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BRIEF REPORT

# Mycobacterial Immune Reconstitution Inflammatory Syndrome in HIV is Associated With Protein-Altering Variants in Hemophagocytic Lymphohistiocytosis-Related Genes

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**(See the Editorial Commentary by French on pages 106–10.)**

People with HIV (PWH) and mycobacterial infections can develop immune reconstitution inflammatory syndrome (IRIS) after starting antiretroviral therapy. The pathophysiology of mycobacterial-IRIS overlaps with primary hemophagocytic lymphohistiocytosis (pHLH). To assess possible genetic predisposition to IRIS, protein-altering variants in genes associated with HLH were evaluated in 82 PWH and mycobacterial infections who developed IRIS (n = 56) or did not develop IRIS (n = 26). Protein-altering variants in cytotoxicity genes were found in 23.2% of IRIS patients compared to only 3.8% of those without IRIS. These findings suggest a possible genetic component in the risk of mycobacterial IRIS in PWH.

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Mycobacterial immune reconstitution inflammatory syndrome (IRIS) continues to cause significant morbidity in people with human immunodeficiency virus (PWH) after starting antiretroviral therapy (ART) especially in resource-constrained settings [1]. IRIS can be associated with infections by *Mycobacterium tuberculosis* and/or nontuberculous mycobacteria, and a high pathogen burden is known to increase risk [1]. Recently, we reported severe mycobacterial-IRIS has overlapping clinical and pathophysiologic features with another hyperinflammatory syndrome, hemophagocytic lymphohistiocytosis (HLH), characterized by a prominent interferon-γ/interleukin 18 (IFN-γ–IL-18) signature, increased activated T cells, and a deficiency in circulating regulatory T cells (Tregs) [2].

HLH in its primary or genetic form (pHLH) usually develops in children carrying homozygous pathogenic variants of genes involved in lymphocyte cytotoxicity, including perforin [3]. Secondary forms of HLH (sHLH) develop as pathologic inflammatory responses triggered by infections, malignancies, or autoimmune disease. Genetic studies of sHLH in those with systemic juvenile idiopathic arthritis or H1N1 influenza have identified increased frequencies of protein-altering variants in genes implicated in pHLH [3, 4]. It is hypothesized these heterozygous variants lower the threshold for hyperinflammation and increase the risk of sHLH when patients acquire "second-hits" due to infection, malignancy, or autoimmunity [3]. Due to the overlapping pathophysiology between severe mycobacterial-IRIS and HLH [2], we evaluated for similar gene variants in a cohort of 82 PWH and mycobacterial infections.

## METHODS

Eighty-two PWH and mycobacterial infections were enrolled in 1 of 2 National Institute of Allergy and Infectious Diseases institutional review board-approved prospective, longitudinal studies (NCT00286767, NCT02147405) of IRIS in PWH and a CD4 count <100 cells/μL. Detailed clinical and immunologic characteristics have been previously reported [2]. Two individuals recruited after that publication are also included in this analysis. IRIS events were defined using the AIDS Clinical Trials Group IRIS definition criteria (Supplementary Methods). All participants signed informed consent and procedures were in accordance with the Declaration of Helsinki.

Participants underwent gene sequencing with the Immunoplex panel encompassing 464 genes associated with primary immune deficiencies performed by the University of Washington Genetics and Solid Tumors Laboratory (Supplementary Methods). This Clinical Laboratory Improvement Amendments (CLIA)-certified panel involves next-generation sequencing of exons and detects large deletions, duplications, and mosaicism. We specifically evaluated for protein-altering variants, which include missense substitutions and protein-truncating variants, and have the greatest potential to impact protein structure and function. Understanding the functional consequences of these genetic changes can provide insight into the role of their associated proteins in driving inflammation and causing disease. Clinical classification of variants was performed based on American College of Medical Genetics criteria. Variants were prioritized based on minor allele frequencies (MAF) in public databases (ie, Exome Aggregation Consortium, 1000-Genomes project) of less than

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0.001 and in silico and damage prediction scores (combined annotation dependent depletion score greater than the mutational significance cutoff) (Supplementary Methods). Variants in genes previously described as contributing to HLH including those involved in cellular cytotoxicity, inflammasome activation, and immune dysregulation were specifically scrutinized. Fisher exact test was used to compare variables. Logistic regression was performed to determine the impact of protein-altering variants in HLH-associated genes on the development of IRIS. Statistical analyses were performed in R (version 4.1.2).

