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Abstract

Purpose: Our understanding of the inborn errors of immunity that cause immunodeficiencies is increasing however, their contribution to pediatric sepsis is unknown.

Methods: We used whole exome sequencing to characterize variants previously reported in monogenic immunologic disorders in 330 children admitted to intensive care for severe sepsis. Candidate variants were restricted to novel null variants or rare variants classified as pathogenic or potentially pathogenic in Qiagen's Human Genetic Mutation Database in a disease consistent inheritance pattern.

Results: One in two children overall and two of three African American children had immunodeficiency-associated variants. Children with candidate variants had increased odds of isolating a blood or urinary pathogen (blood: OR 2.05, 95% CI 1.16 - 3.65, p -value = 0.014, urine: OR: 2.35, 95% CI 1.02 – 5.41, p -value = 0.04) and laboratory evidence of increased immune activation with increased odds of hyperferritinemia (ferritin $\geq$ 500 ng/ml, OR: 1.92, 95% CI: 1.16 – 3.20, p -value = 0.013) and lymphopenia (minimum lymphocyte count $<1000/\mu\text{L}$, OR: 1.62, 95% CI: 1.03 – 2.55, p -value = 0.038).

Conclusion: Herein, we describe the genetic findings in this pediatric sepsis cohort and their microbiologic and immunologic significance providing rationale for screening children with life-threatening infection for potential inborn errors of immunity.

Keywords: *sepsis, inborn errors of immunity, hyperinflammation, primary immunodeficiency*

Introduction

Severe sepsis remains a leading cause of morbidity and mortality with greater than 40 million annual cases worldwide and contributing to over 60% of pediatric deaths, emphasizing a critical need for insight into its pathobiology [1]. Inborn errors of immunity (IEI) have been hypothesized to underlie individual vulnerability to life-threatening infection, not just in primary immunodeficiencies, but also in sporadic cases of severe sepsis [2]. While these links have been explored in individual cases and pathogens, such as influenza [3], invasive pneumococcus [4], pseudomonas [5] and SARS-CoV2 infection [6], the prevalence of IEI has not been systematically evaluated among prospective pediatric severe sepsis cohorts.

Advances in next generation sequencing (NGS) have led to an expanding understanding of the molecular basis of many IEI. In fact, the International Union of Immunologic Societies (IUIS) updates their catalogue of immunologic disorders with monogenic causes biannually, and currently describes over 300 genetic defects.[7] Due to the disorders' widespread phenotypic and genetic heterogeneity, exome sequencing (ES) is commonly used in their diagnosis. However, the broader application of ES to children with life-threatening infection has been hindered by challenges in variant interpretation. In addition to limited understanding of variant pathogenicity, on an individual level even known pathogenic variants are impacted by penetrance, expressivity and environment such that it may not cause disease in all individuals harboring it. This leads to a critical knowledge gap in our understanding of the prevalence and relevance of these inborn errors of immunity in pediatric severe sepsis.

In the neonatal intensive care unit, genetic disease is recognized as a significant contributor to morbidity and mortality and rapid NGS sequencing has demonstrated reduced time to diagnosis and direct clinical impact [8,9]. In older children with concern for underlying genetic disease, comparable diagnostic rates to neonates have been observed nearing 40% with a median time to diagnosis of under 2 weeks. The two largest series utilizing sequencing to achieve molecular diagnosis in children admitted to the pediatric intensive care unit both included cases of unappreciated genetic immunodeficiency in the setting of life-threatening infection [10,11].

Building on these results we used ES to test the hypothesis that variants in genes in the 2017 IUIS classification of Primary Immunodeficiency Diseases are common among children with severe sepsis and may represent potential cases of immunodeficiency.

Methods

Subject Selection

All pediatric severe sepsis admissions between 44 weeks Post Gestational Age and 18 years old admitted to one of nine Pediatric Intensive Care Units in the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Collaborative Pediatric Critical Care Research Network (NICHD-CPCCRN) between 2015-2017 with a central venous or arterial catheter and a commitment to aggressive care were eligible. The study was approved by the central Institutional Review Board and all 9 individual site Institutional Review Boards. Written informed consent was obtained from one or more parents/guardians for each child. Assent was garnered when the child was able. Sepsis was defined as the presence of suspected or documented infection AND evidence of two of the following Systemic Inflammatory Response criteria (1. Tachycardia (heart rate > 90th percentile for age, 2. Tachypnea (respiratory rate > 90th percentile for age), 3. Abnormal temperature (< 36°C or > 38.5°C), and 4. Abnormal WBC count (> 12,000/mm³ or < 4,000/mm³ or > 10% immature neutrophils). Severe sepsis was defined by the previously mentioned criteria AND at least one organ failure defined using the organ failure index (OFI) (cardiovascular: need for cardiovascular infusion support; pulmonary: need for mechanical ventilation support with the ratio of the PaO₂/FIO₂ < 300 without this support; hepatic—total bilirubin > 1.0 mg/dL and alanine aminotransferase > 100 U/L; renal: serum creatinine > 1.0 mg/dL and oliguria [urine output < 0.5 mL/kg/hr]; hematologic: thrombocytopenia < 100,000/mm³ and international normalized ratio > 1.5 × normal; and CNS: Glasgow Coma Scale < 12 in absence of sedatives) [12].

