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Adults with septic shock and extreme hyperferritinemia exhibit pathogenic immune variation

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Abstract

Post-hoc subgroup analysis of the negative trial of interleukin-1β receptor antagonist (IL1RA) for septic shock suggested that patients with features of macrophage activation syndrome (MAS) experienced a 50% relative risk reduction for mortality with treatment. Here we seek a genetic basis for this differential response. From 1341 patients enrolled in the ProCESS trial of early goal directed therapy for septic shock, we selected 6 patients with MAS features and the highest ferritin, for whole exome sequencing (mean 24,030.7 ηg/ml, +/SEM 7,411.1). Eleven rare (minor allele frequency <5%) pathogenic or likely pathogenic variants causal for the monogenic disorders of Familial Hemophagocytic Lymphohistiocytosis, atypical Hemolytic Uremic Syndrome, Familial Mediterranean Fever, and Cryopyrin-associated Periodic Fever were identified. In these conditions, seven of the identified variants are currently targeted with IL1RA and four with anti-C5 antibody. Gene-targeted precision medicine may benefit this subgroup of patients with septic shock and pathogenic immune variation.

Introduction

A landmark study of Danish adoptees showed a near 6-fold increase in the risk of death from infection before age 50 for adoptees whose biological parents also died from infection under 50.[1] Multiple familial, case control and genome wide association studies have sought to identify genetic variation that contributes to sepsis outcome.[2–6] However, despite great variation in host response, attempts to identify genetic variants that contribute to sepsis outcomes has proven challenging. Typically, genomic studies in sepsis have treated all patients as a single group, assuming shared genetic risk factors. They have also focused on

Conflicts of Interest

Drs. Angus and Kellum wish to disclose a consultancy for Sobi, Inc.

correlations between common polymorphisms and sepsis outcome with limited functional studies to support associations.[4,7,8]

MAS is a fulminant form of multi-organ dysfunction presenting with fever, cytopenia, hepatosplenomegaly, hemophagocytosis, and extremely elevated serum ferritin, that can be triggered by sepsis.[9] Although classically described in children, septic hyperferritinemic adults are also at risk of poor outcome.[9,10] Additionally, while overall, interleukin-1β receptor antagonist (IL1RA) therapy was ineffective for septic shock, post-hoc subgroup analysis showed that patients with MAS features experienced a 50% relative risk reduction for mortality when treated with IL1RA.[11,12] As the hyperferritinemic sepsis phenotype overlaps with other inherited immunologic disorders, including hemophagocytic lymphohistiocytosis (HLH), Cryopyrin Associated Periodic Syndromes (CAPS), Familial Mediterranean Fever, and atypical Hemolytic Uremic Syndrome (aHUS), we hypothesized that known pathogenic disease variants for these disorders would be identified in individuals with this sepsis phenotype. This work uses whole exome sequencing (WES) to identify causal mutations for these diseases.[13–17] Here we focus on a subset of adults with septic shock with MAS features to identify rare pathogenic variants known to cause monogenic immunologic disorders, potentially underlying a shared phenotype leading to multi-organ failure.

Results and Discussion

Because MAS is characterized by extremely high serum ferritin levels, we identified 6 patients (0.5%) with the highest concentrations from the ProCESS multicenter trial of protocolized early goal directed therapy for adult septic shock[18] to undergo WES. We hypothesized that patients with hyperinflammation identified by the highest serum ferritin during macrophage activation syndrome would have pathogenic variants known to cause other single gene disorders of defective inflammation control, in a shared genotype-phenotype hypothesis. All variants with a minor allele frequency (MAF) >5% as reported by the 1000 Genomes, ExAC and NHLBI Esp6500 databases were treated as polymorphisms and removed from further analysis. Additionally, to bolster claims of disease relevance, previous literature reports of clinical phenotype related to pathogenic or likely pathogenic variant was required in the filtering scheme. Subsequently, only variants with MAF <5% that have been previously reported as pathogenic or likely pathogenic for heritable immunologic disorders are discussed here. Reference to the general population allows identification of all pathogenic and likely pathogenic variants, which are unlikely to be identified in a smaller sepsis controls sample. The mean ferritin among these 6 patients was 24,031 +/− 7,411 ηg/ml (+/− SEM). As hemophagocytic phenotypes are rare in adults, soluble IL-2 receptor, triglyceride and NK cell function studies are not available. The 6 patients had a mean APACHE II score of 27 +/− 4, corresponding to a mortality risk of 60.5% and multiple system organ failure including hepatic dysfunction and coagulopathy typical of MAS.[19] Five of 6 patients died by 30 days, with subject 3 being the sole survivor. Halacli et al. recently reported a cohort of adults with severe sepsis where 0.7% had ferritin over 15,000 ηg/ml with an observed mortality was 100%.[10] Clinical characteristics of these 6 patients are shown in Table 1.

