Supplementary Methods

Research-Based Whole Exome Sequencing

Whole exome sequencing was performed at the Human Genome sequencing center (HGSC) at Baylor College of Medicine through the Baylor-Hopkins Center for Mendelian Genomics (CMG) initiative. Using 1 μg of DNA, an Illumina paired-end pre-capture library was constructed according to the manufacturer's protocol (Illumina Multiplexing_SamplePrep_Guide_1005361_D) with modifications as described in the *BCM-HGSC Illumina Barcoded Paired-End Capture Library Preparation* protocol. Pre-capture libraries were pooled into 4-plex library pools and then hybridized in solution to the HGSC-designed Core capture reagent[1] (52 Mb, NimbleGen), or 6-plex library pools used the custom VCRome 2.1 capture reagent[1] (42 Mb, NimbleGen) according to the manufacturer's protocol (*NimbleGen SeqCap EZ Exome Library SR User's Guide*) with minor revisions. The sequencing run was performed in paired-end mode using the Illumina HiSeq 2000 platform, with sequencing-by-synthesis reactions extended for 101 cycles from each end and an additional 7 cycles for the index read. With a sequencing yield of 9.1 Gb, the sample achieved 91% of the targeted exome bases covered to a depth of 20X or greater. Illumina sequence analysis was performed using the HGSC Mercury analysis pipeline (https://www.hgsc.bcm.edu/software/mercury)[2,3], which moves data through various analysis tools from the initial sequence generation on the instrument to annotated variant calls (SNPs and intra-read in/dels).

Computational Analyses of Potential Digenic fHLH Gene Variants and Dysregulated Immune Activation and Proliferation-Associated Genes

For multilocus variation/mutational burden or "digenic" inheritance analysis, for each two-gene combination of fHLH genes *PRF1*, *STX11*, *STXBP2*, and *UNC13D* together with *LYST* and *RAB27A*, the number of alleles containing potential protein-altering variants (frameshift insertions/deletions, stopgain/stoploss, splicing defects, non-frameshift insertions/deletions, and missense variants) in HLH affected cases (*n* = 48) was compared to the number in other samples in the Baylor-Hopkins CMG database (total *n* = 6,677; analyzed *n* = 5,981), excluding unaffected relatives in the HLH cohort (*n* = 51) and individuals in the "Immunodeficiency" cohort (*n* = 645). The Baylor-Hopkins CMG database contains exomes from well-phenotyped diseased and healthy individuals (recorded within the PhenoDB database)[4] collected among over 380 phenotypic cohorts. Variants were excluded from this analysis if less than 5 variant reads were observed or if present in homozygous state. For each gene combination, the allele count was increased by 1 if a variant was present in both genes within the exome examined. It was not increased if the exome had no variants in either gene or if it had a variant in only one gene in the gene combination.

For the DIAP gene variant analysis, the number of alleles containing potential protein-altering variants (frameshift insertions/deletions, stopgain/stoploss, splicing defects, non-frameshift insertions/deletions, and missense variants) in *NLRC4*, *NLRP12*, *NLRP4*, *NLRP13,* and *NLRC3* in HLH affected cases (*n* = 48) was compared to the number in other samples in the Baylor-Hopkins CMG database (total *n* = 6,677; analyzed *n* = 5,981), excluding unaffected relatives in the HLH cohort (*n* = 51) and individuals in the "Immunodeficiency" cohort (*n* = 645). The Baylor-Hopkins CMG

database contains exomes from well-phenotyped diseased and healthy individuals (recorded within the PhenoDB database)[4] collected among over 380 phenotypic cohorts. For all analyses, variants were excluded if less than 5 variant reads were observed. For dominant model testing, variants were included for analysis if present in heterozygous state and observed at an allelic frequency of less than or equal to 0.0002 in the Baylor-Hopkins CMG database. For recessive model testing, variants were included for analysis if present in compound heterozygous or homozygous state and observed at an allelic frequency of less than or equal to 0.005 in the Baylor-Hopkins CMG database.

We then performed random sampling without replacement (bootstrapping method) to test the significance of differences in allelic counts between cases and controls. For both analyses, the allele counts in cases and controls were permuted for 100,000 iterations for each gene combination or DIAP-associated gene, and a *p*-value was calculated for the difference in allele counts in cases vs. controls.

