Clinical, immunologic, and genetic characteristics of 148 patients with natural killer cell deficiency

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Background: Natural killer (NK) cell deficiency (NKD) is an immunodeficiency phenotype in which abnormality of NK cells is the major clinically relevant immune defect.

Objective: We sought to define the clinical, immunologic, and genetic characteristics of patients with NKD to aid in the understanding of these individuals and this cell type and guide future research and clinical practice.

Methods: During 2006-2022, 168 individuals with a suspected diagnosis of NKD were enrolled, with comprehensive clinical, immunologic, and genetic data collected and analyzed. Research exome sequencing was performed to identify both known and novel genetic associations.

Results: NK cell abnormalities consistent with NKD were confirmed in 148 participants. Most presented during childhood (median age 13 years, range 0-76 years), though 34% were adults. All tested individuals exhibited reduced NK cell cytotoxic function; 44% also had decreased NK cell numbers and/or mature NK cells. Herpesvirus and/or papillomavirus infections were observed in 71%, malignancies were observed in 7%, and a 5% case-fatality rate was noted. Among the 99 participants who underwent research exome sequencing, 29% were considered solved for a likely contributing variant allele, with 52% of these cases involving known genes and 48% involving novel genes.

ConclusionsNKD is a phenotypic immunodeficiency associated with increased susceptibility to certain viral infections and cancer with multiple genetic etiologies, revealing key biological pathways for NK cell development and function. This research underscores the role of NK cells in human immune defenses and helps advance the identification of at-risk populations, precise genetic diagnoses, and informed clinical management for patients with NKD. (J Allergy Clin Immunol 2025;155:1623-34.)

Key words: *NK cells, inborn errors of immunity, primary immunodeficiency, herpesvirus, cytotoxicity, immunogenetics, gene pathogenic alleles*

Natural killer (NK) cells are key components of the innate immune system, serving vital roles in antiviral defense and tumor surveillance, as evidenced in animal models and human studies.[1-3] They have many functions in human immunity, including production of soluble mediators and costimulation of immune processes, but are renowned for their ability to mediate cytotoxicity: direct cell killing through contact-dependent mechanisms.[4] NK cell cytotoxicity is triggered by the preferential ligation of germline-encoded activating receptors, which are dominant over inhibitory receptors and can be amplified by soluble and cell surface molecules.[5] The cytotoxic process involves the formation of a lytic immunologic synapse through which lytic granules containing perforin and pro-apoptotic granzymes are released onto the target cell.[6,7]

NK cells originate from hematopoietic stem cells and mature through stages in the bone marrow and secondary lymphoid tissues, with the most mature forms highly represented in peripheral blood.[8,9] These subsets include the CD56^{bright} and more mature CD56^{dim} subsets, typically present in blood at a 1:10 ratio.[10,11] In a proposed linear model of differentiation, CD56^{bright} give rise to CD56^{dim} NK cells, although this progression is not fully understood.[12] CD56^{bright} NK cells express low levels of perforin, killer immunoglobulin-like receptors, and CD16a (FcγRIIIa), and are potent cytokine producers. In contrast, CD56^{dim} NK cells express high levels of perforin, CD16a, killer immunoglobulin-like receptors, and, in their most mature form, CD57, making them

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Abbreviations used
CMV: Cytomegalovirus
cNKD: Classical NKD
ES: Exome sequencing
fNKD: Functional NKD
HD: Healthy donor
HLH: Hemophagocytic lymphohistiocytosis
HPV: Human papillomavirus
HSV: Herpes simplex virus
IEI: Inborn error of immunity
IRB: Institutional Review Board
NEAR: Natural Killer Cell Evaluation and Research
NK: Natural killer
NKD: NK cell deficiency
NK-IEI: NK abnormality as part of an IEI
RR: Relative risk
VZV: Varicella-zoster virus

highly cytotoxic. Many factors, including IL-2, promote NK cell maturation and activation, both *in vitro* and *in vivo*.[13,14]

Many inborn errors of immunity (IEIs) can impair NK cell development or function.[15] For example, IL-2 receptor γ chain deficiency causes $T^-B^+NK^-$ severe combined immunodeficiency, disrupting NK cell development,[13,16] whereas perforin deficiency can lead to familial hemophagocytic lymphohistiocytosis and a loss of NK cell cytotoxicity.[17] In all these cases, the NK cell abnormalities are not the primary clinical concern, although they may contribute to disease. The majority of IEIs do not affect NK cells; thus, we refer to the subset that does as an NK abnormality as part of an IEI (NK-IEI).

NK cell deficiency (NKD) is a rarer subset still of IEIs, in which the deficiency of NK cells is the major clinically relevant immunodeficiency and the patient's presentation is not suitably explained by any other immunologic findings. The history of NKD, similar to many IEIs, evolved from compelling case reports[18-21] and advanced to genetic explanations. A feature of NKD, consistent with the known function of NK cells, is an increased susceptibility to viral infections, particularly those caused by herpesviruses. Currently, 10 genes are associated with NKD: *ACTB, ELF4, FCGR3A, GATA2, GINS1, GINS4, IRF8, MCM4, MCM10,* and *RTEL1*.[22-31] Some genes are exclusively linked to NKD, whereas others can cause broader immunodeficiency under specific circumstances. Before the identification of these genetic causes, NKD was categorized into 2 phenotypic subtypes: classical (or developmental) and functional.[32] Classical NKD (cNKD) is characterized by reduced NK cell numbers or maturity due to impaired differentiation or survival, leading to decreased cytotoxic capacity. Functional NKD (fNKD) involves normally developed NK cells that are present in normal percentages in peripheral blood, but are functionally deficient.

Research into NKD and NK-IEI has enhanced our understanding of NK cell biology and the role of NK cells in human host defense. Our program, initiated in 2006 and later formalized as the Natural Killer Cell Evaluation and Research (NEAR) clinical program, has focused on studying individuals with a suspected diagnosis of NKD. Overall, the NEAR program has contributed to understanding the genetic basis of these conditions and derivative NK cell biology. Here, we present the clinical, immunologic, and genetic findings from the first 16 years of the program including 148 individuals with complete data, 99 of whom had research exome sequencing (ES).

METHODS

Study design

This observational, noninterventional study began in 2006. The study received approval from the Children's Hospital of Philadelphia Institutional Review Board (IRB) (2006-7-4885) and subsequently Baylor College of Medicine IRB (H-30487) and Columbia University Medical Center IRB (AAAR7377). Eligible patients presented with a phenotype suggestive of NKD,[15] such as recurrent, severe, or atypical viral infections and persistently abnormal NK cell numbers/function as indicated by clinical laboratory assessments, which were not adequately explained by any other immunologic findings. When possible, family members and age-matched healthy donors (HDs) were also included.[10] Clinical and laboratory data were anonymized and compiled in a REDcap database.

