

CASE REPORT

Open Access



Identification of a novel nonsense mutation in the *UNC13D* gene from a patient with hemophagocytic lymphohistiocytosis: a case report

Xijiang Hu^{1†}, Dongling Liu^{2†}, Xiwen Jiang³, Bo Gao^{4*} and Changying Chen^{2*} 

Abstract

Background: Hemophagocytic lymphohistiocytosis (HLH) is a heterogeneous and potentially fatal disease that presents symptoms of persistent fever, splenomegaly and cytopenia. Primary HLH is identified as an autosomal recessive disorder with causative genes including *HPLH1*, *PRF1*, *UNC13D*, *STX11* and *STXBP2*.

Case presentation: Here, we reported an 8-month-old female patient with compound heterozygosity in the *UNC13D* gene. The patient, who presented typical symptoms, was diagnosed with HLH based on HLH-2004 guidelines. High-throughput amplicon sequencing for the full-length exon, including a 5 bp padding region and 6 HLH-related genes, was performed to identify the pathogenic mutations in this patient. In all, 9 heterozygous variations were detected, namely, 7 nonpathogenic SNPs, one nonsense mutation (NM_199242.2:c.2206C > T, p.Gln736X), and one splicing mutation (NM_199242.2:c.2709 + 1G > A). These two mutations were considered pathogenic according to previous studies and functional prediction. A two-generation pedigree analysis based on Sanger sequencing was performed to confirm the result.

Conclusion: Compound heterozygosity in the *UNC13D* gene was identified in trans and considered a causative mutation in a female patient with HLH. The nonsense mutation (NM_199242.2:c.2206C > T, p.Gln736X) was novel in cases of HLH. Our data expand the spectrum of HLH-related mutations in China and demonstrate the potential of high-throughput amplicon sequencing in the diagnosis of HLH.

Keywords: *UNC13D*, Nonsense mutation, HLH, Amplicon sequencing, Molecular diagnosis

Background

Hemophagocytic lymphohistiocytosis (HLH) is a heterogeneous and potentially fatal disease that presents with symptoms of persistent fever, splenomegaly and cytopenia. The first case was reported in 1952 by Farquhar et al. [1]; uncontrolled proliferation of activated macrophages and T lymphocytes are hallmarks of this syndrome [2]. Two types of HLH were described: a primary and a secondary form [3]. Primary HLH, also termed familial hemophagocytic lymphohistiocytosis (FHL), is an autosomal recessive

genetic disorder with five major causative genes, namely, *HPLH1*, *PRF1*, *UNC13D*, *STX11* and *STXBP2* (Table 1) [4]. The incidence of FHL was estimated to approximately 1:100000 and 1:50000, respectively, according to two independent studies [5, 6]; however, worldwide, the incidence of FHL is still unclear. The secondary HLH, or acquired HLH, is mostly caused by the strong immunological activation of the immune system (e.g., severe infection of virus) rather than genetic mutations, but it may also develop during malignancies. It is difficult to distinguish these two types of HLH from each other, which necessitates an approach to differential diagnoses.

Here, we performed high-throughput amplicon sequencing to detect mutations in whole exons of 6 HLH-related genes for an 8-month-old female patient, who was

* Correspondence: fromzero1121@hotmail.com; changying0000@126.com

†Equal contributors

⁴Department of Laboratory Medicine, Taihe Hospital, Hubei University of Medicine, Shiyan, Hubei, China

²School of Nursing, Zhengzhou University, Zhengzhou 450052, Henan, China
Full list of author information is available at the end of the article



Table 1 Subtypes of familial hemophagocytic lymphohistiocytosis and X-linked lymphoproliferative syndrome

