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Frequency and spectrum of disease-causing variants in 1892 patients with suspected genetic HLH disorders

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Key Points

- A definite genetic diagnosis was made in 10.4% of 1892 patients with suspected HLH by a panel approach including 15 HLH-associated genes.
- HLH next-generation sequencing panels were ~400 test orders per year; single-gene tests related to HLH have drastically decreased.

This article explores the distribution and mutation spectrum of potential disease-causing genetic variants in hemophagocytic lymphohistiocytosis (HLH)–associated genes observed in a large tertiary clinical referral laboratory. Samples from 1892 patients submitted for HLH genetic analysis were studied between September 2013 and June 2018 using a targeted next-generation sequencing panel approach. Patients ranged in age from 1 day to 78 years. Analysis included 15 genes associated with HLH. A potentially causal genetic finding was observed in 227 (12.0%) samples in this cohort. A total of 197 patients (10.4%) had a definite genetic diagnosis. Patients with pathogenic variants in familial HLH genes tended to be diagnosed significantly younger compared with other genes. Pathogenic or likely pathogenic variants in the *PRF1* gene were the most frequent. However, mutations in genes associated with degranulation defects (*STXBP2*, *UNC13D*, *RAB27A*, *LYST*, and *STX11*) were more common than previously appreciated and collectively represented >50% of cases. X-linked conditions (*XIAP*, *SH2D1A*, and *MAGT1*) accounted for 17.8% of the 197 cases. Pathogenic variants in the *SLC7A7* gene were the least encountered. These results describe the largest cohort of genetic variation associated with suspected HLH in North America. Merely 10.4% of patients were identified with a clearly genetic cause by this diagnostic approach; other possible etiologies of HLH should be investigated. These results suggest that careful thought should be given regarding whether patients have a clinical phenotype most consistent with HLH vs other clinical and disease phenotypes. The gene panel identified known pathogenic and novel variants in 10 HLH-associated genes.

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is an aggressive and life-threatening syndrome of hyperinflammation characterized by pathologic activation and proliferation of T cells and macrophages. Although HLH frequently affects infants, it is also observed in children and adults of all ages. HLH can occur as a typical or principal manifestation of several genetically heterogeneous disorders. A group of diseases known as familial HLH types 2-5 are caused by pathogenic variants in *PRF1*, *UNC13D*, *STX11*, and *STXBP2*, respectively, which are all critical for normal cytotoxic lymphocyte granule-mediated cytotoxicity.[1-5] In addition, loss-of-function mutations in the *LYST*, *RAB27A*, and *AP3B1* genes cause problems in the formation of the cytotoxic granules or transport of the granules through the cytoplasm[6-8] and can also lead to HLH as well as other problems such as pigmentary dilution in the disorders known as Chediak-Higashi syndrome, Griscelli syndrome type 2, and Hermansky-Pudlak syndrome type 2,

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The full-text version of this article contains a data supplement.

respectively. Other genetic disorders with more complex mechanisms of diseases that are associated with a high risk of HLH include X-linked lymphoproliferative syndrome type 1 and 2 (XLP1 and XLP2) caused by mutations in the *SH2D1A*[9] and *XIAP*[10] genes, respectively, X-linked immunodeficiency with magnesium defect, Epstein-Barr virus infection and neoplasia caused by defects in the *MAGT1* gene,[11] CD27 deficiency from loss of function in *CD27*,[12] and interleukin-2 inducible T-cell kinase (ITK) deficiency from *ITK* dysfunction.[13] Some metabolic disorders can also be complicated by the development of HLH, notably including lysinuric protein intolerance caused by mutations in the *SLC7A7* gene.[14]

Defects in these genes are sometimes indistinguishable from each other clinically. Historically, genetic investigations started with Sanger sequencing of the most commonly defective gene, *PRF1.* Sequential examination of other HLH-related genes was then pursued if *PRF1* testing was negative. This process not only prolonged the diagnosis in many cases but was also expensive. Next-generation sequencing (NGS) technology has allowed the creation of targeted gene panels in which several genes can be interrogated at once in a time and cost-efficient manner. At Cincinnati Children's Hospital Medical Center (CCHMC), an NGS-based HLH sequencing panel including 15 HLH-associated genes was launched in September 2013. In this study, we aimed to examine the impact of NGS HLH panel on genetic testing ordering patterns and examine the distribution and details of genetic variants observed in 1892 patient samples submitted for NGS HLH panel sequencing.

Materials and methods

Patient samples and clinical information

The present study was approved by the institutional review board at Cincinnati Children's Hospital, Cincinnati, OH. A total of 1892 patient samples and submitted clinical information were analyzed and reviewed after HLH sequencing panel testing was submitted to the Molecular Diagnostic Laboratory at CCHMC between September 2013 and June 2018. Of these, 33 orders clearly stated that the reason for testing was for mutation carrier status evaluation and these were excluded from further analysis for molecular diagnosis. Of the remaining 1859 samples, 1632 had only variants classified as benign, likely benign, or variants of uncertain significance. At least 1 pathogenic or likely pathogenic variant was detected in 227 samples. Among them, 197 samples were identified with either homozygous or compound heterozygous pathologic variants in an autosomal recessive condition, or a hemizygous pathologic variant in an X-linked disorder. In addition, 30 patient samples were identified carrying only 1 heterozygous pathogenic or likely pathogenic variant in a recessive condition (supplemental Figure 1). Patients were referred by physicians from 300 institutions across North America and the included patients were either referred with a clinical diagnosis of HLH or suspected related conditions. Patient age at time of referral ranged from 1 day to 78 years. Forty-seven percent (895/1892) were female and 53% (997/1892) were male. The reported cohort of 197 patients ranged from 1 day to 57.8 years, including 44% (86/197) female and 56% (111/197) male. Fifty-eight percent (114/197) were younger than 2 years old, and 30% (59/197) were between 2 and 18 years old (Table 1). Clinical information was collected using a standardized clinical checklist completed by the ordering physician that captured information such as age of onset, and general clinical history such as fever, liver and spleen abnormalities, infections, skin abnormalities, laboratory findings, neurological abnormalities, family history, and results of previous testing. Our laboratory did not systematically confirm clinical characteristics or prior laboratory investigations of patients reported by referring clinicians.

HLH NGS panel

Fifteen genes that have been associated with HLH or HLH-like conditions were included in our HLH NGS panel: *PRF1*, *UNC13D*, *STX11*, *STXBP2*, *ITK*, *CD27*, *SH2D1A*, *XIAP*, *MAGT1*, *LYST*, *RAB27A*, *AP3B1*, *BLOC1S6*, *SLC7A7*, and *GATA2*. Their associated OMIM diseases, transcripts, and inheritance pattern are listed in supplemental Table 1. A typical turnaround time for this clinical testing is 4 weeks. Expedited turnaround time is available upon request.

NGS, data analysis, and Sanger confirmation

NGS was performed on the genomic DNA isolated from the patient samples using microdroplet polymerase chain reaction technology (RainDance Technologies Inc.) and sequenced on an Illumina HiSeq2500 instrument (Illumina Inc.). All exons, flanking intronic (±20 base pairs) and 5′ and 3′ untranslated regions of the 15 genes in the HLH panel (supplemental Table 1) were captured. Data for each sample were assessed for quality and confirmed they had at least 20× read depth at every target base. Sanger sequencing was performed to rescue all low coverage (<20× read depth) regions. Variants within those regions were identified and evaluated using a validated, custom bioinformatic pipeline. The American College of Medical Genetics and Genomics (ACMG) guidelines for sequence variant classification were used to categorize variants. All reported variants were confirmed by Sanger sequencing. In addition, allele-specific analysis for the 253-kb inversion as well as targeted analysis of the c.118-308 and c.118-307 regions in the *UNC13D* gene were performed for each sample because these variants have been reported to disrupt *UNC13D* transcription in lymphocytes and abolish Munc13-4 expression.[15]

We reviewed the results of the 1892 patient samples, excluded potential carriers based on clinical information provided, and reported the number of samples that were abnormal with pathogenic or likely pathogenic variants associated with HLH. Samples were classified according to the genes affected, types of mutations, and predicted impact on protein sequencing or structure. Pathogenic or likely pathogenic variants were identified in 10 genes: *PRF1*, *STXBP2*, *UNC13D*, *LYST*, *RAB27A*, *STX11*, *SLC7A7*, *XIAP*, *SH2D1A*, and *MAGT1*.

Results

At CCHMC, a 15-gene NGS panel for the molecular diagnosis of HLH disorders was offered from September 2013. Since then, the number of orders for traditionally sequential single-gene tests related to HLH disorders drastically decreased. As shown in Figure 1, from 2013 to 2018, the orders for single-gene Sanger sequencing such as *PRF1*, *UNC13D*, *STXBP2*, *RAB27A*, *XIAP*, and *SH2D1A* decreased from 308, 302, 277, 249, 132, and 104 in 2013 to 21, 3, 4, 1, 9, and 10 in 2018, respectively. On the other hand, the orders of HLH NGS panel jumped and maintained ~400 test orders per year from 2014 to 2018.