Table S1. Diagnostic criteria for HLH used in the HLH-2004 trial.[5]

1. Fever ≥38.5°C
2. Splenomegaly
3. Cytopenias (affecting at least 2 of 3 lineages in the peripheral blood)
   - *Hemoglobin <9 g/dL* (in infants <4 weeks: hemoglobin <10 g/dL)
   - *Platelets <100 x 10^3/ml*
   - *Neutrophils <1 x 10^3/ml*
4. Hypertriglyceridemia (fasting, >265 mg/dl) and/or hypofibrinogenemia (<150 mg/dL)
5. Hemophagocytosis in bone marrow, spleen, lymph nodes, or liver
6. Low or absent NK cell activity
7. Ferritin >500 ng/ml
8. Elevated sCD25 (>2 standard deviations above mean)

Abbreviations: NK, natural killer

Table S2. Variants in familial HLH genes identified in research whole exome sequencing analyses

ID	Gene	Chromosomal coordinates (hg19)	Zygosity	Mutation type	Reference sequence and effect	CMG[a]	ExAC[b]	CADD[c]	Candidate(s)[d]	*P*[e]
HLH_002	*LYST*	1:235826382_TA>T	Het	Splicing defect	NM_000081:exon54: c.11268-5delT	0.24	0.51	N/A	*NLRC4*	N/A
HLH_004	*LYST*	1:235826382_TA>T	Het	Splicing defect	NM_000081:exon54: c.11268-5delT	0.24	0.51	N/A	*NLRC3*	N/A
HLH_005	*LYST*	1:235826382_TA>T	Het	Splicing defect	NM_000081:exon54: c.11268-5delT	0.24	0.51	N/A	*NLRP4*	N/A
	STXBP2	19:7705300_A>ATG	Het	Intronic	N/A	0.02	0.34	N/A		
HLH_006	*LYST*	1:235826382_TA>T	Het	Splicing defect	NM_000081:exon54: c.11268-5delT	0.24	0.51	N/A		N/A
HLH_007	*STXBP2*	19:7704894_GTGTC>G	Het	Intronic	N/A	0.003	0.005	N/A	*NRAS*	
HLH_008	*LYST*	1:235826382_TA>T	Het	Splicing defect	NM_000081:exon54: c.11268-5delT	0.24	0.51	N/A	*NLRP4*	N/A
HLH_014	*LYST*	1:235826382_TA>T	Het	Splicing defect	NM_000081:exon54: c.11268-5delT	0.24	0.51	N/A		NS
	UNC13D	17:73826146_T>C	Het	Missense	NM_199242:exon30: c.2917A>G:p.K973E	0.0002	0.0003	22.4		
HLH_016	*LYST*	1:235856663_G>A	Het	Missense	NM_000081:exon47: c.10688C>T:p.S3563L	0.00008	0.00002	26		NS
	PRF1	10:72358822_A>T	Het	Missense	NM_001083116:exon3: c.655T>A:p.Y219N	0.00008	0	26.8		
	STXBP2	19:7705383_ CATCTGTGTGTGTGT>C	Het	Intronic	N/A	0.03	0.04	N/A		
HLH_017	*LYST*	1:235826382_TA>T	Het	Splicing defect	NM_000081:exon54: c.11268-5delT	0.24	0.51	N/A		NS
	UNC13D	17:73826167_G>A	Het	Missense	NM_199242:exon30: c.2896C>T:p.R966W	0.003	0.01	7		
HLH_024	*RAB27A*	15:55520906_G>A	Hom	Missense	NM_183236:exon5: c.244C>T:p.R82C	0.00008	0.000008	34	*RAB27A*	N/A
HLH_040	*LYST*	1:235826382_TA>T	Het	Splicing defect	NM_000081:exon54: c.11268-5delT	0.24	0.51	N/A		N/A
HLH_043	*STXBP2*	19:7705300_A>ATG	Het	Intronic	N/A	0.02	0.34	N/A		N/A
HLH_050	*LYST*	1:235826382_TA>T	Het	Splicing defect	NM_000081:exon54: c.11268-5delT	0.24	0.51	N/A		N/A
	STXBP2	19:7705383_ CATCTGTGTGTGTGT>C	Het	Intronic	N/A	0.03	0.04	N/A		
HLH_057	*LYST*	1:235826382_TA>T	Het	Splicing defect	NM_000081:exon54: c.11268-5delT	0.24	0.51	N/A		N/A
	STXBP2	19:7705461_C>A	Het	Intronic	N/A	0.002	0.0009	4.3		
	STXBP2	19:7705487_T>G	Het	Intronic	N/A	0.002	0.007	0.3		
	STXBP2	19:7705502_T>A	Het	Intronic	N/A	0.002	0.007	1.3		