DNA Extraction and Exome Sequencing

A total 401 pediatric patients with severe sepsis were enrolled, of whom 381 parents (95%) provided consent for sequencing. Whole blood DNA extraction was performed and 330 individuals completed ES (median DNA yield 39.24 μg, IQR: 20.19 μg - 71.49 μg) and 51 would have insufficient DNA (median yield 1.120 μg, IQR: 0.205 μg - 3.455 μg, p -value < 0.0001). Those with insufficient DNA extraction had a lower median lymphocyte count on the day of sequencing (230 versus 1200 cells per μl, $p = 1.43 \times 10^{-10}$), were older (median age 8.5y v 5.3y, p -value = 0.0008), less likely to be previously healthy (13.7% versus 47.9%, p -value = 2.8×10^{-6}), more likely to have malignancy (47.1% versus 7.0%, p -value = 9.21×10^{-12}), and suffered higher mortality (23.5% versus 8.5%, p -value = 0.0028).

DNA was extracted from whole blood using standard methods. ES was performed on the Ion Torrent Platform at the University of Pittsburgh Genomics Research Core Laboratory. Libraries were constructed using the Ampliseq Exome RDY (ThermoFischer) with 100x target coverage. FASTQ files were aligned to *homo sapiens* reference sequence GRCh37/hg19 to generate VCF files. The Fabric Genomics Opal software platform (Fabric Genomics Inc, CA) was used to identify nonsynonymous variants including missense, nonsense, frameshift, start site, or splice site (+/- 2bp) mutations. Candidate variants were filtered for a minimum coverage >10x and a PHRED score >20 for quality control.

Candidate Gene Filter

The IUIS' report of Inborn Errors of Immunity currently lists 328 genes as causes of over 350 monogenic primary immunodeficiencies [7] (Table S1). Candidate variants were restricted this gene list, with a minor allele frequency (MAF) less than 0.05 in the ExAC, 1000 Genome, NHLBI-ESP 6500 and gnomAD databases. Variants were required to exhibit a disease-consistent inheritance pattern (one variant for autosomal dominant disorders or X-linked disorders in males, and two variants for autosomal recessive disorders). For disorders with evidence supporting both recessive and dominant inheritance, only a single heterozygous variant was required. Additionally, missense variants were limited to only those classified as disease mutation (*DM*) or disease mutation? (*DM?*) in Qiagen's Professional Human Genetic Mutation Database (HGMD) based on peer-reviewed literature of the variant in human disease. The *DM?* designation indicates uncertainty in the link between variant and disease phenotype, and is a potential rather than definitive association between the variant and immunodeficiency. In this manuscript, these *DM* and *DM?* variants will be defined as pathogenic and potentially pathogenic respectively. Therefore, for all candidate missense variants, the specific amino acid change observed in our cohort was also seen in a prior report of human immune deficiency in the peer-reviewed literature. Unique null variants (nonsense, frameshift, canonical +/-1 or 2 splice sites and initiation codon) found in a disease-causing inheritance pattern were treated as potentially pathogenic per the American College of Medical Genetics (ACMG) standards and guidelines for the interpretation of sequence variants [13]. Highly recurrent null variants were filtered from the dataset, due to high likelihood that they represented sequencing error or had no impact on gene function.

Statistical Methods

Comparisons between baseline characteristics and outcome were made between groups of children with and without identified inborn errors of immunity using χ^2 testing for categorical variables and Wilcoxon rank sum for continuous variables using a p -value threshold of 0.05.

For individual identified variants, a cohort MAF was computed (Table S2) and compared using χ^2 testing to gnomAD (<https://gnomad.broadinstitute.org/v2.1.1>) a publicly available reference database of sequencing data from 141,456 individuals from which individuals with severe pediatric diseases are actively excluded [14]. This approach has been used to identify overrepresented monogenic variants in cohorts of individuals with rare diseases [15]. Additional MAF comparisons were made between 1.) African Americans and non-African Americans in the pediatric sepsis cohort, and 2.) African American in the sepsis cohort and individuals of African heritage in gnomAD. P -values were adjusted for multiple testing using the Benjamini-Hochberg method for the number of identified variants. Significance threshold was a p -value of 0.05 after multiple test corrections. All frequency calculations and statistical comparisons were completed using R version 3.5.1.