Subtype	OMIM ID	Causative gene	HGNC ID	Cytogenetic location	Inheritance
FHL1	267700	<i>HPLH1</i> ^a	HGNC:23824	9q21.3 - q22	AR
FHL2	603553	<i>PRF1</i>	HGNC:9360	10q22.1	AR
FHL3	608898	<i>UNC13D</i>	HGNC:23147	17q25.1	AR ^b
FHL4	603552	<i>STX11</i>	HGNC:11429	6q24.2	AR
FHL5	613101	<i>STXBP2</i>	HGNC:11445	19p13.2	AR ^b
XLP1	308240	<i>SH2D1A</i>	HGNC:10820	Xq25	XLR
XLP2	300635	<i>XIAP</i>	HGNC:592	Xq25	XLR

AR autosomal recessive, XLR X-linked recessive

^a The HGNC locus types of *HPLH1* is phenotype only, meaning mapped phenotypes where the causative gene has not been identified (SO:0001500)

^b Both FHL3 and FHL5 could be caused by homozygous or compound heterozygous mutations in causative gene

diagnosed with HLH based on HLH-2004 guidelines [7]. As a result, 9 heterozygous variations, namely, 7 SNPs, one nonsense mutation (c.2206C > T) and one splicing mutation (c.2709 + 1G > A), in the *UNC13D* gene were detected. The nonsense mutation was reported in HLH for the first time. The compound heterozygous mutations in the *UNC13D* gene were considered causative mutations for the patient based on previous studies and functional predictions. A two-generation pedigree analysis showed that they were inherited from the parents. The result suggests great potential of high-throughput amplicon sequencing in the diagnosis of HLH.

Case presentation

This study was approved by the ethics committee on the use of human subjects at Wuhan Children's Hospital. Informed consent from the patient's parents was obtained before collecting blood samples. The patient and her parents are all Han Chinese from Hubei province of China.

This 8-month-old female patient was admitted to Wuhan Children's Hospital after 2 days of fever, with a temperature measured at 39.0 °C on regular examination. Blood analysis showed low platelets (PLT, $45 \times 10^9/L$) and low hemoglobin (Hb, 7.7 g/100 mL). Low NK cell activity (4.45%) and plasma albumin (18.2 g/L) were also observed. Moderate splenomegaly was revealed by ultrasound examination. The bone marrow examination suggested hemophagocytosis (Fig. 1), and no features of malignancy were observed. Since six of the eight criteria of HLH-2004 were fulfilled [7], this patient was diagnosed with HLH. To confirm the subtype of HLH and to investigate causative mutations in this patient, high-throughput amplicon sequencing and two-generation pedigree analysis were performed.

Genomic DNA was purified from peripheral blood mononuclear cells (PBMCs) with a commercial kit purchased from TIANGEN Biotech (Beijing, China). Multiple PCR primers were designed for 6 genes using Ion AmpliSeq™ Designer (Table 1); the *SH2D1A* and *XIAP* genes were included for distinguishing X-linked

lymphoproliferative syndrome (XLP), which has a strong resemblance to HLH [8]. All exons of these genes were covered by 198 amplicons, including 5 bp of padding region. High-throughput amplicon sequencing was performed using an ION S5XL genetic analyzer (Thermo Fisher Scientific, MA, USA). VariantCaller V1.0 was used to detect mutations from sequencing data.

As a result, 9 heterozygous variations were detected, namely, 5 synonymous mutations, one missense mutation, one noncoding region mutation, one nonsense mutation and one splicing mutation (Table 2). All mutations were considered to be benign according to the dbSNP database except for the nonsense mutation NM_199242.2:c.2206C > T and the splicing mutation NM_199242.2:c.2709 + 1G > A. The splicing mutation c.2709 + 1G > A has been reported in patients with HLH with compound heterozygous mutations in the *UNC13D* gene [9, 10], it was predicted to be broken wild-type donor site and most probably affecting splicing, according to the human splicing finder (HSF, version 3.1). However, the nonsense mutation c.2206C > T, which resulted in the introduction of a premature stop codon and produced truncated

