Whole-exome sequencing for the identification of rare variants in primary immunodeficiency genes in children with sepsis - a prospective population-based cohort study

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Summary

In this population-based cohort, whole-exome sequencing identified known and unknown rare predicted pathogenic variants in primary immunodeficiency genes in one of five children with proven sepsis. Future studies need to validate whether these variants contribute to sepsis susceptibility.
Abstract

**Background:** The role of primary immunodeficiencies (PID) in susceptibility to sepsis remains unknown. It is unclear whether children with sepsis benefit from genetic investigations. We hypothesized that sepsis may represent the first manifestation of underlying PID. We applied whole-exome sequencing (WES) to a national cohort of children with sepsis to identify rare, predicted pathogenic variants in PID genes.

**Methods:** Multicenter population-based prospective study including previously healthy children ≥28 days and <17 years admitted with blood culture-proven sepsis. Using a stringent variant filtering procedure, analysis of WES data was restricted to rare, predicted pathogenic variants in 240 PID genes for which increased susceptibility to bacterial infection has been reported.

**Results:** 176 children presenting with 185 sepsis episodes underwent WES (median age 52 months, IQR 15.4-126.4). 41 unique predicted pathogenic PID variants (1 homozygous, 5 hemizygous, and 35 heterozygous) were found in 35/176 (20%) patients, including 3/176 (2%) patients carrying variants which were previously reported to lead to PID. The variants occurred in PID genes across all 8 PID categories as defined by the International Union of Immunological Societies. We did not observe a significant correlation between clinical or laboratory characteristics of patients and the presence or absence of PID variants.

**Conclusions:** Applying WES to a population-based cohort of previously healthy children with bacterial sepsis detected Variants of Uncertain Significance in PID genes in one out of five children. Future studies need to investigate the functional relevance of these variants to determine whether variants in PID genes contribute to pediatric sepsis susceptibility.

**Keywords**  child; exome sequencing; genomics; immunodeficiency; sepsis; variant; variants of uncertain significance
Introduction

The incidence of sepsis is highest at the extremes of age, in the very young and the very old [1-3]. In high income countries, 35 to 50% of pediatric sepsis deaths occur in previously healthy infants and children despite access to vaccination, health care, and effective antibiotics [2, 4]. Sepsis-related morbidity in the absence of risk factors remains high and is associated with significant sequelae such as amputations and neurodevelopmental disability [5]. The mechanisms underlying susceptibility to severe infectious diseases in otherwise healthy patients remain largely unknown, but epidemiological studies suggest a strong genetic contribution [6]. It has been proposed that severe infections in childhood are more likely to be the manifestation of rare, single-gene disorders, compared with infections occurring in adults [7, 8]. Case reports anecdotally show that life-threatening infections of childhood may represent the first manifestation of primary immunodeficiency (PID) [8]. However, little is known about the prevalence of PID gene variants in patients with sepsis, and there is a lack of guidance to advise on genetic or immunologic investigations after a first sepsis episode [9, 10].

Whole-exome sequencing (WES) has emerged as a powerful tool to identify disease-causing genetic variants [11, 12]. We recently reported successful application of WES in a highly selected cohort of pediatric sepsis cases [9]. WES has never been used to assess the genetic contribution to sepsis in larger population-based cohorts.

Although WES is insufficient to determine the functional relevance of genetic variants, it enables their identification in PID genes previously reported to be associated with bacterial infection. We hypothesized that rare and predicted pathogenic genetic variations in PID genes which are associated with bacterial infection are more common in previously healthy children who develop blood culture-positive community-acquired sepsis compared with genomic reference cohorts.
Methods

Study design

We conducted a prospective observational multicenter cohort study enrolling children presenting with blood culture-proven bacterial sepsis in the ten major pediatric hospitals in Switzerland between September 1, 2011, and December 31, 2015. The study protocol and details of the Swiss Pediatric Sepsis Study have been reported elsewhere [13, 14]. Written informed consent for genetic analyses was obtained from parents or legal guardians. The ethical review board approved the study (Cantonal Ethics Committee, University of Bern, KEK-029/11).

Inclusion criteria: Previously healthy children aged >28 days and <17 years with community-acquired, blood culture-proven bacterial sepsis due to *S. pneumoniae*, *S. aureus*, Group A and B streptococcus, *N. meningitidis*, or *H. influenzae*, as defined by 2005 pediatric sepsis consensus definitions [15] were eligible. These pathogens were selected because they represent the most common pathogens causing community-acquired sepsis in children without comorbidities.