As shown in Table 2, 100% of subjects inherited at least one pathogenic or likely pathogenic variant previously reported in the literature as causal for heritable immunologic diseases. Despite the similarity in phenotype, the variants found on WES represent diverse genetic disorders. Three of the six patients had *UNC13D* variants, where mutations are known to cause abnormal NK cell degranulation and cytolytic killing[20] and when inherited as autosomal recessive AR (Bi-allelic mutations) cause Familial HLH Type 3. Accumulating evidence suggests that adults with sporadic HLH[16] or MAS in the context of rheumatic disease,[17,21] or infections[22–24] often carry heterozygous mutations in NK cell degranulation pathways including *UNC13D*. The heterozygous *UNC13D* (c.1579C>T; p.Arg527Trp) mutation in subject 1 has been reported in an 18-year-old female with familial HLH with normal *PRF1* and *STXBP2* sequencing[25] as well as a 12 year HLH patient who also carried a *PRF1* c.50delT variant[26]. The *UNC13D* (c.2782C>T; p.Arg928Cys) variant in subject 3 has been described by Aricò et al in 3 individuals with ALPS[27] a predisposing condition for FHLH, and by Kaufman in a heterozygous individual with MAS related to systemic Juvenile Idiopathic Arthritis[28]. The variants described in subject 5, *UNC13D* (c.2983G>C; p. Ala995Pro) and (c.2542A>C; p.Ile848Pro) have also been described in an ALPS patient without identified FAS, FASL, or CASP10 mutation with functional studies of transfected HMC-1 cells showing decrease in secretory granule fusion with the plasma membrane as measured by a CD63 expression assay following stimulation with fMLP, a macrophage activating chemokine[27].

Among those with pathogenic mutations for recurrent fever syndromes, causal mutations for CAPS and FMF were identified in *MEFV* and *NLRP3*.[29–33] Interestingly, subject 4 carried both *NLRP3* (c.2113 C>A; p.Gln705Lys) and *MEFV* (c.250 G>A; p.Glu84Lys) variants. Screening of individuals with clinical suspicion for CAPS demonstrated that the *NLRP3* (p.Gln705Lys) variant is associated with a mild autoinflammatory phenotype characterized by skin lesions, arthralgia and myalgia.[34,35] This variant increases IL-1β and IL-18 release by cultured monocytes in an IL-1 receptor-dependent manner.[36] Similarly, the *MEFV* (p.Glu84Lys) variant has been reported in both heterzogygous and compound heterozygous states in FMF.[37–39] Other studies have investigated potential interaction of *NLRP3* and *MEFV* variants in digenetic inheritance of abnormal Il-1 driven FMF.[33,40] While there are multiple treatment strategies for CAPS/FMF[15] and MAS,[16,17] both can be targeted with IL1RA.

Complement pathway mutations causal for aHUS were identified in three subjects: two in *CD46*, both with (c.1058C>T; p.Ala353Val) and one in *C3* (c.1407G>C; p.Glu469Asp) and *CFHR5* (c.832G>A; p.Gly278Ser). aHUS is a thrombotic microangiopathy resulting from uncontrolled complement activation causing anemia, thrombocytopenia and kidney failure.[13] This is markedly similar to Thrombocytopenia Associated Multiple Organ Failure, a sepsis phenotype of new onset thrombocytopenia and acute kidney injury where we have recently reported increased incidence of hyperferritinemia.[41,42] When found in patients with aHUS, these variants are FDA approved indications for the C5 inhibitor eculizumab.[13,14]

Our study also identified individuals with multiple pathogenic variants, affecting different aspects of the inflammatory response. This heterogeneity may explain the observed absence of effect of immunomodulatory agents in sepsis, where clinical trials have applied agents

without consideration of potential variation in underlying disease process, leading to overall negative results.[43]

While all the variants reported here have been classified as pathogenic or likely pathogenic in CAPS, aHUS, and HLH/MAS, their identification in hyperferritinemic sepsis is of interest, but cannot be claimed as causal. Additionally, WES sequencing will miss pathogenic variants in regulatory and intronic gene regions that could be relevant to clinical phenotype. This has been shown in some individuals with HLH.[44] Further, the application of immunomodulatory therapies to septic individuals with these variants is of unclear benefit or harm. However, these findings provide evidence that screening select sepsis patients can identify unappreciated heritable disease, and could facilitate a genome-driven precision medicine.