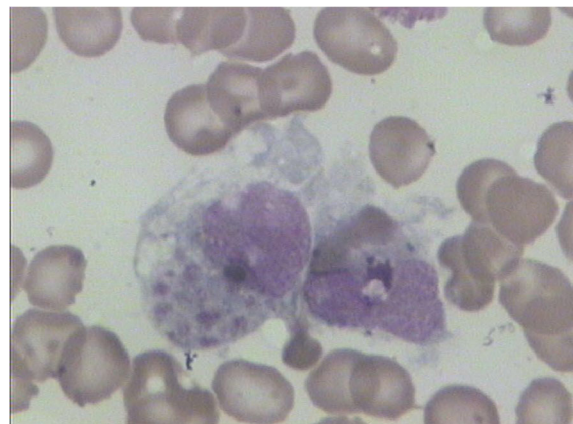


Fig. 1 The bone marrow examination. Phagocytosis was clearly observed without any evidence of malignancy

Table 2 Detected variations in the patient with HLH

Mutation	Position in reference genome GRCh37	Amino acid change	Type	Frequency (%)	Zygoty	ID in dbSNP	Allele Frequency in ExAC
<i>PRF1</i> :c.900C > T	NC_000010.10:g.72358577	p.His300His	Synonymous	49	Heterozygous	rs885822	0.6357
<i>UNC13D</i> :c.3198A > G	NC_000017.10:g.73824121	p.Glu1066Glu	Synonymous	53	Heterozygous	rs7210574	0.4844
<i>UNC13D</i> :c.2599A > G	NC_000017.10:73827205	p.Lys867Glu	Missense	50	Heterozygous	rs1135688	0.3666
<i>UNC13D</i> :c.1977C > T	NC_000017.10:73831016	p.Thr659Thr	Synonymous	43	Heterozygous	rs2290770	0.0318
<i>UNC13D</i> :c.888G > C	NC_000017.10:73836162	p.Pro296Pro	Synonymous	49	Heterozygous	rs3744026	0.1525
<i>UNC13D</i> :c.279C > T	NC_000017.10:73839137	p.Pro93Pro	Synonymous	53	Heterozygous	rs3744007	0.0518
<i>XIAP</i> :c.*12A > G	NC_000023.10:g.123041043	–	Non-coding region	51	Heterozygous	rs28382740	0.2695
<i>UNC13D</i> :c.2709 + 1G > A	NC_000017.10:73826658	–	Splicing	49	Heterozygous	not applicable	not applicable
<i>UNC13D</i> :c.2206C > T	NC_000017.10:73830498	p.Gln736X	Nonsense	48	Heterozygous	not applicable	not applicable

mRNA (p.Gln736X), was reported in HLH for the first time. The compound heterozygosity was considered to be causative in this case, based on functional alteration analysis and previous reports.

Both mutations were confirmed by Sanger sequencing, and a two-generation pedigree analysis was performed with the parents' samples. The results showed these two mutations were inherited from the father and mother (Fig. 2), presenting a typical autosomal recessive mode of inheritance.

Discussion and conclusions

In this case, an 8-month-old female patient was admitted to Wuhan Children's Hospital and diagnosed with HLH based on clinical features, including persistent fever, moderate splenomegaly, low PLT and Hb, decreased NK-cell activity, and hemophagocytosis with no evidence of malignancy. Compound heterozygous mutations consisting of a nonsense mutation (c.2206C > T) and a splicing mutation (c.2709 + 1G > A) in the *UNC13D* gene were identified in this patient using high-throughput amplicon sequencing. A two-generation pedigree analysis based on Sanger sequencing confirmed the results and revealed a typical autosomal recessive inheritance mode in this family.

HLH is a heterogeneous disease with two major conditions and five subtypes; additionally, many other conditions can lead to the clinical picture of HLH, such as malignancies, rheumatoid disorders, and XLP [7]. Molecular diagnosis based on genetic tests provide a powerful tool for differential diagnoses of HLH, which is important for appropriate treatment. Bianca et al. reported an approach for the genetic screening of patients with HLH using targeted high-throughput sequencing of 12 related genes, which resulted in a diagnosis in 22 of 58 patients in the prospective cohort [11]. Further in-depth investigations of the phenotype-genotype relationship of

HLH are required to develop better sequencing panels for diagnosis.