Exclusion criteria: Prematurely born neonates, infants <28 days (or <44 weeks of gestational age) and children with comorbidities, including any chronic inborn or acquired medical conditions, known primary or secondary immunodeficiency, recent trauma, surgery or burns, and children with hospital-acquired infection (>48h after admission), or with pathogens other than those listed under inclusion criteria were not considered for WES.

WES, read mapping, variant calling and variant annotation

Blood samples were collected and processed as reported elsewhere [9]. WES was performed on Illumina HiSeq 2500 at the Lausanne Genomic Technology Facility (Supplementary Methods and supplementary tables 1, 2 and 3). Sequencing reads were mapped to the human reference genome hg19 using BWA-MEM v0.7.15 [16, 17]. The Genome Analysis ToolKit v3.8 Sequence Data Processing Tools and Variant Discovery Tools were used according its best practices [18, 19]. These included base quality score recalibration and per-
sample, simultaneous calling of single nucleotide variants and small insertions and deletions (indels) with the HaplotypeCaller tool, followed by joint genotyping and quality filtering (only variants with genotype quality >100 and read depth >20 were kept for downstream analysis). Genetic and functional annotation included putative effect prediction for Sequence Ontology terms, putative loss-of-function prediction, and effect predictions from multiple algorithms. Each variant was annotated with information on minor allele frequency (MAF) in several public databases, including the 1000 genomes project phase 1 (n=1092), the NHLBI exome sequencing project (n=7520), the Genome Aggregation Database (gnomAD, n=138632), and 519 in-house control exomes.

Filtering and identification of candidate genomic variants in PID genes

Analyses were restricted to non-synonymous exonic variants that are rare in the general population (MAF <0.01 for homozygous/hemizygous and <0.0001 for heterozygous variants in gnomAD database). We also filtered out variants with a MAF >0.01 in our in-house exome database. We restricted the analysis to variants in 240 PID genes previously reported to be associated with bacterial disease as defined by the International Union of Immunological Societies (IUIS) [20]. We further prioritized variants by filtering for predicted pathogenicity, keeping only variants with a putative deleterious impact according to Combined Annotation Dependent Depletion (CADD) v1.3 score [21], resulting in putatively pathogenic variants. For every putatively pathogenic variant, PubMed entries between 1.1.1970 and 30.6.2019 were searched for reports on patients with PID and bacterial infections carrying the exact variant. The study did not include functional validation. Therefore, the filtered rare and predicted pathogenic variants in the selected PID genes were labelled “variants of uncertain significance” (VUS).
Statistical analysis

Descriptive statistics are presented as median and interquartile ranges (IQR) for continuous variables and frequencies and percentages for categorical variables. We used the $\chi^2$ test of proportions and the Wilcoxon non-parametric rank sum test to compare subgroups. We considered a 2-sided p-value of <0.05 as significant. Analyses and graphs were done using R (R Core Team (2019). R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/).
Results

Cohort description

During the study period, out of 1,275 blood culture-proven sepsis episodes, 383 occurred in 380 previously healthy children (Figure 1). *S. pneumoniae*, *S. aureus*, Group A and B streptococcus, *N. meningitidis*, and *H. influenzae* accounted for 271/383 (70%) of sepsis episodes. 94 patients were excluded, predominantly because of lack of consent or lack of access to DNA. One case of *H. influenzae* B sepsis in an unvaccinated child was excluded. The final WES cohort consisted of 176 children including 8 (5%) with recurrent bacterial sepsis (Table 1). 38% of patients developed organ dysfunction and 37% required PICU admission for sepsis. 5/176 (2.8%) children died.

Identification of putatively causative PID variants

WES was successfully performed in all 176 study participants. In total, 8,463,144 variants were identified. After filtering based on predicted function impact, predicted pathogenicity and MAF, 35,966 variants were retained (supplementary figure 1). We then restricted analyses to rare variants in 240 PID genes known to be associated with bacterial infection: 41 unique rare and predicted pathogenic VUS were identified in 35 out of 176 participants (20%) across 24 PID genes (Table 2 and supplementary figure 2). Biallelic (homozygous) variants were found in 1 participant in 1 gene, X-linked variants in 5 participants in 5 genes, and monoallelic variants were found in 35 study participants in 18 genes. In 30/35 patients (86%), only one variant was found whereas in 5/35 (14%) patients two or three variants were discovered. Out of the 41 variants, 3 exact variants in 3/176 participants (2%) were identical to previously reported variants from patients with a PID phenotype with bacterial infection (Table 3).