Patients and Methods

Subjects were taken from the ProCESS multicenter trial cohort of protocolized resuscitation strategies in the emergency department as DNA was collected from the enrollees.[18] Immunomodulatory therapy was not systematically studied in this trial. While the Shakoory study of interleukin-1 receptor blockade in sepsis patients with MAS features laid the groundwork for this study, no DNA samples were available from this initial study. Subsequently, DNA from individuals from the ProCESS trial who met the Shakoory et al. MAS definition underwent WES in the present study.[11] The trial was approved by Institutional Review Board at each enrolling institution. All patients or their legally authorized representatives provided written informed consent. Briefly, individuals were 18 years of age or older and enrolled based on 1.) clinical suspicion for septic shock and 2.) either refractory hypotension (SBP less than 90mmHg or vasopressor requirement following fluid challenge) or evidence of poor perfusion (lactate level > 4mmol per liter).[18] We defined features of MAS as the combination of coagulation dysfunction (platelet count < 100K or INR > 1.5) plus hepatobiliary dysfunction (total bilirubin level > 1.2 mg/dL).[13] Eighty two of the 1341 ProCESS patients met these criteria. While serum ferritin levels were not available a priori, the levels were measured retrospectively from banked serum with the highest level recorded. Among those meeting MAS criteria, the median serum ferritin was 601.9ng/ml (IQR 268.33–2013.45). From these we selected the six patients with the highest ferritin levels (range 7,259–55,314ng/mL) for whole exome sequencing. A recent study from the Hellenic Sepsis Study Group identified serum ferritin greater than 4420 ng/ml as a marker for mortality and increased inflammation as measured by levels of IL-18, INF-y and sCD163.[45] The individuals with the 6 highest ferritin level samples were selected as a proof of concept that pathogenic variants for immunologic disorders with overlapping phenotype could be identified among enriched populations.

DNA was extracted from whole blood samples using standard methods and WES performed at the University of Pittsburgh Genomic Research Core with the Ion Torrent Platform. Libraries were constructed using Ion Ampliseq Exome RDY (ThermoFisher) with target of 100X coverage per sample. FASTQ files were aligned to *homo sapiens* reference sequence hg19 to create VCF files. VCF files were analyzed in the Fabric Genomics Opal 5.2.2 software platform (Fabric Genomics Inc, CA, https://www.fabricgenomics.com) to identify

missense, nonsense or frameshift mutations. Variants were filtered for minor allele frequency less than 5% in the ExAC [46], 1000 Genome [47] and NHLBI-ESP 6500 databases [48]. ExAC database frequencies are reported in Table 2. While no control group was sequenced, all identified variants are previously reported as pathogenic or likely pathogenic, and are rare in the general population.[49,50] Identified variants were restricted to candidates in an immune disorder panel to enhance relevance (Table 3). Each identified variant was evaluated in the HGMD professional database [51] with manual literature review. All identified variants were confirmed via Sanger sequencing. Specific primer pairs can be found in the supplementary table S1.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Sorensen TI, Nielsen GG, Andersen PK, Teasdale TW. Genetic and environmental influences on premature death in adult adoptees. N Engl J Med. 1988; 318:727–32. [PubMed: 3347221]
2. Obel N, Christensen K, Petersen I, Sorensen TIA, Skytthe A. Genetic and environmental influences on risk of death due to infections assessed in Danish twins, 1943–2001. Am J Epidemiol. 2010; 171:1007–13. [PubMed: 20375195]
3. Davila S, Wright VJ, Khor CC, Sim KS, Binder A, Breunis WB, et al. Genome-wide association study identifies variants in the CFH region associated with host susceptibility to meningococcal disease. Nat Genet. 2010; 42:772–6. [PubMed: 20694013]
4. Rautanen A, Mills TC, Gordon AC, Hutton P, Steffens M, Nuamah R, et al. Genome-wide association study of survival from sepsis due to pneumonia: An observational cohort study. Lancet Respir Med. 2015; 3:53–60. [PubMed: 25533491]
5. Kellum JA, Kong L, Fink MP, Weissfeld LA, Yealy DM, Pinsky MR, et al. Understanding the inflammatory cytokine response in pneumonia and sepsis: results of the Genetic and Inflammatory Markers of Sepsis (GenIMS) Study. Arch Intern Med. 2007; 167:1655–63. [PubMed: 17698689]
6. Yende S, Kammerer CM, Angus DC. Genetics and proteomics: deciphering gene association studies in critical illness. Crit Care. 2006; 10:227. [PubMed: 16934133]
7. Nelson CL, Pelak K, Podgoreanu MV, Ahn SH, Scott WK, Allen AS, et al. A genome-wide association study of variants associated with acquisition of Staphylococcus aureus bacteremia in a healthcare setting. BMC Infect Dis. 2014; 14:83. [PubMed: 24524581]
8. Scherag A, Schöneweck F, Kesselmeier M, Taudien S, Platzer M, Felder M, et al. Genetic Factors of the Disease Course after Sepsis: A Genome-Wide Study for 28Day Mortality. EBioMedicine. 2016; 12:239–46. [PubMed: 27639821]
9. Grangé S, Buchonnet G, Besnier E, Artaud-Macari E, Beduneau G, Carpentier D, et al. The Use of Ferritin to Identify Critically Ill Patients With Secondary Hemophagocytic Lymphohistiocytosis. Crit Care Med. 2016; 44:e1045–53. [PubMed: 27441901]
10. Halacli B, Unver N, Halacli SO, Canpinar H, Ersoy EO, Ocal S, et al. Investigation of hemophagocytic lymphohistiocytosis in severe sepsis patients. J Crit Care. 2016; 35:185–90. [PubMed: 27481757]

