Genetic errors of immunity distinguish pediatric nonmalignant lymphoproliferative disorders

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Background: Pediatric nonmalignant lymphoproliferative disorders (PLPDs) are clinically and genetically heterogeneous. Long-standing immune dysregulation and lymphoproliferation in children may be life-threatening, and a paucity of data exists to guide evaluation and treatment of children with PLPD.
Objective: The primary objective of this study was to ascertain the spectrum of genomic immunologic defects in PLPD. Secondary objectives included characterization of clinical outcomes and associations between genetic diagnoses and those outcomes.
Methods: PLPD was defined by persistent lymphadenopathy, lymph organ involvement, or lymphocytic infiltration for more than 3 months, with or without chronic or significant Epstein-Barr virus (EBV) infection. Fifty-one subjects from 47 different families with PLPD were analyzed using whole exome sequencing.
Results: Whole exome sequencing identified likely genetic errors of immunity in 51% to 62% of families (53% to 65% of affected children). Presence of a genetic etiology was associated with younger age and hemophagocytic lymphohistiocytosis. Ten-year survival for the cohort was 72.4%, and patients with viable genetic diagnoses had a higher survival rate (82%) compared to children without a genetic explanation (48%, $P = .03$). Survival outcomes for individuals with EBV-associated disease and no genetic explanation were particularly worse than outcomes for subjects with EBV-associated disease and a genetic explanation (17% vs 90%; $P = .002$). Ascertainment of a molecular diagnosis provided targetable treatment options for up to 18 individuals and led to active management changes for 12 patients.
Conclusions: PLPD defines children at high risk for mortality, and whole exome sequencing informs clinical risks and therapeutic opportunities for this diagnosis. (J Allergy Clin Immunol 2022;149:758-66.)

Key words: *Lymphoproliferation, pediatric, whole exome sequencing, genomic, Epstein-Barr virus*

Lymphadenopathy is common during normal childhood and noted at physical examination for approximately half of all children

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Abbreviations used
ALPS: Autoimmune lymphoproliferative syndrome
CAEBV: Chronic active EBV
EBV: Epstein-Barr virus
EBV-PLPD: EBV-associated PLPD
HGSC: Human Genome Sequencing Center
HLH: Hemophagocytic lymphohistiocytosis
HSCT: Hematopoietic stem cell transplantation
IUIS: International Union of Immunological Societies
LPD: Lymphoproliferative disorder
PIDD: Primary immunodeficiency disease
PIRD: Primary immune regulatory disorder
PLPD: Pediatric nonmalignant LPD
WES: Whole exome sequencing

visiting a medical provider for either well or sick visits.[1] Although transient lymphadenopathy in children is rarely dangerous, long-standing lymphoproliferation may reflect underlying immune dysregulation, increase the risk for developing malignant disease or hemophagocytic lymphohistiocytosis (HLH), and/or drive life-threatening lymphoproliferative disease.[1-3]

Nonmalignant pediatric lymphoproliferative disorders (PLPD) constitute a clinically and genetically heterogeneous group of conditions associated with a wide range of clinical consequences. PLPD are characterized by proliferating (and/or persistent) clonal or polyclonal lymphoid cells that may arise as aberrant responses to immune stimuli or represent intrinsic immune dysregulation.[4] Clinical presentations include chronic or recurrent lymphadenopathy, splenomegaly, or symptoms resulting from organ infiltration by abnormal lymphoid cells. In some cases, patients may develop pathologic inflammation consistent with HLH or macrophage activation syndrome. PLPD are also associated with an increased predisposition toward developing hematopoietic malignancies, specifically lymphoma.[5-7] When a lymph node biopsy sample is found to be negative for malignancy, the diagnostic and therapeutic paths forward for children with evidence of lymphoproliferation remain poorly defined.

Although several inherited diseases of immune dysregulation have been associated with PLPD, the frequency and distribution of primary immunodeficiency diseases (PIDDs) and primary immune regulatory disorders (PIRDs) in children with PLPD are unknown. PIRDs encompass immune-mediated disease leading to autoimmune disease and autoinflammatory conditions.[8,9] Errors in more than 400 genes are now ascribed to PIDD and PIRD,[2,8] and a significant number of these conditions present with clinical features consistent with PLPD.

PLPD associated with Epstein-Barr virus (EBV) can represent *de novo* infection, reactivation, and/or malignant transformation.[7,10] PIDD patients who have impaired natural killer cell cytotoxic function may have increased susceptibility to primary infection or reactivation of viruses, including EBV.[11] Patients with chronic active EBV (CAEBV), a rare form of EBV disease characterized by persistent and/or proliferative EBV-infected lymphocytes during primary or reactivated EBV infection,[12] have poor outcomes, especially individuals with EBV specifically detected in natural killer and T cells.[12,13]

Optimal management of PLPD patients requires understanding of underlying pathogenic drivers. Given the rare occurrence of PLPD and its overlapping features with ordinary reactive lymphadenopathy in children, diagnosis is often quite challenging. We therefore sought to determine the utility of whole exome sequencing (WES) in children with PLPD with a focus on impact on treatment and prognosis.