Characterization and distribution of PID gene variants in children with sepsis

The identified variants were distributed across all PID groups (Table 2, supplementary figures 2 and 3 and supplementary table 4)[20]. They were most commonly found in *CHD7* (n=6 patients), and *SAMD9*, *TCF3* and *BACH3* (n=3 each). The 3 previously reported PID variants related to
combined immunodeficiencies with associated symptoms, phagocyte defects, and complement defects (Table 3).

We did not observe any association of variants with specific pathogens or focus of infection (Table 1, p>0.10). They were found in 13 out of 54 patients with S. pneumoniae infection (24%), in 7 out of 44 patients with S. aureus infection (16%), in 6 out of 33 patients with Group A streptococcal infection (18%), in 4 out of 17 patients with Group B streptococcal infection (24%), in 2 out of 16 patients with N. meningitidis infection (13%), and in 3 out of 12 patients with H. influenzae infection (25%) (Table 2, supplementary figures 2 and 3). There was no significant difference in the proportion of children found to carry PID variants between children <2 years (12/56, 21%) and children >2 years of age (23/120, 19%, p = 0.8). Figure 2 and supplementary figure 4). No significant differences were observed when comparing the proportion of children with and without different PID variants in relation to presence of organ dysfunction, PICU admission, frequency of leukopenia or the maximum C-reactive protein level measured during sepsis (Table 1). Variants were found in 4/8 (50%) patients with recurrent sepsis versus 31/168 (18%) in children without recurrent sepsis (p = 0.051 Fisher test).
Discussion

In this prospective, population-based cohort of previously healthy children presenting with blood culture-proven bacterial sepsis, rare variants in genes previously reported to lead to PID associated with bacterial infection were substantially more common than previously assumed. It is important to note that the variants identified in our study are predicted but not functionally confirmed to affect gene function and lead to immunodeficiency. Therefore, they must be considered variants of uncertain significance (VUS). Importantly, around one out of fifty (3/176; 2%) of children with community-acquired sepsis that underwent WES carried exact variants that have been previously shown to result in PID associated with bacterial infections, which is higher than the estimated prevalence of PID in the population [22]. The variants identified in PID genes were distributed across all main IUIS categories [20]. We did not observe specific patterns in terms of pathogens, severity, inflammatory markers, or age at presentation that associated with the likelihood to detect such variants. These VUS in genes previously linked to susceptibility to bacterial infections within the context of clearly defined PID were found in otherwise apparently healthy children with bacterial sepsis. This finding may be explained either by lack of functional relevance or by pleiotropy. WES represents a promising first-step to investigate children with sepsis for potentially underlying PID. Future studies should combine WES with in-depth functional assays.

Understanding why a minority of children become very ill in the course of an infection remains one of the mysteries of sepsis and may harbor clues for future targeted interventions [23]. Casanova et al previously stated the hypothesis that life-threatening infections of childhood may represent the first manifestation of PID [8]. Identification of PID in children with sepsis due to community-acquired bacteria has been reported in highly selected cases caused by *Pseudomonas aeruginosa* [9, 24, 25] or *S. pneumoniae*. A French study investigated 163 children with pneumococcal disease using conventional immunologic screening reported underlying PID in 10% of cases [26], with a higher proportion of PID found in children >2 years of age. Monogenic diseases resulting in PID have shown to be associated with selected invasive bacterial infections, including Group B streptococcal and
staphylococcal infections [27]. Several non-conventional PID, such as IRAK-4, MyD88 and TIRAP deficiency [28-30], are characterized by incomplete penetrance and variable pathogen specificity. The effect of some PID on increased susceptibility to infection may decrease with age if patients survive infections during childhood [28]. In our study, we did not find any association between presence of PID VUS and increased disease severity, measured by need for PICU admission, number of organ dysfunctions, or septic shock [31]. Blood culture-proven sepsis represents an extreme phenotype from an evolutionary perspective given the high fatality rate of bacteremia in the absence of antimicrobial therapy. In contrast, extreme phenotypes as defined by contemporary medicine with the availability of intensive care support are subject to different host genetic, pathogen, and environmental factors. Importantly, time to antibiotics represents a major determinant of severity [4, 32], and the specific setting in Switzerland has to be considered, including a low proportion of socioeconomically disadvantaged children, and a high-density pediatric hospital network, potentially resulting in earlier treatment.

