

# RFI Gene Mutation in Familial Hemophagocytic Lymphohistiocytosis 2: A Family Report and Literature Review

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**Objective:** Gene mutation analysis was performed on a family with familial hemophagocytic lymphohistiocytosis (FHL) so as to provide an accurate etiological diagnosis, leading to genetic counseling for the family members.

**Methods:** The clinical data of two probands (siblings) with FHL in one family were analyzed, and eight genes related to the onset of the primary hemophagocytic lymphohistiocytosis (pHLH) (*PRF1*, *UNC13D*, *STX11*, *STXBP2*, *SH2D1A*, *BIRC4/XIAP*, *Rab27a*, *LYST*) were detected and analyzed in the probands and their parents with whole exome sequencing.

**Results:** Proband 1 was a two-year-old male with the clinical manifestations of fever, hepatosplenomegaly, and a decreased peripheral blood cell count, sCD25: 12504pg/mL. The results of genetic testing showed that there was a c.1349C>T heterozygous missense mutation and a c.853\_855del heterozygous mutation in the *PRF1* in proband 1. Proband 2 was an eight-year-old female with the clinical manifestations of convulsions and disturbance of consciousness with fever. The genetic test results were the same as those of proband 1. There was a single heterozygous mutation in the parents of the probands, and both probands had compound heterozygous mutations.

**Conclusion:** According to the clinical manifestations, laboratory tests, and results of the family molecular genetic testing, the probands could be clinically diagnosed as FHL2. The results of gene sequencing revealed that this was an autosomal recessive family with familial hemophagocytic syndrome. A rare pathogenic mutation (c.853\_855del) in the *PRF1* was discovered in the two patients with HLH.

**Keywords:** FHL, *PRF1*, novel mutation

Hemophagocytic lymphohistiocytosis (HLH) is also referred to as hemophagocytic syndrome (HPS). HLH is a disease caused by the dysfunction of the immune system. The etiologies include molecular genetic abnormalities, infections, tumors, and autoimmune diseases. The main clinical manifestations are repeated fever, a decreased blood cell count to varying degrees, hepatosplenomegaly, and hepatic function abnormalities,<sup>1</sup> together with manifestations of the central nervous system in many cases.<sup>2</sup> Studies have found that children with HLH have cytotoxic cell dysfunction, meaning that antigens cannot be cleared in the normal manner.<sup>3</sup> In turn, the monocytes and macrophages are constantly stimulated by antigens and are abnormally activated to produce a large number of cytokines, causing tissue damage. HLH progresses quickly with potentially dangerous consequences. If it is not treated in time, or treated ineffectively, survival duration is short and the

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prognosis is poor.<sup>4</sup> HLH is traditionally divided into two subtypes, according to whether there are genetic defects: primary (hereditary) HLH and secondary (acquired) HLH. Hereditary HLH comprises the familial HLH (FHL) types 1–5 and HLH caused by primary immunodeficiency syndrome, and mainly occurs in infants or during early childhood. Acquired HLH is secondary to infections, rheumatic diseases and tumors.<sup>5</sup>

FHL2 is the most common familial form of HLH with immune deficiency. FHL2 patients have mutations in genes affecting the expression of perforin which contribute to excessive activation and hyperplasia of CD8<sup>+</sup> cytotoxic T lymphocytes (CTL) and natural killer (NK) cells. Perforin, a cytolytic protein, is a major factor responsible for the cytolytic activities of CTL and NK cells. Perforin, a pore-forming protein, creates pores in the target cytomembrane, generating apoptotic death through granzymes. *PRF1* mutations cause decreased or absent expression of perforin protein, resulting in cytotoxic immunodeficiency. The loss of cytotoxic immune function contribute to an uncontrolled and persistent immune stimulus response in FHL2 patients, accompanied by high levels of cytokine release. If the immune system and excessive cytotoxic T-cell and NK-cell stimulation incontrollable. They can cause systemic inflammation and multiorgan failure.<sup>6</sup>

In 2004, the International Society of Tissue Cells revised the diagnostic criteria for familial and reactive HLH.<sup>7</sup> In the present study, gene mutation analyses were performed on one family, consisting of two children with FHL and their parents, with a view to providing an accurate etiological diagnosis, followed by etiological diagnosis and genetic counseling for the family members.

