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LETTER TO EDITOR

A Cry for the Development of Newborn Screening for Familial Hemophagocytic Lymphohistiocytosis

Akihiro Hoshino[1,2] · Yang Xi[1,3] · Tomohei Nakao[4,5] · Keisuke Kato[4] · Satoshi Fujiyama[6,7] · Katsuyoshi Koh[8] · Toshihiko Imamura[9] · Hirokazu Kanegane[1,10]

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To the Editor,

The current clinical approach for identifying inborn errors of immunity (IEI) has been dramatically changed by two diagnostic and screening modalities: the next-generation sequencing (NGS) and newborn screening (NS) for severe combined immunodeficiency (SCID). The ability of NGS technology to use genetic information for diagnoses has significantly contributed to improved clinical judgment in choosing the best therapeutic approach in IEI patients. T cell receptor excision circle-based NS programs have enabled presymptomatic patients with SCID to be identified, leading to early treatment with good outcomes. These achievements are inspiring for the desire to continue to improve outcomes in other severe IEI. Recently, new technologies including bead array or proteomic analysis using dried blood spot (DBS) samples have demonstrated the potential of early diagnosis of IEI through NS [1–3]. On the other hand, there are quite limited reports that directly mention their importance in the clinical setting.

Familial hemophagocytic lymphohistiocytosis (FHL) is a severe IEI; it is characterized by hyperinflammation due to impaired cytotoxicity of $CD8^+$ T cells and NK cells. Although an allogenic hematopoietic stem cell transplantation (HSCT) is curative, early mortality before HSCT and late neurological sequelae have not been resolved for more than two decades. Two patients, who are now deceased, presented with FHL before the introduction of NGS; the generic diagnosis was confirmed by the diagnosis of FHL in their younger siblings. Herein, the current NGS approach, as well as what the future of NS may bring, is discussed.

A 4-month-old boy (P1.1) presented with fever and splenomegaly (Supplementary Fig. A). He did not fulfill the hemophagocytic lymphohistiocytosis (HLH)-2004 criteria with normal serum ferritin levels. He displayed bicytopenia with normal neutrophil count. Bone marrow aspiration revealed normal cellular marrow without evidence of malignancy or hemophagocytosis. However, soluble IL-2 receptor levels were elevated. PCR analyses were negative for Epstein-Barr virus, cytomegalovirus, human parvovirus B19, herpes simplex viruses 1 and 2, and human herpesviruses 6 and 7. His clinical course was unremarkable until day 10 of hospitalization. But, abnormal coagulation tests and hyperferritinemia were observed, which were consistent with HLH. The patient was treated with chemotherapy; however, he died of multi-organ failure (MOF) on day 15 of hospitalization. His younger sibling (P1.2) developed fever and

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✉ Hirokazu Kanegane
hkanegane.ped@tmd.ac.jp

1 Department of Pediatrics, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan

2 Department of Pediatrics and Developmental Biology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan

3 Division of Rheumatology and Immunology, Children's Hospital of Chongqing Medical University, Chongqing, China

4 Department of Pediatric Hematology and Oncology, Ibaraki Children's Hospital, Mito, Japan

5 Department of Pediatrics, Dokkyo Medical University Saitama Medical Center, Saitama, Japan

6 Department of Neonatology, Ibaraki Children's Hospital, Mito, Japan

7 Department of Pediatrics, University of Tsukuba Hospital, Tsukuba, Ibaraki, Japan

8 Department of Hematology/Oncology, Saitama Children's Medical Center, Saitama, Japan

9 Department of Pediatrics, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan

10 Department of Child Health and Development, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan

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cytopenia when 1-month-old. He fulfilled the HLH-2004 criteria. Perforin expression was absent (Supplementary Fig. B). Sanger sequencing identified compound heterozygous *PRF1* mutations (p.C381X, p.D430GfsX28), leading to the diagnosis with FHL2 (Supplementary Fig. C). He received chemotherapy following the HLH-2004 protocol; however, he died of HLH/MOF before HSCT. The identical compound heterozygous mutations were retrospectively found using a stored DNA sample from P1.1 (Supplementary Fig. C).

P2.1 showed hydrops fetalis at 30 weeks of gestation and was delivered by emergency cesarean section (Supplementary Fig. A). The parents were not consanguineous. The patient had diffuse subcutaneous edema and massive hepatosplenomegaly. Her white blood cell count was 25.1×10^9/L with 4% segmented neutrophils, 14% immature neutrophils, 32% lymphocytes, 4% monocytes, 6% atypical lymphocytes, and 40% blasts. Although there was no anemia, there was thrombocytopenia with prolonged coagulation time. General care was given to the patient, including an exchange transfusion; however, she died of MOF, pulmonary hemorrhage, and cerebral hemorrhage on day six after birth. Postmortem examination revealed hepatocellular injury with lymphocyte infiltrations, but not hematopoiesis. Her younger sibling (P2.2) developed HLH at 2 months of age. He was diagnosed with FHL2 because of the absent expression of perforin and homozygous *PRF1* mutation (p.L364EfsX92) (Supplementary Fig. B, C). The patient was treated following the HLH-2004 protocol but died of HLH/MOF. Genetic analysis using a stored DNA sample from P2.1 showed an identical homozygous mutation (Supplementary Fig. C). Their younger sibling (P2.3) was also born and had the same mutation. He was diagnosed in the asymptomatic phase, followed by successful HSCT.