Sample collection

After obtaining informed consent, blood samples were collected and encoded. PBMCs were isolated through density centrifugation and subsequently used for immunologic assays and/or cryopreserved for future use as described.[33] Repeat blood collection was performed in many cases for validation, genomics, and further evaluation.

NK cell evaluations

To assess NK cells, flow cytometric analyses were performed including quantitation of CD56, CD16, CD94, and CD57 markers on $CD3^-$ lymphocytes by measuring both mean fluorescence intensity and percent positivity of both $CD56^{dim}$ and $CD56^{bright}$ populations (with percentages among lymphocytes measured to identify relative NK cell deficits where they were present).[11] NK cell function was assessed using a 4-hour ^{51}Cr-release assay with PBMCs and labeled K562 erythroleukemia target cells.[33] Lytic units were used to summarize cytotoxic function and represent the inverse of the number of PBMCs required to lyse 10% of target cells (higher lytic units mean greater function). In some cases, the causative gene or a case report was published with the NK cell data presented here.

ES and analysis

Research-based ES was performed on genomic DNA isolated from blood or saliva under the Baylor-Hopkins Center for Mendelian Genomics (IRB protocol H-29697)[34] with immunologic and NK cell–related discovery focus, as previously reported.[28,29,35] Some participants' ES findings were featured in individual publications (elaborated below) or aggregate immunodeficiency reports.[35]

Statistical analysis

Categorical variables were summarized by proportions and continuous variables by means and SDs if normally distributed (Shapiro-Wilk $P > .05$), and by medians and interquartile ranges otherwise. Group comparisons were performed by χ^2 or Fisher exact tests for categorical variables and by unpaired *t* tests (if normal) or by Wilcoxon rank sum tests (if not normal). All statistical analyses

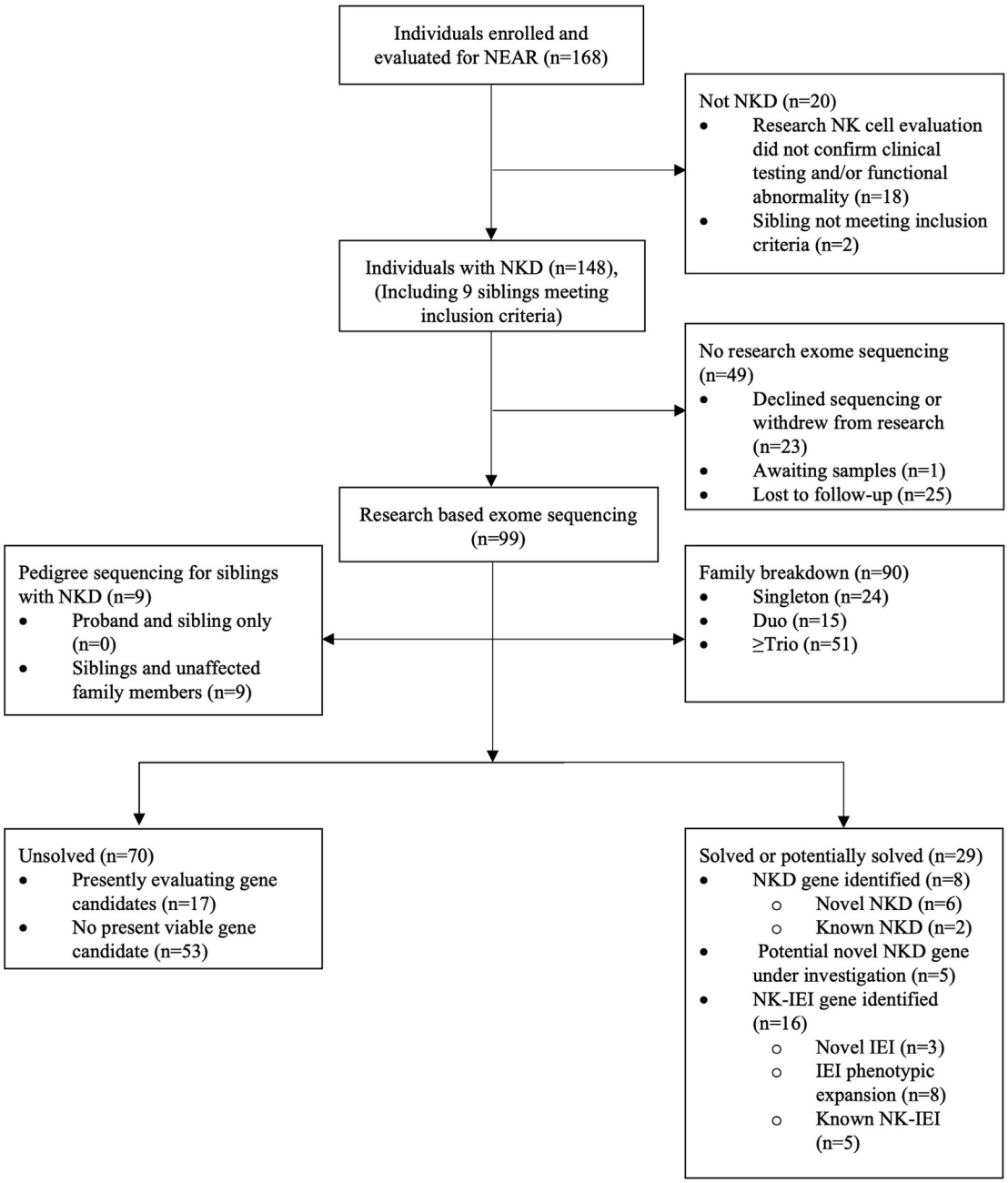

FIG 1. Diagnostic outcome of 168 participants enrolled in the NEAR program from 2006 to 2022. Participants not meeting the inclusion criteria for NKD were excluded (n = 20). Data analysis was performed for 148 participants. Of these, 99 had research sequencing, and 29 were considered solved genetically.

were carried out using R version 4.3.1 with the cufunctions package.[36] No adjustments were made for multiple comparisons.

RESULTS

Patient enrollment and demographics

Our protocol to evaluate patients with a suspected diagnosis of NKD was initiated in 2006 and included 168 individuals from 157 unrelated families enrolled through October 2022 (Fig 1). Individuals were enrolled based on clinical features of NKD and/or persistently abnormal NK cell counts/function as determined by clinical laboratory testing. Research evaluation involved comprehensive assessment of clinical data, NK cell cytotoxicity testing via ^{51}Cr-release assay, and NK cell immunophenotyping. Patients with confirmed abnormalities in research laboratory tests were included in the NKD cohort comprising 148 patients. Twenty individuals, including 2 sibling pairs, were excluded from further analyses as they did not exhibit abnormal research laboratory tests.