## RESULTS

Overall, 82 PWH with mycobacterial infections were followed at the National Institutes of Health from 2006 to 2022. Median age at initial evaluation was 38 years (interquartile range, 34–45 years) with 29 (35%) women (Supplementary Table 1). Most individuals were black (African/African-American) (n = 51; 62.2%) or non-white Hispanic (n = 20; 24.4%). Mycobacterial infections were primarily due to *M. tuberculosis* (n = 38) or *Mycobacterium avium* complex (MAC) (n = 35). In total, 56 (68.3%) patients developed mycobacterial IRIS. At IRIS onset, 26 (45%) patients met at least 1 of the published criteria for HLH/MAS [2]. The age, sex, and race/ethnicity did not differ between the groups. Thirty-six patients with IRIS were treated with corticosteroids. Eight individuals required additional immunosuppression (infliximab or tocilizumab) due to refractory inflammation or steroid-associated adverse effects, all of whom met HLH/MAS criteria.

We first evaluated for rare, predicted protein-altering variants in genes associated with pHLH (*PRF1, UNC13D, STX11, STXBP2, RAB27A, LYST, AP3B1*) [5]. We identified 11 unique variants in 11 (13.4%) individuals. Ten occurred in those with mycobacterial-IRIS (n = 56; 17.9%) compared to only 1 in non-IRIS patients (n = 26; 3.8%). Another 3 individuals were found to carry the *PRF1* p.A91V variant, which has been shown to impair protein function and has been associated with sHLH in other cohorts [3]. In total, 13 of 56 (23.2%) patients with IRIS carried protein-altering variants in pHLH genes compared to 1 of 26 (3.8%) in non-IRIS patients ($P = .03$). Of these patients, 7 had *M. tuberculosis* (50%) and 7 had nontuberculous mycobacteria infections (50%) (Table 1). There was no significant difference in the race/ethnicity of those carrying protein-altering variants with 7 African/African-American (50%), 4 non-white Hispanic (28.6%), and 3 white non-Hispanic patients (21.4%). Linear regression controlling for age, sex, race/ethnicity, disseminated infection, and CD4 T-cell count demonstrated that carrying 1 of these variants increased the risk of IRIS with an odds ratio of 8.64 (95% confidence interval, 1.47–166.3). The prevalence of protein-altering variants in pHLH genes was similar in HLH-IRIS patients (6/26; 23.1%) compared to those with IRIS not meeting HLH criteria (7/30; 23.3%).

The genetic risk of HLH, however, is complex and can involve multiple immunoregulatory pathways in an independent and synergistic manner [5, 6]. Therefore, we expanded our search to 55 additional genes implicated in primary immune deficiencies involving immune dysregulation or inflammasome activation. Using the same search strategy, we identified potential second hits in 4 patients with HLH-IRIS that also carried a protein-altering variant in a cytotoxicity gene (Table 1). Two of these second hits occurred in *TREX1,* which encodes a protein that cleaves intracytoplasmic DNA, and homozygous defects lead to autoinflammatory disease [7]. Multiple protein-altering variants were also identified in *LRBA* and *CARMIL2*, which encode proteins involved in T-cell homeostasis and can cause regulatory T-cell deficiencies when present in homozygosity [7, 8]. Additional rare variants were identified in 8 of 26 (30.7%) patients in the HLH-IRIS cohort compared to only 6 of 56 (10.7%) of the remaining patients with IRIS (no HLH) or no IRIS. Gene variant characteristics and potential functional consequences are reported in Supplementary Table 2 and Supplementary Figure 1.

