STIMULUS REPORT

blood advances

Identification of germline variants in adults with hemophagocytic lymphohistiocytosis

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Key Points

- Some germline variants are predicted to disrupt protein function in HLH-associated genes.
- Such variants are neither enriched in adult-onset HLH nor associated with specific clinical or laboratory features of HLH.

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening disorder of immune system overactivation that occurs as familial and acquired forms.[1] HLH is characterized by excessive cytokine production and inflammation, mediated by multiple immune cells including persistently activated macrophages. Familial HLH (FHL) is typically diagnosed in childhood and is often caused by inherited biallelic, deletion, or truncating variants in genes regulating the cytotoxic function of T lymphocytes and natural killer cells.[2,3] By contrast, acquired HLH usually occurs in the setting of malignancy, infection, or autoimmune disease, and may be diagnosed at any age. Prior studies using in silico prediction algorithms have concluded that germline HLH-associated variants are enriched in adult patients with HLH but have been limited in the number of genes analyzed, incomplete clinical annotation to confirm true HLH diagnoses, and the relatively small size of the adult cohorts. Finally, the comparatively young ages at the time of HLH onset have made distinguishing FHL that occurs in early adulthood from true adult-onset HLH difficult.[4-6] To overcome these issues, we sought to identify potential pathogenic germline variants in 17 genes implicated in FHL or other inherited immune disorders in a highly annotated cohort of patients diagnosed with HLH in adulthood.[7]

Methods

After obtaining institutional review board approval, genomic DNA was isolated from bone marrow aspirate or peripheral blood samples from adults treated in our hospital systems between 2001 and 2018 who met HLH-2004 diagnostic criteria; although there was incomplete information for some patients (particularly natural killer cell activity; see supplemental Figure 1A), all patients reported here met HLH-2004 criteria.[8] Hybrid capture was performed on the samples using a custom SureSelect system (Agilent Technologies) targeting the exons of 17 genes implicated in FHL that occur in the context of other inherited immune disorders and have been described in the literature as associating with HLH, or are tested on commercial HLH panels (*AP3B1*, *BLOC1S6*, *BTK*, *CD27*, *IL2RG*, *ITK*, *LYST*, *MAGTI*, *PRF1*, *RAB27A*, *SH2D1A*, *SLC7A7*, *STX3*, *STX11*, *STXBP2*, *UNC13D*, *XIAP*).[2,7,9] Sequencing was performed on the Illumina platform, and germline variant calling was done using the GATK Haplotype caller pipeline.[10] Common variants were excluded by removing alterations with a genome aggregation database (gnomAD)[11] frequency of >0.05. We predicted the effect of variants on protein function using 5 in silico tools (PON-P2, PROVEAN, FATHMM, M-CAP, and REVEL). Variants classified as disruptive (predicted to impair normal protein function) by at least 2 tools were considered disruptive if none of the tools classified the variant to be nondisruptive. Variants classified as nondisruptive by a single tool were still considered disruptive if 3 or more tools classified the variant as

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The original sequencing data are available upon request to the corresponding author (benjamin_ebert@dfci.harvard.edu).

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Table 1. Characteristics of 88 patients studied in cohort

Total patients	N = 88
Median age (range), y	54 (18-81)
Median age, n (%), y	
<40	23 (26)
40-59	33 (38)
>60	32 (36)
Sex, n (%)	
Female	40 (45)
Male	48 (55)
Self-reported ethnicity, n (%)	
White or Caucasian	68 (77)
Hispanic or Latino	5 (6)
Asian	5 (6)
Black or African American	2 (2)
South Asian/Indian	1 (1)
Unknown/not reported	7 (8)
Precipitating etiology, n (%)	
Malignancy	43 (49)
Infection	26 (30)
Autoimmune disease	15 (17)
Multiple	14 (16)
Idiopathic	20 (23)
HLH-04 criteria, n (%)	
5*	26 (30)
6	38 (43)
7	21 (24)
8	3 (3)

*Three patients were determined to meet at least 5 HLH-04 criteria by the treating clinician but had 4 documented criteria in the clinical database due to incomplete information (see "Methods" and supplemental Figure 1A).

likely disruptive.[12] The effect of splice site variants was predicted as previously described.[13] Statistical analysis for the enrichment of variants in the HLH cohort compared with the control population was performed using a Fisher's exact test with $P < .05$ considered significant.

