff S (2003) SIFT: predicting amino acid changes that affect protein function. Nucleic Acids Res 31:3812–3814
5. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR (2010) A method and server for predicting damaging missense mutations. Nat Methods 7:248–249
6. Jin Z, Wang Y, Wang J, Zhang J, Wu L, Gao Z, Lai W, Wang Z (2018) Primary hemophagocytic lymphohistiocytosis in adults: the utility of family surveys in a single-center study from China. Orphanet J Rare Dis 13:17
7. Beken B, Aytac S, Balta G, Kuskonmaz B, Uckan D, Unal S, Cetin M, Gumruk F (2018) The clinical and laboratory evaluation of familial hemophagocytic lymphohistiocytosis and the importance of hepatic and spinal cord involvement: a single center experience. Haematologica 103:231–236
8. Mahlaoui N, Ouachée-Chardin M, de Saint BG, Neven B, Picard C, Blanche S, Fischer A (2007) Immunotherapy of familial hemophagocytic lymphohistiocytosis with antithymocyte globulins: single-center retrospective report of 38 patients. Pediatrics 120:e622–e628
9. Deiva K, Mahlaoui N, Beaudonnet F, de Saint BG, Caridade G, Moshous D, Mikaeloff Y, Blanche S, Fischer A, Tardieu M (2012) CNS involvement at the onset of primary hemophagocytic lymphohistiocytosis. Neurology 78:1150–1156
10. Xu XJ, Wang HS, Ju XL, Xiao PF, Xiao Y, Xue HM, Shi HY, Gao YJ, Jia GC, Li XR, Zhao WH, Wang NL, Tang YM (2017) Clinical presentation and outcome of pediatric patients with hemophagocytic lymphohistiocytosis in China: a retrospective multicenter study. Pediatr Blood Cancer 64:e26264
11. Zhao YZ, Zhang Q, Li ZG, Zhang L, Lian HY, Ma HH, Wang D, Zhao XX, Wang TY, Zhang R (2018) Central nervous system involvement in 179 Chinese children with hemophagocytic lymphohistiocytosis. Chin Med J 131:1786–1792
12. Koh KN, Im HJ, Chung NG, Cho B, Kang HJ, Shin HY, Lyu CJ, Yoo KH, Koo HH, Kim HJ, Baek HJ, Kook H, Yoon HS, Lim YT, Kim HS, Ryu KH, Seo JJ (2015) Clinical features, genetics, and outcome of pediatric patients with hemophagocytic lymphohistiocytosis in Korea: report of a nationwide survey from Korea Histiocytosis Working Party. Eur J Haematol 94:51–59
13. Cetica V, Sieni E, Pende D, Danesino C, De Fusco C, Locatelli F, Micalizzi C, Putti MC, Biondi A, Fagioli F, Moretta L, Griffiths GM, Luzzatto L, Aricò M (2016) Genetic predisposition to hemophagocytic lymphohistiocytosis: report on 500 patients from the Italian registry. J Allergy Clin Immunol 137:188–196.e4
14. Sepulveda FE, Garrigue A, Maschalidi S, Garfa-Traore M, Ménasché G, Fischer A, de Saint BG (2016) Polygenic mutations in the cytotoxicity pathway increase susceptibility to develop HLH immunopathology in mice. Blood 127:2113–2121
15. Prykhozhij SV, Fuller C, Steele SL, Veinotte CJ, Razaghi B, Robitaille JM, McMaster CR, Shlien A, Malkin D, Berman JN (2018) Optimized knock-in of point mutations in zebrafish using CRISPR/Cas9. Nucleic Acids Res 46:e102
16. Castel SE, Cervera A, Mohammadi P, Aguet F, Reverter F, Wolman A, Guigo R, Iossifov I, Vasileva A, Lappalainen T (2018) Modified penetrance of coding variants by cis-regulatory variation contributes to disease risk. Nat Genet 50:1327–1334
17. Gratton SM, Powell TR, Theeler BJ, Hawley JS, Amjad FS, Tornatore C (2015) Neurological involvement and characterization in acquired hemophagocytic lymphohistiocytosis in adulthood. J Neurol Sci 357:136–142
18. Cai G, Wang Y, Liu X, Han Y, Wang Z (2017) Central nervous system involvement in adults with haemophagocytic lymphohistiocytosis: a single-center study. Ann Hematol 96:1279–1285
19. Yang S, Zhang L, Jia C, Ma H, Henter JI, Shen K (2010) Frequency and development of CNS involvement in Chinese children with hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 54:408–415
20. Ladisch S, Poplack DG, Holiman B, Blaese RM (1978) Immunodeficiency in familial erythrophagocytic lymphohistiocytosis. Lancet 1:581–583
21. Featherstone C, Jackson SP (1999) DNA double-strand break repair. Curr Biol 9:R759–R761
22. Kanaar R, Hoeijmakers JHJ, van Gent DC (1998) Molecular mechanisms of DNA double strand break repair. Trends Cell Biol 8:483–489

23. Ma S, Qin J, Wei A, Li X, Qin Y, Liao L, Lin F (2018) Novel compound heterozygous SPTA1 mutations in a patient with hereditary elliptocytosis. Mol Med Rep 17:5903–5911
24. Da Costa L, Galimand J, Fenneteau O, Mohandas N (2013) Hereditary spherocytosis, elliptocytosis, and other red cell membrane disorders. Blood Rev 27:167–178
25. Zhang K, Chandrakasan S, Chapman H, Valencia CA, Husami A, Kissell D, Johnson JA, Filipovich AH (2014) Synergistic defects of different molecules in the cytotoxic pathway lead to clinical familial hemophagocytic lymphohistiocytosis. Blood 124:1331–1334
26. Chinn IK, Eckstein OS, Peckham-Gregory EC, Goldberg BR, Forbes LR, Nicholas SK, Mace EM, Vogel TP, Abhyankar HA, Diaz MI, Heslop HE, Krance RA, Martinez CA, Nguyen TC, Bashir DA, Goldman JR, Stray-Pedersen A, Pedroza LA, Poli MC, Aldave-Becerra JC, McGhee SA, Al-Herz W, Chamdin A, Coban-Akdemir ZH, Jhangiani SN, Muzny DM, Cao TN, Hong DN, Gibbs RA, Lupski JR, Orange JS, McClain KL, Allen CE (2018) Genetic and mechanistic diversity in pediatric hemophagocytic lymphohistiocytosis. Blood 132:89–100
27. Das R, Guan P, Sprague L, Verbist K, Tedrick P, An QA, Cheng C, Kurachi M, Levine R, Wherry EJ, Canna SW, Behrens EM, Nichols KE (2016) Janus kinase inhibition lessens inflammation and ameliorates disease in murine models of hemophagocytic lymphohistiocytosis. Blood 127:1666–1675
28. Broglie L, Pommert L, Rao S, Thakar M, Phelan R, Margolis D, Talano J (2017) Ruxolitinib for treatment of refractory hemophagocytic lymphohistiocytosis. Blood Adv 1:1533–1536
29. Zandvakili I, Conboy CB, Ayed AO, Cathcart-Rake EJ, Tefferi A (2018) Ruxolitinib as first-line treatment in secondary hemophagocytic lymphohistiocytosis: a second experience. Am J Hematol 93:E123–E125
30. Slostad J, Hoversten P, Haddox CL, Cisak K, Paludo J, Tefferi A (2018) Ruxolitinib as first-line treatment in secondary hemophagocytic lymphohistiocytosis: a single patient experience. Am J Hematol 93:E47–E49