METHODS

Subject enrollment

Patients and family members at Texas Children's Hospital or collaborating referral centers who met criteria for PLPD between 1994 to 2018 were offered participation in this study. Studies were performed under research protocols approved by the Baylor College of Medicine institutional review board. All procedures involving human participants were performed in accordance with institutional and international ethical standards.

Clinical data and study criteria

PLPD was defined as persistent lymphadenopathy, lymph organ involvement, or organ lymphocytic infiltration with duration >3 months, with or without chronic or significant EBV infection in children and young adults (≤21 years). Chronic or significant EBV infection was defined as recurrent or persistent EBV viremia with duration >3 months, invasive EBV disease, or >100,000 EBV DNA copy numbers in either whole blood or plasma.[13,14] Exclusion criteria consisted of history of hematopoietic cell transplantation (HSCT), solid organ transplantation, established diagnosis of autoimmune lymphoproliferative syndrome (ALPS), or malignancy before PLPD. Biopsy details are provided in this article's Online Repository at www.jacionline.org. Data regarding comorbidities and clinical outcomes were extracted from the medical record.

WES and data analysis

Clinical WES was conducted by Baylor Genetics Laboratories (Houston, Tex). Research-based WES was performed at the Human Genome Sequencing Center (HGSC) at Baylor College of Medicine through the Baylor-Hopkins Center for Mendelian Genomics initiative. Using 1 μg of DNA, a paired-end precapture library was constructed according to the manufacturer's protocol (Illumina, San Diego, Calif) with modifications as specified by our in-house capture library preparation protocol. Precapture libraries were pooled into 4-plex library pools and then hybridized in solution to the HGSC-designed Core capture reagent[15] (52 Mb) or 6-plex library pools using the custom VCRome 2.1 capture reagent[15] (42 Mb) according to the manufacturer's instructions (NimbleGen, Madison, Wis), with minor revisions. The sequencing run was performed in paired-end mode using the Illumina HiSeq 2000 platform, with sequencing-by-synthesis reactions extended for 101 cycles from each end and an additional 7 cycles for the index read. With a sequencing yield of 9.1 Gb, the sample achieved 91% of the targeted exome bases covered to a depth of 20× or greater. Illumina sequence analysis was performed using the HGSC Mercury analysis pipeline (https://www.hgsc.bcm.edu/software/mercury),[16,17] which moves data through various analysis tools from the initial sequence generation on the instrument to annotated variant calls (single nucleotide polymorphisms and intraread in/dels). Data were analyzed through the Baylor-Hopkins Center for Mendelian Genomics initiative from 2015 to 2019, as previously described.[18,19] Variants were prioritized according to established guidelines,[20,21] with additional attention paid to variants in genes established by the International Union of Immunological Societies (IUIS)[2,8] to be defective in human immunologic disorders or closely associated with these genes in known protein interactions or immunologic pathways (see Table E1 in this article's Online Repository at www.jacionline.org). Genetic variants were ultimately assigned to the following categories describing potential contributions to immune pathogenesis: (1) defective control of lymphocyte activity; (2) impaired activation/cytotoxicity, cytoskeletal organization, and apoptosis; and (3) dysregulated inflammation.

Statistical analysis

Demographic and clinical information were abstracted from medical records. The chi-square test was used if counts exceeded 5; otherwise, the Fisher exact test was used. Kaplan-Meier survival curves were generated to estimate survival from time of disease presentation to end of follow-up, and a log-rank test was used to estimate differences across items of interest. All statistical analyses were conducted by Stata 13.v1 software (StataCorp, College Station, Tex).

RESULTS

Characteristics of PLPD Cohort

Clinical features. Overall, 51 subjects from 47 families met the criteria for PLPD at Texas Children's Hospital and referring centers (Table I). The median age at disease presentation was 3.3 years (range, 4 weeks to 21 years), with nearly equal proportions of male (n = 26) and female (n = 25) subjects. Almost half (49%) of subjects were Hispanic, and 29% were non-Hispanic White. All patients met at least 1 PLPD criterion: 38 patients (74%) had lymphadenopathy for >3 months, 32 patients (63%) had splenomegaly, and 12 patients (23%) had nonmalignant lymphoproliferation at tissue biopsy. Therapeutic strategies ranged from observation to HSCT. Maximum interventions in ascending order included observation (21.6%), steroids only (15.7%), biologics (19.6%), chemotherapy (21.6%), and HSCT (15.7%).