Results

Prevalence of Pathogenic and Potentially Pathogenic Variants Associated with Inborn Errors of Immunity in Children with Severe Sepsis

Among the 330 sequenced individuals, we limited our inquiry to those genes known to cause monogenic IEI as catalogued by the IUIS. Candidate variants were further restricted to either novel null variants in genes where nonsense mutations are a known disease mechanism or other variants classified in HGMD [16] as pathogenic or potentially pathogenic in a disease causing inheritance pattern. As shown in Fig. 1, the identified variants exhibited both locus and allele diversity, with 317 total variants observations at 117 loci in 191 individuals (57.9%) including 85 previously healthy children (Table 1, Fig. 1). Additionally, more than 2/3 of African American or Black children with severe sepsis (51 of 70, 72%, p -value = 0.0042) were found to have a genetic variant previously associated with IEI (Table 1). While most individuals harbored single IEI (N=114, 59.7% of positive ES), 47 children had at two variants in an immune system gene, 17 had 3 (5%), and 13 had 4 or more (4%, range 1-7, Fig. 2, Table S3).

Among these 317 variants, 181 variants were associated with autosomal dominant disorders (N = 139 individuals), 7 patients were homozygous for variants in conditions with autosomal recessive inheritance and 18 had

two variants in these genes (N = 15 individuals) and two X-linked recessive disorders (N = 2 individuals). 109 variants were associated with disorders that can be inherited in either an autosomal dominant or autosomal recessive manner (N = 87 individuals). For these conditions, only a single monoallelic variant was required.

Association of IEI Status with Infection Site and Laboratory Markers of inflammatory State

Review of microbiologic data showed that the presence of IEI-variants associated with anatomic site of pathogen identification (Fig. 3a). Children with IEI variants had increased odds of culturing a pathogen from either the blood stream or urinary tract (blood culture positive: OR 1.90, 95% CI 1.02 - 3.52, p -value = 0.040; urine culture positive: OR: 2.35, 95% CI 1.02 – 5.41, p -value = 0.04). The strength of this association increased when conventional blood culture results were supplemented with PCR blood pathogen identification (OR 2.05, 95% CI 1.16 - 3.65, p -value = 0.013). Notably, there was no significant difference in odds of having bacteria cultured from the respiratory tract.

Additionally, we sought to identify differences in laboratory markers of immunologic activation between those with and without IEI. As seen in Fig. 3b, Children with IEI-associated variants were more likely to be lymphopenic (minimum lymphocyte count < 1000/ μ L, OR: 1.62, 95% CI: 1.03 – 2.55, p -value = 0.038) and hyperferritinemic (ferritin > 500 ng/ml, OR: 1.92, 95% CI: 1.16 – 3.20, p -value = 0.013) at any point on study, both markers of systemic inflammation and known risk factors for sepsis mortality [17,18]. There were also trends toward increased odds of having CRP > 10mg/dl (OR: 1.57, 95% CI: 1.01 – 2.44, p -value = 0.057) and platelet count < 150 x 10³/ μ L (OR: 1.57, 95% CI: 1.01 – 2.46, p -value = 0.053), also markers of increased inflammation. Children with variants also displayed significant differences in absolute lab values with a lower minimum lymphocyte count (median, [IQR] : 740, [330 – 1300] v. 940/ μ L, [470 - 1520], p -value = 0.02) and minimum platelet count (median, [IQR] : 105.5, [40.3 – 192.0] v. 136.0 x10³/ μ L, [62 – 212.0], p -value = 0.04). A trend towards higher absolute CRP levels (median, [IQR] : 12.0, [4.8 – 20.8] v. 9.2 mg/dL, [3.0 – 17.7], p -value = 0.051) was observed between groups.

IEI Variants According to International Union of Immunologic Society Functional Class

Identified variants occurred across the full spectrum of functional of primary immunodeficiencies classes delineated by the IUIS and included complement disorders, autoinflammatory disorders, combined immunodeficiencies with associated or syndromic defects, congenital defects of phagocyte number and function,

disorders of immune dysregulation, defects in innate and intrinsic immunity, predominantly antibody deficiencies and immunodeficiencies affecting cellular and humoral immunity (Fig. 1, Table S4). Participant variant data including zygosity, previous publications, and gnomad allele frequency can be found in Table S3.

Complement variants previously implicated in atypical hemolytic uremic syndrome (aHUS) were the most common IUIS disease process. In total 137 complement mutations were identified in 92 individuals (27.9%, Fig. 1, Table S4). The most commonly encountered variants in this group included *CFH* (c.3148A>T; p.Asn1050Tyr), *C3* (c.1407G>C; p.Glu469Asp) and *CFHR3* (c.424 C>T; p.Arg142Cys), all previously described in aHUS [19–23]. We also identified several other variants in the alternative complement pathways previously reported in atypical hemolytic uremic syndrome including: *CFHR5*, *CFI*, *CD46*, *CFI*, *CFB*, *CFHR3*, *THBD* and *CFHR4*.