HLH_063	*LYST*	1:235826382_TA>T	Het	Splicing defect	NM_000081:exon54: c.11268-5delT	0.24	0.51	N/A		NS
	STXBP2	19:7711219_G>A	Het	Missense	NM_001272034:exon16: c.1474G>A:p.D492N	0.00008	0	27.5		
HLH_087	*LYST*	1:235950630_C>T	Het	Missense	NM_000081:exon14: c.4732G>A:p.A1578T	0.0002	0	23.2		NS
	UNC13D	17:73830182_C>T	Het	Missense	NM_199242:exon24: c.2341G>A:p.V781I	0.001	0.001	2.4		
HLH_097	*LYST*	1:235850245_C>A	Het	Splicing defect	NM_000081:exon49: c.10800+4G>T	0.007	0.006	N/A		NS
	STX11	6:144507773_C>A	Het	Missense	NM_003764:exon2: c.9C>A:p.D3E	0.0002	0.00003	26.7		
HLH_104	*LYST*	1:235826382_TA>T	Het	Splicing defect	NM_000081:exon54: c.11268-5delT	0.24	0.51	N/A	*IL16*	N/A
HLH_107	*STXBP2*	19:7704886_CTATG>C	Het	Intronic	N/A	0.01	0.006	N/A	*ERCC4, TREM2, or VMAC*	N/A
HLH_109	*STX11*	6:144508353_G>A	Het	Missense	NM_003764:exon2: c.589G>A:p.V197M	0.003	0.0004	24	*RAG2*	N/A
HLH_111	*LYST*	1:235826382_TA>T	Het	Splicing defect	NM_000081:exon54: c.11268-5delT	0.24	0.51	N/A	*STAT2*	N/A
HLH_113	*LYST*	1:235826382_TA>T	Het	Splicing defect	NM_000081:exon54: c.11268-5delT	0.24	0.51	N/A	*PIK3CD*	N/A
HLH_115	*LYST*	1:235826382_TA>T	Het	Splicing defect	NM_000081:exon54: c.11268-5delT	0.24	0.51	N/A	*DOCK8*	N/A
HLH_116	*LYST*	1:235826382_TA>T	Het	Splicing defect	NM_000081:exon54: c.11268-5delT	0.24	0.51	N/A	*RLTPR*	N/A
	LYST	1:235918688_CAAAAG>C	Het	Intronic	N/A	0.001	0	N/A		
	STX11	6:144512671_T>TA	Het	UTR3	N/A	0.004	0	N/A		
	PRF1	10:72357599_TAAA>T	Het	UTR3	N/A	0.0002	0	N/A		
HLH_117	*LYST*	1:235955277_G>A	Het	Missense	NM_000081:exon12: c.4265C>T:p.A1422V	0.0006	0.00008	21.6	*RAG1*	0.03
	RAB27A	15:55516136_G>C	Het	Missense	NM_183236:exon6: c.418C>G:p.Q140E	0.0004	0.0003	10.6		
HLH_121	*LYST*	1:235826382_TA>T	Het	Splicing defect	NM_000081:exon54: c.11268-5delT	0.24	0.51	N/A	*NCF1*	N/A
HLH_122	*LYST*	1:235918688_C>CAAAAG	Het	Intronic	N/A	0.002	0	N/A	*STAT3 & NLRP4*	N/A
	STX11	6:144512671_T>TA	Het	UTR3	N/A	0.004	0	N/A		
	STXBP2	19:7708058_C>T	Het	Missense	NM_001272034:exon13: c.1067C>T:p.T356M	0.00008	0.01	26.3		

[a] Allelic frequency in the Baylor-Hopkins Center for Mendelian Genomics database
[b] Allelic frequency in the Exome Aggregation Consortium database
[c] Combined Annotation Dependent Depletion Phred score

[d] Genes containing likely disease-contributing variants in the subject
[e] Statistical significance in mutational burden/potential digenic inheritance analysis
Abbreviations: Het, heterozygous; Hom, homozygous; ID, identifier; N/A, not available or not applicable; NS, not significant; UTR3, untranslated 3' region
Underlined samples had chromosomal microarray testing performed.