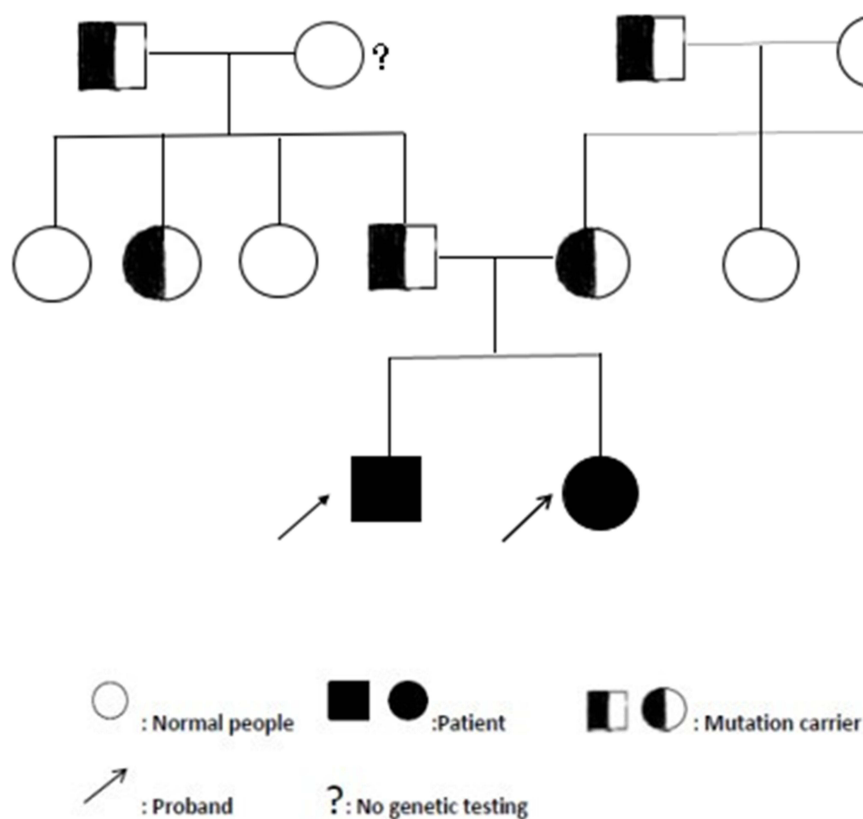
## Case Report

Proband 1, a 2-year-old male, reported “fever and hepatosplenomegaly” at the time of the hospital visit. There was no abnormality in the personal history and past history of the proband. The parents were not consanguineous and had no similar medical history. We perfected the laboratory examination and found: He had decreased peripheral blood cells, bone marrow cell morphology shows: lymphocytes increase, hemophagocytic cells are more common, and rised sCD25: 12504pg/mL. The ferritin level is normal.

Proband 2 (the sibling of proband 1), 8 years old, was first diagnosed with acute disseminated encephalomyelitis (ADEM) in a hospital in Beijing after presenting with “convulsions, disturbance of consciousness and fever for

two days” more than a year ago. Proband 2’s symptoms recovered after immunotherapy. Magnetic resonance imaging (MRI) of the head during the hormone reduction period indicated that there were lesions that were aggravated, and this anomaly was followed by neurological symptoms. With the re-application of hormone therapy, proband 2’s condition improved, but the intracranial lesions still showed progressive aggravation. Transient cytopenia, hepatosplenomegaly, and decreased NKC activity were observed during the course of the disease with normal hepatic function, levels of triacylglyceride and coagulation function in multiple detections. Eventually, after disease analysis and genetic examinations, proband 2 was given the diagnosis of “familial haemophagocytosis syndrome (FHPS) with the onset of the central nervous system”. And the proband 2’s bone marrow cell morphology shows: lymphocytes increase, hemophagocytic cells are more common.