HLH and EBV. Among the 51 subjects, 15 patients (29%) fulfilled at least 5 of 8 HLH-2004[22] diagnostic criteria for HLH; 9 (60%) survived, and 8 (53%) had EBV-associated disease (Table I; and see Table E2 in this article's Online Repository at www.jacionline.org). Among the entire cohort, 21 (41%) had EBV-PLPD, and 14 (67%) of these patients survived (Table I; and see Table E3 in this article's Online Repository at www.jacionline.org). Five (63%) of 8 patients with both EBV-PLPD and HLH survived, and 9 (75%) of 12 patients with EBV-PLPD without HLH survived.

Autoimmune and autoinflammatory conditions. Fifteen subjects (29%) were diagnosed with autoimmune and/or autoinflammatory conditions either before or concurrently with their PLPD diagnosis (Table I; and see Table E4 in this article's Online Repository at www.jacionline.org); this subset of patients had an overall survival rate of 73%. Of the 22 subjects who underwent testing for double-negative alpha–beta T cells, 11 had elevated levels (≥1.5% of total lymphocytes). ALPS was considered at some point in the medical record in 40 patients (78%), but after evaluation, none in this cohort met the diagnostic criteria[23,24] before enrollment, and no functional defects in apoptosis were identified. However, ALPS-associated gene defects were subsequently identified in 2 patients in whom ALPS was not initially suspected or evaluated. For reference, 14 patients were diagnosed with ALPS at our institution during the study period (and were therefore excluded from this cohort).

Malignancy. Subjects with lymphoproliferative disease resulting from malignancy were excluded from this study (Table I; and see Table E5 in this article's Online Repository at www.jacionline.org). Four patients (8%) developed malignancy after meeting the enrollment criteria for nonmalignant PLPD. The median time between PLPD presentation and malignancy diagnosis was 7.75 years (Table E5). All of these patients initially had EBV-associated PLPD with subsequent diagnosis of either mature T-cell lymphoma (n = 1), diffuse large B-cell lymphoma (n = 2), or papillary thyroid carcinoma (n = 1). Notably, only the patient with papillary thyroid carcinoma, which is not typically associated with lymphoproliferative disease, EBV infection, or immune deficiency, survived (25%).

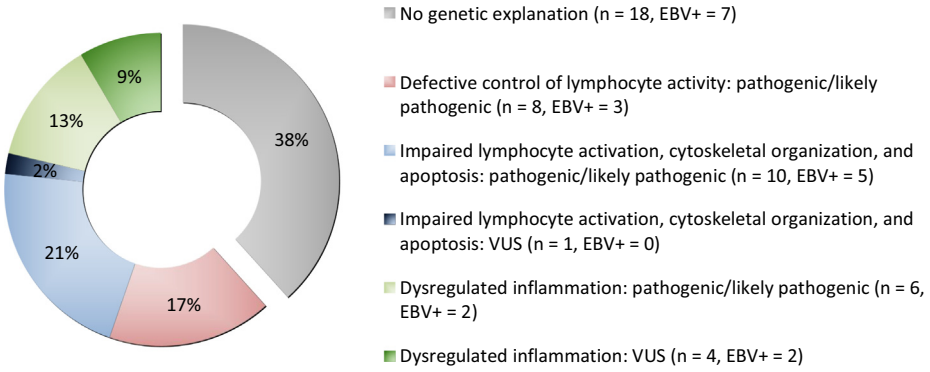
TABLE I. Patient characteristics

Characteristic	Value
Demographics	
Age at presentation (years), median (range)	3.3 (0.08-21)
Sex	
Male	26 (51.0)
Female	25 (49.0)
Race/ethnicity	
Non-Hispanic White	15 (29.4)
Hispanic	25 (49.0)
Non-Hispanic Black	2 (3.9)
Non-Hispanic Asian	6 (11.8)
Non-Hispanic other	2 (3.9)
Unknown	1 (2.0)
LPD characteristics	
Lymphadenopathy >3 months	
Yes	38 (74.5)
No	13 (25.5)
Lymphocyte infiltration found at tissue biopsy	
Yes	12 (23.5)
No	24 (47.0)
Unknown	15 (29.4)
EBV-associated lymphoproliferation	
Yes	21 (41.2)
No	27 (52.9)
Unknown	3 (5.9)
Associated clinical features	
HLH (5 of 8 criteria)	
Yes	15 (29.4)
No	35 (68.6)
Unknown	1 (2.0)
Autoimmune disease diagnosis	15 (29.4)
Malignancy (after LPD)	4 (7.8)
Splenomegaly	32 (62.8)
Therapeutic strategy	
Maximum therapeutic strategy	
Observation only	11 (21.6)
Steroid only	8 (15.7)
Biologics	10 (19.6)
Chemotherapy	11 (21.6)
HSCT	8 (15.7)
Unknown	3 (5.9)
Treated with rituximab	
Yes	10 (19.6)
No	38 (74.5)
Unknown	3 (5.9)
Outcome	
Follow-up time (years), median (range)	5.6 (0.10-26.6)
Alive at end of follow-up	39 (76.5)

Data are presented as no. (%) unless otherwise indicated.