Of the 148 patients, 99 (including 9 sibling pairs) underwent research-based ES; 49 patients were lost to follow-up, declined genetic testing, or opted for clinical testing only. Some individuals who participated in research ES had already had clinical gene sequencing panels (n = 1) or clinical ES (n = 14) without a diagnosis. One deceased male patient was included in the cohort due

TABLE I. Patient demographics and characteristics at enrollment

	All patients (n = 148)	Female children (n = 38)	Male children (n = 59)	Female adults (n = 42)	Male adults (n = 9)
Age, y, median (IQR)	13 (0-76)	9 (0-17)	8 (0-17)	36 (18-76)	33 (18-62)
Age range, no. (%)					
0-3	28 (19)	10 (26)	18 (31)	0	0
4-17	69 (47)	28 (74)	41 (69)	0	0
18-49	41 (28)	0	0	35 (83)	7 (78)
>50	10 (7)	0	0	7 (17)	2 (22)
Race/ethnicity, no. (%)					
Non-Hispanic White	79 (53)	19 (50)	30 (51)	22 (52)	8 (89)
Non-Hispanic other	13 (9)	2 (5)	6 (10)	5 (12)	0
Non-Hispanic Asian	3 (2)	3 (8)	0	0	0
Non-Hispanic Black	1 (1)	0	1 (2)	0	0
Hispanic	10 (7)	4 (11)	5 (8)	1 (2)	0
Other	42 (28)	10 (26)	17 (29)	14 (30)	1 (11)
Patient locations					
US	123 (83)	27 (71)	45 (76)	42	9
Outside US	25 (17)	11 (29)	14 (24)	0	0
Referral status					
Health care provider	139 (94)	37 (97)	58 (98)	37 (88)	7 (78)
Self-referral	9 (6)	1 (3)	1 (2)	5 (12)	2 (22)
Family history					
Early malignancy	24 (16)	3 (8)	5 (8)	13 (31)	3 (33)
Autoimmune disease	22 (15)	6 (16)	7 (12)	8 (19)	1 (11)
Hallmark infection	16 (11)	2 (5)	9 (15)	4 (10)	1 (11)
Immunodeficiency	8 (5)	2 (5)	6 (10)	0	0
Any of the above 4	57 (39)	12 (34)	22 (34)	19 (48)	4 (44)
Consanguinity	8 (5)	4 (11)	3 (5)	1 (2)	0
Adopted	2 (1)	1 (3)	1 (2)	0	0
Outcome					
Alive	141 (95)	34 (89)	58 (98)	42	7 (78)
Deceased	7 (5)	4 (11)	1 (2)	0	2 (22)

to a compelling clinical history, immunophenotype, and the presence of a variant linked to novel NKD in an unrelated proband.[30]

Among the 148 patients confirmed to have an NK cell abnormality, 80 (54%) were female and 68 (46%) were male at birth (Table I). Most patients (83%) were from the United States (Fig E1 in this article's Online Repository available at www.jacionline.org). The median age at enrollment was 13 years (range 0-76 years) with 97 participants (66%) <18 years old (Table I). Where demographic data were available, 79 (53%) identified as non-Hispanic White, and 27 (20%) identified as Hispanic, non-Hispanic Black, Asian, or other race/ethnicity. Most patients (94%) were referred to the NEAR program by a health care provider, with self-referrals accounting for the remaining patients. Familial consanguinity was present in 8 participants, and 2 were adopted. A family history of immunodeficiency, early malignancy, autoimmune disease, or hallmark infection was reported by 57 individuals (39%). By the time of this report, 7 patients had died.

Hallmark infections

The presence of hallmark infections caused by herpesviruses and papillomaviruses is a major indicator for suspected NKD.[15] In our cohort, 105 patients (71%) had experienced 1 or more different hallmark infections at the time of enrollment (Fig 2, *A*). At enrollment, 63 of these patients (61%) had a history of only 1 hallmark infection, 28 (26%) had 2, and 14 (13%) had 3 or more. The most frequent hallmark infections were herpes simplex virus (HSV) (n = 48 [32%]) and EBV (n = 47 [32%]), followed by human papillomavirus (HPV) (21%), varicella-zoster virus (VZV) (17%), and cytomegalovirus (CMV) (9%) (Fig 2, *B*). Adults were more likely than children to have more than 1 hallmark infection (relative risk [RR] 1.73, $P = .054$), as well as higher rates of EBV (RR 1.98, $P = .0067$), VZV (RR 2.85, $P = .0066$), and HPV (RR 2.63, $P = .0038$) infections, and less likely to have recurrent herpes labialis (RR 0.173, $P = .058$) (Fig 2, *C*; Table II; Table E1 in the Online Repository at www.jacionline.org). Female participants were more likely to have had VZV (RR 4.6, $P = .019$), and male participants were more likely to have had herpes labialis (RR 4.6, $P = .019$).

The presentations of these hallmark infections were often atypical, including viremia, recurrent shingles/zoster, recalcitrant warts, mucocutaneous lesions, virus-associated malignancies, hemophagocytic lymphohistiocytosis (HLH), or disseminated infection (Table II; Table E1). Severe or recurrent EBV infections, including severe infectious mononucleosis, recurrent genital herpes, and recurrent shingles, were more prevalent in adults compared with children (RR 7.6, $P = .0002$).

Autoimmune, inflammatory, other infectious, and malignant complications

Patients had histories of other conditions frequently found in individuals with immune disorders (Table II). Recurrent pneumonia was present in 30% of patients, recurrent sinusitis was