The next most frequently encountered group of variants were related to autoinflammatory conditions (Fig. 1, Table S4). They occurred in 72 individuals and included previously identified pathogenic or potentially pathogenic variants in *MEFV*, *NLRP3*, *NLRP12*, *TNFRSF1A*, *CARD14*, *NOD2*, and *PSTPIP1*. The most commonly identified variant in this group, *NLRP3* c.2113C>A; p.Gln705Lys was carried by 21 individuals, and was homozygous in one. Other variants seen in the cohort where the same amino acid change has previously been observed in other autoinflammatory disorders including Familial Mediterranean fever (*MEFV*), familial cold inflammatory syndrome type 2 (*NLRP12*), TNF receptor-associated periodic syndrome (*TNFRSF1A*), Blau Syndrome (*NOD2*), pyogenic sterile arthritis, pyoderma gangrenosum acne syndrome (*PSTPIP1*) and CARD mediated pustular psoriasis (*CARD14*).

The remaining other pathogenic or potentially pathogenic variants according to IUIS Classification of Primary Immunodeficiencies from most to least common include: Combined immunodeficiencies with associated or syndromic features: *KMT2D*, *TERT*, *RTEL1*, *SPINK5*, *TBX1*; congenital defects of phagocyte number and function: *ELANE*, *CFTR*, *ITGB2*, *GATA2*; disorders of immune dysregulation: *AIRE*, *UNC13D*, *PRF1*, *CASP10*, *FAS*, *CTLA4*, *XIAP*; defects of innate and intrinsic immunity: *TINF2*, *IRF3*, *STAT1*, *IL17RA*, *TICAM1*; Predominantly antibody deficiencies: *TNFRSF13B*, *TCF3*, *TTC37*; and mutations affecting cellular and humoral immunity: *IL2RG*, *TAP1*; and (Fig. 1, Table S4).

Specific Variants Overrepresented in the Pediatric Sepsis Cohort Compared to gnomAD

After candidate variant identification, we sought to find statistical evidence of overrepresentation in children with severe sepsis in comparison to participants in the gnomAD database (<https://gnomad.broadinstitute.org/>, v2.1.1), to identify variants that may contribute to severe sepsis risk [14]. A summary of statistically significant findings is provided in Table 2. Several complement variants were significantly over-represented; *C3* (c.1407G>C; p.Glu469Asp), was seen 4.7 times more often in children with sepsis than in the gnomAD database, (N = 15, adjusted p -value = 1.1×10^{-7}) as well as *C3* (c.443G>A; p.Arg148Gln, MAF ratio = 214.2, N = 1, adjusted p -value = 4.1×10^{-7}) both activating variants of the main complement convertase [22]. *CFHR3* was also overrepresented (c.424C>T; p.Arg142Cys, MAF ratio 2.9, N = 15, adjusted p -value = 6.1×10^{-3}), a member of the CFH family of genes with a key role in downregulating complement [24]. In addition, *IRF3* (c.829G>A; p.Ala277Thr), a transcriptional regulator of type I interferon (IFN)-dependent immune responses and variant associated with impaired IFN- β and CXCL10 production after viral stimulation [25], was over-represented in the sepsis cohort with an MAF ratio of 3.4 compared to gnomAD (N = 9, adjusted p -value = 0.04). A complete listing of allele counts, minor allele frequencies and variant-wise comparisons can be found in Table S3.

Specific Variants Overrepresented in African American Children with Severe Sepsis

After showing that children of African American ancestry were at increased odds of having pathogenic or potentially pathogenic variants identified, we sought to identify the specific variants contributing to this association (Fig. 4). Again, complement variants are common but represent a higher proportion of variants identified (Fig. 4 versus Fig. 1). When highlighting ancestry based differences by comparing African American to Non-African American children within our sepsis cohort, the activating complement convertase variant *C3* (c.1407G>C; p.Glu469Asp), complement regulatory variants *CFHR3* (c.424C>T; p.Arg142Cys) and *CFHR5* (c.434G>A; p.Gly145Glu), and *TNFRSF1A* (c.224C>T; p.Pro75Leu) an autoinflammation variant associated with increased NF- κ B p65 activity and IL-8 secretion [26] were overrepresented (Table 2). Next, we compared MAF in children of African American ancestry from the sepsis cohort to individuals of African descent in gnomAD, highlighting potential predisposing factors for severe sepsis among groups of similar ancestral background. While limited by small numbers, we saw that *C3* (c. 443G>A; p.Arg148Gln) and *CFH* (c.2850G>T; p.Gln950His) were both more common than expected in African American children with sepsis (Table 2).

Discussion

Over half of the children with severe sepsis in our sample were found to have at least one variant where the same amino acid change was previously linked to an inborn error of immunity. Further, the presence of these variants associated with increased odds of pathogen identification in blood and urine and increased odds of lymphopenia and hyperferritinemia. While recurrent, severe or atypical infection is a classic characteristic of primary immunodeficiencies, this work suggests that life-threatening infection in the absence of known immunodeficiency may be related to deleterious variants in genes previously associated with inborn errors of immunity. This may be especially true of children in tertiary intensive care units in resource rich settings, as they may represent a significantly enriched population as a result of immunization practices, early and aggressive antibiotic treatment, and the low rates of endemic invasive infection.