Both P1.1 and P2.1 were diagnosed with FHL2, which was screened after the diagnosis of FHL2 in their younger siblings (P1.2 and P2.2). Genetic diagnosis by Sanger sequencing could not be performed during their lifetime. Although P1.1 was diagnosed with HLH, his early death did not allow further examinations for FHL, including perforin expression or degranulation assays. P2.1 showed atypical clinical presentations, which can lead to missed diagnoses, even retrospectively. In addition to our observation, seven patients who had FHL that presented as hydrops fetalis have also been reported [a-e]. P1.1 is an appropriate candidate for the target gene panel (TGP) when utilizing NGS. TGP incurs lower cost, and requires less time, and labor due to the genetic heterogeneity of FHL, when compared with Sanger sequencing. Rapid diagnosis by TGP is within a few days, sometimes 24 h, and has significantly contributed to the early and ample treatment. P2.1, which is a candidate for whole-exome sequencing (WES) or whole-genome sequencing (WGS), could lead to the diagnosis of FHL2. However, preventing their death might not be easy, especially in P2.1. Unfortunately, P1.2 and P2.2 died of HLH/MOF before HSCT despite proper diagnosis after the onset of FHL. Over the two decades, more intensive chemotherapies have not reduced early mortality rates. Alternative therapeutic approaches including targeted therapy, as well as new modalities for presymptomatic diagnosis are therefore being investigated.

FHL is a candidate for NS given the life-threatening nature after onset, coupled with the improved outcome that are seen after early diagnosis and intervention, as well as the current availability of curable therapy. Indeed, P2.3, who was given the presymptomatic diagnosis due to his family history, is doing well after HSCT. Whereas P2.1 may be too severe to be salvaged, her proper diagnosis could have implications for family counseling. Table 1 shows the comparisons of NS for FHL. PCR-based NS has been described for FHL3 due to *UNC13D* inversion, a Scandinavian founder mutation [4].

Table 1 Comparisons of newborn screening methods for familial hemophagocytic lymphohistiocytosis

	Strengths	Limitations
Mass spectrometry-based screening	- None	- No detection of FHL - Normal amino acid/organic acid/fatty acid β-oxidation in FHL
PCR-based screening	- Detection of *UNC13D* inversion, a Scandinavian founder mutation	- No detection of other FHL - Normal TRECs and KRECs in FHL
NGS-based screening (TGP/WES/WGS)	- Potential detection of FHL	- Detection of FHL with milder phenotype - Identification of VUSs/secondary findings
Proteome-based screening	- Potential detection of severe FHL	- Detection of only protein-null mutations - Verification is required
Bead array-based screening	- Potential detection of severe FHL	- Detection of only protein-null mutations - Higher cost than proteome-based screening - Verification is required

FHL familial hemophagocytic lymphohistiocytosis, *KRECs* kappa-deleting recombination excision circles, *NGS* next-generation sequencing, *TGP* target gene panel, *TRECs* T cell receptor excision circles, *VUS* variants of unknown significance, *WES* whole-exome sequencing, *WGS* whole-genome sequencing

Targeting IEI including FHL, NGS-based NS has been conducted using TGP, WES, or WGS [5]. While this is a powerful approach to identify variants, ethical and social issues remain to be resolved for its wide implementation. This approach can identify hypomorphic variants or variants of unknown significance in FHL-causative genes. There are no guidelines on the management of FHL patients having residual cytotoxicity, who show varying clinical courses depending on the type of gene and mutation and might not present until adulthood. Although NS would enable HSCT before the onset, not all patients may benefit from HSCT in infancy, which may cause significant complications, especially in such patients. This approach can also potentially identify pathogenic variants, by chance, known to cause disease for which there is no treatment. On the other hand, protein detection-based NS have been developing. Nakajima et al. recently identified more than 500 disease-related proteins including causative proteins of FHL with proteomic analysis using DBS samples [3]. Although not yet tested in FHL, several causative proteins of IEI also could be identified with bead array [1]. These methods may have the advantage of detecting only patients who should receive HSCT, because the protein defects are identified. Although those approaches should be prospectively evaluated to confirm their usefulness in NS for FHL, presymptomatic diagnosis of FHL by NS and subsequent early treatment for patients expected to have a poor prognosis could contribute to better outcomes. Although NGS has focused on correct diagnoses, work on implementing earlier diagnosis has already begun.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

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