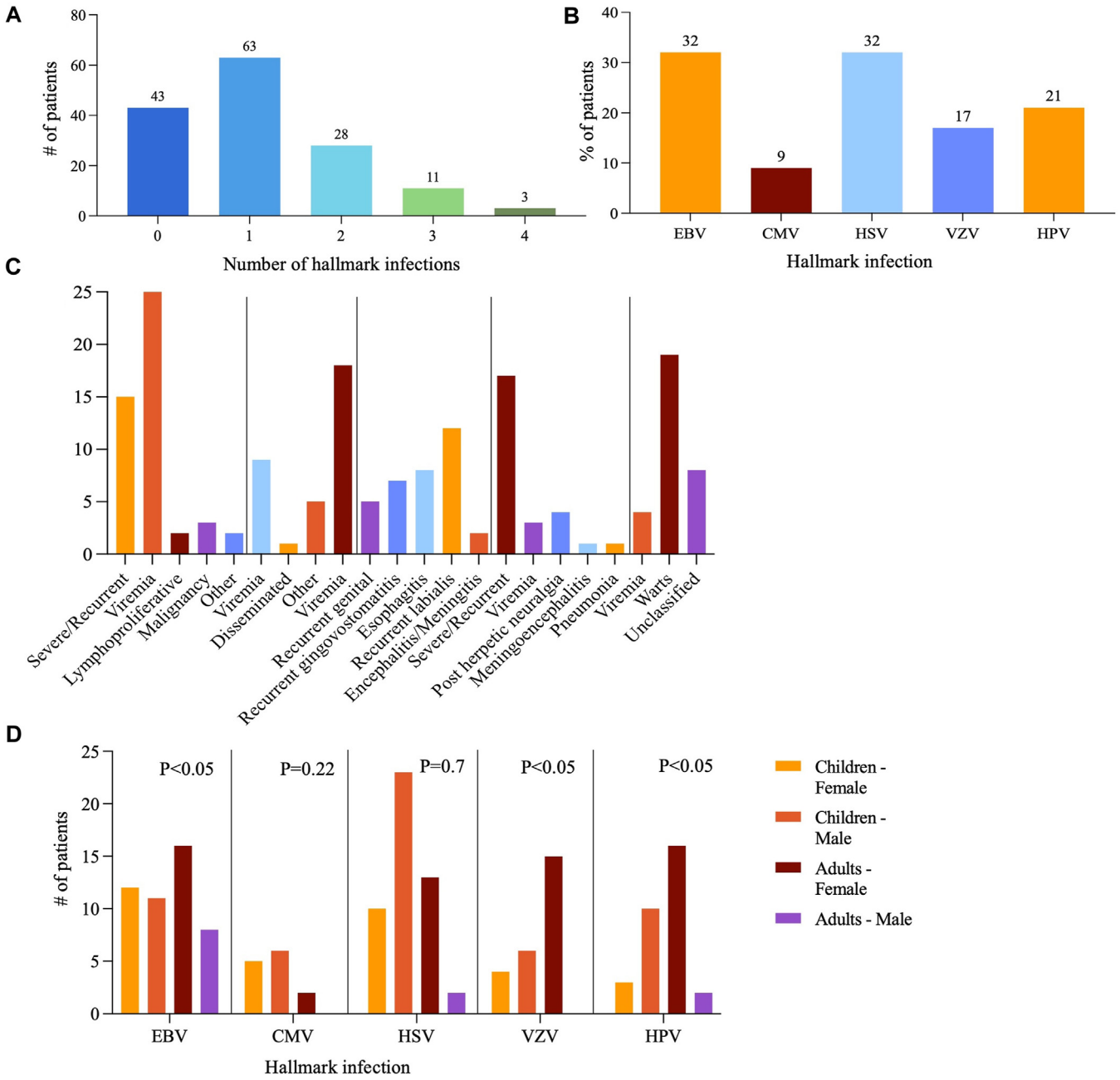

FIG 2. Hallmark infections in 148 patients with NKD. **(A)** Number of historical hallmark infections per patient at time of enrollment. **(B)** Percentages of patients with different hallmark infections. **(C)** Distribution of hallmark infections according to the type of clinical disease. **(D)** Distribution of hallmark infections according to age and sex, with differences in EBV, HSV, and HPV. *P* values used Yates correction for continuity.

present in 32%, and recurrent otitis media was present in 16%; 3 patients (all children) had bronchiectasis. Skin infections and gastrointestinal infections were found in approximately one-third of participants. Recurrent candidiasis and recurrent molluscum occurred in 12% and 5% of patients, respectively. Other presumably noninfectious complications were also present, including cytopenias (25%), neurologic disease (17%), hepatosplenomegaly (10%), and thyroid disease (8%). Likely inflammatory or autoimmune complications included HLH (3%), inflammatory bowel disease (3%), hepatitis (3%), and myocarditis (2%). These conditions were all equally prevalent in adults and children except for thyroid disease, which had a higher prevalence in adults (RR 9.51, $P = .0004$).

Given the established linkages between NK cells and anticancer immunity, attention was given to the presence or development of malignancy. Ten patients (7%) were diagnosed with malignancy at a median age of 16.5 years (range 2-62 years); 5 patients had a hematologic malignancy, and 1 patient had multiple malignancies (Table E2 in the Online Repository at www.jacionline.org). At the time of this report, 7 patients had been treated and were in remission, and 3 had died: 1 of preexisting HLH, 1 of pneumonia during treatment for EBV^+CD30^+

TABLE II. Most common infectious, autoimmune, inflammatory, and other complications in children vs adults with NKD

	Adults (n = 51), no. (%)	Children (n = 97), no. (%)	RR in adults vs children	*P* value
Skin infections/soft tissue abscesses	16 (31)	36 (37)	0.85	ns
Gastrointestinal infections	15 (29)	34 (35)	0.84	ns
Recurrent URTI	12 (24)	31 (32)	0.74	ns
Recurrent pneumonia	17 (33)	27 (28)	1.20	ns
Cytopenia	10 (20)	27 (28)	0.70	ns
Recurrent sinusitis	22 (43)	26 (27)	1.61	.067
Recurrent otitis media	4 (8)	19 (20)	0.4	.100
Hepatosplenomegaly	0	15 (15)	0	.001
Neurologic disease*	12 (24)	13 (13)	1.76	.180
Recurrent candidiasis	6 (12)	12 (12)	0.95	ns
HLH	0	5 (5)	0	.16
Malignancy	5 (10)	5 (5)	1.9	ns
Hepatitis	1 (2)	4 (4)	0.48	ns
Inflammatory bowel disease	1 (2)	4 (4)	0.47	ns
Bronchiectasis	0	3 (3)	0	ns
Myocarditis	1 (2)	2 (2)	0.95	ns
Thyroid disease	10 (20)	2 (2)	9.51	.0004
EBV (all types)	24 (47)	23 (24)	1.98	.0067
Severe/recurrent EBV infection	12 (24)	3 (3)	7.61	.0002
EBV viremia	9 (18)	16 (16)	1.07	ns
CMV (all types)	2 (4)	11 (11)	0.34	ns
CMV viremia	0	9 (9)	0	.028
HPV (all types)	18 (35)	13 (13)	2.63	.0038
HPV unclassified	8 (16)	0	0	.0001
Warts	7 (14)	12 (12)	1.11	ns
HPV viremia	3 (6)	1 (1)	5.71	.120
HSV (all types)	15 (29)	33 (34)	0.86	ns
HSV viremia	6 (12)	12 (12)	0.95	ns
Recurrent genital herpes	4 (8)	1 (1)	7.60	.048
Recurrent herpetic gingivostomatitis	3 (6)	4 (4)	1.43	ns
HSV esophagitis	2 (4)	6 (6)	0.63	ns
Recurrent herpes labialis	1 (2)	11 (11)	0.17	.058
Molluscum (recurrent/severe)	1 (2)	7 (7)	0.27	ns
VZV (all types)	15 (29)	10 (10)	2.85	.0066
Severe/recurrent VZV	10 (20)	7 (7)	2.72	.032

ns, Not significant; *URTI*, upper respiratory tract infection.
*Includes historical diagnoses that may or may not be infectious, autoimmune, or inflammatory (developmental delay, seizures, ataxia, amnesia, neurocognitive impairment, neuropathy, and Guillain-Barré syndrome).