Genetic findings

Genetic errors of immunity are prevalent in PLPD. All 51 participants from the 47 families underwent WES. Clinical WES was completed in 19 of the families (19 probands), resulting in genetic diagnoses for only 4 children (21%). For the other 15 cases and families who underwent clinical WES that did not yield a diagnosis, 12 consented to research-level analyses of the clinical exome data, resulting in identification of an additional 8 candidate



Defective control of lymphocyte activity: pathogenic/likely pathogenic	*BCL6B*, *CTLA4* (x 2), *LRBA*, *PIK3CD* (x 3), *PIK3R1*
Impaired lymphocyte activation, cytoskeletal organization, and apoptosis: pathogenic/likely pathogenic	*CD27* (x 2), *CDC42*, *DOCK4*, *FAS*, *KRAS*, *NCKAP1L*, *NRAS*, *RAB27A*, *ZBTB24*
Impaired lymphocyte activation, cytoskeletal organization, and apoptosis: VUS	*IKZF1*
Dysregulated inflammation: pathogenic/likely pathogenic	*CASP1*, *STAT1*, *STAT3* (x 2), *XIAP* (x 2)
Dysregulated inflammation: VUS	*BIRC6*, *CASP1*, *CASP5*, *PLCG2*

FIG 1. Genetic testing reveals underlying immune defects in children with LPD. Genetic profiles for 47 families who met criteria for PLPD and received WES. The *graph* displays the distribution of families among the 4 broad genetic categories; the *table* provides the list of implicated genes (and number of affected families in parentheses, if greater than 1) associated with each defective immune mechanism.

molecular diagnoses. For one of these families, research WES of 2 additional affected siblings enabled identification of the defect in *PIK3CD* in all 3 children. Research-based WES analyses were also performed without clinical WES for 28 families (30 cases), leading to likely molecular diagnoses in 13 (46%) (14 cases, 47%) and further potential genetic explanations in 4 (14%) (5 cases, 17%). Thus, 29 (62%) of 47 PLPD families, or 33 (65%) of 51 affected children, were found to have likely or plausible disease-associated genetic errors of immunity (Table E1). Note that "genetic errors" serves as a more appropriate term than "inborn errors" because of the identified likely somatic changes to *KRAS* and *NRAS*. Of these 29 families (33 cases) with viable genetic explanations, 21 (23 cases) had disease candidate variants in 15 IUIS-established PIDD and PIRD genes.[2,3,8] One family (LPD019 and LPD034) was discovered to have a novel disease candidate for which the variants (in *NCKAP1L*) were functionally validated.[25] In the remaining 7 families (8 cases) with genetic disease candidates, 1 was hypothesized to have phenotypic expansion of a known disease-associated gene (*CDC42*[26,27]), and 6 (7 cases) had potentially novel genetic causes of human disease. At minimum, 24 (51%) of 47 families, or 27 (53%) of 51 affected children, had pathogenic or likely pathogenic genetic etiologies for LPD. A smaller proportion of patients (21%) who received only clinical WES resulted in likely/potential diagnoses versus 61% who underwent research WES only ($P = .01$). Further, when considering children who underwent clinical WES followed by research-based analysis, 63% obtained likely/potential diagnoses compared to only 21% who had clinical WES only ($P = .003$). Rather than suggesting inferiority of clinical testing, these observations reflect the improvement in WES methodology over the course of the study period. All of the LPD-associated genes were observed to fall broadly into 1 of 3 categories[2,3] according to the immunologic mechanism: (1) defective control of lymphocyte activity; (2) impaired lymphocyte activation/cytotoxicity, cytoskeletal organization, and apoptosis; and (3) dysregulated inflammation (Fig 1).

Genotype/phenotype correlations are present. The proportion of subjects with a potential molecular explanation was inversely correlated with age at presentation (Fig 2, *A*). Patients with suggested genetic abnormalities were significantly younger at presentation compared to subjects who lacked genetic findings ($P = .02$; see Fig E1 in this article's Online Repository at www.jacionline.org). In fact, all 7 children who presented with PLPD when they were <1 year old were found to have a viable genetic explanation for the disease. Of the 28 patients between 1 and 8 years of age, 72% had a potential genetic etiology identified. In contrast, a molecular diagnosis for PLPD was less likely to be identified in the 16 patients (38%) who developed symptoms after 8 years of age.

The proportion of patients with possible genetic explanations did not differ significantly between EBV-PLPD and PLPD without EBV. Of the 21 patients with EBV-PLPD, 67% had potential genetic explanations, and of the 27 patients with PLPD without EBV, 70% had implicated genetic findings ($P = .91$). Likewise, among the 3 immune-mediated genetic categories, the proportion of EBV-affected individuals was evenly distributed (Fig 1).