Children requiring life support due to severe infections in PICUs are currently not routinely investigated for the presence of PIDs. No specific guidelines exist for clinicians when facing the question if a child with sepsis may harbor a PID [10]. There is a need for studies defining indications for PID investigations in children with sepsis; coupled with increased education and awareness of pediatric intensivists, infectious disease specialists, and pediatricians on the topic. Selection rules based on pathogens, severity, or a history of familial reoccurrence alone could be inaccurate, as demonstrated by the absence of clear patterns in our study. In addition, commonly used immunological investigations, such as the measurement of total and specific immunoglobulin, complement protein concentrations and basic lymphocyte populations will miss a substantial proportion of PID in comparison to WES. Importantly, recognizing and accurately diagnosing underlying PID in children presenting with sepsis is important given the risk of recurrence of infection, with potentially fatal but preventable outcome [9]. The challenges in interpreting the findings of our study highlight that while next generation sequencing (NGS) represents a promising diagnostic tool in children with sepsis, NGS-based genetic testing must be followed by specifically
indicated functional tests if WES is to be considered as a routine diagnostic approach. Validation of the findings will require immunological follow-up of the study cohort, including assessment of subsequent invasive infections. Unfortunately, the study approval was restricted to singleton testing. Future studies embarking on unravelling the genetic contribution to childhood sepsis need to incorporate functional analyses both during disease and (if the patient survives) during recovery, including extended investigations of parents and siblings as indicated. In view of our findings of a high number of VUS across all IUlS categories, the availability of immune-phenotyping platforms to assess a broad range of immunological pathways carries strong promise [33].

NGS has been successfully applied both to discover novel disease-causing gene variants and to obtain a molecular diagnosis of monogenic conditions [34, 35], and may be cost effective in comparison to standard genetic diagnostic procedures [35]. Yet, as a result of the increasing number of patients undergoing WES [11, 36], the field of clinical immunology is rapidly evolving, and expanding cohorts demonstrate considerable variability of the phenotypes. The mechanisms underlying incomplete penetrance and variable expressivity remain mostly unexplained and are likely complex, as demonstrated by the recent discovery of the role of anti-LTA antibody deficiency in patients with TIRAP deficiency [27]. We applied stringent filtering criteria including several control cohorts, and restricted analyses to rare variants predicted to have functional impact in genes known to lead to PID phenotypes associated with bacterial infection. Despite this approach, confirming that rare VUS identified by WES are pathogenic remains a challenge [12, 37]. Rare variants in genes previously demonstrated to result in Mendelian disease may also contribute to the risk of infection through complex and polygenic mechanisms [38]. A recent landmark study using electronic health record based phenotype risk scores in large genotyped cohorts identified a substantial number of undiagnosed patients harboring rare variants and phenotypes related to unrecognized Mendelian diseases [39]. Combination of international cohorts will be required to provide the power for statistical validation of enriched variants or pathways, or to test the hypothesis that complex/polygenic predisposition could also play a role in human susceptibility to sepsis.
Despite these challenges, our findings are based on a high-quality prospective population-based study restricted to the gold-standard of invasive infection (bacteraemia). The design of this national cohort minimized inclusion bias in comparison to the majority of immunologic studies, commonly based on highly selected referral patients with severe or recurrent disease. We carefully excluded children with underlying comorbidities, in order to focus on community-acquired sepsis in children that were apparently healthy before hospital admission. We acknowledge that this may have led to the exclusion of children harboring PID associated with congenital disease or syndromes. We restricted WES to the most common pathogens causing community-acquired sepsis [14], and acknowledge that PIDs potentially may be more common in children presenting with unusual bacterial pathogens.

Our study has several additional limitations. Most importantly, we did not functionally verify the pathogenicity of the variants identified in PID genes. While we used stringent filtering and pathogenicity prediction criteria aligned with best practice, and assessed whether variants had been previously demonstrated to lead to PID with bacterial infection, their pathogenicity has not been demonstrated, thus resulting in a possible overestimation of the proportion of PID-related sepsis. Functional validation in our and other independent cohorts will be required to define the true proportion of PID causing sepsis in children. Second, we only focused on PID genes previously demonstrated to be associated with bacterial infection, which may have led to exclusion of causative variants, thereby resulting in an underestimation of the proportion of PID-related sepsis. In addition, the genetic background for several PID, mainly disorders affecting antibody production, is unknown, which may again result in an underestimation of PID. Considering the rapid expansion of recently identified novel PID, it is thus also likely that a subset of patients may harbor disease-conferring yet unidentified novel PID. Experimental validation to define the causal relationship between genotype and phenotype is crucial during the investigation of novel PID candidates. Third, an intrinsic limitation in WES is the inability to detect deep intronic and intergenic genetic variants. Finally, we did not assess microbiological factors determining pathogen virulence, such as toxins.