Table S3. Bioinformatic mutational burden analyses of potential digenic fHLH variants.

Gene 1	Gene 2	Frequency of combination in HLH cohort	Frequency of combination in Baylor-Hopkins CMG	*p*
PRF1	*STX11*	0	0.004	0.66
PRF1	*STXBP2*	0	0.004	0.68
PRF1	*LYST*	0.02	0.06	0.89
PRF1	*UNC13D*	0	0.01	0.82
PRF1	*RAB27A*	0	0.0007	0.57
STX11	*STXBP2*	0	0.001	0.59
STX11	*LYST*	0.02	0.02	0.35
STX11	*UNC13D*	0.02 (note: from clinical testing)	0.005	0.03
STX11	*RAB27A*	0	0.0003	0.55
STXBP2	*LYST*	0.02	0.02	0.52
STXBP2	*UNC13D*	0	0.005	0.70
STXBP2	*RAB27A*	0	0.0008	0.56
LYST	*UNC13D*	0.06	0.07	0.56
LYST	*RAB27A*	0.02	0.005	0.03
UNC13D	*RAB27A*	0	0.001	0.59

Table S4. Potential molecular diagnoses associated with HLH in 28 of 48 subjects evaluated by research whole exome sequencing analyses.

ID	Gene(s)	Chromosomal coordinates (hg19)	Reference sequence and effect	Zygosity	Consanguinity[a]	Inheritance[b]	Mutation type	CMG[c]	ExAC[d]	MSC[e]	CADD[f]	Group[g]
HLH_024	*RAB27A*	15:55520906_G>A	NM_183236:exon5: c.244C>T:p.R82C	Hom	1	(P / M)	Missense	0.0001	0.000008	4.037	34	1
	RAD51C	17:56772522_G>A	NM_058216:exon2: c.376G>A:p.A126T	Hom	1	(P / M)	Missense	0.004	0.004	0.001	21.2	-
HLH_112	*WAS*	X:48547298_C>T	NM_000377:exon10: c.1181C>T:p.P394L	Hemi	2	M	Missense	0.0003	0.0003	0.721	13.6	2A
HLH_120	*TTC7A*	2:47233133_G>A	NM_001288953:exon10: c.1036G>A:p.A346T	Het	0	NT	Missense	0.0001	0.0002	0.001	0.177	2B
	TTC7A	2:47251490_G>A	NM_001288953:exon15: c.1531G>A:p.V511I	Het	0	NT	Missense	0.0002	0.0002	0.001	10.63	2B
	LRBA	4:151242409_T>G	NM_006726:exon51: c.7597A>C:p.T2533P	Het	0	NT	Missense	0.005	0.005	32	7.889	2B
	LRBA	4:151792519_G>A	NM_006726:exon19: c.2345C>T:p.T782I	Het	0	NT	Missense	0.0001	0.000008	32	32	2B
	NLRP12	19:54327394_C>T	NM_144687:exon1: c.35G>A:p.R12H	Het	0	NT	Missense	0.0001	0.00006	23.6	23.7	3
HLH_115	*DOCK8*	9:(24850-379936)x0	-	Hom	1	P / M	Deletion	N/A	N/A	0.001	N/A	2A
HLH_116	*CARMIL2*	16:67681977_G>A	NM_001013838:exon14: c.1094G>A:p.R365H	Het	0	P	Missense	0.0002	0.00005	3.313	21.5	2B
	CARMIL2	16:67688564_A>T	NM_001013838:exon31: c.3551A>T:p.E1184V	Het	0	M	Missense	0.0006	0.0008	3.313	26.5	2B
	CLEC18B	16:74446946_C>G	NM_001011880:exon5: c.665G>C:p.G222A	Het	0	M	Missense	0.0002	0.000008	3.313	23.9	-
	CLEC18B	16:74446997_G>A	NM_001011880:exon5: c.614C>T:p.S205L	Het	0	P	Missense	0.0002	0.00007	3.313	28.4	-
HLH_117	*RAG1*	11:36596065_G>A	NM_000448:exon2: c.1211G>A:p.R404Q	Hom	1	(P / M)	Missense	0.0002	0.000008	1.118	29.4	2A
HLH_109	*RAG2*	11:36614905_C>T	NM_001243786:exon3: c.814G>A:p.V272I	Het	2	Not M	Missense	0.0001	0.0002	0.001	3.491	2A
	RAG2	11:36615697_C>T	NM_001243786:exon3: c.22G>A:p.V8I	Het	2	M	Missense	0.004	0.003	0.001	20.2	2A
HLH_114	*CYBB*	X:37668913_GA>G	NM_000397:exon12: c.1556delA:p.E519fs	Hemi	0	NT	Frameshift deletion	0.0001	0	0.001	N/A	2A
	DOCK11	X:117796732_A>T	NM_144658:exon45: c.5053A>T:p.M1685L	Hemi	0	NT	Missense	0.0001	0.00001	3.313	21.6	-
HLH_121	*NCF1*	7:74197326_A>G	NM_000265:exon6: c.496A>G:p.N166D	Hom	2	(P / M)	Missense	0.001	0.0008	0.001	0.003	2A
	NLRP12	19:54327368_C>T	NM_144687:exon1: c.61G>A:p.E21K	Het	2	NT	Missense	0.0001	0.00004	23.6	24.1	3
HLH_118	*STAT1*	2:191844538_C>T	NM_139266:exon20: c.1687G>A:p.E563K	Het	0	DN	Missense	0.0001	0	7.736	24.1	2A
HLH_111	*STAT2*	12:56750224_C>T	NM_198332:exon3:	Hom	1	(P) / M	Splicing defect	0.0002	0	3.313	28.3	2A