The proband 1 met the following five, out of a total of eight, clinical diagnostic criteria of HLH-2004: fever, splenomegaly, cytopenia, hemophagocytosis and sCD25:12504pg/mL. While the proband 2 also met five: fever, splenomegaly, cytopenia, hemophagocytosis and decreased NKC activity. Since the two probands were strongly suspected of being HLH, genetic testing of 12 subjects in three generations of the family was carried out, and the blood was drawn and sent to the Beijing Kangxu Medical Laboratory. The proband 1 did full exon detection, using the method of next-generation sequencing, while the proband 2 did site verification, using sanger sequencing. The genotypes of these two sites can be determined by sanger sequencing. The results are shown in Figure 1. There was a heterozygous missense mutation in exon 3 c.1349C>T (nucleotide No. 1349 in the coding region was changed from C to T), which was detected in the probands, their father, grandfather and father’s sister, and c.853\_855del (nucleotide No. 853\_855 in the coding region) hybrid combined frameshift mutation, which was detected in the probands, their mother, grandfather, and mother’s brother. The probands’s genes sequencing results in Figure 2. The c.1349C>T heterozygous missense mutation caused amino acid N450 to change from Thr to Met; while the c.853\_855del heterozygous frameshift mutation caused the deletion of amino acid N285. The protein damage prediction results were analyzed by SIFT, Polyphen2 and Mutation Taster. The c.1349C>T heterozygous missense mutation is c. 1349c > t heterozygous missense mutation is harmful and pathogenic. While



**Figure 1** HLH pedigree map.

c.853\_855del heterozygous frameshift mutation is suspected to be pathogenic. The above-mentioned mutations might have caused the protein function to be affected, which in turn would lead to the occurrence of HLH. The mutation site c.1349C>T (p.Thr450Met) has been included in the dbSNP database with the ID of rs18965890, and literature reports related to HLH disease can be retrieved.<sup>8</sup> While the pathogenicity of the mutation c.853\_855del has not been reported in the Chinese literature, nor has it been included in the dbSNP database. Both the maternal and fraternal grandfathers, the maternal grandmother, the parents, the second aunt, and an uncle had single heterozygous mutations. None of the above-mentioned mutations were polymorphic changes, which have an extremely low frequency of occurrence in the population. The compound heterozygous variants found in the *PRF1* gene of the probands were inherited from their parents, and the above variants might be pathogenic variants that caused the onset of the disease in the probands.

The proband 1 was Proband 1 received bone marrow transplantation successfully in January 2020. And proband 2 is scheduled to receive bone marrow transplantation this year.