11. Shakoory B, Carcillo JA, Chatham WW, Amdur RL, Zhao H, Dinarello CA, et al. Interleukin-1 Receptor Blockade Is Associated With Reduced Mortality in Sepsis Patients With Features of Macrophage Activation Syndrome. Crit Care Med. 2016; 44:275–81. [PubMed: 26584195]
12. Fisher CJ, Dhainaut JF, Opal SM, Pribble JP, Balk RA, Slotman GJ, et al. Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome. Results from a randomized, double-blind, placebo-controlled trial. Phase III rhIL-1ra Sepsis Syndrome Study Group. JAMA. 1994; 271:1836–43. [PubMed: 8196140]
13. Bu F, Borsa N, Gianluigi A, Smith RJH. Familial atypical hemolytic uremic syndrome: A review of its genetic and clinical aspects. Clinical and Developmental Immunology. 2012; 2012:9.
14. Westra D, Volokhina E, Van Der Heijden E, Vos A, Huigen M, Jansen J, et al. Genetic disorders in complement (regulating) genes in patients with atypical haemolytic uraemic syndrome (aHUS). Nephrol Dial Transplant. 2010; 25:2195–202. [PubMed: 20106822]
15. Koné-Paut I, Galeotti C. Current treatment recommendations and considerations for cryopyrin-associated periodic syndrome. Expert Rev Clin Immunol. 2015; 11:1083–92. [PubMed: 26312542]
16. Cetica V, Sieni E, Pende D, Danesino C, De Fusco C, Locatelli F, et al. Genetic predisposition to hemophagocytic lymphohistiocytosis: Report on 500 patients from the Italian registry. J Allergy Clin Immunol. 2016; 137:188–96. [PubMed: 26342526]
17. Ravelli A, Grom AA, Behrens EM, Cron RQ. Macrophage activation syndrome as part of systemic juvenile idiopathic arthritis: diagnosis, genetics, pathophysiology and treatment. Genes Immun. 2012; 13:289–98. [PubMed: 22418018]
18. Yealy D, Kellum J, Huang D, Barnato A, Weissfeld L, Pike F, et al. A randomized trial of protocol-based care for early septic shock. N Engl J Med. 2014; 370:1683–93. [PubMed: 24635773]
19. Knaus W, Draper E, Wagner D, Zimmerman J. APACHE II: a severity of disease classification system. Crit Care Med. 1985; 13:818–29. [PubMed: 3928249]
20. Rudd E, Bryceson YT, Zheng C, Edner J, Wood SM, Ramme K, et al. Spectrum, and clinical and functional implications of UNC13D mutations in familial haemophagocytic lymphohistiocytosis. J Med Genet. 2007; 45:134–41. [PubMed: 17993578]
21. Strippoli R, Caiello I, De Benedetti F. Reaching the threshold: A multilayer pathogenesis of macrophage activation syndrome. J Rheumatol. 2013; 40:761–7. [PubMed: 23588947]
22. Schulert GS, Zhang M, Fall N, Husami A, Kissell D, Hanosh A, et al. Whole-exome sequencing reveals mutations in genes linked to hemophagocytic lymphohistiocytosis and macrophage activation syndrome in fatal cases of H1N1 influenza. J Infect Dis. 2016; 213:1180–8. [PubMed: 26597256]
23. Bell CJ, Dinwiddie DL, Miller NA, Hateley SL, Ganusova EE, Mudge J, et al. Carrier Testing for Severe Childhood Recessive Diseases by Next-Generation Sequencing HHS Public Access. Sci Transl Med January. 2011; 12:65–4.
24. Basheer A, Padhi S, Boopathy V, Mallick S, Nair S, Varghese RGB, et al. Hemophagocytic lymphohistiocytosis: An Unusual complication of Orientia tsutsugamushi disease (scrub typhus). Mediterr J Hematol Infect Dis. 2015; 7:e2015008. [PubMed: 25574367]
25. Zhang K, Jordan MB, Marsh RA, Johnson JA, Kissell D, Meller J, et al. Hypomorphic mutations in PRF1, MUNC13-4, and STXBP2 are associated with adult-onset familial HLH. Blood. 2011; 118:5794–8. [PubMed: 21881043]
26. Zhang K, Chandrakasan S, Chapman H, Valencia CA, Husami A, Kissell D, et al. Synergistic defects of different molecules in the cytotoxic pathway lead to clinical familial hemophagocytic lymphohistiocytosis. Blood. 2014; 124:1331–1334. [PubMed: 24916509]
27. Aricò M, Boggio E, Cetica V, Melensi M, Orilieri E, Clemente N, et al. Variations of the UNC13D Gene in Patients with Autoimmune Lymphoproliferative Syndrome. PLoS One. 2013; 8:1–9.
28. Kaufman KM, Linghu B, Szustakowski JD, Husami A, Yang F, Zhang K, et al. Whole Exome Sequencing Reveals Overlap Between Macrophage Activation Syndrome in Systemic Juvenile Idiopathic Arthritis and Familial Hemophagocytic Lymphohistiocytosis. Arthritis Rheumatol (Hoboken, NJ). 2014; 66:3486–95.
29. Moradian MM, Sarkisian T, Ajrapetyan H, Avanesian N. Genotype-phenotype studies in a large cohort of Armenian patients with familial Mediterranean fever suggest clinical disease with heterozygous MEFV mutations. J Hum Genet. 2010; 55:389–93. [PubMed: 20485448]

