Supplemental material

Lam et al., https://doi.org/10.1084/jem.20190147

Table S1. **Clinical laboratory data**

	Pt 1		Pt 2	
	1 mo	**Full-blown HLH**	**1 mo**	**Full-blown HLH**
WBC (10^3/µl)	2,430	410	4,460	920
No. of neutrophils	150	70	660	50
No. of lymphocytes	1,870	260	2,970	610
Hemoglobin (g/dl)	9.9	9.8	10.3	9.3
Platelets (10^3/µl)	10	17	29	38
ALT (IU/liter)	15	3,952	38	403
AST (IU/liter)	17	8,440	25	1,254
LDH (IU/liter)	421	23,966	869	17,705
γGT (IU/liter)	151	75	430	1,189
Fibrinogen (mg/dl)	281	312	489	212
D-dimer (µg/ml)	7.77	10.52	3.43	17.90
Triglycerides (mg/dl)	449	443	358	669
Ferritin (ng/ml)	2,594	155,440	2,763	609,760
CRP (mg/dl)	14.76	42.56	13.10	43.65

Per the referral note from Dr. H.C. Erichsen, Pt 3 had severe anemia and thrombocytopenia at birth, usually increased CRP between 50 and 200 mg/dl, ESR 60 mm/h at birth, and >100 mm/h afterwards. Ferritin at birth ranged between 2,000 and 2,500 ng/ml and increased to >100,000 ng/ml with subsequent HLH episodes. Procalcitonin was low. WBC, white blood cell; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; γGT, gamma-glutamyl transferase; ALT, alanine aminotransferase; AST, aspartate transaminase; LDH, lactic acid dehydrogenase.

Table S3. **WES/WGS metrics and data output**

Pt 1	
WES enrichment kit	Agilent SureSelect AllExon v5+UTR
Sequencing platform	Illumina HiSeq
No. of reads	115,179,795
Mean read length (bases)	100
Target region coverage (%)	
>2×	97.7
>20×	86
>50×	67
Average depth on target	91×
Total number of high-quality variants	127,405
Variants with effect on CDS or affecting splice sites[a]	14,224
Private, clinically associated and low-frequency variants[b]	338
Putative disease genes (autosomal recessive trait)	3
Filtered candidate genes[c]	2[d]
Putative disease genes (de novo)	4
Filtered candidate genes[c]	4[e]
Pt 3	
Sequencing platform	Illumina HiSeq
Capture design	HGSC Core Rebal
Unique aligned MB	9,767
Total MB	10,427
Total read (1.8)	116,780,944
Reads PF (1.8)	103,246,033
Median insert size (bases)	180
Mode insert size (bases)	162
Duplicate reads (%)	7
Average coverage	114
Coverage bases	
≥1×	96
≥10×	91
≥20×	87
≥40×	79
Pt 4	
WGS preparation protocol	TruSeq DNA PCR-free protocol
Sequencing platform	Illumina HiSeq
Target region coverage (%)	
>10×	99.12
>20×	95.4
Average depth on target	34×
Putative disease genes (autosomal recessive trait)	4[f]
Putative disease genes (de novo)	4[g]

Pt 3 was identified through a shared CMG database, and studies for Pt 3 included trio WES (performed in Oslo, Norway). Pt 3 then underwent repeat WES at Baylor College of Medicine (output shown in table above) followed by Sanger sequence confirmation in both parents. HGSC, Human Genome Sequencing Center; MB, megabases; PF, pass-filter.

[a]High-quality nonsynonymous single-nucleotide variants and indels within coding exons and splice sites (±8 bases).

[b]High-quality, functionally relevant variants and unknown, private, or low-frequency variants (gnomAD minor allele frequency <0.1% and frequency <1% within our ∼1,300-exome database).

[c]Functional impact of variants was assessed by CADD v.1.3 (http://cadd.gs.washington.edu/), dbNSFP M-CAP v.1.0 (http://sites.google.com/site/jpopgen/dbNSFP), and Intervar (http://wintervar.wglab.org) v0.1.7. Variants predicted as benign or likely benign by Intervar were discarded, and only those with a CADD score >15 or an M-CAP score >0.025 were retained.

[d]*ATXN7* (c.113_118dupAGCAGC, p.Gln38_Gln39dup) and *NOP9* (c.501_509dupGGAGGAGGA, p.Glu167_Glu169dup).