Overall, there were no differences in sex, race/ethnicity, or type of mycobacteria between carriers and noncarriers of protein-altering variants in any HLH-associated immunoregulatory genes within the IRIS and non-IRIS subgroups (Supplementary Table 3). Within the IRIS subgroup, a greater percentage of patients carrying protein-altering variants required corticosteroids (13/19, 68.4%) and immunosuppression beyond steroids (4/19, 21%) compared to those without variants (corticosteroids, 21/37, 56.8%; or additional immunosuppression, 4/37, 10.8%). These differences were not statistically significant, possibly due to the small sample size. Notably, 3 of 6 patients with HLH-IRIS that carried multiple protein-altering variants required additional immunosuppression beyond corticosteroids for refractory inflammation. No patients with IRIS (no HLH) or those without IRIS carried more than 1 variant in HLH-associated genes.

Using previously reported biomarker and immunophenotyping data from IRIS events [2], we compared IFN-γ–associated biomarkers and T-cell subsets between those with and without protein-altering variants within the HLH-IRIS, IRIS (no HLH), and non-IRIS subgroups. Although there were small increases in these markers in those carrying protein-altering variants compared to noncarriers, these differences were overall small and did not reach statistical significance (Supplementary Figure 2). More detailed analyses interrogating the specific functional impact of each gene variant is needed to determine their individual role and potential contribution to pathologic inflammatory processes.

## DISCUSSION

HIV with mycobacterial coinfection remains common and identifying predictive factors for mycobacterial-IRIS prior to initiation of ART remains essential. High mycobacterial burden and low CD4 T cells are known to increase risk and a genetic predisposition has long been hypothesized but remains incompletely

**Table 1. Protein-Altering Variants Identified in Genes Associated With Cytotoxicity or Immune Dysregulation**

| ID | Clinical Characteristics | | | Genes Implicated in Risk of Hyperinflammatory Syndromes | | | Potential Second Hits or Variants in Other Immunoregulatory Genes | | |
|---|---|---|---|---|---|---|---|---|---|
| | Race/Ethnicity | Mycobacteria Species | Duration of Immunosuppression | Gene | cDNA[a] | Protein[b] | Gene | cDNA | Protein |
| HLH_01 | African | TB | 14 wk | ***RAB27A*** | **c.383T > C** | **p.I128T** | *AP3D1* | c.2650C> T | p.R884W |
| HLH_02 | Hispanic | MAC | 15 wk | ***AP3B1*** | **c.942G > A** | **Splicing** | *TREX1* | c.879_880del | p.L294Vfs*20 |
| HLH_03[c] | African | TB | 136 wk | ***STXBP2*** | **c.508G > A** | **p.G170L** | *TREX1* | c.739G > C | p.A247P |
| HLH_04[c] | Hispanic | TB | 10 wk | ***LYST*** | **c.2963G > A** | **p.R988Q** | *LRBA* | c.1755 + 1G > T | Splicing |
| HLH_05 | Hispanic | *M. marinum* | 10 wk | *LRBA* | c.6401G > A | p.R2134H | *NCF4*[d] *NCF4*[d] | c.647C > T c.1019G > A | p.T216M p.R340K |
| HLH_06[c] | African | TB | 25 wk | *CARMIL2*[d] | c.1987C > T | p.Q663* | *CARMIL2*[d] | c.1919 + 6C > T | Splicing |
| HLH_07 | African-American | *M. kansasii* | 31 wk | ***LYST*** | **c.4637C > T** | **p.A1546V** | … | … | … |
| HLH_08 | African | TB | 36 wk | ***AP3B1*** | **c.1651G > T** | **p.A600S** | … | … | … |
| HLH_09 | African | MAC | 8 wk | *MCM4* | c.787T > C | p.F263L | … | … | … |
| HLH_10[c] | African-American | MAC | 18 wk | *AP3D1* | c.1279C > T | p.R427W | … | … | … |
| IRIS_01 | Hispanic | MAC | None | ***LYST*** | **c.3590del** | **p.P1197Lfs*19** | … | … | … |
| IRIS_02 | African | TB | 7 wk | ***LYST*** | **c.3991G > A** | **P.D1331N** | … | … | … |
| IRIS_03 | African-American | TB | None | ***LYST*** | **c.8083T > G** | **p.S2695A** | … | … | … |
| IRIS_04 | Hispanic | MAC | None | ***UNC13D*** | **c.2180G > A** | **p.R727Q** | … | … | … |
| IRIS_05 | European | MAC | 4 wk | ***PRF1*** | **c.272C > T** | **p.A91V** | … | … | … |
| IRIS_06 | European | MAC | None | ***PRF1*** | **c.272C > T** | **p.A91V** | … | … | … |
| IRIS_07 | European | MAC | 65 wk | ***PRF1*** | **c.272C > T** | **p.A91V** | … | … | … |
| IRIS_08 | African-American | MAC | None | *RAG1* | c.940C > T | p.R314W | … | … | … |
| IRIS_09 | Hispanic | MAC | None | *WAS* | c.463 + 3G > C | Splicing | … | … | … |
| Non-IRIS_01 | African | TB | None | ***STX11*** | **c.745G > A** | **p.V249I** | … | … | … |
| Non-IRIS_02 | Hispanic | TB | None | *LRBA* | c.8315C > T | p.A2772V | … | … | … |
| Non-IRIS_03 | Hispanic | *M. bovis* | None | *AP3D1* | c.2590_2592del | p.K864del | … | … | … |
| Non-IRIS_04 | African | TB | None | *NCF1* | c.579G > A | p.W193* | … | … | … |
| Non-IRIS_05 | African-American | TB | None | *FCGR3A* | c.827G > A | p.W276X | … | … | … |