Genetic findings were more common in patients with HLH compared to patients who eventually developed malignancy, although the proportional differences did not reach a level of statistical significance ($P = .08$). Among the 15 patients who met HLH diagnostic criteria,[13,22] a probable genetic explanation was

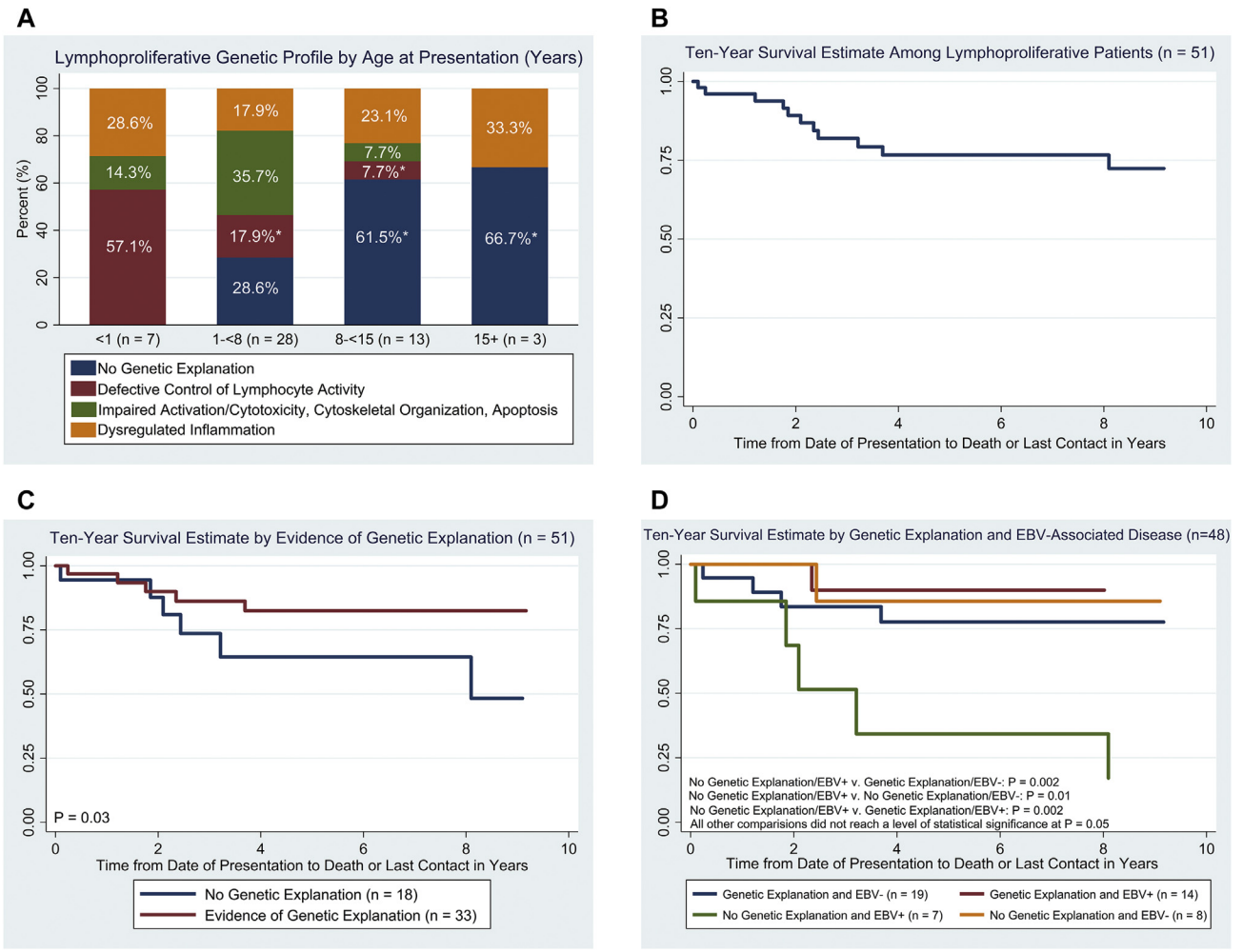


FIG 2. Features of clinical presentation and outcomes. **A,** PLPD genetic profile by age at presentation. Subjects were separated into 4 groups by age in years at presentation (x-axis). A 2-sample test of proportions with a 95% confidence level for each comparison was used to analyze proportional differences in genetic profile by age (n = 51). Asterisks indicate a significant ($P < .05$) difference from the <1-year-old group with the same genetic profile. **B,** Ten-year survival estimate from PLPD presentation to date of death or last contact in years (n = 51). **C,** Ten-year survival estimate from PLPD presentation to date of death or last contact in years by presence of a genetic explanation (n = 51). **D,** Ten-year survival estimate from PLPD presentation to date of death or last contact in years by EBV-associated disease and genetic explanation (n = 51).

present in 11 (73%), 9 of whom were under the age of 8 (Table E2). Fewer patients who developed malignancy subsequent to their PLPD diagnosis (25%) had a genetic disorder (Table E5).