			c.131+1G>A									
HLH_122	*STAT3*	17:40486008_T>C	NM_213662:exon9: c.857A>G:p.E286G	Het	0	DN	Missense	0.0001	0	15.29	26.4	2A
	NLRP4	19:56369040_G>C	NM_134444:exon3: c.281G>C:p.G94A	Hom	0	P / M	Missense	0.002	0.003	3.313	0.154	3
HLH_113	*PIK3CD*	1:9781572_G>A	NM_005026:exon15: c.1882G>A:p.E628K	Het	0	NT	Missense	0.0001	0	23.8	25.1	2B
HLH_110	*MCM3AP*	21:47699982_T>C	NM_003906:exon4: c.1592A>G:p.E531G	Het	0	P	Missense	0.0004	0.0007	3.313	22.8	2B
	MCM3AP	21:47704306_G>C	NM_003906:exon1: c.895C>G:p.R299G	Het	0	M	Missense	0.0001	0	3.313	22.4	2B
	MCM9	6:119137445_C>A	NM_017696:exon12: c.1974G>T:p.Q658H	Het	0	M	Missense	0.008	0.008	0.001	24.1	2B
	MCM9	6:119234579_T>C	NM_017696:exon5: c.911A>G:p.N304S	Het	0	P	Missense	0.007	0.003	0.001	26.4	2B
HLH_119	*CASP10*	2:202074287_T>C	NM_032977:exon9: c.1415+2T>C	Het	0	DN	Splicing defect	0.0001	0.00003	0.001	23.7	2A
	HAVCR2	5:156533787_T>C	NM_032782:exon2: c.245A>G:p.Y82C	Hom	0	P / (M)	Missense	0.003	0.004	3.313	24	-
HLH_002	*NLRC4*	2:32476322_C>T	NM_001199139:exon4: c.611G>A:p.R204H	Het	2	NT	Missense	0.0001	0.00005	12.26	4.759	3
HLH_003	*NLRC4*	2:32476073_A>G	NM_001199139:exon4: c.860T>C:p.I287T	Het	2	DN	Missense	0.0002	0	12.26	23.2	3
HLH_006	*NLRP12*	19:54310804_C>G	NM_144687:exon4: c.2188G>C:p.V730L	Het	0	NT	Missense	0.0001	0.000008	23.6	21	3
HLH_005	*NLRP4*	19:56369244_C>T	NM_134444:exon3: c.485C>T:p.T162M	Het	0	P	Missense	0.004	0.005	3.313	25.5	3
	NLRP4	19:56388513_T>C	NM_134444:exon8: c.2677T>C:p.C893R	Het	0	M	Missense	0.0002	0	3.313	23.6	3
	TNFRSF4	1:1147210_G>A	NM_003327:exon6: c.637C>T:p.R213C	Het	0	DN	Missense	0.0001	0	3.313	22.6	-
	PRKCI	3:169998024_A>G	NM_002740:exon9: c.A715G:p.T239A	Het	0	DN	Missense	0.00008	0.00002	5.744	10.81	-
HLH_008	*NLRP4*	19:56370038_G>A	NM_134444:exon3: c.1279G>A:p.A427T	Het	0	NT	Missense	0.005	0.004	3.313	1.29	3
	NLRP4	19:56370230_G>A	NM_134444:exon3: c.1471G>A:p.A491T	Het	0	NT	Missense	0.0005	0.0007	3.313	0.005	3
	IRF2BP2	1:234743113_G>A	NM_182972:exon2: c.1534C>T:p.R512W	Het	0	NT	Missense	0.0001	0	3.313	32	-
HLH_004	*NLRC3*	16:3613295_T>C	NM_178844:exon5: c.1643A>G:p.E548G	Het	0	M	Missense	0.002	0.002	3.313	0.337	3
	NLRC3	16:3614541_G>A	NM_178844:exon5: c.397C>T:p.R133W	Het	0	P	Missense	0.0002	0.00007	3.313	27.8	3
HLH_001	*NLRP13*	19:56410233_C>T	NM_176810:exon10: c.2860G>A:p.V954M	Het	0	NT	Missense	0.0001	0.00007	3.313	2.101	3