Hodgkin lymphoma with mixed cellularity,[31] and 1 of complications associated with recurrent T-cell lymphoma, including angioimmunoblastic and CD30^{+} anaplastic cutaneous lymphoma.[30]

NK cell function and immunophenotyping

All patients tested had decreased NK cell cytotoxic function, as it was an enrollment criterion. In aggregate, cytotoxicity measured at enrollment using ^{51}Cr-release assay of patient PBMCs against K562 target cells was significantly decreased compared with that found in 205 healthy donors (HDs) (Fig 3, *A*). This was not rescued by short-term *in vitro* IL-2 stimulation, suggesting an absolute defect in NK cell function (Fig 3, *B*). These decreases were also evident and statistically significant when NK cell cytotoxicity was represented as lytic units (Fig 3, *C*).

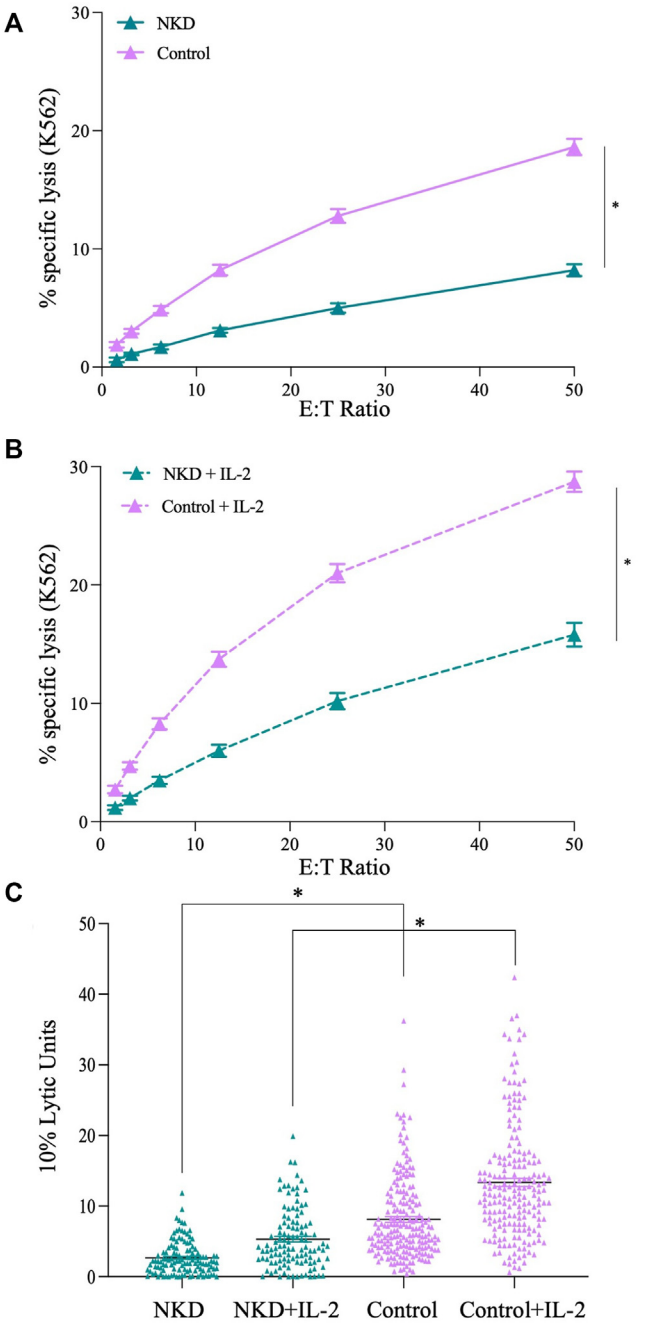

FIG 3. NK cell cytotoxicity in 148 patients with NKD. Four-hour ^{51}Cr-release assay in patient vs control PBMCs against K562 target cells, without **(A)** or with 1000 U/mL of IL-2 **(B)** added during the assay. **(C)** NK cell lytic units from PBMCs without or with added 1000 U/mL of IL-2. Error bars show ±SEM. *$P < .05$ by paired *t* test. *E:T Ratio*, Ratio of effector cells to target cells.

NK cell quantitation by flow cytometry identified 37 patients with low NK cell percentages compared with HDs (Fig E2 in the Online Repository at www.jacionline.org) and that were <2% of total lymphocytes (Table E3 in the Online Repository at www.jacionline.org) and 34 patients with >20% CD56^{bright} NK cells within the NK cell compartment (Table E3), signifying a potential developmental NK cell defect. Collectively, this represented 57 patients (39%), and they were considered to have classical NKD (cNKD) (≤2% NK cells and/or ≥20% CD56^{bright} NK cells),