30. Booty M, Chae J, Masters S, Remmers E, Barham B, Le J, et al. Familial Mediterranean fever with a single MEFV mutation: Where is the second hit? Arthritis Rheum. 2009; 60:1851–61. [PubMed: 19479870]
31. Marek-Yagel D, Berkun Y, Padeh S, Abu A, Reznik-Wolf H, Livneh A, et al. Clinical disease among patients heterozygous for familial mediterranean fever. Arthritis Rheum. 2009; 60:1862–6. [PubMed: 19479871]
32. Delvaeye M, Noris M, De Vriese A, Esmon CT, Esmon NL, Ferrell G, et al. Thrombomodulin mutations in atypical hemolytic-uremic syndrome. N Engl J Med. 2009; 361:345–57. [PubMed: 19625716]
33. Neocleous V, Byrou S, Toumba M, Costi C, Shammas C, Kyriakou C, et al. Evidence of digenic inheritance in autoinflammation-associated genes. J Genet. 2016; 95:761–6. [PubMed: 27994174]
34. Naselli A, Penco F, Cantarini L, Insalaco A, Alessio M, Tommasini A, et al. Clinical Characteristics of Patients Carrying the Q703K Variant of the NLRP3 Gene: A 10-year Multicentric National Study. J Rheumatol. 2016; 43:1093–100. [PubMed: 27036377]
35. Vitale A, Lucherini O, Galeazzi M, Frediani B, Cantarini L. Long-term clinical course of patients carrying the Q703K mutation in the NLRP3 gene: a case series. Clin Exp Rheumatol. 2012; 30:943–6. [PubMed: 22935299]
36. Verma D, Särndahl E, Andersson H, Eriksson P, Fredrikson M, Jönsson J-I, et al. The Q705K polymorphism in NLRP3 is a gain-of-function alteration leading to excessive interleukin-1β and IL-18 production. PLoS One. 2012; 7:e34977. [PubMed: 22529966]
37. Tomiyama N, Higashiuesato Y, Oda T, Baba E, Harada M, Azuma M, et al. MEFV mutation analysis of familial Mediterranean fever in Japan. Clin Exp Rheumatol. 2008; 26:13–7. [PubMed: 18328141]
38. Kitade T, Horiki N, Katsurahara M, Totoki T, Harada T, Tano S, et al. Usefulness of Small Intestinal Endoscopy in a Case of Adult-onset Familial Mediterranean Fever Associated with Jejunoileitis. Intern Med. 2015; 54:1343–7. [PubMed: 26027984]
39. Migita K, Agematsu K, Yazaki M, Nonaka F, Nakamura A, Toma T, et al. Familial Mediterranean fever: genotype-phenotype correlations in Japanese patients. Medicine (Baltimore). 2014; 93:158–64. [PubMed: 24797171]
40. Singh-Grewal D, Chaitow J, Aksentijevich I, Christodoulou J. Coexistent MEFV and CIAS1 mutations manifesting as familial Mediterranean fever plus deafness. Ann Rheum Dis. 2007; 66:1541. [PubMed: 17934081]
41. Carcillo JA, Halstead ES, Hall MW, Nguyen TC, Reeder R, Aneja R, et al. Three Hypothetical Inflammation Pathobiology Phenotypes and Pediatric Sepsis-Induced Multiple Organ Failure Outcome. Pediatr Crit Care Med. 2017; 18:513–23. [PubMed: 28410274]
42. Nguyen TC, Cruz MA, Carcillo JA. Thrombocytopenia-Associated Multiple Organ Failure and Acute Kidney Injury. Crit Care Clin. 2015; 31:661–74. [PubMed: 26410136]
43. Prescott HC, Calfee CS, Taylor Thompson B, Angus DC, Liu VX. Toward smarter lumping and smarter splitting: Rethinking strategies for sepsis and acute respiratory distress syndrome clinical trial design. Am J Respir Crit Care Med. 2016; 194:147–55. [PubMed: 27244481]
44. Cichocki F, Schlums H, Li H, Stache V, Holmes T, Lenvik TR, et al. Transcriptional regulation of Munc13-4 expression in cytotoxic lymphocytes is disrupted by an intronic mutation associated with a primary immunodeficiency. J Exp Med. 2014; 211:1079–91. [PubMed: 24842371]
45. Kyriazopoulou E, Leventogiannis K, Norrby-Teglund A, Dimopoulos G, Pantazi A, Orfanos SE, et al. Macrophage activation-like syndrome: An immunological entity associated with rapid progression to death in sepsis. BMC Med. 2017; 15:1–10. [PubMed: 28049467]
46. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature. 2016; 536:285–91. [PubMed: 27535533]
47. Auton A, Abecasis GR, Altshuler DM, Durbin RM, Abecasis GR, Bentley DR, et al. A global reference for human genetic variation. Nature. 2015; 526:68–74. [PubMed: 26432245]
48. NHLBI Grand Opportunity Exome Sequencing Project (ESP). Available from: https://esp.gs.washington.edu/drupal/