Results and discussion

Our cohort included 88 adult patients with HLH (Table 1). The median age at diagnosis was 54 years old (range, 18-81 years) and the majority were male (55%) (supplemental Figure 1B). All patients met HLH-2004 criteria.[8,14,15] A precipitating etiology was identified in most patients (77%) including malignancy (49%), infection (30%), and autoimmune disease (17%).

Among the 17 genes sequenced, a total of 42 variants with a gnomAD frequency of <0.05 were identified in 45 patients. Of these, 7 variants were considered disruptive (predicted to have an impact on protein function) in 18 patients (20% of the cohort) (Figure 1A). The most common variant was *PRF1* A91V (n = 12; 14%), an alteration commonly reported in adult HLH that has been shown to impair lymphocyte cytotoxicity.[16,17] Three patients had *SLC7A7* A91V, whereas the disruptive variants were restricted to individuals, including *PRF1* H222Q, *ITK* R581Q, *LYST* R2624W, *STX3* splicing, and a male patient with *SH2D1A* R55Q (*SH2DA1* is on chromosome X). Three patients harbored multiple disruptive variants (*PRF1* A91V and *PRF1* H222Q, *PRF1* A91V and *SLC7A7* A91V, and biallelic *PRF1* A91V). We did not identify disruptive variants in the other FHL genes (*UNC13D*, *STX11*, or *STXBP2*) (Figure 1B).

We next examined whether the 7 unique disruptive variants were enriched in our cohort relative to whole-exome data from 2504 patients in the 1000 Genome Project (TGP; Figure 1C). Disruptive variants were identified in the TGP using the same gene set, pipeline, filtering, and algorithms used in our cohort. The specific variants in our cohort were significantly less common in the TGP (20% vs 5%; $P < .001$), an unsurprising result given the rarity of individual variants and requirement for a gnomAD frequency of <0.05. To avoid this selection bias, we compared the frequency of disruptive variants in our cohort to the frequency of any disruptive variant in the 17 tested genes in the TGP. Again, our cohort had a higher frequency compared with the TGP (20% vs 9%; $P < .01$). However, this difference was no longer significant when restricting our cohort to patients who reported as "white" or "Caucasian" (n = 68, the most common ancestry in our cohort, hereafter referred to as "white") and comparing those to European populations in the TGP (n = 503; 20% vs 13%; $P = .13$). Furthermore, although the *PRF1* A91V variant was more common in our cohort compared with the TGP (14% vs 3%; $P < .001$), it was not more common when restricting to European populations (17% vs 9%; $P = .08$). Furthermore, after excluding patients with a *PRF1* A91V variant, there was no difference in the frequency of patients with disruptive variants between our cohort and the TGP (4.4% in both groups; Figure 1C). Of note, there was no difference in the frequency of variants among whites (14 of 68; 21%) and nonwhites (4 of 20; 20%) in the cohort.

Finally, we investigated whether the presence of a disruptive variant was associated with any clinical feature. The presence of a disruptive variant was not associated with age (mean age, 53 vs 51 years; $P = .64$), sex (33% vs 49% female; $P = .22$; supplemental Figure 2), or a specific precipitating etiology including malignancy (19% vs 22%; $P = .79$). Nor was there a significant difference in peak ferritin levels, soluble interleukin 2 receptor (sIL-2R) levels, or the sIL-2R-to-ferritin ratio (a metric often elevated in patients with lymphoma-associated HLH).[18] We did not find a difference in the frequency of variants between patients meeting 5 to 6 HLH-04 criteria (13 of 64; 20%) and those meeting 7 to 8 criteria (5 of 24; 21%). Similarly, no differences were noted in those with and without a *PRF1* A91V variant (supplemental Figure 3). Survival analysis was not possible given the number of potential covariates (such as age, sex, ancestry, precipitating etiology, prior/subsequent treatment).