In conclusion, applying WES to a population-based cohort of previously healthy children presenting with bacterial sepsis allowed the detection of VUS in previously reported PID genes in a substantial
proportion of sepsis cases. Our study explores the possible contribution of host genetic variation to sepsis susceptibility. Future studies combining NGS with functional testing are required to validate whether unrecognized PIDs contribute significantly to sepsis at the populational level. Our findings indicate an urgent need for evidence-based recommendations on the optimal selection of children with sepsis for genetic and functional investigations to identify PIDs.
**Author Contributions** LJS designed and supervised the study, oversaw analyses, and wrote the first draft, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. AB, SA, JF contributed to study design, performed analyses of bioinformatics data, revised the manuscript and approved the final version. JT performed analyses, contributed to the first draft, and approved the final version. CB, CA, PKA, KP, EG, MS, UH, SB, CR, TR, AN, and CK were involved in study design, patient recruitment, data analysis, and manuscript preparation, and approved the final version. VSS and EB performed analyses of bioinformatics data in meningococcal patients. VSS, EB, FMT, VW, JH, LC, ML contributed to study design, manuscript revision, and approved the final version.

For the full list of investigators in the EUCLIDS consortium and the Swiss Pediatric Sepsis Study see Supplement Material.

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**Conflict of interest** U.H. reports personal fees from Sanofi Pasteur USA and Sanofi France for the Global Pertussis Initiative and Collaboration of European Experts on Pertussis Awareness Generation (CEEPAG), outside the submitted work. All authors have declared any conflict of interest.
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Figure legends

Figure 1. Enrolment flow diagram of whole-exome sequencing (WES) cohort. Patients undergoing WES were previously healthy children with blood-culture proven sepsis caused by *S. pneumoniae*, *S. aureus*, Group A streptococcus, Group B streptococcus, *N. meningitidis* and *H. influenzae*. CVC, central venous line; GBS, Group B streptococcus; PCR, polymerase chain reaction; SPSS, Swiss Pediatric Sepsis Study.

Figure 2. Number of previously healthy children with blood-culture proven sepsis in relation to age and severity at presentation. Children testing positive for a rare Variant of Uncertain Significance (VUS) associated with primary immunodeficiency are shown in blue, in comparison to children where no VUS associated with primary immunodeficiency was found, shown in grey. HI, *Haemophilus influenzae*; NM, *Neisseria meningitidis*; GBS, Group B streptococcus; GAS, group A streptococcus; SA, *Staphylococcus aureus*; SP, *Streptococcus pneumoniae*. 
Table 1 - Demographic and clinical characteristics of children with blood culture-proven bacterial sepsis in relation to whole exome sequencing findings of putatively pathogenic variants in known primary immunodeficiency (PID) genes. Characteristics of children without a primary immunodeficiency (PID) variant (n=141) are compared to children in whom a PID Variant of Uncertain Significance was found (n=35, including novel and previously reported PID variants), and children in whom a previously reported PID variant was found (n=3).