A total of 1892 HLH panel testing results were analyzed, and clearly pathogenic and likely pathogenic variants were identified in 227

Table 1. Pathogenic or likely pathogenic variants identified in 197 HLH patients with a definite genetic diagnosis

Patient no.	Sex	Age at testing, y (unless indicated otherwise)	Ethnicity	Gene	Variant	Zygosity	Population frequency (gnomAD*), %	Symptoms/immunology testing/family history†
1	Female	53 d	African American	*PRF1*	c.50del(p.Leu17fs)	Homozygous	0.033	Symptoms of HLH
2	Male	63 d	Unknown	*PRF1*	c.50del(p.Leu17fs)	Homozygous	0.033	Symptoms of HLH
3	Male	0.4	Middle Eastern	*PRF1*	c.50del(p.Leu17fs)	Homozygous	0.033	Symptoms of HLH
4	Female	27 d	Unknown	*PRF1*	c.50del(p.Leu17fs)	Homozygous	0.033	Absent perforin expression
5	Female	32 d	African American	*PRF1*	c.50del(p.Leu17fs)	Homozygous	0.033	Absent perforin expression
6	Male	4 d	African American	*PRF1*	c.50del(p.Leu17fs)	Homozygous	0.033	Absent perforin expression; sibling died of HLH
7	Female	0.4	African American	*PRF1*	c.50del(p.Leu17fs)	Homozygous	0.033	Symptoms of HLH
8	Male	6 d	African American + European-white	*PRF1*	c.50del(p.Leu17fs)	Homozygous	0.033	Absent perforin expression
9	Male	59 d	African American	*PRF1*	c.50del(p.Leu17fs)	Homozygous	0.033	Symptoms of HLH
10	Male	13 d	African	*PRF1*	c.50del(p.Leu17fs)	Homozygous	0.033	Symptoms of HLH
11	Female	7 d	Unknown	*PRF1*	c.50del(p.Leu17fs)	Heterozygous	0.033	Absent NK cell function
				PRF1	c.266C>T(p.Pro89Leu)	Heterozygous	ND	
12	Female	0.3	African American	*PRF1*	c.50del(p.Leu17fs)	Heterozygous	0.033	NA
				PRF1	c.350_356delinsATGC (p.Val117_Arg119delinsAspAla)	Heterozygous	ND	
13	Male	0.4	Unknown	*PRF1*	c.50del(p.Leu17fs)	Heterozygous	0.033	NA
				PRF1	c.445G>A(p.Gly149Ser)	Heterozygous	0.014	
14	Male	3.3	African American	*PRF1*	c.50del(p.Leu17fs)	Heterozygous	0.033	Absent perforin expression; brother with HLH
				PRF1	c.527G>A(p.Cys176Tyr)	Heterozygous	ND	
15	Female	0.5	Latino-Hispanic	*PRF1*	c.50del(p.Leu17fs)	Heterozygous	0.033	Absent perforin expression
				PRF1	c.659G>A(p.Gly220Asp)	Heterozygous	0.0008	
16	Female	0.2	African American	*PRF1*	c.50del(p.Leu17fs)	Heterozygous	0.033	Absent NK cell function
				PRF1	c.853_855del(p.Lys285del)	Heterozygous	0.0056	
17	Male	66 d	Unknown	*PRF1*	c.50del(p.Leu17fs)	Heterozygous	0.033	NA
				PRF1	c.895C>T(p.Arg299Cys)	Heterozygous	0.0012	
18	Male	20.6	Latino-Hispanic	*PRF1*	c.50del(p.Leu17fs)	Heterozygous	0.033	NA
				PRF1	c.902C>T(p.Ser301Leu)	Heterozygous	ND	
19	Male	54 d	African American	*PRF1*	c.50del(p.Leu17fs)	Heterozygous	0.033	Symptoms of HLH
				PRF1	c.916G>T(p.Gly306Cys)	Heterozygous	ND	
20	Female	45 d	African American	*PRF1*	c.50del(p.Leu17fs)	Heterozygous	0.033	Absent perforin expression
				PRF1	c.916G>T(p.Gly306Cys)	Heterozygous	ND	
21	Male	32 d	Latino-Hispanic	*PRF1*	c.50del(p.Leu17fs)	Heterozygous	0.033	Symptoms of HLH
				PRF1	c.985dup(p.Val329fs)	Heterozygous	ND	
22	Male	0.3	African American	*PRF1*	c.50del(p.Leu17fs)	Heterozygous	0.033	Absent perforin expression
				PRF1	c.1385C>A(p.Ser462*)	Heterozygous	ND	
23	Male	36.1	Unknown	*PRF1*	c.116C>A(p.Pro39His)	Heterozygous	0.00081	NA
				PRF1	c.445G>A(p.Gly149Ser)	Heterozygous	0.014	
24	Female	1.2	Asian-American	*PRF1*	c.133G>A(p.Gly45Arg)	Homozygous	0.0012	Absent NK cell function
25	Female	37 d	Non-Hispanic white	*PRF1*	c.150del(p.Thr51fs)	Heterozygous	0.0004	Absent perforin expression
				PRF1	c.227G>A(p.Cys76Tyr)	Heterozygous	0.00071	
26	Male	69 d	Latino-Hispanic	*PRF1*	c.218G>C(p.Cys73Ser)	Homozygous	0.0004	Symptoms of HLH

NA, no data; ND, no data; NK, natural killer.

*gnomAD v2.1.1 total population frequency.

†HLH hemophagocytic lymphohistiocytosis, symptoms of HLH reported included any or all of the following "fever, hepatosplenomegaly, anemia/cytopenias, neutropenia/leukopenia, elevated ferritin/triglycerides, and/or decreased fibrinogen."

‡According to the ACMG guideline, c.272C>T(p.Ala91Val) in *PRF1* was classified as a variant of unknown significance.

Table 1. (continued)

Patient no.	Sex	Age at testing, y (unless indicated otherwise)	Ethnicity	Gene	Variant	Zygosity	Population frequency (gnomAD*), %	Symptoms/immunology testing/family history†
27	Female	20	European-American	*PRF1*	c.227G>A(p.Cys76Tyr)	Heterozygous	0.00071	Absent perforin expression
				PRF1	c.626A>C(p.Gln209Pro)	Heterozygous	0.0012	
28	Female	21.8	Unknown	*PRF1*	c.272C>T(p.Ala91Val)‡	Heterozygous	2.92	Absent NK cell function, decreased perforin expression
				PRF1	c.445G>A(p.Gly149Ser)	Heterozygous	0.014	
29	Male	17	European-American	*PRF1*	c.272C>T(p.Ala91Val)‡	Heterozygous	2.92	Absent perforin expression
				PRF1	c.635A>C(p.Tyr212Ser)	Heterozygous	ND	
30	Female	41.2	European-American	*PRF1*	c.272C>T(p.Ala91Val)‡	Heterozygous	2.92	Absent NK cell function, decreased perforin expression
				PRF1	c.666C>A(p.His222Gln)	Heterozygous	0.0039	
31	Female	8.3	European-American	*PRF1*	c.443C>G(p.Ala148Gly)	Heterozygous	0.0004	NA
				PRF1	c.666C>A(p.His222Gln)	Heterozygous	0.0039	
32	Male	2.6	Unknown	*PRF1*	c.445G>A(p.Gly149Ser)	Homozygous	0.014	Absent NK cell function
33	Male	0.8	Latino-Hispanic	*PRF1*	c.445G>A(p.Gly149Ser)	Homozygous	0.014	Symptoms of HLH
34	Female	6	Latino-Hispanic	*PRF1*	c.445G>A(p.Gly149Ser)	Homozygous	0.014	NA
35	Female	0.3	European-American	*PRF1*	c.445G>A(p.Gly149Ser)	Heterozygous	0.014	Family history of HLH
				PRF1	c.614A>G(p.Asn205Ser)	Heterozygous	0.0043	
36	Male	42 d	Latino-Hispanic	*PRF1*	c.445G>A(p.Gly149Ser)	Heterozygous	0.014	NA
				PRF1	c.938A>T(p.Asp313Val)	Heterozygous	0.0012	
37	Male	4.6	Unknown	*PRF1*	c.445G>A(p.Gly149Ser)	Heterozygous	0.014	Absent perforin expression
				PRF1	c.1081A>T(p.Arg361Trp)	Heterozygous	0.0011	
38	Female	32 d	Middle Eastern	*PRF1*	c.501C>G(p.Tyr167*)	Homozygous	ND	Symptoms of HLH
39	Female	0.3	Unknown	*PRF1*	c.512C>A(p.Thr171Asn)	Homozygous	0.0028	Absent perforin expression
40	Male	9.5	European-American	*PRF1*	c.786_801del(p.Gln263fs)	Heterozygous	ND	Absent NK cell function
				PRF1	c.886T>C(p.Tyr296His)	Heterozygous	0.0012	
41	Male	59 d	Unknown	*PRF1*	c.853_855del(p.Lys285del)	Heterozygous	0.0057	NA
				PRF1	c.921del(p.His308fs)	Heterozygous	0.002	
42	Female	0.7	Middle Eastern	*PRF1*	c.880del(p.Gln294fs)	Homozygous	ND	Symptoms of HLH
43	Female	2	Middle Eastern	*PRF1*	c.895C>T(p.Arg299Cys)	Homozygous	0.0012	Symptoms of HLH
44	Female	0.2	Latino-Hispanic	*PRF1*	c.904G>T(p.Glu302*)	Homozygous	ND	Absent perforin expression
45	Female	1.8	Unknown	*PRF1*	c.949G>A(p.Gly317Arg)	Homozygous	0.0008	Symptoms of HLH
46	Female	10.3	European-American	*PRF1*	c.973T>C(p.Tyr325His)	Heterozygous	ND	Absent perforin expression
				PRF1	c.1326_1328del(p.Phe443del)	Heterozygous	ND	
47	Male	1.1	Middle Eastern	*PRF1*	c.1070G>C(p.Arg357Pro)	Homozygous	ND	Symptoms of HLH
48	Male	12.5	Middle Eastern	*PRF1*	c.1081A>T(p.Arg361Trp)	Homozygous	0.0011	Abnormal brain lesions and seizures
49	Female	2.6	Unknown	*PRF1*	c.1229_1230delinsCC (p.Arg410Pro)	Homozygous	ND	NA
50	Female	0.2	African American	*PRF1*	c.1304C>T(p.Thr435Met)	Heterozygous	0.0028	Absent perforin expression
				PRF1	c.1314T>A(p.Tyr438*)	Heterozygous	0.0032	
51	Female	2.6	Latino-Hispanic	*PRF1*	c.1337A>C(p.Gln446Pro)	Homozygous	0.0016	NA
52	Female	2.6	Unknown	*PRF1*	c.1337A>C(p.Gln446Pro)	Homozygous	0.0016	Symptoms of HLH
53	Female	0.4	Middle Eastern	*STXBP2*	c.37+2T>C	Heterozygous	ND	Absent NK cell function
				STXBP2	c.1430C>T(p.Pro477Leu)	Heterozygous	0.00074	
54	Male	0.6	Unknown	*STXBP2*	c.37+5G>A	Heterozygous	ND	NA
				STXBP2	c.1057T>C (p.Cys353Arg)	Heterozygous	0.0004	

NA, no data; ND, no data; NK, natural killer.