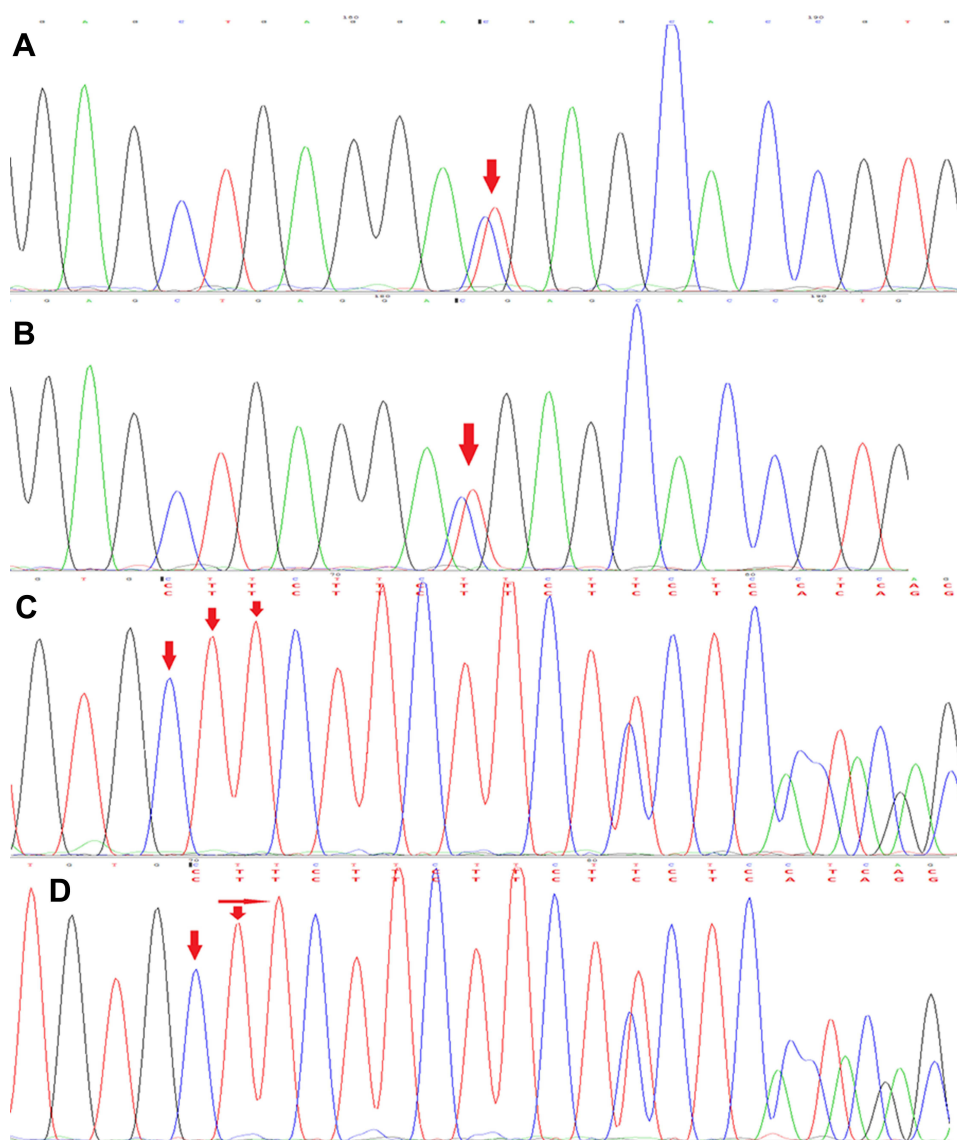
Informed written consent was obtained from patients and their guardians for the publication of the case details and any accompanying images.

## Literature Review

The CNKI and Wanfang databases were searched using the keywords of “familial hemophagocytic syndrome”, “*PRF1*” and “novel mutation”, and the PubMed database was searched using the keywords of “FHL”, “*PRF1*” and “novel mutation” from January 1st, 2009 to June 30th, 2020.

From a comprehensive database of gene mutations related to the disease, the four genes for FHL, namely, *PRF1*, *UNC13D*, *STXBP2*, and *STX11*, were investigated. There were 433 different mutations of these genes to be found in the literature review, of which *UNC13D* had the maximum amount of mutations (189), followed by *PRF1* (157), *STXBP2* (66), and then *STX11* (21).<sup>9</sup>

Thus, it was shown that mutations in the *PRF1* accounted for the main proportion of the occurrences of FHL. Genotypic characteristics and immune indicators were evaluated in a study concerning 311 cases of HPS in China. It was reported that in the 128 cases where



**Figure 2** The probands's genes sequencing results (A). The sequencing peak of c.1349C>T of Proband 1. (B) The sequencing peak of c.1349C>T of Proband 2. (C) The sequencing peak of c.853\_855del of Proband 1. (D) The sequencing peak of c.853\_855del of Proband 2.