|                          | All children (n=176, 100%) | Children without PID variants (n=141, 80%) | Children with PID Variants of Uncertain Significance (n=35, 20%) | Children with previously reported PID variant (n=3, 2%) |
|--------------------------|-----------------------------|---------------------------------------------|-----------------------------------------------------------------|------------------------------------------------------|
| **Demographics**         |                             |                                             |                                                                 |                                                      |
| Age at sepsis onset (months) | 51.9 (15.3 - 126.5)         | 54.5 (15.4 - 127.2)                        | 40.2 (9.1 - 111.7)                                               | 128.3 (27.5 - 170.4)                                  |
| Age group                |                             |                                             |                                                                 |                                                      |
| 28-365 days              | 41 (23%)                    | 32 (23%)                                   | 9 (26%)                                                        | 1 (33%)                                              |
| 1-4 years                | 53 (30%)                    | 42 (30%)                                   | 11 (31%)                                                       |                                                      |
| 5-9 years                | 35 (20%)                    | 28 (20%)                                   | 7 (20%)                                                        |                                                      |
| 10-16 years              | 47 (27%)                    | 39 (28%)                                   | 8 (23%)                                                        | 2 (67%)                                              |
| Male                     | 112 (64%)                   | 87 (62%)                                   | 25 (71%)                                                       | 2 (67%)                                              |
| Ethnicity *              |                             |                                             |                                                                 |                                                      |
| Caucasian                | 159 (90%)                   | 127 (90%)                                  | 32 (91%)                                                       | 3 (100%)                                             |
| Asian                    | 4 (2%)                      | 3 (2%)                                     | 1 (3%)                                                         |                                                      |
| African                  | 4 (2%)                      | 4 (3%)                                     |                                                               |                                                      |
| other                    | 3 (2%)                      | 3 (2%)                                     |                                                               |                                                      |
| **Site of infection**    |                             |                                             |                                                                 |                                                      |
| Primary bloodstream infection | 22 (12%)                    | 19 (13%)                                   | 3 (9%)                                                         |                                                      |
| Pneumonia                | 47 (27%)                    | 39 (28%)                                   | 8 (23%)                                                        | 1 (33%)                                              |
| Bone and joint infection | 37 (21%)                    | 30 (21%)                                   | 7 (20%)                                                        | 1 (33%)                                              |
| Central nervous system infection | 33 (19%)                   | 23 (16%)                                   | 10 (29%)                                                       | 1 (33%)                                              |
| Skin and soft tissue infection | 15 (9%)                    | 12 (9%)                                    | 3 (9%)                                                         |                                                      |
| Ear, Nose, and Throat infection | 9 (5%)                     | 6 (4%)                                     | 3 (9%)                                                         |                                                      |
| Toxic shock Syndrome     | 4 (2%)                      | 4 (3%)                                     |                                                               |                                                      |
| Gastrointestinal system infection | 2 (1%)                     | 2 (1%)                                     |                                                               |                                                      |
|                                | All children (n=176, 100%) | Children without PID variants (n=141, 80%) | Children with PID Variants of Uncertain Significance (n=35, 20%) | Children with previously reported PID variant (n=3, 2%) |
|--------------------------------|-----------------------------|-------------------------------------------|---------------------------------------------------------------|-----------------------------------------------------|
| Urinary tract infection        | 1 (1%)                      | 1(1%)                                     |                                                               |                                                     |
| Other specific infection type  | 6 (3%)                      | 5(4%)                                     | 1(3%)                                                         |                                                     |
| **Pathogens**                  |                             |                                           |                                                               |                                                     |
| Gram positive bacteria         |                             |                                           |                                                               |                                                     |
| S. pneumoniae                  | 54 (31%)                    | 41(29%)                                   | 13(37%)                                                      | 2(67%)                                              |
| S. aureus                      | 44 (25%)                    | 37(26%)                                   | 7(20%)                                                       | 1(33%)                                              |
| Group A streptococci           | 33 (19%)                    | 27(19%)                                   | 6(17%)                                                       |                                                     |
| Group B streptococci           | 17 (10%)                    | 13(9%)                                    | 4(11%)                                                       |                                                     |
| Gram negative bacteria         | 28 (16%)                    | 23(16%)                                   | 5(14%)                                                       |                                                     |
| N. meningitidis                | 16 (9%)                     | 14(10%)                                   | 2(6%)                                                        |                                                     |
| H. influenzae                  | 12 (7%)                     | 9(6%)                                     | 3(9%)                                                        |                                                     |
| **Severity of sepsis**         |                             |                                           |                                                               |                                                     |
| Organ dysfunction              |                             |                                           |                                                               |                                                     |
| No organ dysfunction present   | 108 (61%)                   | 89(63%)                                   | 19(54%)                                                      | 1(33%)                                              |
| ≥ 1 organ dysfunction          | 68 (39%)                    | 52(37%)                                   | 16(46%)                                                      | 2(67%)                                              |
| C-reactive protein (mg/L) \(^a\) | 192 (94 - 260)              | 186 (95 - 266)                            | 198 (88 - 237)                                               | 192 (165 - 200)                                    |
| Leukopenia present \(^c\)      | 30 (17%)                    | 21(15%)                                   | 9(26%)                                                       | 1(33%)                                              |
| PICU/ NICU admission           | 66 (38%)                    | 53(38%)                                   | 13(37%)                                                      | 2(67%)                                              |
| Length of PICU stay (days)     | 5 (2 - 9)                   | 4 (2 - 9)                                 | 7 (2 - 12)                                                   | 19 (2 - 36)                                        |
| Length of hospital stay after sepsis onset (days) | 10 (7 - 15)             | 10 (7 - 15)                              | 11 (7 - 15)                                                  | 10 (6 - 55)                                        |
| Invasive ventilation           | 36 (20%)                    | 29(21%)                                   | 7(20%)                                                       | 1(33%)                                              |
All children (n=176, 100%) | Children without PID variants (n=141, 80%) | Children with PID Variants of Uncertain Significance (n=35, 20%) | Children with previously reported PID variant (n=3, 2%)
---|---|---|---
Inotrope requirement | 39 (22%) | 31(22%) | 8(23%) | 2(67%)
Case fatality | 6 (3%) | 5(4%) | 1(3%) | 