Our data suggest that disruptive germline variants do not drive adult HLH, unlike FHL. In contrast to cases of FHL, biallelic alterations, deletions, nonsense, and frameshift mutations were extremely rare, consistent with data from animal models that either biallelic loss or polygenic mutations can drive disease.[19] Furthermore, disruptive variants were not enriched in adult HLH compared with control when accounting for ancestry, particularly when considering

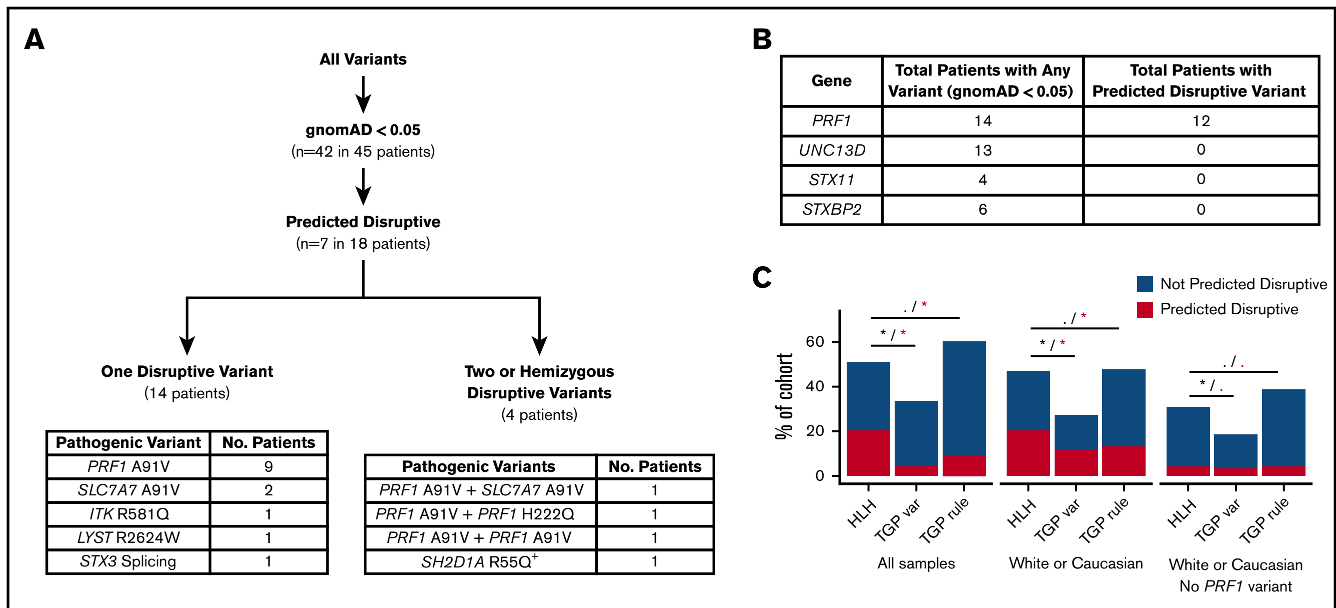

Figure 1. Frequency and distribution of predicted disruptive variants in the HLH cohort. (A) Schematic of germline variant frequency within cohort among 17 HLH-associated genes sequenced. (B) Frequency of total and predicted disruptive germline variants observed in FHL genes in cohort. (C) Percentage of individuals in HLH and the 1000 Genomes Project (TGP) cohort carrying variants in HLH-associated genes. TGP var indicates the percentage of individuals in the TGP cohort carrying variants identified in the HLH cohort; TGP rule indicates the percentage of individuals in the TGP cohort carrying any variants identified by the same rules as for the HLH cohort. Percentages compared whole data (HLH [n = 88] and TGP [n = 2503]) and white or Caucasian individuals (HLH [n = 68] and TGP [n = 503]). Among white or Caucasian individuals, similar analysis was performed by excluding the *PRF1* A91V variant. The percentage of individuals carrying predicted disruptive variants is shown in red. $^*P < .05$; $\bullet P \geq .05$. The significance test result for carriers of all variants is indicated in black; the same for carriers of predicted disruptive variants is shown in red. + indicates the *SH2D1A* R55Q mutation, which is encoded on the X chromosome, was present in a male patient.

non-*PRF1* A91V variants. Additionally, there were no clinical differences between patients with and without disruptive variants. Finally, the contrast between the high frequency of disruptive variants in the control population (9%) and the rarity of adult HLH suggests that either the in silico algorithms overestimate the disruptive effects of variants or these disruptive variants are not strong drivers of disease. Either interpretation raises questions about the role and routine testing of germline variants in adults with HLH. Indeed, the fact that some of the predicted disruptive mutations including *PRF1* A91V are considered of uncertain significance by other metrics like the American College of Medical Genetics (ACMG) further calls into question the role of these mutations in adult HLH pathogenesis.[20] Our data, however, do not negate the importance of testing for genetic variants in cases in which FHL is highly suspected (including strong family history and young age of onset) and may influence treatment decisions (such as donor selection for allogeneic hematopoietic stem cell transplant).