Overall, the landscape of potential IEI in children with severe sepsis was highly heterogeneous at both the gene and allele level, reflecting cohort diversity, which included all children with severe sepsis. As such, we identified a wide range of defects affecting diverse aspects of host immune response with variably severe phenotypes. Previously, in six of six adults with extreme hyperferritinemic sepsis we also observed these inborn errors of immunity including variants previously described in atypical hemolytic uremic syndrome, hemophagocytic lymphohistiocytosis, familial Mediterranean fever and cryopyrin associated periodic syndrome, suggesting that these findings may also be relevant to a subset of adults with hyperinflammatory sepsis phenotypes [27].

Both in the cohort as a whole, and in children of African American ancestry complement variants were frequent. Complement is a part of the innate immune system that functions in early response to pathogens. Inactivating variants lead to increased susceptibility to bacterial pathogens such as meningococcus [28], invasive pneumococcal disease [4] and can be selected against in humans in the setting of endemic infection [29]. However, this improved pathogen clearance may come with a cost of increased tendency towards inflammation and thrombotic microangiopathy [30]. *C3* c.1407G>C and *CFHR3* c.424C>T, the most common in our cohort, have been reported as causal variants for aHUS [22,24] and were statistically overrepresented in comparison to gnomAD. In addition to being reported in aHUS (required for their inclusion), other identified complement variants have been associated with thromboembolic phenotypes [31] including recurrent pregnancy loss (*C3* c.2203C>T) [32], HELLP syndrome

(*CD46* c.1058C>T, *CD46* c.38C>T) [33,34] and drug-induced thrombotic microangiopathy (*CFH* c.2850G>T, *CFH* c.3148A>T) [35]. This suggests the variants may convey risk for a hyperactivated complement response following infection or other immunologic triggers.

When considering the frequency of complement variation in the study population, it is important to emphasize the ancestry-specific differences. For *C3* c.1407G>C and *CFHR3* c.424C>T, the overrepresentation may be explained in part due to their frequency in African American children. *CFHR5* c.434G>A and *TNFRSF1A* c.224C>T, a gene that encodes a TNF- α innate immune receptor that can directly activate complement signaling [36], were also more common among African American children with sepsis than in children of other ancestral backgrounds. Still, these effects do not seem completely explained by population stratification, as in comparison to African gnomAD participants, African American children with sepsis more commonly carried *CFH* c.2850G>T and *C3* c.443G>A variants. While gnomAD participants of African background are an imperfect control as genetic variation can differ significantly by geographic region, previous reports of disease association argue for their functional relevance and emphasize the role of complement in sepsis pathobiology in African American children. These findings raise the question as to whether differences in pediatric sepsis outcome and severity of illness that associate with ancestral background may in some instances be genetically mediated, perhaps related to differences in inflammatory response. Regardless, they emphasize the need for diverse cohorts in future studies of genetic risk in pediatric sepsis.

The second most commonly encountered functional group were variants related to Autoinflammatory conditions. Genetic mutations in *NLRP3* cause the cryopyrin-associated periodic syndromes including familial cold inflammatory syndrome, Muckle-Wells syndrome and neonatal-onset multisystem inflammatory disorder (NOMID). These autoinflammatory disorders are monogenic inflammasomopathies inherited in an autosomal dominant pattern with incomplete penetrance. The specific *NLRP3* p.Gln705Lys variant leads to constitutive hyperactivation with increased IL-1 β and IL-18 synthesis [37] that has been linked to an intensified acute phase response [38]. MEFV variants, previously reported in Familial Mediterranean Fever were also commonly encountered and have been associated with an exaggerated immune response to infection with larger increases in WBC count, ESR and LDH levels accompanied by measurable differences in tachycardia and hypotension [39].

IRF3 (c.829G>A; p.Ala277Thr) variants were also encountered 3.4 times more commonly in the pediatric sepsis cohort than expected in gnomAD (adjusted *p*-value = 0.04). Heterozygous *IRF3* variants (c.829G>A;

p.Ala277Thr) have been described in herpes simplex encephalitis. As a regulator of the type-1 interferon response, peripheral blood mononuclear cells isolated from individuals with *IRF3* c. 829G>A have significantly lower CXCL10 and IFN- β levels, following poly(I:C), HSV-1 (a DNA virus) and RNA virus infection, demonstrating impaired innate antiviral response [25]. Additionally, other *IRF3* variants have been shown to be overrepresented among individuals with life-threatening COVID-19 infection, where they are postulated to contribute to impaired viral clearance [40]. As viral infections are a common cause of sepsis in children, this leads us to hypothesize that genetic interferon pathway variation may be related to risk for severe viral illness in general.