49. Kryukov GV, Pennacchio LA, Sunyaev SR. Most Rare Missense Alleles Are Deleterious in Humans: Implications for Complex Disease and Association Studies. Am J Hum Genet. 2007; 80:727–39. [PubMed: 17357078]
50. Tennessen JA, Bigham AW, O'Connor TD, Fu W, Kenny EE, Gravel S, et al. Evolution and functional impact of rare coding variation from deep sequencing of human exomes. Science. 2012; 337:64–9. [PubMed: 22604720]
51. Stenson PD, Mort M, Ball EV, Shaw K, Phillips A, Cooper DN. The Human Gene Mutation Database: building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic medicine. Hum Genet. 2014; 133:1–9. [PubMed: 24077912]
52. Schramm EC, Roumenina LT, Rybkine T, Chauvet S, Vieira-Martins P, Hue C, et al. Functional mapping of the interactions between complement C3 and regulatory proteins using atypical hemolytic uremic syndrome-associated mutations. Blood. 2015; 125:2359–70. [PubMed: 25608561]
53. Zuber J, Fakhouri F, Roumenina LT, Loirat C, Frémeaux-Bacchi V. French Study Group for aHUS/C3G. Use of eculizumab for atypical haemolytic uraemic syndrome and C3 glomerulopathies. Nat Rev Nephrol. 2012; 8:643–57. [PubMed: 23026949]
54. Asif A, Nayer A, Haas CS. Atypical hemolytic uremic syndrome in the setting of complement-amplifying conditions: case reports and a review of the evidence for treatment with eculizumab. J Nephrol. 2016; 30:347–62. [PubMed: 27848226]
55. Fakhouri F, Hourmant M, Campistol JM, Cataland SR, Espinosa M, Gaber AO, et al. Terminal Complement Inhibitor Eculizumab in Adult Patients with Atypical Hemolytic Uremic Syndrome: A Single-Arm, Open-Label Trial. Am J Kidney Dis. 2016; 68:84–93. [PubMed: 27012908]
56. Boom V, Anton J, Lahdenne P, Quartier P, Ravelli A, Wulffraat NM, et al. Evidence-based diagnosis and treatment of macrophage activation syndrome in systemic juvenile idiopathic arthritis. Pediatr Rheumatol Online J. 2015; 13:55. [PubMed: 26634252]
57. Caprioli J, Noris M, Brioschi S, Pianetti G, Castelletti F, Bettinaglio P, et al. Genetics of HUS: the impact of MCP, CFH, and IF mutations on clinical presentation, response to treatment, and outcome. Blood. 2006; 108:1267–79. [PubMed: 16621965]
58. Bu F, Maga T, Meyer NC, Wang K, Thomas CP, Nester CM, et al. Comprehensive Genetic Analysis of Complement and Coagulation Genes in Atypical Hemolytic Uremic Syndrome. J Am Soc Nephrol. 2014; 25:55–64. [PubMed: 24029428]
59. Gül A. Approach to the patients with inadequate response to colchicine in familial Mediterranean fever. Best Pract Res Clin Rheumatol. 2016; 30:296–303. [PubMed: 27886801]
60. Šedivá A, Horváth R, Manásek V, Gregorová A, Plevová P, Horáčková M, et al. Cluster of patients with Familial Mediterranean fever and heterozygous carriers of mutations in MEFV gene in the Czech Republic. Clin Genet. 2014; 86:564–9. [PubMed: 24251727]
61. Pollard KS, Hubisz MJ, Rosenbloom KR, Siepel A. Detection of nonneutral substitution rates on mammalian phylogenies. Genome Res. 2010; 20:110–21. [PubMed: 19858363]
62. Ng PC, Henikoff S. Predicting Deleterious Amino Acid Substitutions Predicting Deleterious Amino Acid Substitutions. Genome Res. 2001; 11:863–74. [PubMed: 11337480]