Our study has several limitations. Like most prior studies, our cohort is limited in size given the rarity of HLH and difficulty obtaining usable biospecimens. However, this cohort contrasts with prior adult HLH reports including strict diagnostic inclusion criteria, significantly higher median age (at least 10 years older), expanded gene panel, and incorporation of ancestry (an essential consideration when studying germline genetics). Furthermore, whereas prior studies compared the frequency of the specific disruptive variants in their cohort to control populations, we compared the frequency of any disruptive variant in a gene previously associated with HLH in our cohort to a control population, and further assessed the importance of ancestry and *PRF1* A91V in this comparison. Another limitation is the use of in silico algorithms to predict the effect of mutations on protein function, an approach used in nearly all prior studies of adult HLH. However, we used newer algorithms that, although still limited, have shown better performance than those previously used.[12] We also note that both ACMG and commercial testing often incorporate these predictive algorithms so understanding their relevance has clinical implications.[20] To highlight the importance of these issues and the variability across studies, we contrast our results with a recent report of 112 adults with HLH in China that concludes that germline variants are enriched in this population (43%); though in agreement with our study, they were predominantly missense and monoallelic.[6] The studies differ in the ancestral composition of the cohort, older age of our population (which excludes cases of pediatric HLH), differing algorithms to predict disruption of protein function, and our approach to look for any disruptive variant in control populations (not just the frequency of those found in the group with HLH). In contrast, another study from China found that just 5.1% of adults with HLH had a germline variant; but again, the average patient age, gene panel sequenced, and disruptive prediction algorithm differed, and there was no formal comparison with a control population.[21] Ultimately, additional efforts to better define disruptive variants in larger numbers of genes, including whole-exome/genome analyses, combined with expanded cohorts of adults with HLH of varying ancestries, will further refine our understanding of genetic contribution to the disease. Our data suggest that familial and

adult-onset HLH have a distinct pathophysiology and do not support routine germline testing of adults with HLH.

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Authorship

Contribution: P.G.M., N.B., and B.L.E. initiated the project, designed the research, and wrote the paper with input from other authors; P.G.M., A.N., J.C., C.J.G., A.S.S., M.A., and A.M.S. performed the research; P.G.M., A.N., and C.J.G. analyzed the data; and S.B., F.P., J.A., G.P., R.P.H., M.S.T., J.C.A., E.A.M., P.L.R., and S.N. contributed vital new reagents or analytical tools.

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