*gnomAD v2.1.1 total population frequency.

†HLH hemophagocytic lymphohistiocytosis, symptoms of HLH reported included any or all of the following "fever, hepatosplenomegaly, anemia/cytopenias, neutropenia/leukopenia, elevated ferritin/triglycerides, and/or decreased fibrinogen."

‡According to the ACMG guideline, c.272C>T(p.Ala91Val) in *PRF1* was classified as a variant of unknown significance.

Table 1. (continued)

Patient no.	Sex	Age at testing, y (unless indicated otherwise)	Ethnicity	Gene	Variant	Zygosity	Population frequency (gnomAD*), %	Symptoms/immunology testing/family history†
55	Female	63 d	Asian-American	*STXBP2*	c.193C>T(p.Arg65Trp)	Homozygous	0.00071	Absent NK cell function
56	Female	5.5	Unknown	*STXBP2*	c.194G>A(p.Arg65Gln)	Heterozygous	0.0028	Absent NK cell function
				STXBP2	c.560C>T (p.Pro187Leu)	Heterozygous	0.00064	
57	Male	4.1	European-American	*STXBP2*	c.194G>A(p.Arg65Gln)	Heterozygous	0.0028	Symptoms of HLH
				STXBP2	c.1621G>A(p.Gly541Ser)	Heterozygous	0.023	
58	Female	4.2	European-American	*STXBP2*	c.326-30_326-23del	Heterozygous	0.0068	Symptoms of HLH
				STXBP2	c.1621G>A(p.Gly541Ser)	Heterozygous	0.023	
59	Male	0.6	Latino-Hispanic	*STXBP2*	c.389T>C(p.Leu130Ser)	Homozygous	0.0032	Symptoms of HLH
60	Male	45 d	African American	*STXBP2*	c.389T>C(p.Leu130Ser)	Heterozygous	0.0032	Symptoms of HLH; family history of HLH
				STXBP2	exon 14-19 deletion	Heterozygous	ND	
61	Male	0.7	Middle Eastern	*STXBP2*	c.481del(p.Arg161fs)	Homozygous	ND	Symptoms of HLH
62	Male	0.4	Unknown	*STXBP2*	c.481del(p.Arg161fs)	Homozygous	ND	Symptoms of HLH
63	Female	11	European-American	*STXBP2*	c.539_540delinsAA(p.Cys180*)	Heterozygous	ND	Symptoms of HLH
				STXBP2	c.1247-1G>C	Heterozygous	0.02	
64	Male	0.3	Latino-Hispanic	*STXBP2*	c.703C>G(p.Arg235Gly)	Homozygous	0.00071	Absent NK cell function
65	Female	52.7	European-American	*STXBP2*	c.752C>T(p.Ala251Val)	Heterozygous	ND	Symptoms of HLH
				STXBP2	c.1621G>A(p.Gly541Ser)	Heterozygous	0.023	
66	Male	0.9	Unknown	*STXBP2*	c.902+5G>A	Heterozygous	0.0036	NA
				STXBP2	c.1247-1G>C	Heterozygous	0.02	
67	Male	3.1	Unknown	*STXBP2*	c.1247-1G>C	Homozygous	0.02	Symptoms of HLH
68	Female	22.7	Latino-Spanish	*STXBP2*	c.1247-1G>C	Homozygous	0.02	Decreased NK cell function
69	Male	26.4	European-American	*STXBP2*	c.1247-1G>C	Homozygous	0.02	Symptoms of HLH
70	Male	25.6	European-American	*STXBP2*	c.1247-1G>C	Homozygous	0.02	Symptoms of HLH
71	Female	29.7	European-American	*STXBP2*	c.1247-1G>C	Homozygous	0.02	NA
72	Male	4	European-American	*STXBP2*	c.1247-1G>C	Heterozygous	0.02	Absent NK cell function
				STXBP2	c.1621G>A(p.Gly541Ser)	Heterozygous	0.023	
73	Female	15.8	European-American	*STXBP2*	c.1247-1G>C	Heterozygous	0.02	Absent NK cell function
				STXBP2	c.1621G>A(p.Gly541Ser)	Heterozygous	0.023	
74	Female	19	Unknown	*STXBP2*	c.1247-1G>C	Heterozygous	0.02	Decreased NK cell function
				STXBP2	c.1621G>A(p.Gly541Ser)	Heterozygous	0.023	
75	Female	26.9	European-American	*STXBP2*	c.1247-1G>C	Heterozygous	0.02	Symptoms of HLH
				STXBP2	c.1621G>A(p.Gly541Ser)	Heterozygous	0.023	
76	Female	57.8	European-American	*STXBP2*	c.1247-1G>C	Heterozygous	0.02	Absent NK cell function
				STXBP2	c.1621G>A(p.Gly541Ser)	Heterozygous	0.023	
77	Female	0.2	Middle Eastern	*STXBP2*	c.1430C>T(p.Pro477Leu)	Homozygous	0.00074	Symptoms of HLH
78	Female	0.3	Middle Eastern	*STXBP2*	c.1430C>T(p.Pro477Leu)	Homozygous	0.00074	NA
79	Female	0.3	Middle Eastern	*STXBP2*	c.1430C>T(p.Pro477Leu)	Homozygous	0.00074	Symptoms of HLH
80	Female	0.6	Middle Eastern	*STXBP2*	c.1430C>T(p.Pro477Leu)	Homozygous	0.00074	Symptoms of HLH
81	Male	0.2	Middle Eastern	*STXBP2*	c.1430C>T(p.Pro477Leu)	Homozygous	0.00074	NA
82	Male	0.7	Middle Eastern	*STXBP2*	c.1430C>T(p.Pro477Leu)	Homozygous	0.00074	Symptoms of HLH
83	Female	0.8	Middle Eastern	*STXBP2*	c.1430C>T(p.Pro477Leu)	Homozygous	0.00074	Symptoms of HLH
84	Male	0.8	Unknown	*STXBP2*	c.1430C>T(p.Pro477Leu)	Homozygous	0.00074	Symptoms of HLH

NA, no data; ND, no data; NK, natural killer.

*gnomAD v2.1.1 total population frequency.

†HLH hemophagocytic lymphohistiocytosis, symptoms of HLH reported included any or all of the following "fever, hepatosplenomegaly, anemia/cytopenias, neutropenia/leukopenia, elevated ferritin/triglycerides, and/or decreased fibrinogen."

‡According to the ACMG guideline, c.272C>T(p.Ala91Val) in *PRF1* was classified as a variant of unknown significance.

Table 1. (continued)

Patient no.	Sex	Age at testing, y (unless indicated otherwise)	Ethnicity	Gene	Variant	Zygosity	Population frequency (gnomAD*), %	Symptoms/immunology testing/family history†
85	Male	0.6	Unknown	*STXBP2*	c.1430C>T(p.Pro477Leu)	Homozygous	0.00074	Family history of HLH
86	Male	1.6	Middle Eastern	*STXBP2*	c.1430C>T(p.Pro477Leu)	Homozygous	0.00074	Symptoms of HLH
87	Male	0.3	Middle Eastern	*STXBP2*	c.1430C>T(p.Pro477Leu)	Homozygous	0.00074	Symptoms of HLH
88	Female	63 d	Middle Eastern	*STXBP2*	c.1430C>T(p.Pro477Leu)	Homozygous	0.00074	Family history of HLH
89	Male	0.5	Unknown	*STXBP2*	c.1430C>T(p.Pro477Leu)	Homozygous	0.00074	Symptoms of HLH
90	Male	0.2	Middle Eastern	*STXBP2*	c.1430C>T(p.Pro477Leu)	Homozygous	0.00074	Symptoms of HLH
91	Male	11.1	Unknown	*STXBP2*	c.1430C>T(p.Pro477Leu)	Homozygous	0.00074	Absent NK cell function
92	Female	10.1	Unknown	*STXBP2*	c.1430C>T(p.Pro477Leu)	Heterozygous	0.00074	Symptoms of HLH
				STXBP2	c.1696+5G>T	Heterozygous	ND	
93	Male	0.5	Middle Eastern	*STXBP2*	c.1452+1G>A	Homozygous	ND	Symptoms of HLH
94	Male	1	Middle Eastern	*STXBP2*	c.1452+1G>A	Homozygous	ND	Abnormal NK cell function
95	Female	49 d	European-American	*UNC13D*	c.118-308C>T	Heterozygous	0.019	Dysmorphic facies, decreased NK cell function
				UNC13D	c.2258_2267delins TACCTTGTTCGA (p.Gly753fs)	Heterozygous	ND	
96	Male	0.7	European-American	*UNC13D*	c.118-308C>T	Heterozygous	0.019	Decreased NK cell function
				UNC13D	c.2346_2349del(p.Arg782fs)	Heterozygous	0.01	
97	Male	0.2	European-American + Latino-Spanish	*UNC13D*	c.118-308C>T	Heterozygous	0.019	Decreased NK cell function
				UNC13D	c.2346_2349del(p.Arg782fs)	Heterozygous	0.01	
98	Female	1.3	Non-Hispanic white	*UNC13D*	c.118-308C>T	Heterozygous	0.019	Symptoms of HLH, seizures, normal NK cell function
				UNC13D	c.2867C>T(p.Pro956Leu)	Heterozygous	ND	
99	Female	1	Non-Hispanic white	*UNC13D*	c.118-308C>T	Heterozygous	0.019	Absent NK cell function
				UNC13D	c.3193C>T(p.Arg1065*)	Heterozygous	0.0011	
100	Female	3.2	European-American	*UNC13D*	c.118-308C>T	Heterozygous	0.019	Absent NK cell function
				UNC13D	253Kb inversion	Heterozygous	ND	
101	Male	2.6	Unknown	*UNC13D*	253Kb inversion	Heterozygous	ND	Decreased NK cell function
				UNC13D	c.154-1G>C	Heterozygous	ND	
102	Male	0.2	Unknown	*UNC13D*	253Kb inversion	Heterozygous	ND	Symptoms of HLH
				UNC13D	c.551G>A(p.Trp184*)	Heterozygous	0.0011	
103	Female	0.2	European-American	*UNC13D*	253Kb inversion	Heterozygous	ND	NA
				UNC13D	c.1389+1G>A	Heterozygous	0.0071	
104	Female	0.2	European-American	*UNC13D*	253Kb inversion	Heterozygous	ND	Absent NK cell function
				UNC13D	c.2447+1G>A	Heterozygous	0.00051	
105	Female	0.6	Non-Hispanic white	*UNC13D*	253Kb inversion	Heterozygous	ND	Decreased NK cell function
				UNC13D	c.2695C>T(p.Arg899*)	Heterozygous	0.0018	
106	Male	11.4	Hispanic white	*UNC13D*	c.182A>G(p.Tyr61Cys)	Heterozygous	ND	Symptoms of HLH
				UNC13D	c.778T>C(p.Trp260Arg)	Heterozygous	ND	
107	Male	0.4	European-American	*UNC13D*	c.262-1G>A	Heterozygous	ND	Symptoms of HLH, abnormal NK cell function, family history of HLH
				UNC13D	c.766C>T(p.Arg256*)	Heterozygous	0.0025	
108	Male	0.4	Unknown	*UNC13D*	c.321+1_321+2del	Heterozygous	ND	Decreased NK cell function
				UNC13D	c.753+1G>T	Heterozygous	0.0044	

NA, no data; ND, no data; NK, natural killer.