genetic testing was complete, the different types of genetic variation could be clarified. The mutation rate of the *UNCI3D* gene was said to be the highest (29%), and then *LYST* (21%), *PRFI* (17%) and *STXBP2* (10%). The most common of the main pathogenic genes in 39 cases of primary HPS was *PRFI* (16/39, 41%), follow by *UNCI3D* (10/39, 26%) and *BIRC4* (6/39, 15%). Also, there were differences in the genes related to primary HPS in children and adolescents, in that *PRFI* was more prominent in children and adolescents while in grown-up *UNCI3D* was the most common, followed by *PRFI*.<sup>10</sup>

There was a report in X of a new fatal case of congenital pyrimidine metabolic abnormality and rapid progression of multiple organ failure. It was found that the

patient had two single gene defects at the same time, including the c.853\_855del. At present, this gene defect has not been reported in China. The mixed phenotype of the two coexisting single gene defects might have been the main reason for the rapid deterioration of the condition in the patient, leading to multiple organ failure and early death.<sup>11</sup>

## Discussion

HLH is a group of clinical syndromes in which lymphocytes and macrophages are excessively activated and then proliferate due to multiple etiologies. A large number of inflammatory cytokines are released causing a “cytokine storm”, resulting in multiple organ dysfunction. Current

studies believe that the main pathogenesis of the disease is an immune regulation disorder or immune imbalance, causing the excessive secretion of lymphokines and monocyte factors by T lymphocytes and mononuclear macrophages. In addition, a cascade of inflammatory mediators will lead to corresponding clinical symptoms. The highly activated macrophages, which have strong cell activation, proliferation, and hemophagocytic abilities, together with the increased levels of inflammation, can inhibit blood cell production and reduce the amount of blood cells.<sup>1</sup>

Classify FHL as five subtypes, types 1 to 5, all of which are autosomal recessive diseases. Except for FHL1, in which the gene defect is unknown, four different pathogenic genes have been identified: *PFRI* (FHL2), *UNC13D* (FHL3), *STX11* (FHL4), and *STXBP2* (FHL5).<sup>12</sup> The incidence of gene defects in FHL, of which FHL2 and FHL3 are the most common, is largely correlated with race.<sup>13</sup> The *PRFI* is located at 10q21-22 and contains three exons. The coding regions are the 2nd and 3rd exons. The perforin encoded in *PRFI* contains 555 amino acids, including three trans-membrane regions, the EGF-like region and three functional regions in the C2 region. The perforin can produce cytotoxic particles in the cytoplasm of target cells, which can lead to apoptosis. The NK cells and CTL cells mainly kill the target cells through the perforin/granzyme pathway. When this gene is mutated, the expression of perforin is down-regulated, and so the stability is reduced. When cytotoxic cells make contact with the target cell, the damaged perforin cannot smoothly form a channel on the membrane of the target cell. This will prevent the perforin from delivering the granzyme into the target cell to induce the apoptosis and osmotic dissolution, and, eventually, lead to impaired function in the killing of the target cells.

The *PRFI* mutation is the most frequency mutation, accounting for 20–40% of all FHL cases. The highest rate of *PRFI* mutation was reported in African-American families (>50%), and then the population of Turkey (43%), Japan (42%), and India (41%).<sup>14</sup> According NCBI clinvar database (<https://www.ncbi.nlm.nih.gov/clinvar>), a total of 173 *PRFI* mutations have been reported now, among which 28 mutations were definitely pathogenic, all occurring in the second and third exons of the coding region. Some special mutation sites are more common in subjects from specific races and regions.<sup>14</sup> The nonsense mutations