Variants were compared against the human reference genome: GRCh37/hg19. The ID reports the clinical patient category whether they developed HLH-IRIS, IRIS (no HLH), or no IRIS. Variants in cytotoxicity genes directly associated with primary hemophagocytic lymphohistiocytosis are listed in bold.

Abbreviations: cDNA, complementary deoxyribonucleic acid; del, deletion; fs, frameshift; HLH, hemophagocytic lymphohistiocytosis; IRIS, immune reconstitution inflammatory syndrome; MAC, *Mycobacterium avium* complex; TB, tuberculosis; wk, weeks.

[a]Gene location and description of the nucleotide change in the complementary DNA strand for each variant identified.

[b]Location and description of the amino acid change within the protein for each variant.

[c]Individuals with HLH-IRIS that required additional immunosuppression beyond corticosteroids due to refractory inflammation or steroid-associated adverse effects.

[d]Two variants identified in the same gene in the same individual—it is unknown if these variants exist in cis or trans on their associated chromosome.

explored. Specific single-nucleotide polymorphisms in key innate immune proteins, including proinflammatory cytokines (tumor necrosis factor-α [TNF-α], IL-6) and the inflammasome, have been found to associate with an increased risk of IRIS [9–11]. Using a different approach, we interrogated genes involved in lymphocyte cytotoxicity and other immunoregulatory pathways related to pHLH to identify protein-altering variants that could impact protein function and potentially exacerbate inflammatory pathways in severe mycobacterial-IRIS.