	NLRP13	19:56424008_C>T	NM_176810:exon5: c.1175G>A:p.R392Q	Het	0	NT	Missense	0.0002	0.0005	3.313	7.246	3
HLH_007	*NRAS*	1:115258748_C>T	NM_002524:exon2: c.34G>A:p.G12S	Het	0	DN	Missense	0.0001	0	24.9	25.6	3
HLH_104	*IL16*	15:81571896_C>T	NM_001172128:exon8: c.865-3C>T	Hom	1	P / (M)	Splicing defect	0.002	0.002	3.313	N/A	4
	KIFC1	6:33365944_G>C	NM_002263:exon2: c.150+1G>C	Het	1	M	Splicing defect	0.0002	0	3.313	24.8	-
	KIFC1	6:33372764_C>T	NM_002263:exon7: c.892C>T:p.R298W	Het	1	Not M	Missense	0.0002	0.00002	3.313	34	-
HLH_105	*STAT4*	2:191899278_C>T	NM_003151:exon18: c.1616G>A:p.C539Y	Het	0	NT	Missense	0.0001	0.000008	3.313	31	4
	GATA4	8:11606426_A>G	NM_002052:exon3: c.617-2A>G	Het	0	NT	Splicing defect	0.0001	0	0.001	24.3	-
HLH_106	*ARHGEF6*	X:135750306_C>A	NM_004840:exon22: c.2213G>T:p.C738F	Hemi	0	NT	Missense	0.0001	0.00001	3.313	23	4
	PLCG2	16:81973606_G>A	NM_002661:exon30: c.3423G>A:p.M1141I	Het	0	NT	Missense	0.0001	0.00002	3.313	23.6	4
	AZU1	19:830804_G>A	NM_001700:exon4: c.457G>A:p.G153S	Hom	0	NT	Missense	0.0002	0.00007	3.313	26.2	-
HLH_107	*G3BP1*	5:151179538_C>T	NM_005754:exon9: c.932C>T:p.P311L	Hom	1	P / M	Missense	0.0003	0	4.363	26.2	4
	ERCC4	16:14029418_TTTCGGAATCCTGAAAGAACCCCTCACTATCATCCATCCGC>T	NM_005236:exon8: c.1630_1669del: p.544_557del	Het	1	P	Frameshift deletion	0.0002	0	0.001	N/A	4
	ERCC4	16:14041570_T>C	NM_005236:exon11: c.2117T>C:p.I706T	Het	1	M	Missense	0.002	0.001	0.001	26.9	4
	TREM2	6:41127543_G>A	NM_001271821:exon3: c.469C>T:p.H157Y	Hom	1	P / M	Missense	0.005	0.004	0.001	23.1	4
	TSPAN6	X:99888957_T>G	NM_003270:exon4: c.421A>C:p.S141R	Hemi	1	DN	Missense	0.0001	0	5.380	20.5	-
HLH_108	*RASGRP3*	2:33745018_C>A	NM_170672:exon6: c.174-1C>A	Het	0	M	Splicing defect	0.0002	0	3.313	23.2	4
	RASGRP3	2:33752240_G>A	NM_170672:exon11: c.844G>A:p.G282S	Het	0	Not M	Missense	0.009	0.009	3.313	24.4	4

[a] Known or suspected consanguinity: 0 – none, 1 – present, 2 – potential distant consanguinity