Table 1

Clinical phenotypes of subjects enrolled in the study. Lab values represent baseline values at time of enrollment. BSI: blood stream infection, PNA: pneumonia, UTI: urinary tract infection. Culture negative indicates that no positive culture from any site was obtained.

Subject	Age	Sex	SBP (mmHg)	Lactate (mmol/L)	WBC ($\times 10^9$ /L)	Hgb (g/dL)	Plt ($x10^9$/L)	INR	PTT (s)	Tbili (mg/dL)	Cr (g/dL)	Ferritin (ηg/ml)	Infection	APACHE II	Dead at 30d
1	32	M	80	3.9	2.9	8.4	44	1.5		2.5	3.1	14,949	Culture negative	24	Yes
2	73	M	83	16	10.5	17.4	57	1.2	26.0	1.5	2.7	36,240	UTI/BSI	42	Yes
3	64	F	91	7.4	2.9	14.8	33			1.7	3.3	7,259	BSI	18	No
4	44	F	140	9.5	6.4	9.1	25	1.8		6.2	0.8	8,329	PNA/BSI	20	Yes
5	51	M	70	6.3	4.5	13.9	50		47.1	1.8	3.5	55,314	PNA/BSI	37	Yes
6	70	F	102	3.9	8.4	5.1	88	3.2	48.0	6.4	5.1	11,850	Culture negative	22	Yes
MEAN	56		94	7.8	5.9	11.5	50	1.9	40.4	3.4	3.1	24,031		27	
+/− SEM	6.6		10	1.9	1.3	1.9	9	0.4	0.9	0.9	0.6	7,411		4	

Table 2

Table of previously reported pathogenic variants identified during whole exome sequencing of sepsis patients with extreme hyperferritinemia. Numbers 1–6 indicate study subjects where rows represent individual variants. Columns indicate genes with mutations identified and the specific variant. Reference numbers in column 3 refer to original reports of human pathogenicity, and column 4 the genetic disorder associated with it. In order to support claims of disease relevance, previous literature reports of clinical phenotype was required as part of the variant filtering scheme. Subsequently, all eleven variants have been previously reported in individuals with either aHUS, HLH, or the periodic fever syndromes of CAPS or FMF as indicated. MAF per the ExAC database is provided. PhyloP computes conservation or evolutionary acceleration. Values are between −11.764 and +6.424. Positive scores indicate conservation; negative scores fast-evolution[61]. SIFT scores are between 0–1 with scores less than 0.05 predicted to be deleterious, those greater than or equal to 0.05 are predicted to be tolerated[62]. Putative targeted therapies have been suggested based on the identification of these variants in the context of aHUS, HLH, MEFV and CAPS, with citations provided. aHUS: atypical hemolytic uremic syndrome; CAPS: cryopyrin associated periodic fever syndrome, FMF: familial Mediterranean fever, HLH: hemophagocytic lymphohistiocytosis; MAF: Minor allele frequency.