The *UNC13D* gene, which encodes a protein involved in the cytotoxic activity of T lymphocytes, was confirmed as a causative gene of familial HLH type 3. Pathogenic mutations in the *UNC13D* gene are very common in patients with HLH, as 17–19% of FHL patients from Turkey and Germany, 89% from Korea, and 30% from Japan were identified to have pathogenic mutations in the *UNC13D* gene [12–14]. Compound heterozygosity in the *UNC13D* gene was also reported, especially in the splicing site [9, 10]. However, there has been no genetic investigation on a substantial number of HLH patients in China. In this case, we identified the causative mutations of this patient in 24 h, using our customized panel which was designed automatically with online tool and purchased from Thermo Fisher Scientific (MA, USA). Average base coverage depth of the 55 kb target region was 1070-fold, 96.44% of total base was sequenced more than 100-fold. The concordance rate of amplicon sequencing and sanger sequencing is 100% in this case. The cost of this approach is about 1/5 to 1/10 of whole exome sequencing. The application of high-throughput amplicon sequencing would be helpful to diagnose genetic disease with an efficient and cost-saving way.

In conclusion, compound heterozygosity consisting of a nonsense mutation (c.2206C > T) and a splicing mutation (c.2709 + 1G > A) in the *UNC13D* gene was identified in a patient with HLH using high-throughput amplicon sequencing. Both mutations were confirmed by Sanger sequencing and were inherited from the parents. Our results expand the spectrum of *UNC13D* mutations in Chinese patients with HLH and demonstrate the potential of high-throughput amplicon sequencing in the diagnosis of heterogeneous genetic disorders.

