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Published online 2023 January 09 **Short report**

Compound heterozygous mutation in PRF1 gene in a Patient with familial hemophagocytic lymphohistiocytosis-2

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Abstract

Background: Familial Hemophagocytic lymphohistiocytosis is an autosomal recessive lethal disorder caused by mutations in several genes. The immune system is overactivated in this disease and several organs such as liver and spleen are affected. Since Haematopoitic stem cell transplantation is essencial for surviving the patients with familial hemophagocytic lymphohistiocytosis, the early diagnosis is critical.

Objectives

The aim of this study was to determine the probable genetic cause of disease in an infant with unclear primary diagnosis and suspected to suffer from blood cancer, Hemophagocytic lymphohistiocytosis or polycyctic liver disease.

Methods

Whole exome sequencing and Sanger sequencing were used to investigate the mutation and its confirmation respectively.

Results

We found a compound heterozugous mutation in PRF1 gene including one novel nonsense and one previously reported missense mutations.

Conclusions

Due to the disease and clinical heterogeneity, next generation sequencing is the recommended method to find the disease causing mutations and confirm the disease.

Keywords: Hemophagocytic lymphohistiocytosis, PRF1, Whole exome sequencing, immune system

Background

Hemophagocytic lymphohistiocytosis (HLH) is a severe lifethreatening syndrome of defective apoptosis in which the uncontrolled activated lymphocytes and macrophages secrete more cytokines than is necessary. The clinical symptoms include prolonged fever, pancytopenia, hepatosplenomegaly, hemophagocytosis, multi-organ failure and neurological problems. High fever and pancytopenia in HLH result in the liver and spleen enlargement and liver dysfunction (1-4). There are two forms of HLH: The primary form is a familial autosomal recessive disease caused by mutations in genes encoding proteins that have roles in inactivation of the immune system. The secondary form is related to infections, autoimmune, malignancy, metabolic disorders, transplantations, chimeric antigen receptor T-cell therapies, and etc.

The incidence of primary HLH is almost 1.2 in 1,000,000 under the age of 15 (1). Due to the clinical heterogeneity of HLH, its diagnosis is challenging (1-3).

In order to improve survival, allogenic hematopoietic stem cell transplantation is highly recommended for cases with relapse or resistance to the initial therapy as soon as possible (3).

Here we report an 18-month-old infant boy with initial ambiguous diagnosis who was referred to Tehran Medical Genetics Laboratory to be checked for variants in genes responsible for blood cancer, Hemophagocytic Lymphohistiocytosis (HLH) and polycyctic liver disease (PLD) with whole exome sequencing. His clinical symptoms started with fever at 12 months and aggravated to enlargement of abdominal area and death at 18 months of age. His parents were not relative and they had no other children.

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Methods

Sample Preparation

Informed written consent was obtained from the patient's parents. Peripheral blood was taken from the family members. DNA extraction was performed using column based kits from GeneAll Biotechnology Co., Ltd.

Whole Exome Sequencing and Bioinformatic Analysis

The patient's DNA was sent to BGI Company in China for whole exome sequencing. Agilent SureSelect V7 was used as capture kit. Base calling converted the images generated by sequencers into nucleotide sequences, which are called raw data or raw reads and are stored in FASTQ format. The raw reads were filtered so the adaptor sequences, contamination and low-quality reads were removed. Mapping the sequencing reads was performed using BWA-MEM. Variant calling process done by GATK HaplotypeCaller and DeepVariant. VCF file was filtered for the genes relevant to the disease. Then the variants were investigated based on databases such as HGMD (academic version), ClinVar, gnomAD, ExAC, Kaviar, ESP6500, GME, 1000G and Iranome. The investigation for relevant variants was focused on coding exons and flanking intronic bases.

All potential modes of inheritance patterns were considered. In addition, provided family history and clinical information were used to evaluate identified variants.

Sanger sequencing confirmation

Sanger sequencing was used to confirm the detected variants and variant - disease segregation in the family. Primers surrounding the mutations were designed using GeneRunner software and Primer designing tool- NCBI (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). The targets were amplified and the Cycle sequencing reactions were performed using ABI BigDye terminator and ABI 3130 genetic analyser.

Results

Whole exome sequencing revealed a compound heterozygous mutation in PRF1 gene in the patient. The c.C977G:p.S326X (NM_005041) in exon 3 of PRF1 gene is a novel nonsense mutation which is classified as likely pathogenic according to ACMG guideline and the c.G445A:p.G149S in exon 2 which is a known pathogenic variant in the same gene (Figure 1). Sanger sequencing confirmed the mutations and its segregation in the family. We did not find any disease causing variant in the PLD relative genes.

Figure 1. chr10:g.70598744G>C (c.C977G:p.S326X) and chr10:g.70600458C>T (c.G445A:p.G149S) compound heterozygous mutations in PRF1 gene in a patient with HLH disease.

Discussion

Hemophagocytic lymphohistiocytosis is a heterogeneous aggressive, potentially fatal disease in which the immune system is overactivated and several organs such as liver and spleen are affected. In order to avoid life threatening complications, early diagnosis and treatment are critical (1, 5).