*gnomAD v2.1.1 total population frequency.

†HLH hemophagocytic lymphohistiocytosis, symptoms of HLH reported included any or all of the following "fever, hepatosplenomegaly, anemia/cytopenias, neutropenia/leukopenia, elevated ferritin/triglycerides, and/or decreased fibrinogen."

‡According to the ACMG guideline, c.272C>T(p.Ala91Val) in *PRF1* was classified as a variant of unknown significance.

Table 1. (continued)

Patient no.	Sex	Age at testing, y (unless indicated otherwise)	Ethnicity	Gene	Variant	Zygosity	Population frequency (gnomAD*), %	Symptoms/immunology testing/family history†
109	Male	0.3	Unknown	*UNC13D*	c.322-2A>T	Heterozygous	0.0024	Symptoms of HLH, decreased NK cell function
				UNC13D	c.2346_2349del(p.Arg782fs)	Heterozygous	0.01	
110	Male	0.3	Unknown	*UNC13D*	c.419T>C(p.Ile140Thr)	Heterozygous	0.0004	NA
				UNC13D	c.460C>T(p.Arg154Trp)	Heterozygous	0.011	
111	Female	5.4	Middle Eastern	*UNC13D*	c.424dup(p.Gln142fs)	Homozygous	ND	Symptoms of HLH
112	Male	7.7	Latino-Hispanic	*UNC13D*	c.518C>T(p.Thr173Met)	Heterozygous	0.0028	NA
				UNC13D	c.1803_1819dup(p.Arg607fs)	Heterozygous	ND	
113	Female	39 d	European-American	*UNC13D*	c.551G>A(p.Trp184*)	Heterozygous	0.0011	Abnormal NK cell function
				UNC13D	c.766C>T(p.Arg256*)	Heterozygous	0.0025	
114	Male	4.9	European-American	*UNC13D*	c.570-2A>T	Heterozygous	ND	Absent NK cell function
				UNC13D	c.3049G>A(p.Glu1017Lys)	Heterozygous	0.00044	
115	Female	0.2	Middle Eastern	*UNC13D*	c.753+1G>T	Homozygous	0.0044	Symptoms of HLH
116	Female	0.6	European-American	*UNC13D*	c.766C>T(p.Arg256*)	Heterozygous	0.0025	Symptoms of HLH, abnormal NK cell function
				UNC13D	c.2447+1G>A	Heterozygous	0.00051	
117	Female	1.2	Latino-Spanish	*UNC13D*	c.859del(p.Arg287fs)	Homozygous	ND	Decreased NK cell function
118	Female	1	Non-Hispanic white	*UNC13D*	c.1055+1G>T	Heterozygous	ND	Decreased NK cell function, family history of HLH
				UNC13D	c.2346_2349del(p.Arg782fs)	Heterozygous	0.01	
119	Male	0.7	European-American	*UNC13D*	c.1229_1230dup(p.Arg411fs)	Heterozygous	0.00	Symptoms of HLH, hypertelorism
				UNC13D	c.2298+1G>T	Heterozygous	ND	
120	Female	0.2	European-American	*UNC13D*	c.1259_1260del(p.Ser420fs)	Heterozygous	ND	symptoms of HLH, decreased NK cell function
				UNC13D	c.1848+1G>C	Heterozygous	ND	
121	Male	18.3	European-American	*UNC13D*	c.1387C>T(p.Gln463*)	Heterozygous	ND	symptoms of HLH, decreased NK cell function
				UNC13D	c.1820G>C(p.Arg607Pro)	Heterozygous	0.011	
122	Female	58 d	European-American + African American	*UNC13D*	c.1389+1G>A	Heterozygous	0.0071	Symptoms of HLH
				UNC13D	c.1848+1G>C	Heterozygous	ND	
123	Female	0.2	Middle Eastern	*UNC13D*	c.1423C>T(p.Gln475*)	Homozygous	ND	NA
124	Male	10 d	Pacific Islander	*UNC13D*	c.2296C>T(p.Gln766*)	Homozygous	ND	Decreased NK cell function
125	Female	13.2	Unknown	*UNC13D*	c.2346_2349del(p.Arg782fs)	Heterozygous	0.01	NA
				UNC13D	c.2588G>A(p.Gly863Asp)	Heterozygous	0.029	
126	Female	2	Unknown	*UNC13D*	c.2346_2349del(p.Arg782fs)	Heterozygous	0.01	NA
				UNC13D	c.3065T>C(p.Leu1022Pro)	Heterozygous	ND	
127	Female	0.2	Middle Eastern	*UNC13D*	c.2553+1G>T	Homozygous	ND	Symptoms of HLH
128	Female	0.3	Middle Eastern	*UNC13D*	c.2553+1G>T	Homozygous	ND	Symptoms of HLH
129	Female	1	Asian	*UNC13D*	c.2588G>A(p.Gly863Asp)	Homozygous	0.029	Decreased NK cell function
130	Female	0.4	African American	*UNC13D*	c.2695C>T(p.Arg899*)	Homozygous	0.0018	Symptoms of HLH, absent NK cell function, dysmorphic facies
131	Male	0.7	Non-Hispanic white	*UNC13D*	c.2819del(p.Leu940fs)	Homozygous	ND	symptoms of HLH
132	Female	0.6	Middle Eastern	*UNC13D*	c.3048dup(p.Glu1017fs)	Homozygous	ND	NA
133	Female	0.7	Unknown	*UNC13D*	c.3053C>A(p.Ala1018Asp)	Homozygous	0.00088	2 affected siblings
134	Male	13	European-American	*RAB27A*	c.121A>G(p.Thr41Ala)	Heterozygous	ND	NA
				RAB27A	c.352C>T(p.Gln118*)	Heterozygous	ND	
135	Male	9.6	Middle Eastern	*RAB27A*	c.244C>T(p.Arg82Cys)	Homozygous	0.0016	NA

NA, no data; ND, no data; NK, natural killer.

*gnomAD v2.1.1 total population frequency.

†HLH hemophagocytic lymphohistiocytosis, symptoms of HLH reported included any or all of the following "fever, hepatosplenomegaly, anemia/cytopenias, neutropenia/leukopenia, elevated ferritin/triglycerides, and/or decreased fibrinogen."

‡According to the ACMG guideline, c.272C>T(p.Ala91Val) in *PRF1* was classified as a variant of unknown significance.

Table 1. (continued)