aData not available in 6 episodes, bData not available in 9 episodes, cData not available in 3 episodes. PICU=pediatric intensive care unit. NICU=neonatal intensive care unit. Categorical variables are given as frequencies and percentages, continuous variables as median and interquartile range. Column percentages are given. Percentages are based upon available data for each variable.
Table 2. Clinical characteristics of children with blood culture-proven bacterial sepsis where a Variant of Unknown Significance in a primary immunodeficiency (PID) gene was found (n=35) are shown for each PID according to the IUIS classification.

| Gene   | Inheritance | Unique variants | Unique patients | Age in mo [mean, range] | Sex | Clinical focus | Organ dysfunction | Primary pathogen cultured |
|--------|-------------|-----------------|-----------------|-------------------------|-----|----------------|-------------------|--------------------------|
|        |             |                 |                 |                         |     | Pneumonia      | Bone                | M F CN EN S T I M S S A P |
|        |             |                 |                 |                         |     | Cellulitis     | Other               | No ≥1 H N GB GA S S A P  |
|        |             |                 |                 |                         |     |                |                   |                          |
| CID/CID+ |             |                 |                 |                         |     |                |                   |                          |
| BCL11B | X 2 A 2 A 2 | 2               | 84 (75,93)      | 2                       | 1   | 1              | 1                 | 1 1 1 1 1 1 1 1 1 1 1 1 |
| CHD7   | X 6 A 6 A 6 | 6               | 61 (2,148)      | 5 1                     | 2   | 1 1 2          | 2                 | 2 4 1 1 1 2 1 1 1 1 1 1 |
| KDM6A  | X 3 A 1 A 1 | 1               | 31 (31,31)      | 1                       | 1   | 1              | 1                 | 1 1 1 1 1 1 1 1 1 1 1 1 |
| KMT2D  | X 2 A 2 A 100 | 2              | (34,167)        | 1                       | 1   | 1              | 1                 | 1 1 1 1 1 1 1 1 1 1 1 1 |
| SAMD9  | X 3 A 3 A 16 | 3               | 16 (1,40)       | 2 1                     | 1   | 2              | 1                 | 1 1 2 1 1 1 1 1 1 1 1 1 |
| SEMA3E | X 1 A 1 A 14 | 1               | 14 (14,14)      | 1                       | 1   | 1              | 1                 | 1 1 1 1 1 1 1 1 1 1 1 1 |
| STAT3  | X 1 A 1 A 34 | 1               | 34 (3,43)       | 1                       | 1   | 1              | 1                 | 1 1 1 1 1 1 1 1 1 1 1 1 |
| TBX1   | X 1 A 1 A 6  | 1               | 6 (6,6)         | 1                       | 1   | 1              | 1                 | 1 1 1 1 1 1 1 1 1 1 1 1 |
| WAS    | X 1 A 1 A 7  | 1               | 7 (7,7)         | 1                       | 1   | 1              | 1                 | 1 1 1 1 1 1 1 1 1 1 1 1 |
| PAD/PIR D |             |                 |                 |                         |     |                |                   |                          |
| IRF2BP2| X 1 A 1 A 148 | 1              | 148 (148,148)   | 1                       | 1   | 1              | 1                 | 1 1 1 1 1 1 1 1 1 1 1 1 |
| NFKB2  | X 2 A 2 A 88 | 2               | 88 (43,132)     | 2                       | 2   | 2              | 2                 | 2 1 1 1 1 1 1 1 1 1 1 1 |
| PTEN   | X 1 A 1 A 132| 1               | 132 (132,132)   | 1                       | 1   | 1              | 1                 | 1 1 1 1 1 1 1 1 1 1 1 1 |
| TCF3   | X 3 A 3 A 63 | 3               | 63 (2,100)      | 3                       | 1   | 1              | 1                 | 1 3 1 1 1 1 1 1 1 1 1 1 |
| TNFRSF13B | X 1 A 1 A 64 | 1               | 64 (64,64)      | 1                       | 1   | 1              | 1                 | 1 1 1 1 1 1 1 1 1 1 1 1 |
| BACH2  | X 3 A 3 A 109 | 3              | 109 (23,176)    | 3                       | 1   | 1              | 1                 | 1 1 1 1 1 1 1 1 1 1 1 1 |
| NFAT5  | X 1 A 1 A 5  | 1               | 5 (5,5)         | 1                       | 1   | 1              | 1                 | 1 1 1 1 1 1 1 1 1 1 1 1 |
| PID/IID|             |                 |                 |                         |     |                |                   |                          |
| CYBB   | X 1 A 1 A 128| 1               | 128 (128,128)   | 1                       | 1   | 1              | 1                 | 1 1 1 1 1 1 1 1 1 1 1 1 |
| CXCR4  | X 2 A 2 A 41 | 2               | 41 (34,48)      | 2                       | 1   | 1              | 1                 | 1 2 2 2 2 2 2 2 2 2 2 2 |
| IL17F  | X 1 A 1 A 112| 1               | 112 (112,112)   | 1                       | 1   | 1              | 1                 | 1 1 1 1 1 1 1 1 1 1 1 1 |
| Gene     | Associated Symptoms | M | F | CNS | ENT | LRTI | HI | NM | GBS | GAS | SA | SP |
|----------|---------------------|---|---|-----|-----|------|----|-----|-----|-----|----|----|
| IRAK1    |                     | 1 | 1 | 4   | 1   | 1    |    |     |     |     |    |    |
| AID/CD   |                     |   |   |     |     |      |    |     |     |     |    |    |
| PLCG2    |                     | 2 | 2 | 1   | 1   | 2    | 1  | 1   | 2   |     |    |    |
| POLA1    |                     | 1 | 1 | 1   | 1   | 1    | 1  | 1   | 1   |     |    |    |
| C4A      |                     | 1 | 1 | 1   | 1   | 1    | 1  | 1   | 1   |     |    |    |
| CFH      |                     | 2 | 2 | 1   | 1   | 1    | 2  | 1   | 1   |     |    |    |