Lack of genetic diagnosis is associated with increased risk for mortality. The estimated 10-year survival for the entire cohort was 72.4% with a median follow-up of 5.6 years (range, 0.10-26.6 years, Fig 2, *B*). When we analyzed the cohort as a whole (Fig 2, *C,* and see Fig E2 in this article's Online Repository at www.jacionline.org), patients without an identified possible genetic etiology had significantly lower 10-year survival compared to patients with a potential genetic explanation (48% vs 82%, respectively, $P = .03$). The 10-year survival estimate for children with EBV-PLPD trended lower compared to children without EBV (56% vs 80%; $P = .13$). Children with EBV-PLPD frequently had complicated courses: 5 had HLH, 4 developed malignancy, and 1 developed both. Presence of EBV-PLPD did not predict an underlying genetic defect. Most notably, however, subjects with EBV-PLPD without a viable genetic explanation had significantly lower estimated survival than children with a suggested genetic explanation (17% vs 90%, $P = .002$; Fig 2, *D*). In fact, the group of patients who had EBV-PLPD without a genetic explanation was the category associated with the highest risk of death.

Genetic testing impacts therapeutic decisions. Identification of an underlying genetic diagnosis in PLPD patients informs therapeutic opportunities (Figs 3 and 4; and see Table E6 in this article's Online Repository at www.jacionline.org). Currently, targeted therapies are available or show promise for treatment of at least 11 of the genetic conditions diagnosed in this cohort (potentially benefitting up to 18 patients from 16 families).[28] Furthermore, successful outcomes have been reported after HSCT in 10 of the 15 IUIS-recognized genetic errors of immunity reported here (which could treat up to 20 patients from 18 families). Before the availability of genetic testing

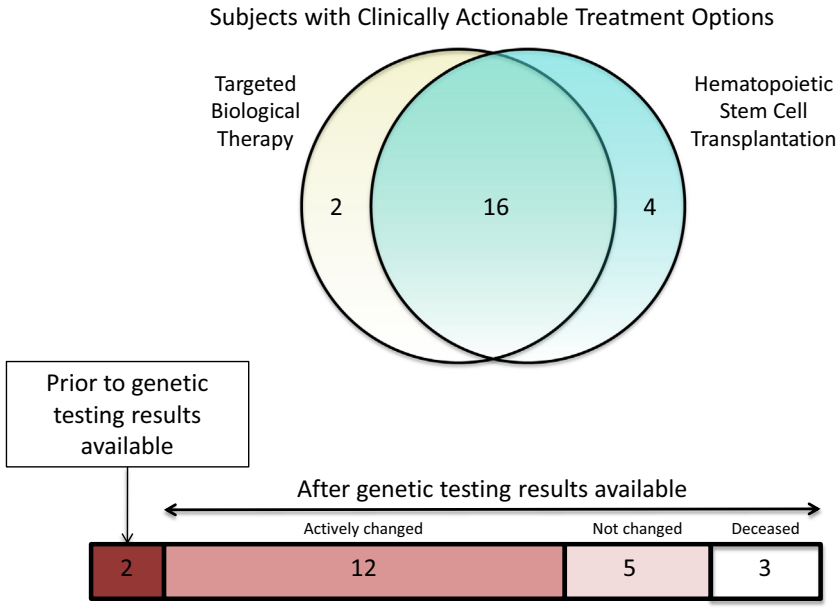


FIG 3. Treatment altered by genetic diagnoses. *Top* shows the number of subjects eligible for targeted biological therapy alone, HSCT alone, or either therapy based on the discovered genetic diagnosis. *Bottom* depicts numbers of patients who were treated according to these strategies before and after genetic testing results became available.