Other key findings of the study include that if offered in the intensive care unit, genetic testing for immunologic disease is agreed to by 95% of parents of children with sepsis. The acceptability of genomic testing is important in light of current questions regarding patient and family preferences regarding genetic diagnosis. We also found that insufficient sampling of DNA from peripheral blood in the setting of lymphopenia was common, suggesting a role for alternative sampling techniques including buccal mucosa, saliva or uroepithelial cells. This is of considerable importance, as sepsis patients with lymphopenia are known to be at greater risk of morbidity and mortality [17].

The main limitation of our study is that while the presence of a pathogenic or potentially pathogenic variant in a disease consistent inheritance pattern is remarkable, on an individual basis a variant cannot be equated with immunodeficiency. While candidate variants were restricted to those with prior associations with disease in humans, literature-based classifications are likely to misclassify a portion of variants. Therefore, even autosomal dominant conditions represent cases of potential rather than confirmed immunodeficiency. While it is unlikely that the majority of children with candidate variants represent missed diagnoses of primary immunodeficiency, the repeated observation of previously reported variants in a disease consistent inheritance pattern argues against interpretation as incidental. While a fraction of these children may currently or in the future meet classic immunodeficiency definitions, the remainder may reflect a phenotypic spectrum, where genetic heterogeneity impacts sepsis-risk or immunologic phenotype during infection, short of overt immunodeficiency. This phenotypic spectrum is impacted by penetrance, expressivity, epistasis, gene-gene interactions and environmental factors. Subsequently, the molecular findings of inborn errors of immunity are related to, but remain distinct from the clinical diagnosis of immunodeficiency. Other notable limitations include the 15 cases of potential autosomal recessive biallelic disease, where ES is unable to differentiate *in cis* from *in trans* variants in the absence of parental sampling. ES also fails to

identify regulatory, structural and copy number variants that may contribute to the genetic landscape of pediatric sepsis. Future study is needed to clarify the role that these genetic findings play in predisposition to infection, differential immune response, severity of illness and recurrence risk between ancestry groups and among individuals with shared genetic risk.

Conclusions

In conclusion genetic variation previously linked to inborn errors of immunity is common in our pediatric severe sepsis cohort. These variants were associated with infection site and laboratory markers of reflective of activated inflammatory states. This suggests that screening of children with severe sepsis for unappreciated heritable immunologic disease is warranted with subsequent evaluation by clinical geneticists to determine clinical significance. In the future, the pathobiologic insights afforded by this approach could facilitate a genome-driven precision medicine approach for children with severe sepsis.

Declarations

Ethical Approval

The study was approved by the central Institutional Review Board and all 9 individual site Institutional Review Boards

Consent to Participate

Written informed consent was obtained from one or more parents/guardians for each child. Written assent was garnered when the child was able.

Consent to Publish

This consent provided consent for participation in the study, as well as consent for publication of study results.

Authors Contributions

This study was conceptualized by JAC, DAN, JMD, JL, UC and JV. Data curation was performed by KK, RS, DH, UC and RB. KK, LGG, RB, HJP, RWR performed formal analysis. Funding was acquired by JAC. The Investigation was performed by KK, JL, DH, UC, RS, JAC, RAB, DW, MMP, KM, MH, CN, JCL, AD, TS, TC, REH, AFZ, JMD. Methodology was created by JAC, JV, JL, JMD, DAN. Supervision of the project was performed by JAC, JV, LGG, JMD, DAN. Visualization and original draft writing was performed by KK. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Competing Interests

Drs. Carcillo's, Berg's, Wessel's, Pollack's, Meert's, Hall's, Doctor's, Cornell's, Harrison's, Zuppa's, Reeder's, Banks's, and Holubkov's institutions received funding from the National Institutes of Health (NIH). Drs. Carcillo's, Newth's, Shanley's, and Dean's institutions received funding from the National Institutes of Child Health and Human Development. Drs. Carcillo, Berg, Wessel, Polack, Meert, Hall, Newth, Doctor, Shanley, Cornell, Harrison,

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Availability of Data and Material

All data used in this analysis is available in the Main and Extended Tables.

Code Availability

Not applicable.

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Figure/Table Legends

Table 1 Characteristics for all study participants, individuals with and without inborn errors of immunity on whole exome sequencing

Categorical data are expressed as *N* (%). Continuous variables are presented as median and (IQR). Statistical comparison between groups was performed using Wilcoxon-rank sum for continuous variables and Fisher's exact test for categorical variables. There were no significant differences identified between the complete cohort and the IEI positive or IEI negative groups. In comparing children with severe sepsis with IEI variants to those without, children of African American ancestry were noted to have increased odds of having an IEI variant identified.