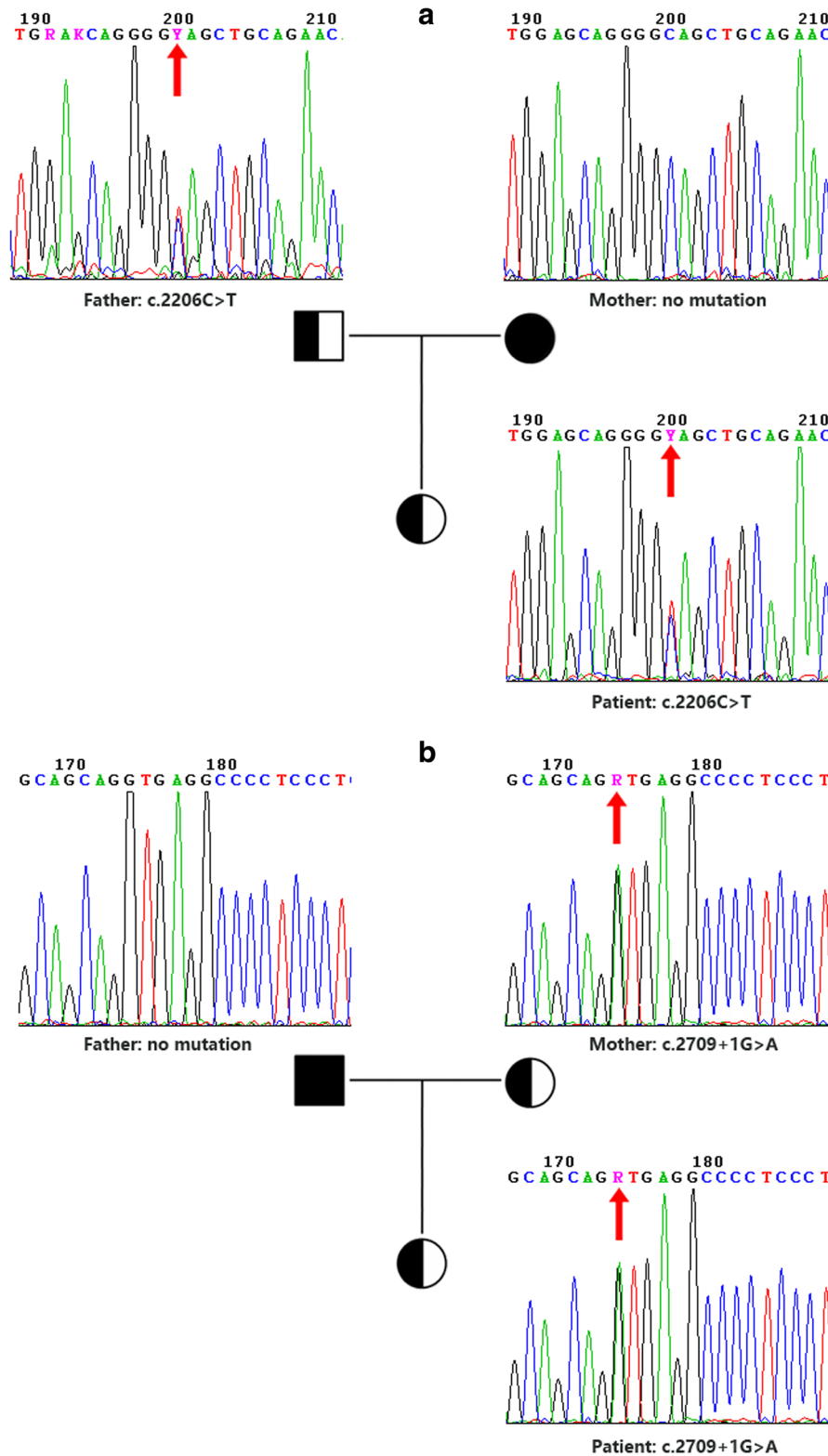


Fig. 2 Two-generation pedigree analysis for two heterozygous mutations in the *UNC13D* gene. **a** Novel nonsense mutation (c.2206C > T) in *UNC13D*-exon24; **b** Reported splicing mutation (c.2709 + 1G > A) in *UNC13D*-exon28. The position of the mutation is marked with a red arrow. The results showed that the nonsense mutation was inherited from father and the splicing mutation was inherited from mother. Zygosity was indicated by letters of degenerate bases (Y: C/T, R: A/G)

Abbreviations

FHL: Familial hemophagocytic lymphohistiocytosis; Hb: Hemoglobin; HLH: Hemophagocytic lymphohistiocytosis; PBMC: Peripheral blood mononuclear cells; PLT: Platelets; XLP: X-linked lymphoproliferative syndrome

Acknowledgments

The authors appreciate the participation of the patient's family in this clinical study. This work was supported by the Wuhan Children's Hospital and Taihe Hospital. We are grateful to Cheng Wan from Peking University for helping with the manuscript and providing meaningful discussion.

Funding

Key Research Projects of Henan Provincial Department of Education. ID: 17A320051.

Availability of data and materials

The sequencing data were deposited into the NCBI Short Read Archive under accession number SRP124825.

Authors' contributions

XH, DL and XJ performed the experiments. BG carried out the blood analysis and analyzed the data. XH and DL analyzed the data and wrote the manuscript. XJ revised the manuscript. BG and CC designed this study and edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved according to the guidelines of the Committee on the Use of Human Subjects in Wuhan Children's Hospital.

Consent for publication

Written informed consent for the publication of the patient's medical data was obtained from the parents before collecting samples. A copy of the written consent is available for review by the editor of this journal.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details

¹Wuhan Children's Hospital (Wuhan Maternal and Child Healthcare Hospital), Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430016, Hubei, China. ²School of Nursing, Zhengzhou University, Zhengzhou 450052, Henan, China. ³DaAn Gene Co., Ltd. Of Sun Yat-sen University, The Medicine and Biological Engineering Technology Research Center of the Ministry of Health, Guangzhou, Guangdong, China. ⁴Department of Laboratory Medicine, Taihe Hospital, Hubei University of Medicine, Shiyan, Hubei, China.