We identified an enrichment of protein-altering variants in cytotoxicity genes in patients with mycobacterial-IRIS (23.2%) compared to those without IRIS (3.8%). These variants occurred in heterozygosity and were primarily missense mutations, similar to those found at higher rates in sHLH cohorts triggered by autoimmune diseases or influenza infections [3, 4]. In pHLH, digenic inheritance is well described, supporting the mechanism that heterozygous variants can lead to haploinsufficiency, a hypothesis supported by mouse models [5, 12]. It is possible that partially damaging heterozygous variants may act as genetic modifiers and decrease the threshold for IRIS. This is mechanistically supported by the overlapping pathophysiology between mycobacterial-IRIS and HLH, which produce similar dysregulated T-cell phenotypes characterized by an expansion of activated T cells and decrease in Tregs. It is hypothesized that partial defects could amplify this immune dysregulation in an at-risk population [2, 13]. Similar genetic modifiers have been recognized in PWH in other situations such as the presence of heterozygous *CCR5-Δ32* or *CCR2-64I* alleles associating with protection against HIV-1 progression [14]. Additional genetic studies and detailed functional analyses are required to further validate these novel findings. Notably, there was no increase in the prevalence of pHLH variants specifically in those with HLH-IRIS; however, these potential genetic modifiers do not represent the only risk factor for IRIS and other variables such as pathogen burden could dampen or exacerbate a genetic predisposition.

Additionally, the genetic risk of HLH is complex and multifactorial with many immunoregulatory and inflammasome genes implicated in predisposing to HLH in primary immunodeficiencies [5]. Therefore, we broadened our search to include these genes and identified additional rare, protein-altering variants in genes involved in T-cell homeostasis and innate immune activation. Notably, these occurred as second-hits in multiple HLH-IRIS patients that already carried protein-altering cytotoxicity variants. These findings raise the possibility of digenic inheritance contributing to IRIS severity. The impact of these variants could be amplified in the setting of profound immune dysregulation driven by HIV/AIDS and ART-mediated immune reconstitution, leading to higher rates of severe IRIS in these patients despite having minimal impact in an otherwise healthy population.

Notable variants were discovered in *LRBA* and *CARMIL2* in 2 individuals with HLH-IRIS that required infliximab due to steroid-refractory inflammation. These genes are involved in T-cell activation/signaling and when found in homozygosity can lead to a dysregulated T-cell phenotype with an imbalance in the activated T-cell to Treg ratio [7, 8] resembling the immunophenotype described in HLH-IRIS patients. Two other individuals with heterozygous variants in cytotoxicity genes also carried protein-altering variants in *TREX1* that can amplify innate immune responses [7]. Due to the known impact of innate immune signaling and T-cell activation in HLH and mycobacterial-IRIS, it is possible these risk factors could be synergistic. In fact, increased inflammasome activation and defects in cytotoxicity have been shown to be independent and synergistic factors contributing to hyperinflammation in mouse models of HLH [6].

Considering the potential impact of protein-altering variants in HLH-associated genes in addition to previously described associations with single-nucleotide polymorphisms in innate immune proteins [9–11], suggest that susceptibility to mycobacterial-IRIS is polygenic. Future studies with larger sample sizes and whole-genome sequencing may identify the involvement of new genes that are more predictive of pathologic inflammatory syndromes. The impact of these genetic modifiers is not limited to HIV and IRIS. For example, severe coronavirus disease 2019 (COVID-19) also involves activation of the inflammasome and the myeloid cell compartment [15], and protein-altering variants along similar inflammatory pathways could impact COVID-19 severity.

Our study has limitations, our findings represent associations and detailed functional analyses are required to confirm the impact of these variants. Additionally, interpreting MAF in our cohort is limited by the underrepresentation of African and Hispanic ancestry in public databases. We attempted to control for this key factor by utilizing a strict MAF cutoff of $<0.001$, and our study population was representative of those most likely to benefit from these findings, increasing its generalizability.

In summary, we describe a novel enrichment of rare, protein-altering variants in lymphocyte cytotoxicity genes in individuals with mycobacterial-IRIS. Those with HLH-IRIS were more likely to carry multiple variants in immunoregulatory genes including those involved in T-cell homeostasis and innate immune signaling. These findings reinforce the overlapping pathophysiology between mycobacterial-IRIS and HLH. Functional confirmation and validation in larger cohorts will be important, as genetic predisposition may provide a novel mechanism for risk stratification and treatment optimization of PWH and mycobacterial infections.

## Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not

copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

## Notes

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