XL, X-linked; AR, autosomal-recessive; AD, autosomal-dominant; M, male; F, female; CNS, central nervous system (infection); ENT, ear nose throat (infection); LRTI, lower respiratory tract infection; HI, *Haemophilus influenzae*; NM, *Neisseria meningitidis*; GBS, Group B streptococcus; GAS, Group A streptococcus; SA, *Staphylococcus aureus*; SP, *Streptococcus pneumoniae*.

CID, combined immunodeficiencies; CID+, CID with associated symptoms; PAD, predominantly antibody disorders; PIRD, primary immune dysregulatory disorders; PD, phagocyte defects; IID, innate immune defects; AID, autoinflammatory disorders; CD, complement defects.
Table 3. Rare variants previously reported in patients with primary immunodeficiency (PID) and bacterial infections which were found in children with blood culture-proven sepsis. Host and pathogen characteristics of the sepsis episodes (n=3) where a patient was found to carry a previously reported PID variant associated with bacterial infection, and details of the identified variants are shown.

| IUIS category | Sex | Age (days) | Pathogen | Clinical focus | Gene | HGVS.p | Inheritance | Read depth | Frequency in gnomAD | Pubmed ID |
|---------------|-----|------------|----------|----------------|------|--------|-------------|------------|---------------------|-----------|
| CID+          | M   | 223        | SP       | CNS            | WAS  | E131K  | XL          | 25         | 0.002600            | 66        |
|               |     |            |          |                |      | (p.Glu131Lys) |            |            |                     |           |
| PD            | M   | 3905       | SA       | bone/joint     | CYB  | G364R  | XL          | 40         | 0.003946            | 64        |
|               |     |            |          |                |      | (p.Gly364Arg) |            |            |                     |           |
| CD            | F   | 5443       | SP       | pneumonia      | CFH  | P503A  | AD          | 176        | 1.63E-05            | 66        |
|               |     |            |          |                |      | (p.Pro503Ala) |            |            |                     |           |

F, female; M, male; HI, Haemophilus influenzae; NM, Neisseria meningitidis; GBS, Group B streptococcus; GAS, group A streptococcus; SA, Staphylococcus aureus; SP, Streptococcus pneumoniae; AD, autosomal-dominant; AR, autosomal-recessive; XL, X-linked
Figure 2

No organ dysfunction

1 or more organ dysfunction

Variants found

No

Yes

Number of patients

Primary pathogen cultured

Primary pathogen cultured

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