Patient no.	Sex	Age at testing, y (unless indicated otherwise)	Ethnicity	Gene	Variant	Zygosity	Population frequency (gnomAD*), %	Symptoms/immunology testing/family history†
136	Male	9.9	Middle Eastern	*RAB27A*	c.244C>T(p.Arg82Cys)	Homozygous	0.0016	NA
137	Female	2	Middle Eastern	*RAB27A*	c.244C>T(p.Arg82Cys)	Homozygous	0.0016	Failure to thrive, bone marrow failure
138	Female	9.4	Middle Eastern	*RAB27A*	c.244C>T(p.Arg82Cys)	Homozygous	0.0016	Symptoms of HLH
139	Male	10.6	Middle Eastern	*RAB27A*	c.244C>T(p.Arg82Cys)	Homozygous	0.0016	Symptoms of HLH
140	Male	19.5	Middle Eastern	*RAB27A*	c.244C>T(p.Arg82Cys)	Homozygous	0.0016	Symptoms of HLH
141	Female	0.3	Latino-Hispanic	*RAB27A*	c.335del(p.Asn112fs)	Homozygous	0.0044	Symptoms of HLH
142	Female	0.4	Middle Eastern	*RAB27A*	c.400A>C(p.Lys134Gln)	Homozygous	ND	NA
143	Female	53.9	Pacific Islander	*RAB27A*	c.476A>G(p.Tyr159Cys)	Homozygous	ND	Symptoms of HLH, absent NK cell function
144	Male	1.2	Middle Eastern	*RAB27A*	c.598C>T(p.Arg200*)	Homozygous	0.0004	NA
145	Female	0.4	European-American	*RAB27A*	c.638_642del(p.Glu213fs)	Homozygous	0.0008	Symptoms of HLH
146	Female	0.7	African American	*LYST*	c.925C>T(p.Arg309*)	Heterozygous	ND	Oculocutaneous albinism, neutropenia
				LYST	c.2015dup(p.Tyr672*)	Heterozygous	ND	
147	Male	3.4	Unknown	*LYST*	c.3194del(p.Leu1065*)	Homozygous	0.0004	NA
148	Female	0.3	Middle Eastern	*LYST*	c.4159dup(p.Thr1387fs)	Homozygous	ND	Premature gray hair, anemia
149	Male	0.9	European-American	*LYST*	c.5715del(p.Asn1905fs)	Heterozygous	ND	Oculocutaneous albinism, neutropenia, absent NK cell function
				LYST	c.8802-2A>G	Heterozygous	ND	
150	Female	6.9	Unknown	*LYST*	c.5784+1G>T	Homozygous	ND	Oculocutaneous albinism, dysmorphic facies, neutropenia
151	Male	3.2	Non-Hispanic white	*LYST*	c.6159_6160del(p.Met2053fs)	Homozygous	ND	NA
152	Male	1.8	Middle Eastern	*LYST*	c.7291del(p.Leu2431fs)	Homozygous	ND	Hypopigmentation, anemia
153	Male	1.2	African American	*LYST*	c.8770C>T(p.Gln2924*)	Heterozygous	ND	Silver hair, hypopigmented skin lesions, pancytopenia
				LYST	c.9844_9845del(p.Ser3282fs)	Heterozygous	ND	
154	Female	7.1	Middle Eastern	*LYST*	c.10776C>G(p.Tyr3592*)	Homozygous	ND	Abnormal pigmentation, neutropenia
155	Female	1.2	Unknown	*STX11*	c.73G>T(p.Glu25*)	Heterozygous	0.0004	Decreased NK cell function
				STX11	c.748C>T(p.Gln250*)	Heterozygous	0.00081	
156	Female	5.6	Middle Eastern	*STX11*	c.173T>C(p.Leu58Pro)	Homozygous	0.0008	Symptoms of HLH, grayish hair
157	Female	11.6	Middle Eastern	*STX11*	c.173T>C(p.Leu58Pro)	Homozygous	0.0008	NA
158	Male	2.9	European-American + Latino-Hispanic	*STX11*	c.462_463delinsA(p.Asp155fs)	Heterozygous	ND	Decreased NK cell function
				STX11	c.784C>T(p.Gln262*)	Heterozygous	ND	
159	Male	1.4	Asian-Indian	*STX11*	c.687dup(p.Gln230fs)	Homozygous	ND	NA
160	Female	12 d	Middle Eastern	*SLC7A7*	c.1429+1G>C	Homozygous	ND	Family history of HLH
161	Male	1.2	African American	*SLC7A7*	c.701del(p.Ser234fs)	Heterozygous	0.0016	NA
				SLC7A7	c.895-1G>A	Heterozygous	ND	
162	Female	13.7	European-American	*SLC7A7*	c.360_361delinsAA (p.Trp121Arg)	Homozygous	ND	NA
163	Male	5.4	African American	*XIAP*	c.145C>T(p.Arg49*)	Hemizygous	ND	Markedly decreased XIAP expression
164	Male	18.7	European-American	*XIAP*	c.345C>G(p.Tyr115*)	Hemizygous	ND	Symptoms of HLH
165	Male	30.3	European-American	*XIAP*	c.608G>T(p.Cys203Phe)	Hemizygous	ND	NA
166	Male	2	European-American	*XIAP*	c.664C>T(p.Arg222*)	Hemizygous	ND	Symptoms of HLH
167	Male	8.9	European-American	*XIAP*	c.738del(p.Asp247fs)	Hemizygous	ND	NA

NA, no data; ND, no data; NK, natural killer.

*gnomAD v2.1.1 total population frequency.

†HLH hemophagocytic lymphohistiocytosis, symptoms of HLH reported included any or all of the following "fever, hepatosplenomegaly, anemia/cytopenias, neutropenia/leukopenia, elevated ferritin/triglycerides, and/or decreased fibrinogen."

‡According to the ACMG guideline, c.272C>T(p.Ala91Val) in *PRF1* was classified as a variant of unknown significance.

Table 1. (continued)

Patient no.	Sex	Age at testing, y (unless indicated otherwise)	Ethnicity	Gene	Variant	Zygosity	Population frequency (gnomAD*), %	Symptoms/immunology testing/family history†
168	Male	16	European-American	*XIAP*	c.738del(p.Asp247fs)	Hemizygous	ND	Absent XIAP expression
169	Male	3.6	African American	*XIAP*	c.889A>T(p.Lys297*)	Hemizygous	ND	NA
170	Male	0.4	Unknown	*XIAP*	c.894_898del(p.Lys299fs)	Hemizygous	ND	NA
171	Male	17.2	European-American	*XIAP*	c.894_898del(p.Lys299fs)	Hemizygous	ND	Symptoms of HLH
172	Male	19.6	African American	*XIAP*	c.926_929del(p.Asp309fs)	Hemizygous	ND	Symptoms of HLH
173	Male	17.2	European-American	*XIAP*	c.969G>A(p.Trp323*)	Hemizygous	ND	Decreased XIAP expression
174	Male	22.6	Unknown	*XIAP*	c.1021_1022del(p.Asn341fs)	Hemizygous	ND	NA
175	Male	4.5	Unknown	*XIAP*	c.1056+1G>A	Hemizygous	ND	NA
176	Male	2.7	Latino-Hispanic	*XIAP*	c.1141C>T(p.Arg381*)	Hemizygous	ND	NA
177	Male	11.8	Unknown	*XIAP*	c.1141C>T(p.Arg381*)	Hemizygous	ND	Absent XIAP expression
178	Male	1 d	Pacific-Islander	*XIAP*	c.1239_1242dup(p.Val415fs)	Hemizygous	ND	Markedly decreased XIAP expression
179	Male	1.9	Unknown	*XIAP*	c.1239_1242dup(p.Val415fs)	Hemizygous	ND	NA
180	Male	1.1	Unknown	*XIAP*	c.1301-1G>A	Hemizygous	ND	Symptoms of HLH
181	Male	1.5	Unknown	*XIAP*	c.1445C>G(p.Pro482Arg)	Hemizygous	ND	NA
182	Male	35	European-American	*XIAP*	c.1456dup(p.Thr486fs)	Hemizygous	ND	Symptoms of HLH
183	Male	4.5	European-American	*SH2D1A*	c.20A>G(p.Tyr7Cys)	Hemizygous	ND	Absent SAP in $CD8^+$ T cells
184	Male	1.8	Unknown	*SH2D1A*	c.117C>T(p.Gly39Gly)	Hemizygous	ND	NA
185	Male	3.5	Unknown	*SH2D1A*	c.130T>C(p.Cys44Arg)	Hemizygous	ND	Absent SAP in $CD8^+$ T cells
186	Male	27	European-American	*SH2D1A*	c.163C>T(p.Arg55*)	Hemizygous	ND	History of pneumonia
187	Male	8 d	Unknown	*SH2D1A*	c.172C>T(p.Gln58*)	Hemizygous	ND	NA
188	Male	6.8	African American	*SH2D1A*	c.172C>T(p.Gln58*)	Hemizygous	ND	Absent SAP in $CD8^+$ T cells
189	Male	4.7	Latino-Hispanic	*SH2D1A*	c.199_201+19del(p.Glu67del)	Hemizygous	ND	Absent SAP in $CD8^+$ T cells
190	Male	1.3	African American	*SH2D1A*	c.201G>A(p.Glu67Glu)	Hemizygous	ND	Absent SAP in $CD8^+$ T cells
191	Male	7 d	European-American	*SH2D1A*	c.245del(p.Asn82fs)	Hemizygous	ND	Absent SAP in $CD8^+$ T cells
192	Male	34 d	European-American + Pacific-Islander	*SH2D1A*	c.295C>T(p.Gln99*)	Hemizygous	ND	Absent SAP in $CD8^+$ T cells
193	Male	5.4	Middle-Eastern	*MAGT1*	c.154_161delinsC(p.Ile52fs)	Hemizygous	ND	Symptoms of HLH, bone pain in low extremities
194	Male	10.4	European-American	*MAGT1*	c.223C>T(p.Gln75*)	Hemizygous	ND	NA
195	Male	18.4	African American	*MAGT1*	c.407G>A(p.Trp136*)	Hemizygous	ND	NA
196	Male	27.6	European-American	*MAGT1*	c.443_444del(p.Phe148fs)	Hemizygous	ND	Symptoms of HLH
197	Male	17.3	European-American	*MAGT1*	c.774del(p.Phe258fs)	Hemizygous	ND	NA

NA, no data; ND, no data; NK, natural killer.
*gnomAD v2.1.1 total population frequency.
†HLH hemophagocytic lymphohistiocytosis, symptoms of HLH reported included any or all of the following "fever, hepatosplenomegaly, anemia/cytopenias, neutropenia/leukopenia, elevated ferritin/triglycerides, and/or decreased fibrinogen."
‡According to the ACMG guideline, c.272C>T(p.Ala91Val) in *PRF1* was classified as a variant of unknown significance.

patients. Of these, 197 samples had a definite molecular genetic diagnosis: 87 samples with homozygotes and 75 with compound heterozygotes observed in a recessive condition, respectively, and 35 samples with hemizygotes observed in an X-linked disorder. This resulted in a positive molecular diagnostic rate of 10.4% (supplemental Figure 1). Table 1 lists the genetic variants identified in these 197 patients. Pathogenic or likely causal variants in the *PRF1* gene were the most frequent and were identified in 26.4% (52/197) of patients (Figure 2A). Mutations in the genes associated with degranulation defects were more common than previously appreciated: 21.3% (42/197) of the patients had pathogenic or likely causal variants in *STXBP2*, 19.8% (39/197) in *UNC13D*, 6.1% (12/197) in *RAB27A*, 4.6% (9/197) in *LYST*, and 2.5% (5/197) in *STX11*. Pathogenic variants in the lysinuric protein intolerance gene *SLC7A7* were identified in 1.5% (3/197) of the patients, the least frequent group of patients in our cohort. X-linked conditions accounted for 17.8% (35/197) of the patients: 20 (10.2%) patients had pathogenic or suspected diagnostic variants in *XIAP*, 10 (5.1%) in *SH2D1A*, and 5 (2.5%) in *MAGT1* (Figure 2A). In addition, 30 of 227 patients with clinically suspected