^a. OR 2.30 95% CI 1.25 - 4.36, *p* = 0.0042

IEI: Inborn Errors of Immunity; IQR: Interquartile range; N: number; ICU: intensive care unit; LOS: length of stay; MV: mechanical ventilation; ECMO: extracorporeal membrane oxygenation; PLEX: plasma exchange; CRRT: continuous renal replacement therapy

Table 2 Summary table of statistical assessment of over representation inborn errors of immunity allele frequency differences by ancestry group

Summary table of statistically significant frequency comparisons following multiple test corrections between the following groups: 1.) Sepsis cohort and gnomAD to identify variants more common in the sepsis population as a whole; 2.) African American and Non-African American children in the sepsis cohort to identify variants that may explain the increased odds of probable IEI identified on whole exome sequencing of African American children; 3.) African Americans in the sepsis cohort and individuals of African ancestry in gnomAD to identify probable IEI over-represented variants in comparison to a specific ancestral background. Table shows ratio of minor allele frequency in statistically significant comparison of interest, number of individuals within the subgroup where the variant was identified. *p*-values shown are χ^2 comparisons with Benjamini-Hochberg adjustment for the 117 total variants observed in the dataset

Fig. 1 Genomic landscape of inborn errors of immunity in severe pediatric sepsis.

This figure displays every pathogenic or likely pathogenic variant identified in the cohort and from inner-most ring to outer-most ring indicates its International Union of Immunologic Societies Classification, the disease where it has been previously identified, the gene locus affected and finally the specific allele change. 117 unique variants were identified in 191 individuals. Representatives of all IUIS disease classes were identified in the cohort including from most to least common: complement deficiencies (complement, blue), autoinflammatory disorders (autoinflammatory, yellow), combined immunodeficiency with associated or syndromic features (syndromic features, gray), congenital defects of phagocyte number and function (phagocyte defects, green), diseases of immune dysregulation (immune dysregulation, peach), defects of innate and intrinsic immunity (innate immunity, purple), predominantly antibody deficiencies (antibody, orange), immunodeficiencies affecting cellular and humoral immunity (T & B cell, teal). Variants observed a single time are labeled at the margin for legibility. Subsequently, the wedge size allotted to an individual variant, gene, disease and immune disorder classification is proportional to the relative frequency of its observation in the dataset

Fig. 2. Number of variants per subject in the severe pediatric sepsis cohort

Shows the number of inborn errors of immunity variants identified per subject, which ranged from zero to seven

Fig. 3 Immunologic and Infectious Characteristics for individuals with and without inborn errors of immunity

a. Shows the odds ratios and 95%CI for positive microbiologic testing in children with inborn errors of immunity compared to those without genetic variants. Microbiologic data is taken from any day on study. Blood culture represents positive bacterial cultures only, while blood pathogen includes blood culture and blood PCR pathogen identification. Respiratory cultures include specimens taken from tracheal aspirate, pleural fluid culture, bronchial brush and bronchoalveolar lavage. Children with variants were noted to have significantly increased odds of positive conventional blood culture, combined PCR and culture positive blood pathogen testing and urine culture b. Shows the odds ratios and 95%CI for laboratory characteristics in children with inborn errors of immunity compared to children without. Comparison is of the most extreme values from any day on study. Children with potential inborn error of immunity variants were noted to have significantly increased odds of lymphopenia and hyperferritinemia
*Fisher's exact test p -value < 0.05

Fig. 4 Genomic landscape of inborn errors of immunity in African American children with severe pediatric sepsis

This figure displays every pathogenic or likely pathogenic variant identified in African American children with severe sepsis. From inner-most ring to outer-most ring it indicates the variants' International Union of Immunologic Societies Classification, the human disease where it has been previously identified, the gene locus affected and finally the specific allele change. Representatives of all IUIS disease classes from most to least common: complement deficiencies (complement, blue), autoinflammatory disorders (autoinflammatory, yellow), congenital defects of phagocyte number and function (phagocyte defects, green), combined immunodeficiency with associated or syndromic features (syndromic features, gray), diseases of immune dysregulation (immune dysregulation, peach), defects of innate and intrinsic immunity (innate immunity, purple) and predominantly antibody deficiencies (antibody, orange). The wedge size allotted to an individual variant, gene, disease and immune disorder classification is proportional to the relative frequency of its observation in the dataset

Supplemental Figure/Table Legends**Table S1 Candidate Gene List**

Table provides the genes included in this study, the IUIS Classification, its associated immunologic defect, inheritance pattern taken from On Mendelian Inheritance in Man (OMIM), as well as OMIM phenotype number

Table S2 Variant Counts and MAF comparisons

Counts of identified variants in children within the cohort, gnomAD and their comparisons. Table shows the total numbers of individual variants identified in the cohort, as well as their counts and frequencies in gnomAD, African American children with sepsis in the cohort, and participants of African descent in the gnomAD database