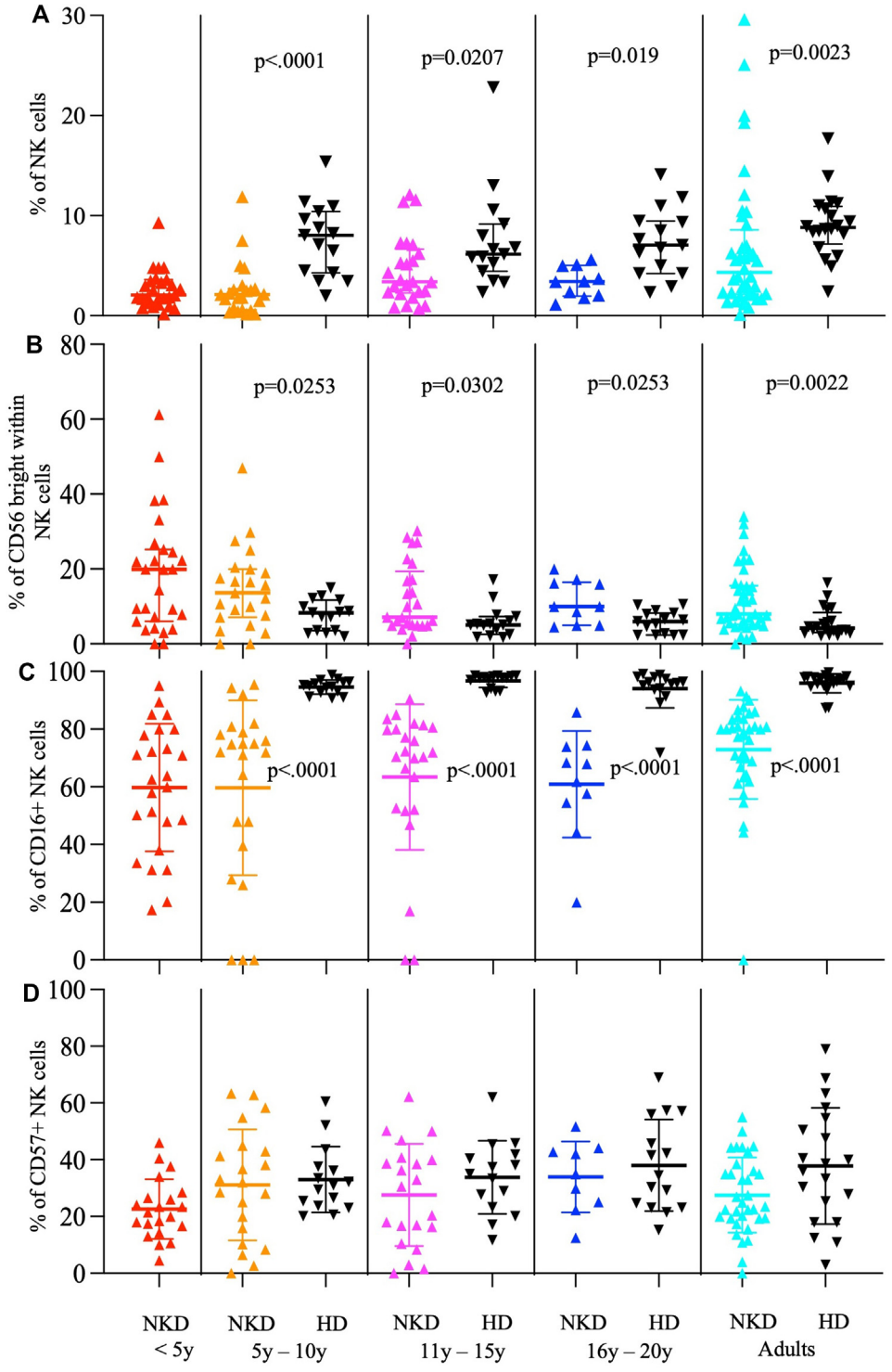

FIG 4. Detailed NK cell phenotype in 124 of 148 patients with NKD. Percentage of **(A)** $CD56^{+}/CD3^{-}$ NK cells (of total lymphocytes), **(B)** $CD56^{bright}$, **(C)** CD16, and **(D)** CD57 of NK cells in patients vs age-matched HDs. *Horizontal lines* denote means and error bars ± SD. *P* values represent differences between NKD patients and age-matched HDs by Wilcoxon rank-sum test. Where no *P* values are shown, the values were $P > .05$.

whereas 91 (61%) had normal NK cell percentages and $CD56^{bright}$-to-$CD56^{dim}$ ratios and thus were considered to have functional NKD (fNKD) (≥2% NK cells or ≤20% $CD56^{bright}$ NK cells). Patients with cNKD were younger (median age 9 vs 14 years; $P = .008$), had a lower NK cell percentage (median 1.7% vs 5.1%; $P < .0001$), higher $CD56^{bright}$ (median 21.7% vs 8.95%; $P < .0001$), and lower lytic units (median 1.29% vs 2.28%, $P = .0236$) (Table E3).

As a cohort with both cNKD and fNKD, patients overall demonstrated significantly reduced peripheral NK cell frequencies compared with age-matched HDs across different age groups (Fig 4, *A*). Furthermore, patients overall had NK cells with a higher percentage of $CD56^{bright}$ immature and lower percentages of $CD16^{+}$ mature NK cells compared with age-matched HDs (Fig 4, *B* and *C*). The percentage of $CD57^{+}$ (terminally mature) NK cells, however, was not significantly different between the NKD cohort and age-matched HDs (Fig 4, *D*), although there were patients with NKD who were lacking $CD16^{+}$ or $CD57^{+}$ NK cells altogether (likely reflecting subsets of cNKD patients). NK cells expressing CD94 were significantly reduced only in adult and 11- to 15-year-old patients compared with HDs (Fig E3 in the Online Repository at www.jacionline.org), although there were clearly patients at all ages who had low percentages of $CD94^{+}$ NK cells. Overall, patients with NKD appear to have relative deficits and impaired terminal maturation of NK cells compared with HDs.

Although the NK cell abnormalities were the major clinically relevant immunologic abnormality in enrolled patients with NKD, there were other immunologic parameters out of normal range in a minority of patients (Table E4 in the Online Repository at www.jacionline.org). The most common other immune phenotypes of note were low $CD19^{+}$ B cells (16%), low IgA (13%), and decreased pneumococcal titers (13%). All other immunologic findings were in ≤10% of patients. T-cell percentages among patients and age-matched HDs overlapped without statistically significant differences (Fig E4 in the Online Repository at www.jacionline.org).

Research ES and genetic diagnoses

Research ES was offered to patients with a confirmed NK cell functional abnormality and was conducted on 99 patients (67%) from 90 unrelated families (Fig 1). A total of 49 patients did not participate in research ES because they specifically declined, withdrew from research, or were lost to follow-up; 15 patients did have clinical ES that did not establish a genetic diagnosis. Most of the research ES performed for the 99 patients was done as familial analyses, with only 23% done as proband only. The overall diagnostic yield of research ES was 29% at the time of this report, with an additional 17% of patients having some viable gene candidate presently under evaluation. In 53 patients (54%), their condition remained unsolved without any viable gene candidates. There was no statistically significant difference in the rate of confirmation of genetic diagnosis between family and proband sequencing or between NKD subtypes.

The genes associated with NKD identified through our research ES in the 29 patients whose condition was considered to be solved all relied on confirmatory approaches that resulted in a peer-reviewed publication of that finding (Table E5 in the Online Repository at www.jacionline.org), a publication in progress, or identity to a previously reported finding (Table E6 in the Online Repository at www.jacionline.org). The distribution of causative genes identified covered 6 categories (Fig 5, *A*) including 21 individual genes (Fig 5, *B*): (1) novel NKD gene defects (*MCM10, IRF8, GINS4, ELF4, ACTB*); (2) known NKD gene defects (*FCGR3A*); (3) potentially novel NKD genes (n = 3); (4) known NK-IEI (*NFAT5, RAB27A, NLRP4, PLCG2*); (5) novel NK-IEI (*CDC42, CDC45, POMP*); (6) NK cell abnormality as a phenotypic expansion of a known IEI, an IEI previously not known to affect NK cells (*PIK3CD, NFKB2, TTC7A, ZBTB24, DOCK8*). The specific clinical and immunophenotypic features of these patients have been published (Table E5) or are summarized here (Table E6); there were no significant differences in gender, age, clinical history, or outcome between our patients with NKD having an NKD or NK-IEI genetic explanation (not shown).

Treatment and outcomes

Treatments provided for patients in this cohort were as directed by their referring center and focused on treatment and prophylaxis of infection as well as treatment for autoimmune and inflammatory diagnoses. Hematopoietic stem cell transplantation was performed in 6 patients having a confirmed genetic diagnosis (Table E7 in the Online Repository at www.jacionline.org) and based on specific indications for each case as recommended and directed by the treating physicians/center. One child with *MCM10* deficiency had CMV-associated HLH,[28] whereas another child with *NLRP4* deficiency had B-cell non-Hodgkin lymphoma and EBV-associated HLH. Two patients with *CDC42* and *POMP* deficiency had HLH.[37-39] A child with *TTC7A* deficiency experienced refractory debilitating warts. A female patient with *DOCK8* deficiency had EBV/CMV viremia, recurrent infections, and sclerosing cholangitis.[40] The overall transplant survival rate was 50% as of the time of this report.