[e]*CDC42* (c.556C>T, p.Arg186Cys), *TTN* (c.57305T>C, p.Ile19102Thr), *GPR158* (c.1976A>G, p.Tyr359Cys), and *PLEKHG3* (c.2650C>A, p.Leu884Met).

[f]Including the following genes: *DHTKD1*, *LPL*, *GALC*, and *CHSY1*.

[g]Including the following genes: *LAMA3*, *ADAMTSL2*, *CDC42*, and *FTCD*.

Table S4. **Vulval phenotypes in *C. elegans* expressing WT CDC-42 or the disease-associated mutants**

Genotype	Transgene	Pvl (%)	Muv (%)	Vul (%)	*n*
WT	—	0.5	0	0	>1,000
WT	Empty vector	1	0	0	201
WT	*cdc-42^{WT}*	21.1[a]	3.3[b]	0	399
WT	*cdc-42^{R68Q}*	11.6[a,c]	3.2[b]	0	249
WT	*cdc-42^{A159V}*	38.8[a,d]	7.1[b,e]	0	196
WT	*cdc-42^{K186C}*	10.6[a,d]	2.7[b]	0	698
let-23(sy1)	—	0	0	79.1	134
let-23(sy1)	*cdc-42^{WT}*	0	0	50.1[f]	192
let-23(sy1)	*cdc-42^{R68Q}*	0	0	48.7[f]	150
let-23(sy1)	*cdc-42^{A159V}*	0	0	16.1[f,g]	186
let-23(sy1)	*cdc-42^{K186C}*	0	0	61.7[h,i]	135

Cdc-42 alleles were expressed under the control of the inducible *hsp16.41* promoter. Animals were grown at 20°C and heat shocked at late-L2/early-L3 larval stages. *n* indicates the number of animals scored. Pvl, Muv, and Vul phenotypes are expressed as percentage of adult animals with a Pvl, displaying ectopic pseudovulvae, or completely lacking the vulva, respectively. *Let-23(sy1)* is a hypomorphic allele of *let-23/EGFR*.
In all comparisons, P values were calculated using a two-tailed Fisher's exact test.
[a]Significantly different from animals expressing the empty vector ($P < 1.2e^{-6}$).
[b]Significantly different from animals expressing the empty vector ($P < 0.05$).
[c]Significantly different from animals expressing *cdc-42^{WT}* ($P < 0.005$).
[d]Significantly different from animals expressing *cdc-42^{WT}* ($P < 0.00002$).
[e]Significantly different from animals expressing *cdc-42^{WT}* ($P < 0.05$).
[f]Significantly different from *let-23(sy1)* animals ($P < 7.8e^{-7}$).
[g]Significantly different from *let-23(sy1)* animals expressing *cdc-42^{WT}* ($P < 3.2e^{-6}$).
[h]Significantly different from *let-23(sy1)* animals ($P < 0.005$).
[i]Significantly different from *let-23(sy1)* animals expressing *cdc-42^{WT}* ($P < 0.05$).

Table S5. **Clonogenic assay of BM cells from Pt 1**

	BFU-E	CFU-GM	CFU-G	CFU-M	CFU-GEMM
Pt 1 (CFU/2 × 10^5 BM MNCs)	6	1.5	0	2.5	0
Controls (BM MNCs)	15–45	25–50	5–10	5–10	1–3
Pt 1 (CFU/1 × 10^3 BM CD34$^+$)	10	4	ND	ND	ND
Controls (BM CD34$^+$)	8-84	25-93	ND	ND	ND

Mononuclear cells (MNCs) or purified CD34$^+$ cells from BM aspirate were plated in a clonogenic assay in the presence of Methocult and 0.9% methylcellulose and scored for hematopoietic colonies after 14 d. Colony number was greatly reduced compared to normal BM cultures of age-matched healthy subjects. BFU-E, erythroid burst-forming units; CFU-GM, CFU-granulocyte/monocyte; CFU-G, CFU-granulocyte; CFU-M, CFU-monocyte; CFU-GEMM, CFU-granulocyte/erythrocyte/monocyte/megakaryocyte.

Table S2 is provided online as a separate Excel file and shows hematological and immunological profiles.