Received: 22 January 2018 Accepted: 1 May 2018

Published online: 21 May 2018

References

- Bell RJ, Brafield AJ, Barnes ND, France NE. Familial Haemophagocytic Reticulosis. *Arch Dis Child*. 1952;27(136):519–25.
- Filipovich AH. Hemophagocytic lymphohistiocytosis (HLH) and related disorders. *Hematology*. 2009;2009(1):127–31.
- Janka GE. Familial and acquired hemophagocytic lymphohistiocytosis. *Annu Rev Med*. 2012;63(1):233–46.
- Mehta RS, Smith RE. Hemophagocytic lymphohistiocytosis (HLH): a review of literature. *Med Oncol*. 2013;30(4):740.
- Henter J, Elinder G, Soder O, Ost A. Incidence in Sweden and clinical features of familial Hemophagocytic Lymphohistiocytosis. *Acta Paediatr*. 1991;80(4):428–35.
- Niece JA, Rogers ZR, Ahmad N, Langevin AR, McClain KL. Hemophagocytic lymphohistiocytosis in Texas: observations on ethnicity and race. *Pediatr Blood Cancer*. 2010;54(3):424–8.
- Henter J, Horne A, Egeler RM, Filipovich AH, Imashuku S, Ladisch S, McClain K, Webb D, Winiarski J, Janka G. HLH-2004: diagnostic and therapeutic

- guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer*. 2007;48(2):124–31.
- Arico M, Imashuku S, Clementi R, Hibi S, Teramura T, Danesino C, Haber DA, Nichols KE. Hemophagocytic lymphohistiocytosis due to germline mutations in SH2D1A, the X-linked lymphoproliferative disease gene. *Blood*. 2001;97(4):1131–3.
- Zhizhuo H, Junmei X, Yuelin S, Qiang Q, Chunyan L, Zhengde X, Kunling S. Screening the PRF1, UNC13D, STX11, SH2D1A, XIAP, and ITK gene mutations in Chinese children with Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer*. 2012;58(3):410–4.
- Liu D, Hu X, Jiang X, Gao B, Wan C, Chen C. Characterization of a novel splicing mutation in UNC13D gene through amplicon sequencing: a case report on HLH. *BMC Med Genet*. 2017;18(1):135.
- Tesi B, Lagerstedt-Robinson K, Chiang SC, Ben Bdira E, Abboud M, Belen B, Devecioglu O, Fadoo Z, Yeoh AE, Erichsen HC, et al. Targeted high-throughput sequencing for genetic diagnostics of hemophagocytic lymphohistiocytosis. *Genome Med*. 2015;7:130.
- Stadt UZ, Beutel K, Kolberg S, Schneppenheim R, Kabisch H, Janka G, Hennies HC. Mutation spectrum in children with primary hemophagocytic lymphohistiocytosis: molecular and functional analyses of PRF1, UNC13D, STX11, and RAB27A†. *Hum Mutat*. 2006;27(1):62–8.
- Yoon HS, Kim H, Yoo K, Sung K, Koo H, Kang HJ, Shin HY, Ahn HS, Kim J, Lim Y. UNC13D is the predominant causative gene with recurrent splicing mutations in Korean patients with familial hemophagocytic lymphohistiocytosis. *Haematologica*. 2010;95(4):622–6.
- Yamamoto K, Ishii E, Sako M, Ohga S, Furuno K, Suzuki N, Ueda I, Imayoshi M, Yamamoto S, Morimoto A. Identification of novel MUNC13-4 mutations in familial haemophagocytic lymphohistiocytosis and functional analysis of MUNC13-4-deficient cytotoxic T lymphocytes. *J Med Genet*. 2004;41(10):763–7.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