(c.1122G>A) of *PRFI* mutations are more common in the Turkish population, the frameshift mutations (c.1090\_1091delCT and c.50delT) are more common in Japanese and African-American populations, while the missense mutations, p.Ala91Val and p.Trp129Ser, have appeared in the Italian and Indian populations, respectively. The missense mutations (c.445G>A) are common in Spanish patients while in Japanese patients with HLH, the most common mutation is c.1090\_1091delCT (accounting for approximately 62.5% of the *PRFI* mutations), followed by c.207delC (about 37.5% of *PRFI* mutations).<sup>15,16</sup>

In addition, mutations and variants of the *PRFI* have also been reported in other diseases, including perforin deficiency syndrome, multiple sclerosis, type 1 diabetes, non-Hodgkin's lymphoma, and leukemia. So far, more than 115 pathogenic gene variants have been reported for *PRFI*, which mainly is the missense and nonsense mutations. Generally, the severity of the disease depends on the residual activity of perforin (Table 1).<sup>17</sup>

According to data from European and American HLH registration centers, FHL3 accounts for about 30–50% of FHL, second only to FHL2. However, data from Japan, South Korea, and other countries in Asia show that FHL3 is the most common and can account for up to 89% of FHL in this part of the world.<sup>18</sup> According to the statistics from the Histiocytosis Research Group of the Chinese Academy of Pediatrics in 2016, there were 323 cases of HLH in 12 hospitals in China. Among these cases, FHL accounted for 27.9%, of which 41.7% were FHL3, exceeding FHL2, which accounted for 29.2%.<sup>19</sup> These data indicate that the mutation type in FHL has a geographical location and race difference. FHL3 is an autosomal recessive inherited disease. So far, 112 mutations have been discovered. The *UNC13D* gene mutation causes the abnormal function of the Munc13-4 protein, leading to severe damage to the killing function of the NK cells. The mutation sites are all over the gene, and nonsense/missense mutations predominate (53.6%).<sup>20</sup>

## Conclusion

In the present case, both the two probands in the family were confirmed with a diagnosis of familial HPS. A pathogenic mutation in the *PRFI* was found in the probands, and there have been related literature reports concerning the heterozygous mutation of c.1349C>T. The pathogenicity of the c.853\_855del heterozygous mutation has not been reported in China.



**Table 1** Mutation of PRF1 Gene in Database

Mutation Site	Amino Acid Change
c.694C>T	Arg232Cys <sup>21</sup>
c.1191insTG	His398fsX32 <sup>21</sup>
c.1228C>T	Arg410Trp <sup>22</sup>
c.1349C>T	Thr450Met <sup>22</sup>
c.1A>G	Met1Val <sup>22</sup>
c.283T>C	Trp94Arg <sup>23</sup>
c.658G>A	Gly220Ser <sup>23</sup>
c.662C>T	Thr221Ile <sup>23</sup>
c.673C>T	Arg225Trp <sup>23</sup>
c.1182insT	Gly394FsX <sup>23</sup>
c.50delIT	Leu17fsX34 <sup>24</sup>
c.207delC	Asp70fsX37 <sup>24</sup>
c.1090-1091delCT	Leu364fsX93 <sup>24</sup>
c.3G>A	Met1Val <sup>24</sup>
c.185_195delII	Asp62fsX12 <sup>24</sup>
c.112G>A	Val38Met <sup>24</sup>
c.272C>T	Ala91Val <sup>24</sup>
c.445G>A	Gly149Ser <sup>24</sup>
c.786_801delI6	Ala262fsX22 <sup>24</sup>
c.601C>A	Pro201Thr <sup>24</sup>
c.853_855delAAG	Lys285del <sup>24</sup>
c.666C>A	His222Gln <sup>24</sup>
c.695G>A	Arg232His <sup>24</sup>
c.718C>G	Arg240Gly <sup>24</sup>
c.1122G>A	Trp374X <sup>24</sup>
c.1576delT	Leu526fsX87 <sup>24</sup>
c.847-852del6	L283-L284del <sup>25</sup>
c.657C>A	Tyr219X <sup>25</sup>
c.1288G>T	Asp430Tyr <sup>26</sup>
c.949G>A	Glu317Arg <sup>26</sup>
c.671T>A	Ile224Asp <sup>27</sup>
c.148G>A	Val50Met <sup>27</sup>
c.133G>A	Gly45Arg <sup>28</sup>
c.160C>T	Arg54Ser <sup>28</sup>
c.38G>C	Val38Leu <sup>28</sup>
c.284_287delI2	Lys284-Lys287del <sup>28</sup>
c.1304C>T	Thr435Met <sup>29</sup>
c.1262T>G	Phe421Cys <sup>29</sup>
c.914G>A	Gly305Asp <sup>29</sup>
c.1066C>T	Arg356Trp <sup>29</sup>
c.755A>G	Asn252Ser <sup>29</sup>
c.577T>C	Phe193Leu <sup>29</sup>
c.1229G>C	Arg410Pro <sup>29</sup>
c.116C>T	Pro39His <sup>29</sup>
c.1083delG	Arg361X <sup>30</sup>
c.1491T>A	Cys497X <sup>30</sup>
c.1289insG	Asp430Gly <sup>30</sup>
c.1246C>T	Gln416X <sup>30</sup>
c.757G>A	Glu253Lys <sup>30</sup>
c.253G>A	Leu85Ile <sup>30</sup>
c.190C>T	Gln64X <sup>31</sup>