As for overall outcomes, 7 individuals in the cohort have died, giving a case fatality ratio of 5% (Table I; Table E8 in the Online Repository at www.jacionline.org). Two patients with *MCM10* or *NFKB2* deficiency died of disseminated CMV disease causing end organ dysfunction.[28,41] One patient with *ACTB* deficiency died of complications of refractory EBV^{+} Hodgkin lymphoma.[31] One patient with *NLRP4* deficiency died of large B-cell non-Hodgkin lymphoma, and one with *CDC42* deficiency died at 1 year of age following transplant for HLH.[38] One patient with *ELF4* deficiency died of complicated lymphoma.[30] Finally, 1 patient without a known genetic explanation died of sepsis. A fatal or life-threatening clinical course, defined as death, malignancy, HLH, or requirement for transplant, was associated with lower NK cell percentages; lower lytic activity; confirmed genetic diagnosis; and presence of autoimmune or inflammatory complications, EBV malignancy, and CMV disease (Table III). Univariate analysis based on confirmed genetic diagnosis (solved) identified greater likelihood for CMV infection and fatality (Table E9 in the Online Repository at www.jacionline.org).

A number of factors were associated with the composite outcome positively or negatively, and logistic regression was performed to see which remained significant when looked at in combination. Four factors remained significant (solved, NK cells <1%, presence of autoimmune or inflammatory complications, HPV viremia), and genetically solved was the most significant. The logistic regression formula had high accuracy (receiver operating characteristic area under the curve = 0.836) (Fig E5 in the Online Repository at www.jacionline.org), with excellent specificity (all 129 good outcomes correctly classified), but not good sensitivity (only 4 of the 19 patients with severe outcome correctly classified). One may summarize the logistic regression result as the presence of no more than one of the 4 risk factors meant a nonsevere outcome (with 100% specificity).

DISCUSSION

This study reports our 16-year experience studying patients with NKD with the aim of characterizing their clinical and

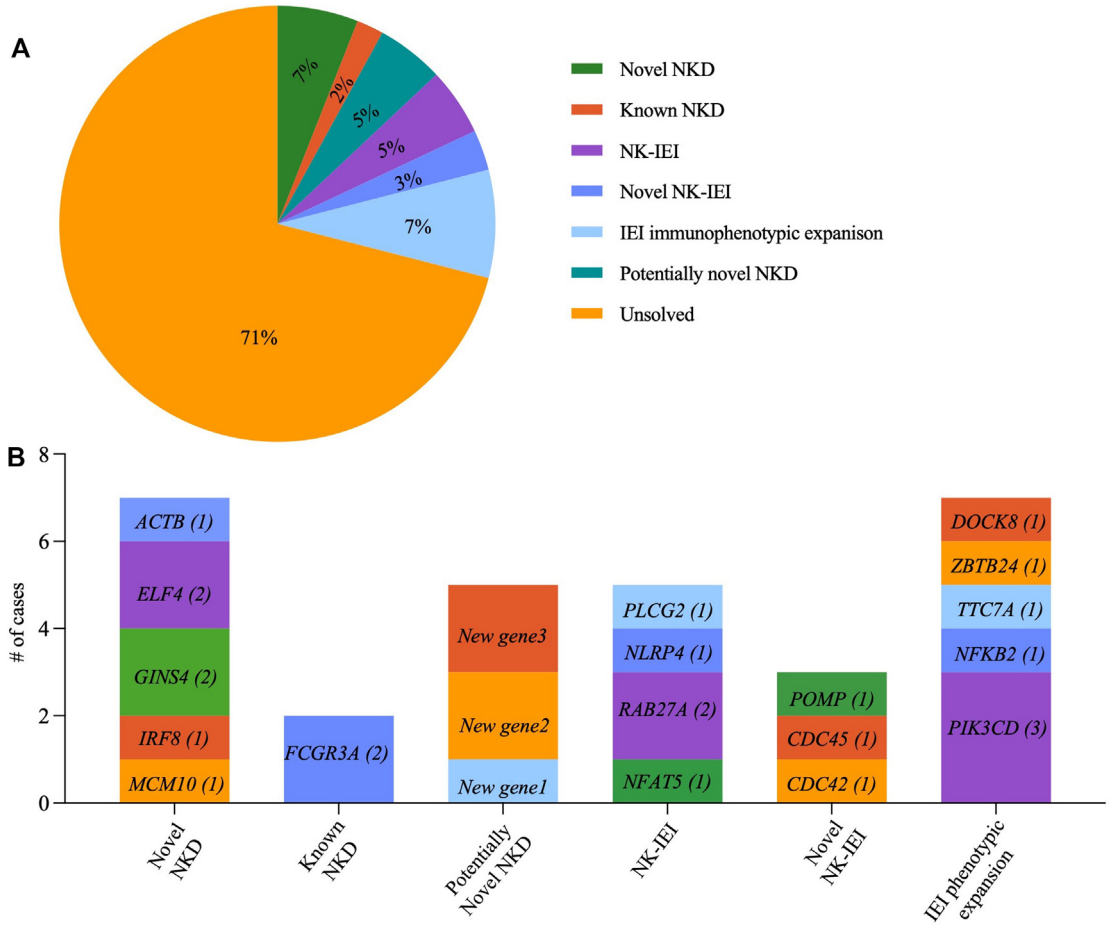

FIG 5. Molecular diagnoses in 99 patients with NKD via research ES. **(A)** Outcome categories of ES: NK-IEI; IEI immunophenotypic expansion, referring to IEI gene not previously associated with NK cell abnormality; potentially novel NKD, referring to genes currently being confirmed. **(B)** Distribution and identity of genes within the NKD cohort considered causative across outcome categories. All of the NKD genes listed cause cNKD except *ACTB* and *FCGR3A*, which cause fNKD.

molecular features. It does not provide information on the effectiveness of treatments or interventions, and no recommendations can be made based on it. That said, this study describes the characteristics of a group of patients in whom an NK cell abnormality was held to be the major clinically relevant immunodeficiency.

Before this report, NKD cases were relegated to individual case reports, genetic discovery works, or perspective overviews. Notably, a case from the 1980s with multiple herpesvirus infections has often been hailed as the first relevant NKD example.[18] Additionally, a family with severe EBV described in the 1980s[19] and another with CMV from the 2000s suggested heritable genetic underpinnings for NKD.[20] These and other related reports[42] inspired the creation of a program to identify and study similar individuals in greater detail.[43,44] Most were identified via referral from other subspecialists, but some contacted us directly. As NKD is considered rare, it is not possible to estimate a prevalence from our work, given the small sample size and potential errors common to referral studies. Initially, it was unclear what this program would achieve, but it did identify additional cases of NKD and has resulted in genetic explanations for both NKD and NK-IEI in individuals who initially presented with features of an abnormal NK cell clinical phenotype. Though many of these were previously reported, the collective experience has taught us about common features and findings of these individuals, which we have attempted to describe here in aggregate and ideally inform future research.