[b] Presence of variant in genomic DNA extracted from parent(s); designations in parentheses denote samples that could not be tested due to insufficient quantity or unavailability but predicted due to confirmed homozygosity

[c] Allelic frequency in the Baylor-Hopkins Center for Mendelian Genomics database

[d] Allelic frequency in the Exome Aggregation Consortium database

[e] Rockefeller University Mutations Significance Cutoff for CADD Phred score

[f] Combined Annotation Dependent Depletion Phred score

[g] Group classifications: 1 - familial HLH, 2 - primary immunodeficiency disease, 3 - dysregulated immune activation and proliferation, 4 - other gene candidates; A - likely, B - possible

Abbreviations: DN, *de novo*; Hemi, hemizygous; Het, heterozygous; Hom, homozygous; ID, identifier; M, maternal; N/A, not available; NT, not tested due to insufficiency/unavailability of sample(s); P, paternal

Table S5. Genetic Findings and Kaplan-Meier Survival Estimates

Genetic Category	Total	Survivors (*N*)	Overall Survival Estimate	*p*	*p*	*p*	*p*
fHLH	19	12	54%	0.49	0.48	0.81	0.30
PIDD Only	11	7	88%	0.17	0.81	0.50	Reference
DIAP Only	8	5	67%	0.44	0.60	Reference	
Other Candidate Defects	5	3	67%	0.38	Reference		
No Genetic Explanation	76	41	47%	Reference			

Abbreviations: DIAP, dysregulated immune activation or proliferation; fHLH, familial HLH; PIDD, primary immunodeficiency disease.

Table S6. Clinical and Genetic Features by Associated Trigger

Associated Trigger	Total	Overall Survivors (*N*)	1-Year Survival Estimate[a]	3-Year Survival Estimate[a]	Overall Survival Estimate[a]	*p* (Overall Survival Estimate[a])	*p* (Overall Survival Estimate[a])	*p* (Overall Survival Estimate[a])	Maximum Level of Therapy	Genetic Profile
Autoimmune	32	23	75%	69%	69%	0.58	0.13	0.13	Observation/None: 3.1% Biologics/Steroids: 31.3%* Immunochemotherapy: 53.1% HSCT: 12.5%*	fHLH: 0.0%* PIDD: 3.1% DIAP: 15.6% PIDD/DIAP: 3.1% Other Candidate Defects: 0.0% No Genetic Explanation: 78.1%*
Infection	44	23	52%	49%	44%	0.02	0.70	Reference	Observation/None: 11.4% Biologics/Steroids: 13.6% Immunochemotherapy: 36.4% HSCT: 34.1% Unknown: 4.6%	fHLH: 15.9% PIDD: 11.4% DIAP: 0.0% PIDD/DIAP: 2.3% Other Candidate Defects: 4.6% No Genetic Explanation: 65.9%
Malignancy	14	6	44%	44%	44%	0.02	Reference		Observation/None: 7.1% Biologics/Steroids: 0.0% Immunochemotherapy: 78.6%* HSCT: 7.1%* Unknown: 7.1%	fHLH: 7.1% PIDD: 7.1% DIAP: 7.1% PIDD/DIAP: 0.0% Other Candidate Defects: 14.3% No Genetic Explanation: 64.3%
No Associated Trigger	32	25	93%	78%	58%	Reference			Observation/None: 6.3% Biologics/Steroids: 6.3% Immunochemotherapy: 31.3% HSCT: 56.3% Unknown: 0.0%	fHLH: 34.4% PIDD: 12.5% DIAP: 6.3% PIDD/DIAP: 3.1% Other Candidate Defects: 3.1% No Genetic Explanation: 40.6%

Abbreviations: DIAP, dysregulated immune activation or proliferation; fHLH, familial HLH; HSCT, hematopoietic stem cell transplantation; PIDD, primary immunodeficiency disease.

[a] By Kaplan-Meier analysis

* Significantly different compared to No Associated Trigger group ($p < 0.05$)

Supplemental Figures

Figure S1. Summary of workflow and genetic findings for 101 subjects who met the HLH-2004 criteria and received genetic testing. Abbreviations: DIAP, dysregulated immune activation or proliferation; fHLH, familial hemophagocytic lymphohistiocytosis; PIDD, primary immunodeficiency disease.