(Continued)

**Table 1** (Continued).

Mutation Site	Amino Acid Change
c.548T>G	Val183Gly <sup>31</sup>
c.835G>A	Cys279Tyr <sup>31</sup>
c.1286G>A	Gly429Glu <sup>31</sup>
c.781G>A	Glu261Lys <sup>32</sup>
c.836G>A	Cys279Tyr <sup>32</sup>
c.503G>A	Ser168Asn <sup>33</sup>
c.1177T>C	Cys393Arg <sup>33</sup>
c.83G>A	Arg28His <sup>34</sup>
c.632C>T	Ala211Val <sup>34</sup>
c.93C>G	Cys31Trp <sup>34</sup>
c.145G>A	Asp49Asn <sup>35</sup>
c.1442A > C	Gln481Pro <sup>36</sup>
c.65delC	Arg22fsX29 <sup>37</sup>
c.757G>A	Glu253Lys <sup>38</sup>
c.643C>a	Leu215Ile <sup>38</sup>
c.785C>A	Ala262Asp <sup>38</sup>
c.136G>U	Glu46X <sup>38</sup>
c.355A>U	Arg119Trp <sup>38</sup>
c.1376C>T	Pro459Leu <sup>38</sup>
c.1562A>G	Tyr521Cys <sup>39</sup>
c.996_1000delGCCCG	Pro333fsX <sup>39</sup>
c.1310C>T	Ala437Val <sup>40</sup>
c.992C>T	Ser331Leu <sup>40</sup>
c.1229G>A	Arg410Gln <sup>40</sup>
c.10C>T	Arg4Cys <sup>40</sup>
c.655T>A	Tyr219Asn <sup>40</sup>
c.386G>C	Try129Ser <sup>41</sup>
c.1243G>A	Ala415Thr <sup>41</sup>
c.1471G>A	Asp491Asn <sup>41</sup>
c.833G>A	Ala278Asn <sup>14</sup>
c.658G>C	Gly220Arg <sup>14</sup>
c.1183T>C	Cys395Arg <sup>14</sup>
c.90T>G	Cys31Gly <sup>14</sup>
c.1018G>A	Asp340Asn <sup>14</sup>
c.490C>T	Gln164X <sup>14</sup>
c.528_529delinsAA	Cys176X <sup>14</sup>
c.1288insG	Asp430Gly fsX28 <sup>14</sup>
c.394G>A	Gly132Arg <sup>42</sup>
c.941_948delinsA	L314fsX327 <sup>43</sup>
c.1226C>A	Pro409His <sup>43</sup>
c.443C>A	Ala148Asp <sup>43</sup>
c.590T>G	Leu197Arg <sup>43</sup>
c.1314T>A	Tyr438 <sup>43</sup>
c.1385C>A	Ser462 <sup>43</sup>
c.150delG	Thr51fsX106 <sup>43</sup>
c.1337A>C	Gln446Pro <sup>43</sup>
c.916G>T	Gly306Cys <sup>43</sup>
c.851_862delI2	Lys284_Lys287del <sup>43</sup>
c.350_356delinsATGC	Val117_Arg119 <sup>43</sup>
c.785C>T	Ala262Val <sup>43</sup>