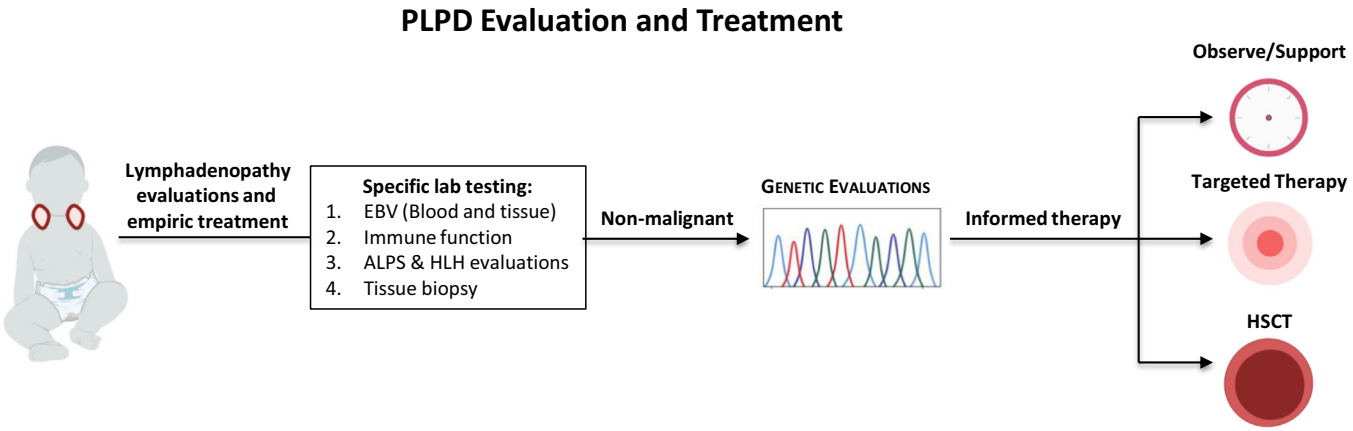


FIG 4. PLPD evaluation and treatment. This schema demonstrates a framework for evaluation and treatment of children with prolonged lymphoproliferation. If symptoms persist or worsen despite standard evaluations and empiric therapies, more extensive laboratory testing characterizing EBV infection status, immune function, and HLH status may be informative. If analysis of biopsy tissue samples reveals nonmalignant lymphoproliferation, our results indicate that genetic evaluations have a high likelihood of identifying a genetic cause of disease that may inform optimal therapy, be it observation, targeted therapy, or HSCT.

results, only 2 patients had received empiric treatment that would have been supported by their ultimate genetic diagnoses. After genetic testing results were available, 12 patients had diagnoses that led to active changes in the treatment plan through either targeted therapies or planning for HSCT. Five patients who had actionable findings after genetic testing did not have changes in their treatment plans, as they were either clinically well or lost to follow-up. Unfortunately, 3 patients died before receiving their genetic diagnoses (*NRAS, KRAS,* and *CASP1*). Importantly, 6 novel disease candidate genes were discovered, which may lead to unique opportunities for precision therapy. It becomes important to note that estimated 10-year survival was greatest (100%, n = 10) among subjects in whom control of disease was achieved using targeted biologic therapies (see Fig E3 in this article's Online Repository at www.jacionline.org).

DISCUSSION

Clinical and genomic landscape of PLPD

Pediatric nonmalignant LPD represents a heterogeneous group of conditions with high risk for mortality characterized by lymphadenopathy and/or lymph organ involvement with or

without chronic, severe, or recurrent EBV infection. HLH has been associated with a range of lymphoproliferative disorders (LPDs)[29,30] and was enriched in this cohort, with 15 (29%) of 51 children meeting the 2004 HLH diagnostic criteria. Children with immune disorders also carry increased risk of malignancy.[31] Despite exclusion of malignancy at presentation, 8% of this PLPD cohort subsequently developed this complication.

In order to improve knowledge of underlying immune pathogenesis mechanisms in PLPD to better inform treatment, we performed WES of 51 subjects from the 47 families in this cohort. This unbiased approach led to a genetic diagnosis in 51% to 62% of families (53% to 65% of affected children) (Fig 1), encapsulating a heterogenous collection of genetic errors of immunity. As a comparison, Stray-Pedersen et al[18] reported a 40% overall genetic diagnostic rate, including potentially novel diseases, in patients with PIDD. Findings from this study support the clinical utility of comprehensive genetic analysis in PLPD, with high likelihood of identifying genetic alterations that inform therapeutic opportunities and clinical risk.

PLPD risk stratification

Overall survival was 72% with a trend toward worse outcomes associated with EBV infection, HLH, and subsequent malignancy. Earlier age at presentation with LPD was positively correlated with the likelihood of identifying a potential genetic diagnosis, especially in children with impaired lymphocyte activation/cytotoxicity, cytoskeletal organization, and apoptosis (see Table E7 in this article's Online Repository at www.jacionline.org). In fact, a molecular explanation was found in all 7 patients <1 year old who were presented for care. These data particularly support the clinical utility of WES for infants and younger children with PLPD. At older ages, acquired factors, such as autoimmune disease and infection, may also contribute to development of PLPD. Even so, for 9 patients above 12 years of age, 3 had a plausible underlying genetic explanation, suggesting that genetic testing can also play a critical role in diagnosis and management of PLPD in adolescents and young adults.