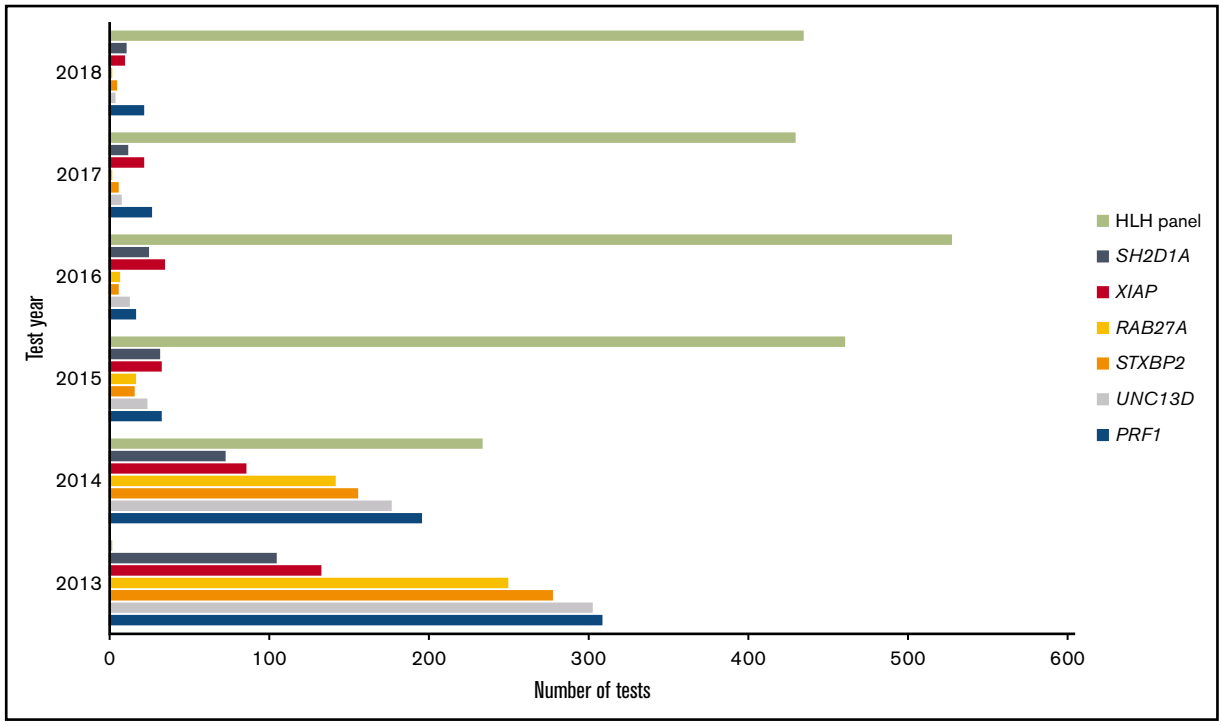

Figure 1. Volume of HLH-related single gene and HLH panel testing in Cincinnati Children's Hospital Medical Center from 2013 to 2018.

HLH were identified to carry only 1 pathogenic or likely pathogenic variant in a recessive condition by this panel approach. Among them, 50% (15/30) had a *PRF1* pathogenic variant, 3 had a *STXBP2* pathogenic variant, 4 had a *UNC13D* pathogenic variant, 5 had a *RAB27* pathogenic variant, 2 had a *LYST* pathogenic variant, and 1 patient had a *STX11* pathogenic variant. Of these 30 patients, 3 (cases S18, S23, and S30) also carried another common *PRF1* variant c.272C>T (p.Ala91Val) in the heterozygous state (Table 2).

When the patients were divided based on age ranges, the diagnostic rates in patients aged 0 to 12 months, 1 to 5 years, 5 to 12 years, 12 to 18 years, and older than 18 years are 28.6% (95/332), 11.3% (43/380), 6.7% (25/371), 3.3% (10/304), and 4.8% (24/505), respectively. Moreover, patients with a molecular diagnosis in familial HLH type 2-5 genes (*PRF1*, *UNC13D*, *STX11*, and *STXBP2*; supplemental Table 1) tended to be referred and diagnosed at an earlier age compared with other genes (median age, 0.7 years [4 days-57.8 years] vs 4.5 years [1 day-53.9 years]; $P = .009$). Patients with X-linked conditions (*XIAP*, *SH2D1A,* and *MAGT1*) were referred and diagnosed at relatively older ages (median age, 5.4 years [1 day-35 years]) (Figure 2B).

Ten of 15 genes in the HLH sequencing panel were identified with pathogenic or likely pathogenic variants in our patient cohort, with the highest allele number and unique variants in the *PRF1* gene (115 alleles; 45 unique variants). The majority of the identified *PRF1* variants (66.7%; 30/45) were missense changes that were distributed along the exons without a particular hot spot. The most frequent pathogenic variant identified in our cohort was c.50del (p.Leu17fs), which appeared in 37 alleles in 27 patients including 10 homozygotes, 12 compound heterozygotes, and 5 heterozygotes. Of the 27 patients carrying this variant, 16 were African American, 3 were Latino-Spanish, 1 was Middle Eastern, and 7 were unknown. The other frequently detected variant was c.445G>A (p.Gly149Ser), which was identified in 19.2% (10/52) of the patients including 3 homozygotes, 6 compound heterozygotes, and 1 heterozygote. Among these 10 patients, 4 were Latino-Spanish, 1 was European-American, and 5 were unknown. (Tables 1 and 2; Figures 3 and 4A).

The second most frequently mutated gene in our cohort was the *STXBP2* gene but with fewer unique variants (87 alleles, 20 unique variants). One-half (10/20) of unique *STXBP2* variants were missense changes. As shown in Figure 4B, the missense variant c.1430C>T (p.Pro477Leu) and the splicing variant c.1247-1G>C were the 2 most frequent variants identified in *STXBP2*, accounting for 33 alleles in 18 patients and 17 alleles in 12 patients, respectively. Of the 18 patients carrying the c.1430C>T (p.Pro477Leu) variant, 13 were Middle Eastern and 5 were unknown. This result implies that this particular mutation in the *STXBP2* gene identified in these patients might be identical by descent (ie, they might be inherited from a common ancestry). On the other hand, among the 12 patients who carry at least 1 splicing variant c.1247-1G>C, 8 were European-American, 1 was Latino-Spanish, and 3 were unknown (Tables 1 and 2; Figures 3 and 4B).

UNC13D was identified with a similar number of causal alleles to *STXBP2* but with the doubled number of unique variants (82 alleles, 45 unique variants). Fourteen of 45 unique *UNC13D* variants were splicing mutations. The intronic variant c.118-308C>T and the 253-kb inversion were each identified in 6 patients in the compound heterozygous state along with a diverse second mutation, much higher than a recently reported Chinese cohort[16] (Table 1; Figures 3 and 4C). As shown in Table 1, among the 39 patients with the *UNC13D* pathogenic or likely pathogenic variants, besides common HLH symptoms, cases 95 and 130 were also reported

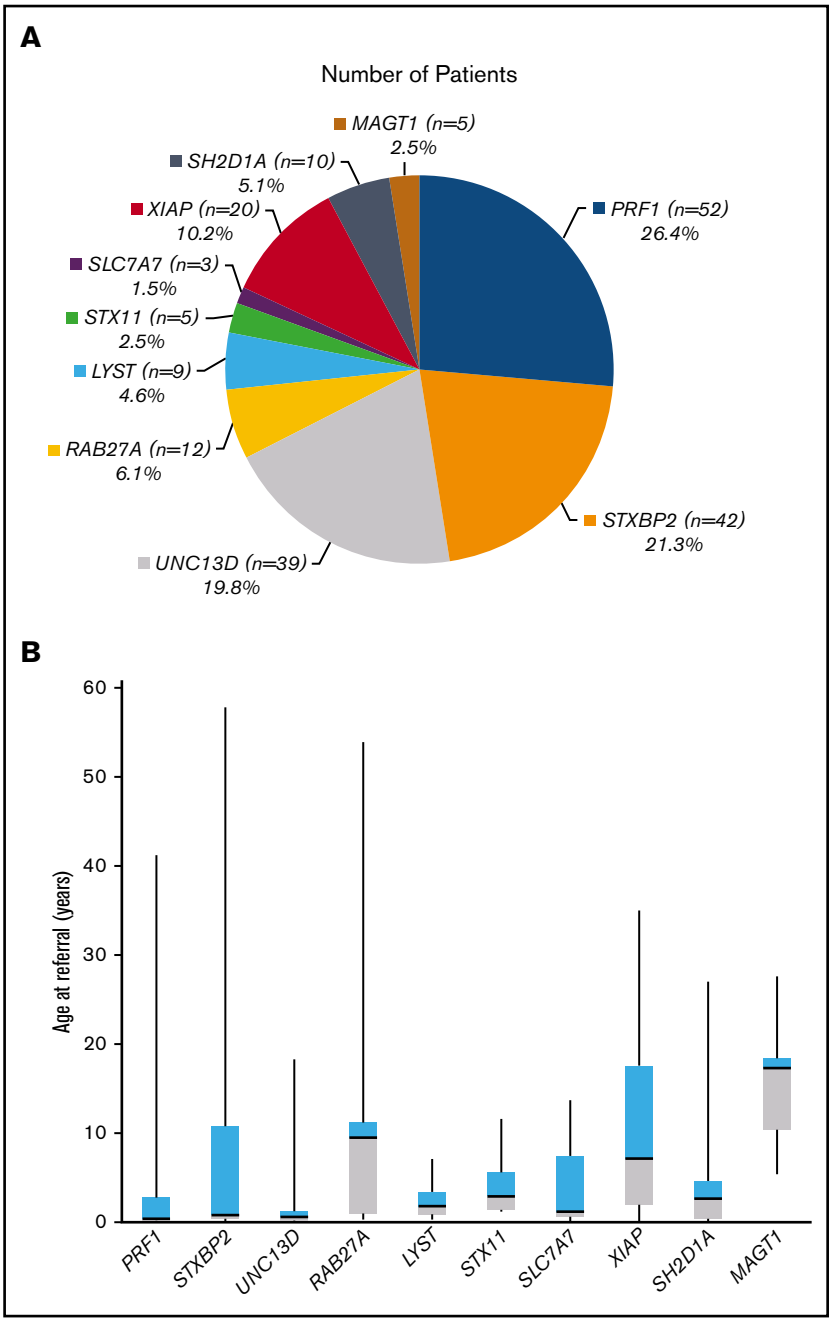

Figure 2. Characteristics of genetic findings and age ranges for 197 HLH patients. (A) Distribution of genetic findings in 197 HLH patients with a definite genetic diagnosis. (B) Whisker-box plot of the age ranges at referral for 197 HLH patients.

to have dysmorphic facies, case 98 seizures, and case 119 hypertelorism. Eleven mutated alleles and 7 unique alterations were identified in the *STX11* gene in our cohort. Although most unique variants (5/7) are truncating variants, the missense variant c.173T>C (p.Leu58Pro) was the most frequently identified in *STX11*, appearing as 2 homozygotes in 2 patients with unknown relationship. Twenty-nine and 20 pathogenic or likely causal variants representing 11 and 13 unique genetic alterations were also identified in *RAB27A* and *LYST*, respectively. Most patients (7/9; 77.8%) with a molecular variation finding in the *LYST* gene were reported to have an albinism phenotype including silver hair, abnormal pigmentation, and oculocutaneous albinism (Tables 1 and 2; Figure 3; supplemental Figure 2).