Table S3 Identified inborn errors of immunity by subject

Table shows the subject, gene, variant, disease phenotype where the variant has been previously seen, HGMD classification, inheritance pattern, protein sequence consequence, zygosity, rsID, gnomAD minor allele frequency, PolyPhen2, Sift Score and PubMed ID of previous publications. Subjects are broken down into groups based on the numbers of variants identified per subject

Table S4 Variants According to International Union of Immunologic Societies Primary Immunodeficiency Diseases Classification Variants are grouped according to International Union of Immunologic Societies (IUIS) classification and gene and listed from most common to least common. Inheritance pattern is taken from On Mendelian Inheritance in Man (OMIM). These genes are classified according to functional grouping according to the International Union of Immunologic Societies and include Complement Disorders, Autoinflammatory Disorders, Cellular and Humoral immune deficiencies, Combined Immunodeficiencies with Syndromic Features, Antibody deficiencies, Diseases of Immune Dysregulation, Defects of Phagocyte Number and Function and Defects of Innate and Intrinsic Immunity. Candidate variant filter was limited to nonsense and missense variants listed in Human Genetic Mutation Database Classification as disease mutation (DM) or likely disease mutation (DM?) and having been previously reported in cases of the related immunologic disorder.

AD: autosomal dominant; AR: autosomal recessive; DM: disease mutation; DM? likely disease mutation; FS: Frameshift; IUIS: International Union of Immunologic Societies; MAF: minor allele frequency; HGMD: Human Genetic Mutation Database

Figure 1

Figure 1

Genomic landscape of inborn errors of immunity in severe pediatric sepsis. This figure displays every pathogenic or likely pathogenic variant identified in the cohort and from inner-most ring to outer-most ring indicates its International Union of Immunologic Societies Classification, the disease where it has been

previously identified, the gene locus affected and finally the specific allele change. 117 unique variants were identified in 191 individuals. Representatives of all IUIS disease classes were identified in the cohort including from most to least common: complement deficiencies (complement, blue), autoinflammatory disorders (autoinflammatory, yellow), combined immunodeficiency with associated or syndromic features (syndromic features, gray), congenital defects of phagocyte number and function (phagocyte defects, green), diseases of immune dysregulation (immune dysregulation, peach), defects of innate and intrinsic immunity (innate immunity, purple), predominantly antibody deficiencies (antibody, orange), immunodeficiencies affecting cellular and humoral immunity (T & B cell, teal). Variants observed a single time are labeled at the margin for legibility. Subsequently, the wedge size allotted to an individual variant, gene, disease and immune disorder classification is proportional to the relative frequency of its observation in the dataset

Figure 2

Number of variants per subject in the severe pediatric sepsis cohort Shows the number of inborn errors of immunity variants identified per subject, which ranged from zero to seven

Figure 3

Immunologic and Infectious Characteristics for individuals with and without inborn errors of immunity a. Shows the odds ratios and 95%CI for positive microbiologic testing in children with inborn errors of immunity compared to those without genetic variants. Microbiologic data is taken from any day on study. Blood culture represents positive bacterial cultures only, while blood pathogen includes blood culture and blood PCR pathogen identification. Respiratory cultures include specimens taken from tracheal aspirate, pleural fluid culture, bronchial brush and bronchoalveolar lavage. Children with variants were noted to have significantly increased odds of positive conventional blood culture, combined PCR and culture positive blood pathogen testing and urine culture b. Shows the odds ratios and 95%CI for laboratory characteristics in children with inborn errors of immunity compared to children without. Comparison is of the most extreme values from any day on study. Children with potential inborn error of immunity variants

were noted to have significantly increased odds of lymphopenia and hyperferritinemia *Fisher’s exact test p-value < 0.05

Figure 4

Genomic landscape of inborn errors of immunity in African American children with severe pediatric sepsis. This figure displays every pathogenic or likely pathogenic variant identified in African American children with severe sepsis. From inner-most ring to outer-most ring it indicates the variants’ International Union of

Immunologic Societies Classification, the human disease where it has been previously identified, the gene locus affected and finally the specific allele change. Representatives of all IUIS disease classes from most to least common: complement deficiencies (complement, blue), autoinflammatory disorders (autoinflammatory, yellow), congenital defects of phagocyte number and function (phagocyte defects, green), combined immunodeficiency with associated or syndromic features (syndromic features, gray), diseases of immune dysregulation (immune dysregulation, peach), defects of innate and intrinsic immunity (innate immunity, purple) and predominantly antibody deficiencies (antibody, orange. The wedge size allotted to an individual variant, gene, disease and immune disorder classification is proportional to the relative frequency of its observation in the dataset

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table2MAFcomparisonsummary.docx](#)
- [SupplementalTable1.GeneList.xlsx](#)
- [SupplementalTable2.FrequencyCohortandgnomAD.xls](#)
- [SupplementalTable3.Variantsbysubjectnumbervar.xlsx](#)
- [SupplementalTable4.VariantsandMAFsbyIUISclass.xlsx](#)
- [Table1ClinicalCharacteristics.docx](#)