Increased mortality in patients with EBV-PLPD and no genetic explanation

EBV is the most common pathogen associated with nonmalignant LPD.[32] In this cohort, patients with EBV-PLPD had pathogenic or likely pathogenic variants in several genes associated with atypical EBV disease: *CTLA4, LRBA, PIK3CD, CD27, RAB27A, ZBTB24,* and *STAT1*.[33] Additionally, somatic *PLCG2* mutations have been correlated with EBV-positive Burkitt lymphoma.[34] Potentially disease-associated variants in *CASP1* and *CASP5* were also discovered in EBV-PLPD patients.[24,35-46] *CASP1* has been provocatively implicated in IRF8-dependent EBV lytic reactivation.[47] EBV status alone, however, did not affect the likelihood of having a potential underlying genetic explanation for LPD (67% of EBV-associated LPD vs 70% of non-EBV–associated LPD). Furthermore, susceptibility to EBV infection was not significantly skewed toward any of the 3 immunologic mechanism categories (Fig 1). However, children with EBV-associated PLPD without an identifiable genetic diagnosis had a much higher risk of mortality (17% estimated 10-year survival) compared to children with EBV-associated PLPD and a plausible genetic etiology (90% estimated 10-year survival; Fig 2, *D*). EBV-LPD may evolve from persistence of EBV-infected lymphocytes as a reflection of immune dysfunction and/or proliferation of EBV-infected lymphocytes that endure despite intact immune function. In this series, the latter was associated with more aggressive disease, including a higher likelihood of HLH, malignancy, and need for HSCT. Early genetic testing may therefore be particularly important for children with EBV-PLPD. Importantly, CAEBV disease is characterized by persistence of EBV without a known immunodeficiency or immune regulation disorder.[12] This distinction underscores the importance of genetic testing in the CAEBV evaluation in order to detect genetic susceptibility to atypical EBV disease/lymphoproliferation and leave CAEBV as a diagnosis of exclusion.

Genetic diagnoses yield treatment opportunities

Early detection of genetic diagnoses in PLPD informs mechanisms of pathogenesis, facilitates assessments of clinical risks, and identifies potential therapeutic targets. In this PLPD cohort, genetic diagnoses offered improved therapeutic opportunities. Empirically, subjects received treatment with corticosteroids, biologic therapies, chemotherapy, and/or HSCT after diagnosis. Results from genetic testing directly led to active changes in the management plan for 12 (24%) of 51 patients. However, 3 subjects died before the potential molecular diagnosis was identified. Specific therapeutic strategies associated with genetic findings are outlined in Table E6. Two children (one with activated PI-3-kinase delta syndrome type 1 and the other with *CTLA4* haploinsufficiency) received HSCT before molecular diagnosis on the basis of clinical features. Overall, our data are consistent with results from a study in which 40% of PIDD patients studied by WES were diagnosed with a genetic cause for disease, leading to changes in the diagnosis and therapeutic management of approximately 25% of patients.

WES also facilitated detection of potential disease-modifying genetic variants. For instance, in addition to a variant of uncertain significance in *CASP1,* siblings LPD010 and LPD023 both carried biallelic variants in *TP53I13* that were computationally predicted to be damaging (Table E1). Although this gene is not currently associated with human disease, its gene product is known to have tumor-suppressive properties.[48] As a result, we cannot exclude disease contribution from these variants. In a second example, LPD035 was found to have *de novo* and paternally inherited variants in *CDC42* and *NLRP12,* respectively. For this child, anakinra resulted in resolution of fevers, rash, and arthritis but did not alleviate the lymphoproliferative disease, unlike the experience reported by others.[27] This observation is not surprising because anakinra does not correct the cytoskeletal and cytotoxic abnormalities caused by defects at p.R186 of *CDC42*.[26] Some of the improvement observed with anakinra therapy may have occurred as a result of mitigation of the effect of the *NLRP12* variant. These examples highlight the potential for characterization of molecular defects by WES to inform personalized therapy that may be more effective and safer than empiric immune suppression strategies or HSCT.

HSCT in PLPD

The children who underwent HSCT had the lowest 10-year survival (38%) compared to subjects who were provided less intense therapies (Fig E3), likely reflecting the severity of their

disease as well as the risks of HSCT in patients with uncontrolled lymphoproliferation. Of the 8 children who underwent HSCT, 3 who lacked a genetic explanation proceeded to HSCT as a result of failure of conventional intervention with empiric steroids, biologics, or cytotoxic chemotherapy. For subjects who survived transplantation, 2 of the 3 survivors had genetic diagnoses (*ZBTB24* and *CTLA4* deficiencies). Genetic testing can therefore help guide the need for this intervention.

Conclusions

Although lymphadenopathy remains a common presentation in children, prolonged and severe symptoms defined by our PLPD criteria characterized a cohort at high risk for mortality for whom no precise diagnostic or therapeutic approach had been established. An unbiased genetic testing approach to delineate the molecular etiologies within our PLPD cohort strongly supports the use of genetic testing to identify potentially actionable disease-causing molecular defects (Fig 4).[8] In particular, significant findings from this study show that genetic testing identified a molecular etiology in 100% of patients with PLPD under 1 year of age. Further, the presence of a genetic error of immunity was associated with improved survival in patients, particularly subjects with EBV-associated disease. Last, early identification of genetic diagnoses allowed for precision therapy and/or definitive HSCT, potentially avoiding the morbidity and mortality associated with uncontrolled disease and broad immunosuppression. As a result, our findings support early WES and genetic characterization of patients who meet the criteria for PLPD both clinically and in prospective cohort studies.

Clinical implications: Genetic evaluation is necessary in PLPD because it not only helps determine the underlying mechanistic etiology of disease and carries prognostic implications but also directs key management decisions.

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