Thirty unique pathogenic and likely pathogenic variants were identified in the 3 genes associated with an X-linked condition: 16, 9, and 5 unique variants in *XIAP*, *SH2D1A*, and *MAGT1* were identified in 20, 10, and 5 male patients, respectively. Of these unique variants, 83% (25/30) were truncating variants which would presumably result in loss of function of the protein products (Table 1; Figures 2A, 3, and 4D; supplemental Figure 2).

Discussion

In this study, we analyzed 1892 samples tested for a panel of 15 HLH-associated genes, which were received between September 2013 and June 2018 at CCHMC. Both known and novel pathogenic or likely pathogenic variants have been identified in this

Table 2. List of 30 patients in whom only 1 heterozygous pathogenic or likely pathogenic variant was identified

Patient no.	Sex	Age at testing, y (unless indicated otherwise)	Ethnicity	Gene	Variant	Zygosity	Population frequency (gnomAD*), %	Symptoms/immunology testing/family history†
S01	Female	28.3	Unknown	*PRF1*	c.35_46del(p.Leu12_Leu15del)	Heterozygous	ND	NA
S02	Male	20 d	Unknown	*PRF1*	c.50del(p.Leu17fs)	Heterozygous	0.033	Decreased NK cell function and perforin expression
S03	Female	0.4	Unknown	*PRF1*	c.50del(p.Leu17fs)	Heterozygous	0.033	Symptoms of HLH
S04	Male	7.8	African American	*PRF1*	c.50del(p.Leu17fs)	Heterozygous	0.033	Decreased perforin expression
S05	Female	11.2	African American	*PRF1*	c.50del(p.Leu17fs)	Heterozygous	0.033	Absent NK cell function
S06	Male	52	African American	*PRF1*	c.50del(p.Leu17fs)	Heterozygous	0.033	HLH, lymphoma
S07	Male	13.3	Native American	*PRF1*	c.112G>A(p.Val38Met)	Heterozygous	0.0073	Symptoms of HLH
S08	Male	16.6	Latino-Hispanic	*PRF1*	c.445G>A(p.Gly149Ser)	Heterozygous	0.014	Symptoms of HLH
S09	Female	9.9	Unknown	*PRF1*	c.563C>T(p.Pro188Leu)	Heterozygous	0.013	Symptoms of HLH
S10	Male	17.8	Unknown	*PRF1*	c.853_855del(p.Lys285del)	Heterozygous	0.0057	Thrombocytopenia, absent NK cell function
S11	Female	3.1	European-American	*PRF1*	c.1066C>T(p.Arg356Trp)	Heterozygous	0.0014	NA
S12	Female	3	Unknown	*PRF1*	c.1117C>T(p.Arg373Cys)	Heterozygous	0.0051	Symptoms of HLH; normal NK cell function
S13	Female	16.7	Unknown	*PRF1*	c.1117C>T(p.Arg373Cys)	Heterozygous	0.0051	Absent NK cell function
S14	Female	12 d	Middle Eastern	*PRF1*	c.1122G>A(p.Trp374*)	Heterozygous	0.0016	NA
S15	Female	9.7	Malaysian-Chinese	*PRF1*	c.1349C>T(p.Thr450Met)	Heterozygous	0.0028	History of HLH
S16	Female	0.5	Middle Eastern	*STXBP2*	c.1430C>T(p.Pro477Leu)	Heterozygous	0.00074	Symptoms of HLH
S17	Female	0.5	Latino-Hispanic	*STXBP2*	c.1717C>T(p.Pro573Ser)	Heterozygous	ND	NA
S18	Male	37 d	European-American	*STXBP2*	c.1717C>T(p.Pro573Ser)	Heterozygous	ND	Absent NK cell function
				PRF1	c.272C>T(p.Ala91Val)‡	Heterozygous	2.92	
S19	Male	14.9	Asian-American	*UNC13D*	c.118-307G>A	Heterozygous	ND	Absent NK cell function
S20	Male	20.3	Unknown	*UNC13D*	c.247C>T(p.Arg83*)	Heterozygous	0.0004	NA
S21	Female	62.9	European-American	*UNC13D*	c.1759C>T(p.Arg587Cys)	Heterozygous	0.019	Symptoms of HLH
S22	Female	13.1	European-American	*UNC13D*	c.2037_2038insG(p.Arg680fs)	Heterozygous	ND	Symptoms of HLH, one sibling deceased due to HLH
S23	Male	0.8	Middle Eastern	*RAB27A*	c.148_149delinsC(p.Arg50fs)	Heterozygous	ND	Gray hair, suspected for GS, consanguinity
				PRF1	c.272C>T(p.Ala91Val)‡	Heterozygous	2.92	
S24	Male	3	European-American	*RAB27A*	c.240-47_240delins20	Heterozygous	ND	Rash, neutropenia
S25	Male	11.1	Indian	*RAB27A*	c.244C>T(p.Arg82Cys)	Heterozygous	0.0016	Symptoms of HLH
S26	Female	24.9	Latino-Hispanic	*RAB27A*	c.335del(p.Asn112fs)	Heterozygous	0.0044	Symptoms of HLH
S27	Male	2.1	Unknown	*RAB27A*	c.400_401del(p.Lys134fs)	Heterozygous	0.0004	Abnormal brain MRI, decreased NK cell function
S28	Male	29	European-American	*LYST*	c.465_466del(p.Asp157fs)	Heterozygous	ND	Pancytopenia, increased ferritin level

*gnomAD v2.1.1 total population frequency.

†HLH hemophagocytic lymphohistiocytosis, symptoms of HLH reported included any or all of the following "fever, hepatosplenomegaly, anemia/cytopenias, neutropenia/leukopenia, elevated ferritin/triglycerides, and/or decreased fibrinogen."

‡According to the ACMG guideline, c.272C>T(p.Ala91Val) in *PRF1* was classified as a variant of unknown significance.

Table 2. (continued)

Patient no.	Sex	Age at testing, y (unless indicated otherwise)	Ethnicity	Gene	Variant	Zygosity	Population frequency (gnomAD*), %	Symptoms/immunology testing/family history†
S29	Male	6.4	Middle Eastern	*LYST*	c.4159dup(p.Thr1387fs)	Heterozygous	ND	Gray hair
S30	Female	23.2	Unknown	*STX11*	c.650T>A(p.Leu217Gln)	Heterozygous	0.0004	NA
				PRF1	c.272C>T(p.Ala91Val)‡	Heterozygous	2.92	

*gnomAD v2.1.1 total population frequency.

†HLH hemophagocytic lymphohistiocytosis, symptoms of HLH reported included any or all of the following "fever, hepatosplenomegaly, anemia/cytopenias, neutropenia/leukopenia, elevated ferritin/triglycerides, and/or decreased fibrinogen."

‡According to the ACMG guideline, c.272C>T(p.Ala91Val) in *PRF1* was classified as a variant of unknown significance.

population, and their frequency and distributions were analyzed. We found that 12% of the patients had at least 1 pathogenic or likely pathogenic variant in 1 of the 15 genes, and 10.4% of the patients had a definite molecular diagnosis in an HLH-associated gene. To our knowledge, this is the largest cohort of genetically diagnosed HLH, with the highest number of targeted HLH disease-causing genes tested simultaneously.

Patients with a definite genetic diagnosis were significantly younger than patients with only 1 heterozygote finding, with a median age of 14 months (197 cases) compared with 10 years (30 cases) (P = .007). When including all patients analyzed, we found that samples from younger patients were more likely to result in a genetic diagnosis (see "Results"). This observation in our cohort is consistent with previous reports in other similar studies.[16-18]

Together, the genes responsible for degranulation defects (*UNC13D*, *STXBP2*, *STX11*, *LYST*, *RAB27A*) accounted for the majority of cases (107/197; 54.3%). As seen in southern Europe,[17] FHL2 and FHL3 account for a significant proportion of our cases (91/197; 46.2%). However, whereas FHL5 accounted for a minor proportion of HLH in Europeans, it is much more prevalent in our cohort (42/197; 21.3%). *PRF1* was the most frequent causal gene (52/197; 26.4%). Although this finding is not surprising, other groups have described *PRF1* defects in 40% to 60% of their cases.[19,20] Nevertheless, all cohorts reported to date used different methods and criteria to define their cases; some included the variants of unknown significance, and others reported only the known significant biallelic or hemizygous variants. These inconsistent criteria make it difficult to compare the results among different groups. The lower percentage of *PRF1* gene mutations described in our cohort may possibly reflect the use of immunological screening (perforin detection by flow cytometry) before sending for genetic diagnosis.