Subject	Gene	Variant	Amino Acid Change	Disease	MAF	PhyloP Score	SIFT Score	Putative Therapy
1	*C3*	c.1407G>C [52] NM_000064.2	p.Glu469Asp	aHUS	0.00394	−0.9	1	Anti-C5 ab [53–55]
	UNC13D	c.1579C>T [25,26] NM_199242.2	p.Arg527Trp	HLH	0.00523	0.45	0.02	IL1-RA [56,17]
2	*CD46*	c.1058C>T [57] NM_172359.2	p.Ala353Val	aHUS	0.01532	−3.19	0.47	Anti-C5 ab [53–55]
	CFHR5	c.832G>A [58] NM_030787	p.Gly278Ser		0.00729	1.39	0.03	
3	*UNC13D*	c.2782C>T [27,28] NM199242.2	p.Arg928Cys	HLH	0.02986	0.65	0.13	IL1RA [56,17]
4	*NLRP3*	c.2113C>A [35] NM_004895.4	p.Gln705Lys	CAPS	0.0495	−0.17	0.22	IL1RA [15]
	MEFV	c.250G>A [37] NM_000243.2	p.Glu84Lys	FMF	0.00012	1.48	0	IL1RA [59]
5	*UNC13D*	c.2983G>C [27] NM_199242.2	p.Ala995Pro	HLH	0.00096	1.52	0.22	IL1RA [56,17]
		c.2542A>C [27] NM_199242.2	p.Ile848Leu		0.00090	−0.72	0.10	
6	*CD46*	c.1058C>T [57] NM_172359.2	p.Ala353Val	aHUS	0.01532	−3.19	0.47	Anti-C5 ab [53–55]
	MEFV	c.2084A>G [60] NM_000243.2	p.Lys695Arg	FMF	0.00550	−0.05	0.13	IL1RA [59]

Table 3

This table shows the gene panel examined in our study. All 6 subjects underwent whole exome sequencing. Identified variants were filtered for minor allele frequency less than 5% based on the 1000 Genomes, ExAC and NHLBI-ESP 6500 databases. Variants were then filtered for those genes in the panel of interest, that were previously reported as pathogenic or likely pathogenic in the corresponding immunologic disorder as reported in the HGMD professional database.

Disease Class	Disease	Genes
Primary Immunodeficiencies	Chronic Granulomatous Disease	*CYBA, CYBB, NCF1, NCF2, NCF4*
	WHIM Syndrome	*CXCR4*
	Bruton's Agammaglobulinemia	*BTK*
	Activated PI3K-Delta Syndrome	*PIK3CD*
	Common Variable Immunodeficiency	*BLK, CD19, CD81, CR2, CTLA4, ICOS, IKZF1, IL21, IL21R, LRBA, IRF2BP2, MS4A1, NFKB1, NFKB2K, PIK3R1, PLCG2, PRKCD, RAC2, TNFRSF13B, TNFRSF13C, TNFSF12, VAV1*
	SCID	*ADA, AK2, CD3D, CD3E, DCLRE1C, FOXN1, IL2RG, IL7R, JAK3, NHEJ1, ORAI1, PNP, PTPRC, RAG1, RAG2, RMRP, STAT5B, STIM1, TBX1, ZAP70*
	HLH	*PRF1, UNC13D, AP3B1, BLOC1S6, CD27, ITK, LYST, RAB27A, SLC7A7, STX11, STXBP2, SH2D1A, XIAP*
Lymphoproliferative Syndromes	ALPS	*CASP10, CASP8, FADD, FAS, FASLG, KRAS, MAGT1, NRAS*
Recurrent Fever Syndromes	Crypopyrin-Associated Periodic Syndrome	*NLRP3*
	Familial Mediterranean Fever	*MEFV*
Complement Coagulation Disorders	aHUS	*PLG, C3, CD46, CBF, CFH, CFHR5, CFI, DGKE, THBD*
	TTP	*ADAMTS13*
Disorders of Iron Handling	Hemochromatosis	*FTH1, HFE, SLC40A1, TFR2*
	Juvenile hemochromatosis	*HAMP, HFE2*