(Continued)

Table I (Continued).

Mutation Site	Amino Acid Change
c.688G>T	Gly230Cys <sup>43</sup>
c.382G>T	Asp128Tyr <sup>43</sup>
c.1153C>T	Arg385Cys <sup>43</sup>
c.626A>C	Gln209Pro <sup>43</sup>
c.886T>C	Tyr296H <sup>43</sup>
c.512C>A	Thr171Asn <sup>43</sup>
c.895C>A	Arg299Ser <sup>43</sup>
c.1081A>T	Arg361Trp <sup>43</sup>
c.614A>G	Asn205Ser <sup>43</sup>
c.285G>T	Trp95Cys <sup>43</sup>
c.140G>T	Gly47Val <sup>44</sup>
c.11G>A	Arg4His <sup>45</sup>
c.403G>A	Val135Met <sup>45</sup>
c.434T>C	Val145Ala <sup>45</sup>
c.796G>A	Ile266Val <sup>45</sup>
c.1313A>G	Tyr438Cys <sup>46</sup>
c.282C>A	Asn94Lys <sup>47</sup>
c.536ins	[48]
c.1133A>G	Asn378Ser <sup>48</sup>
c.937A>G	Asn312Asp <sup>48</sup>
c.1259T>C	Ile420Thr <sup>48</sup>
c.443T>C	Val148Ala <sup>48</sup>
c.422T>G	Ile148Ser <sup>48</sup>
c.286G>C	Gln96Arg <sup>48</sup>
c.626G>A	Arg209Gln <sup>48</sup>
c.1168C>T	Arg390X <sup>49</sup>
c.724G>T	Cys242Gly <sup>49</sup>
c.902C>A	Ser301Tyr <sup>49</sup>
c.528_529delinsAA	Cys176X <sup>50</sup>
c.762delC	Cys255AlafsX73 <sup>50</sup>
c.1822del	Val608CysfsX16 <sup>50</sup>
c.858+1G>A	Splice error <sup>50</sup>
c.1519G>T	Glu507X <sup>50</sup>
c.1321A>G	Leu441Val <sup>51</sup>
c.1081A>T	Arg361Trp <sup>52</sup>
c.163C>T	Arg55Cys <sup>53</sup>

However, it might also cause protein dysfunction, and this might be the cause of HLH in the probands. Meanwhile, detailed etiological diagnosis and genetic counseling has also been provided for the families of the patients.

## Abbreviations

FHL, familial hemophagocytic lymphohistiocytosis; pHLH, primary hemophagocytic lymphohistiocytosis; HLH, Hemophagocytic lymphohistiocytosis; HPS, hemophagocytic syndrome; MRI, Magnetic resonance imaging; FHPS, familial haemophagocytosis syndrome.

## Ethics Approval and Consent to Participate

This study was conducted with approval from the Ethics Committee of The Affiliated Hospital of Inner Mongolia Medical University. This study was conducted in accordance with the declaration of Helsinki. Informed written consent was obtained from patients and the children's guardians for the publication of the case details and any accompanying images.

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## Disclosure

The authors report no conflicts of interest in this work.

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