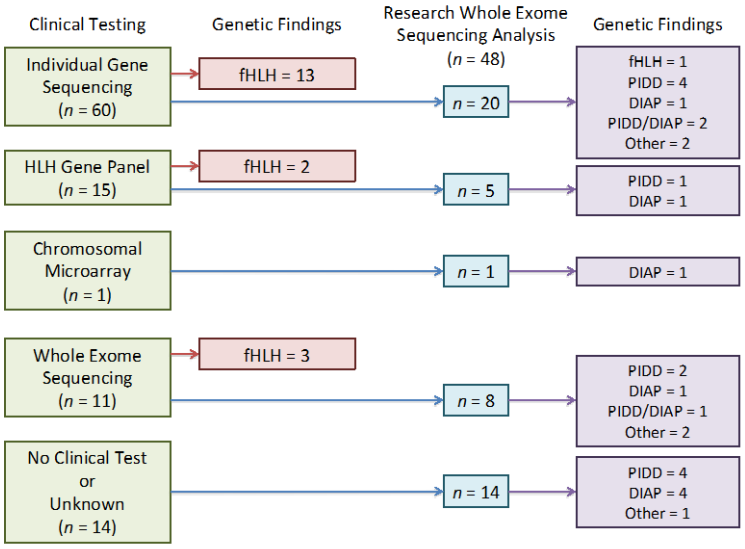
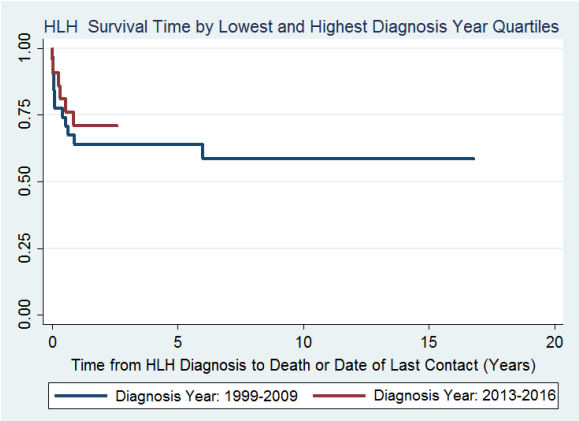
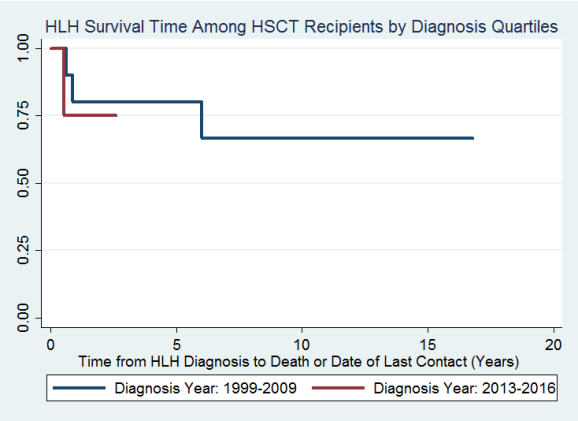


Figure S2. Outcome data (Kaplan-Meier survival curves) for subjects in the earliest (blue) and latest (red) quartiles as determined by date of HLH diagnosis. (A) Overall survival estimates from HLH diagnosis to date of death or last contact in years for the earliest and latest quartiles for all subjects enrolled (p = 0.57). (B) Among subjects who received HSCT, overall survival estimates from HLH diagnosis to date of death or last contact in years for the earliest and latest quartiles (*p* = 0.76). Abbreviation: HSCT, hematopoietic stem cell transplantation.

A



B



References:

1. Bainbridge MN, Wang M, Wu Y, et al. Targeted enrichment beyond the consensus coding DNA sequence exome reveals exons with higher variant densities. Genome Biology 2011;12:R68.

2. Challis D, Yu J, Evani US, et al. An integrative variant analysis suite for whole exome next-generation sequencing data. BMC Bioinformatics 2012;13:8.

3. Reid JG, Carroll A, Veeraraghavan N, et al. Launching genomics into the cloud: deployment of Mercury, a next generation sequence analysis pipeline. BMC Bioinformatics 2014;15:30.

4. Hamosh A, Sobreira N, Hoover-Fong J, et al. PhenoDB: A New Web-Based Tool for the Collection, Storage, and Analysis of Phenotypic Features. Human Mutation 2013;34:566-71.

5. Henter J-I, Horne A, Aricó M, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatric Blood & Cancer 2007;48:124-31.