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Familial hemophagocytic lymphohistiocytosis-2 (FHL2) is one of the primary HLH disorders caused by mutations in the PRF1 gene on chromosome 10q22 which is inherited with an autosomal recessive manner. This gene encodes perforin which is expressed in activated cytotoxic lymphocytes and natural killer cells. It forms membrane pores for releasing of granzymes followed by cytolysis of target cells such as tumor or virus infected cells. Almost 15-50% of familial hemophagocytic lymphohistiocytosis are caused by the Mutations in PRF1 gene (6-9).

PRF1 mutations cause perforin expression to be reduced or abolished on the surface of cytotoxic cells. So the cytotoxic T cells and NK cells became unable to destroy their target cells. This result in T cells and macrophages hyperactivation and increased production of cytokines such as gamma-interferon (IFNG) and TNF-alpha and emerging the HLH symptoms. So hemophagocytosis happen in Lymph nodes, Bone marrow, spleen and liver. Chemotherapy and immunosuppressant therapy may result in Improvement of the disease symptoms, but the disease is lethal without hematopoietic stem cell transplantation (9).

We found a compound heterozygous mutation in PRF1 gene. The c.C977G:p.S326X (NM_005041) in exon 3 of PRF1 gene is a novel nonsense mutation which changes the amino acid Serine in the position 326 to a stop codon. It is classified as likely pathogenic according to ACMG guideline. The c.G445A:p.G149S (rs147462227) is a missense variant in exon 2 of this gene. According to ClinVar this mutation cause Aplastic anemia, Familial hemophagocytic lymphohistiocytosis 2 and inborn genetic diseases (https://www.ncbi.nlm.nih.gov/snp/rs147462227#clinical_significance) (10, 11).

Both mutations happened in MACPF (membrane attack complex/perforin family) domain of Perforin. This domain (amino acids 27-375) includes the central machinery of pore formation (https://www.uniprot.org).

Conclusion

HLH has disease heterogeneity and happens by mutations in several genes. It has also phenotypic heterogeneity which makes the diagnosis difficult. So next generation sequencing is the recommended method to determine the disease causing mutations in suspected HLH patients which provide an early diagnosis and subsequent treatment.

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References

1. Nepesov S, Yaman Y, Elli M, Bayram N, Özdilli K, Kıykım A, et al. Clinical, Genetic, and Outcome Characteristics of Pediatric Patients with Primary Hemophagocytic Lymphohistiocytosis. Turkish Archives of Pediatrics. 2022;57(4):398.
2. Kacar AG, Celkan T. Hemophagocytic lymphohistiocytosis. 2022.
3. Kim YR, Kim D-Y. Current status of the diagnosis and treatment of hemophagocytic lymphohistiocytosis in adults. Blood research. 2021;56(S1):17-25.
4. Ericson KG, Fadeel B, Nilsson-Ardnor S, Söderhäll C, Samuelsson A, Janka G, et al. Spectrum of perforin gene mutations in familial hemophagocytic lymphohistiocytosis. The American Journal of Human Genetics. 2001;68(3):590-7.
5. 5. Wegehaupt O, Wustrau K, Lehmberg K, Ehl S. Cell versus cytokine–directed therapies for hemophagocytic lymphohistiocytosis (HLH) in inborn errors of immunity. Frontiers in Immunology. 2020;11:808.
6. 6. Solomou EE, Gibellini F, Stewart B, Malide D, Berg M, Visconte V, et al. Perforin gene mutations in patients with acquired aplastic anemia. Blood, The Journal of the American Society of Hematology. 2007;109(12):5234-7.
7. 7. Thiery J, Keefe D, Saffarian S, Martinvalet D, Walch M, Boucrot E, et al. Perforin activates clathrin-and dynamin-dependent endocytosis, which is required for plasma membrane repair and delivery of granzyme B for granzyme-mediated apoptosis. Blood, The Journal of the American Society of Hematology. 2010;115(8):1582-93.
8. 8. Praper T, Sonnen A, Viero G, Kladnik A, Froelich CJ, Anderluh G, et al. Human perforin employs different avenues to damage membranes. Journal of Biological Chemistry. 2011;286(4):2946-55.
9. 9. Liu C, Li M, Wu X, Yao X, Zhao L. Type 2 familial hemophagocytic lymphohistiocytosis in half brothers: a case report. Medicine. 2018;97(30).
10. 10. Stadt UZ, Beutel K, Kolberg S, Schneppenheim R, Kabisch H, Janka G, et al. Mutation spectrum in children with primary hemophagocytic lymphohistiocytosis: molecular and functional analyses of PRF1, UNC13D, STX11, and RAB27A. Human mutation. 2006;27(1):62-8.
11. 11. Tesi B, Chiang SC, El-Ghoneimy D, Hussein AA, Langenskiöld C, Wali R, et al. Spectrum of atypical clinical presentations in patients with biallelic PRF1 missense mutations. Pediatric blood & cancer. 2015;62(12):2094-100.

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