Compared with other large cohorts previously reported,[16,17,21] our results show an extremely wide range of different mutations, reflecting the vast multiethnic population living in North America. Among the 227 cases, mutations were found in 10 different genes (*PRF1*, *UNC13D*, *STX11*, *STXBP2*, *RAB27A*, *LYST*, *XIAP*, *SH2D1A*, *MAGT1*, and *SLC7A7*), with a total of 175 different mutations. Of these unique mutations, 105 were found in only 1 patient. Only 4 variants were observed in more than 7 patients (*PRF1*: c.50del, c.445G>A; *STXBP2*: c.1247-1G>C, c.1430C>T). The well-described frameshift variant commonly found in patients with African American background, c.50del in *PRF1*,[19,22] was the most common variant found in our cohort. The second most common mutation in *PRF1* was c.445G>A, which has been previously described by multiple groups in different ethnic backgrounds, including Caucasian, Hispanic, Portuguese, German, and

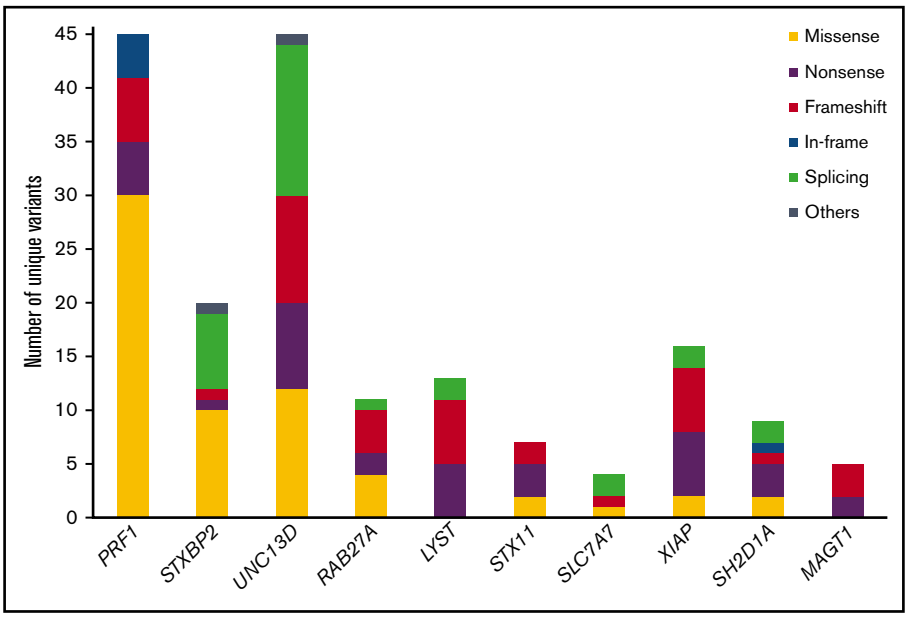

Figure 3. Distribution of unique pathogenic or likely pathogenic variants identified in 10 HLH-associated genes in our patient cohort.

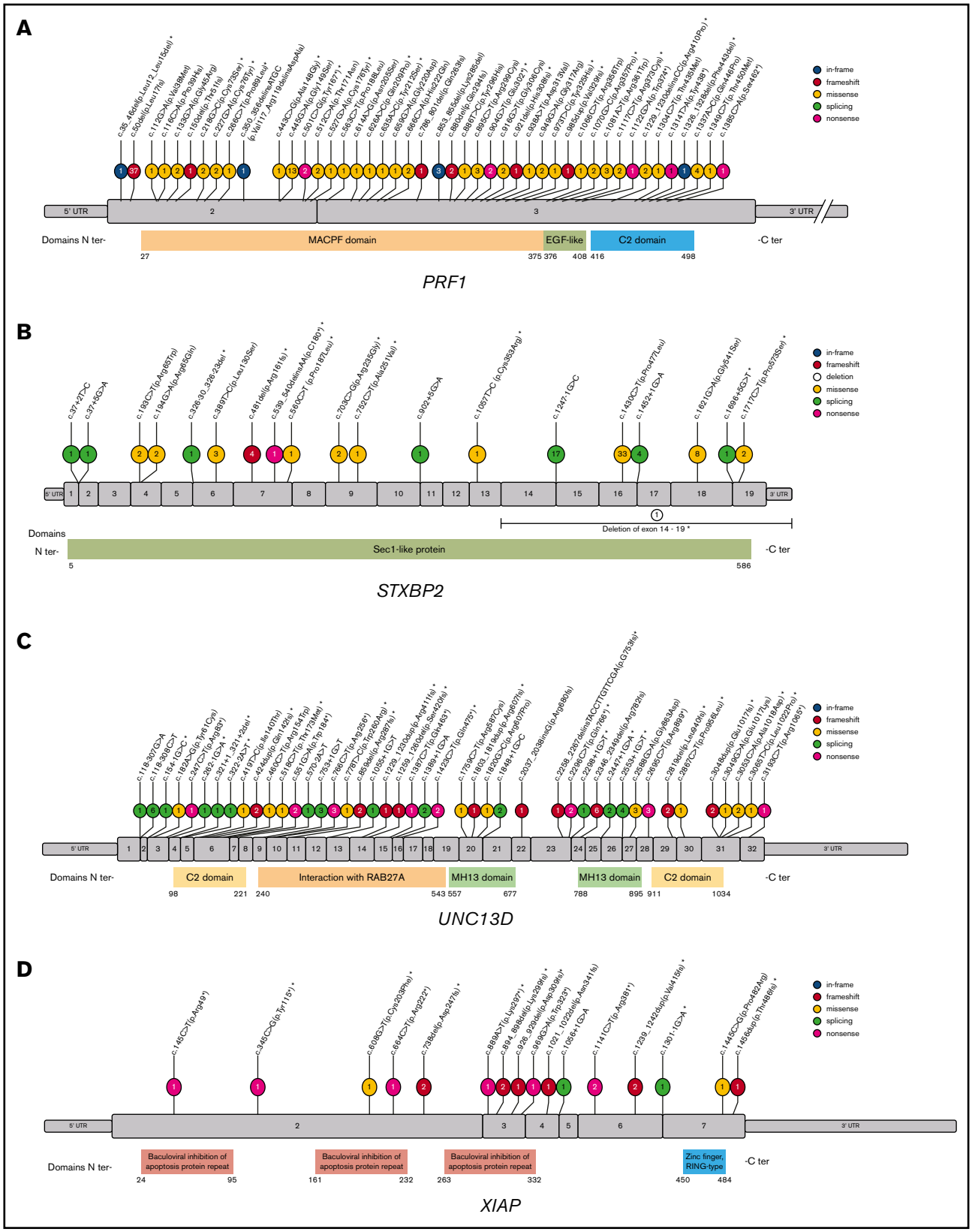

Figure 4. Distributions and frequencies of pathogenic or likely pathogenic variants in the most frequently affected genes associated with HLH in our cohort. (A) Distributions and frequencies of pathogenic or likely pathogenic variants in *PRF1*. (B) Distributions and frequencies of pathogenic or likely pathogenic variants in *STXBP2*. (C) Distributions and frequencies of pathogenic or likely pathogenic variants in *UNC13D*. Note: the 253-kb inversion is not shown in the graph. (D) Distributions and frequencies of pathogenic or likely pathogenic variants in *XIAP*. (A-D) The total number of alleles affected with each variant is indicated in the circles. *Novel variants.

Chinese,[16,19,20,23,24] reflecting the multiethnic population in North America. Interestingly, the commonly reported mutation in the Turkish population,[20,25,26] c.1122G>A, was found in only 1 patient in our cohort. Similarly, we have only 1 case with c.1349C>T, the most common *PRF1* mutation among the Chinese population,[16] and no cases of c.1090_1091del or c.207del, variants commonly found in the Japanese population.[27,28] One of the most prevalent mutations in our cohort, c.1247-1G>C in *STXBP2*, was previously described by other groups and found in multiple ethnic background including Caucasian, Turkish, Northern European, and Pakistani.[5,29,30]

Splicing mutations accounted for an important proportion of our *UNC13D* variants, with 14 of 45 unique mutations,[31,32] which represents a different mutation spectrum of *UNC13D* variation from a Chinese cohort.[16] We observed 3 cases of c.753+1G>T, a predominant mutation found in the German population[20] as well as other parts of Europe (Italy[33] and Croatia[15]). The deep intronic variant c.118-308C>T was observed in 6 unrelated patients, being one of the most frequent *UNC13D* mutations in our cohort. This is one of the most commonly reported mutations among the Korean population, but interestingly we had no cases of c.754-1G>C, the other very common mutation in Korea.[32,34]

The NGS-based approach is highly accurate in capturing point mutations and small deletions and insertions (such as <10 base pair deletions, duplication or insertion), which are the vast majority of sequence changes causing HLH in this 15-gene panel. However, our NGS-based diagnostic pipeline could not reliably identify large structural variations such as large deletions, duplications, or insertions occurring in the genes in this panel because of the limitation of sensitivity and specificity. Better algorithms that could use enriched NGS data to reliably identify these types of variation are warranted. It may be helpful for making a definite genetic diagnosis in the 30 patients in whom only 1 suspicious heterozygous variant in the genes associated with autosomal recessive conditions was identified.

Overall, a genetic diagnosis could be made in 10.4% of the patients using this HLH panel approach. The relatively low diagnostic rate can be explained by multiple factors. Complete clinical information and fulfillment of HLH clinical criteria is crucial to understanding our genetic results. As a heterogeneous disease with different disease mechanisms, many HLH-associated genes have yet to be discovered. In addition, some newly discovered HLH-associated genes such as *NLRC4*[35,36] and *CDC42*[37] were not included in our panel and therefore could cause a missing genetic diagnosis for some of those negative cases. Some additional primary immune deficiencies have been reported to present with HLH, for example severe combined immunodeficiency, DiGeorge syndrome, Wiskott-Aldrich syndrome, chronic granulomatous disease, and STAT1 gain of function, among others.[18,38-40] Several metabolic diseases also predispose to the development of HLH.[41-44] With sequencing costs and analysis times going down, whole exome sequencing and even whole genome sequencing will provide more comprehensive solutions for detecting underlying genetic causes of HLH. Whole exome and whole genome sequencing would also identify genetic disorders that are associated with clinical phenotypes that mimic HLH. Indeed, a broader use of whole exome sequencing has already been recommended for patients with HLH.[18] On the other hand, a significant proportion of the 1859 patients in this cohort likely had a secondary form of acquired HLH that developed in the context of malignancy, autoimmune disease, or infection, and whole exome and whole genome sequencing may not be a cost-effective approach for all patients with HLH at present. As the field of genetics continues to make clinical advances, clinicians should continue to weigh the ease, cost, completeness, and timeliness of genetic panel testing options for patients with HLH.

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Authorship

Contribution: K.A.R., R.A.M., and M.S. designed the research; V.G.-L., L.D., R.S., J.C., K.Z., K.A.R., R.A.M., and M.S. analyzed data; V.G.-L. and M.S. interpreted results; V.G.-L., J.C., R.A.M., and M.S. wrote the manuscript; and all authors edited the manuscript.

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