There were limitations recognized along the way and in retrospect. One is that inclusion was based on abnormal laboratory evaluation of NK cells. With few confirmed patients available at the beginning of this work, defining what was abnormal for NK cell function was difficult. As such, initial findings from this study have been used to inform what is an abnormal result when considering functional and phenotypic NK cell testing. Although we have frequently relied on HDs and age-matched HD ranges, some of which were published elsewhere and now some here, this was imperfect. We now can look retrospectively and offer recommendations for values to indicate consideration of NKD in individuals with a suggestive clinical history and based on the aggregate experience we report here. Specifically, we suggest a heightened index for suspicion for NKD in patients with a relevant clinical history who have <2% NK cells and/or >20% $CD56^{bright}$ NK cells, and PBMC NK cell cytotoxicity < -1 z score relative to HD ranges across multiple

TABLE III. Summary of univariate analysis of factors affecting outcome

	Continuous factor in severe outcome (n = 19)*	Continuous factor in non-severe outcome (n = 129)	*P* value†
Lytic units, median (IQR)	2.15 (1.08-3.86)	0.47 (0.186-5.8)	ns
NK cell (%), median (IQR)	3.3 (1.86-6.16)	2.06 (0.18-3.15)	.011
CD56 bright, median (IQR)	11 (6.1-20)	13.4 (3-21.8)	ns

	Severe outcome risk with categorical factor present (n = 19)	Severe outcome risk with categorical factor absent (n = 129)	RR with factor present	*P* value
Age at enrollment, median (IQR)	12 (7.5-17)	13 (5-30)	—	ns
Biological sex, female, n/N (%)	12/80 (15)	7/68 (10.3)	1.46	ns
Biological sex, male, n/N (%)	7/80 (8.8)	12/68 (17.6)	0.5	ns
History of EBV, n/N (%)	8/47 (17)	11/101 (10.9)	1.56	ns
History of HSV, n/N (%)	5/48 (10.4)	14/100 (14)	0.74	ns
History of CMV, n/N (%)	6/13 (46.2)	13/135 (9.6)	4.79	.002
History of VZV, n/N (%)	1/25 (4)	18/123 (14.6)	0.27	ns
History of HPV, n/N (%)	5/31 (16.1)	14/117 (12)	1.35	ns
fNKD, n/N (%)	8/91 (8.8)	11/57 (19.3)	0.46	.110
cNKD, n/N (%)	11/57 (19.3)	8/91 (8.8)	2.2	.110
<4% NK cells, n/N (%)	13/78 (16.7)	6/70 (8.6)	1.94	ns
<2% NK cells, n/N (%)	8/37 (21.6)	11/111 (9.9)	2.18	.087
<1% NK cells, n/N (%)	7/17 (41.2)	12/131 (9.2)	4.5	.002
>20% CD56 bright cells, n/N (%)	5/34 (14.7)	14/114 (12.3)	1.2	ns
Solved, n/N (%)	10/29 (34.5)	9/119 (7.6)	4.56	.0005
Unsolved, n/N (%)	6/70 (8.6)	13/78 (16.7)	0.51	ns
Chronic EBV viremia, n/N (%)	3/25 (12)	16/123 (13)	0.92	ns
EBV lymphoproliferative, n/N (%)	1/2 (50)	18/146 (12.3)	4.06	ns
EBV malignancy, n/N (%)	3/3 (100)	16/145 (11)	9.06	ns
EBV HLH, n/N (%)	1/1 (100)	18/147 (12.2)	8.17	ns
EBV dacryoadenitis, n/N (%)	0/1 (0)	19/147 (12.9)	0	ns
HSV viremia, n/N (%)	3/18 (16.7)	16/130 (12.3)	1.35	ns
Recurrent herpetic gingivostomatitis, n/N (%)	0/7 (0)	19/141 (13.5)	0	ns
HSV esophagitis and other disseminated infection, n/N (%)	1/8 (12.5)	18/140 (12.9)	0.97	ns
HSV encephalitis and meningitis, n/N (%)	0/2 (0)	19/146 (13)	0	ns
CMV viremia, n/N (%)	5/9 (55.6)	14/139 (10.1)	5.52	.0019
Congenital CMV, n/N (%)	0/2 (0)	19/146 (13)	0	ns
Disseminated CMV disease, n/N (%)	0/1 (0)	19/147 (12.9)	0	ns
VZV viremia, n/N (%)	0/3 (0)	19/145 (13.1)	0	ns
Varicella pneumonia, n/N (%)	0/1 (0)	19/147 (12.9)	0	ns
Varicella meningoencephalitis, n/N (%)	0/1 (0)	19/147 (12.9)	0	ns
Post herpetic neuralgia, n/N (%)	0/1 (0)	19/147 (12.9)	0	ns
HPV viremia, n/N (%)	2/4 (50)	17/144 (11.8)	4.24	ns
Autoimmune and autoinflammatory, n/N (%)	13/49 (26.5)	6/99 (6.1)	4.38	.0012

IQR, Interquartile range; *ns*, not significant.
*Severe outcome: death, malignancy, HLH, or transplant.
†Comparisons by Fisher exact test if any expected <11 or any 0 or by χ^2 test.

effector-to-target cell ratios, all confirmable on repeated independent occasions, and where this appears to be the main clinically relevant immunodeficiency (Fig E6 in the Online Repository at www.jacionline.org).

Additionally, though our cohort was recruited from various national and international locations, there were higher numbers in places where the program has been based (New York, Texas, and Pennsylvania). This is likely related to the direct dissemination of information from the investigative center, but definitely resulted in access and ascertainment biases. This may also be related to the predominance of children in the cohort, given that the program was partially based at children's hospitals. There was also a predominance of White participants and many in whom self-identified ethnicity was not ascertained, which suggests a need for improvement in referral deficits in rare disease work in future studies.

We found that NKD was observed across the age continuum, with a predominance in children. The infectious disease

prevalence in the cohort suggests that there is an element of the timing of exposure that can affect susceptibility to disease, whether that be at a time that T-cell or humoral defenses are not fully mature or when they may have waned with age. We also note in our study the preponderance of VZV history in women with NKD ($P = .019$ compared with men), something we do not understand at present. Importantly, whereas there are many causes of susceptibility to herpesvirus within the broader set of IEIs, why individuals with impaired NK cell responses have certain types of herpesvirus infections and not others, or specific severities of infection, is unclear. It is also unclear to us why some individuals had more severe systemic infections, whereas others had recurrent localized or recrudescent (ie, zoster) infection. Multivariable analyses of the data from our cohort (not shown) did not yield specific associations of these outcomes with any single characteristic in a statistically significant way. That said, we did find significant differences between patients with fNKD and cNKD. Patients with fNKD had a higher NK cell count, were less likely to have a severe outcome, and were older. Though perhaps more challenging to identify, we continue to suggest a relevance of cNKD and fNKD subsets for the overarching NKD label, both of which still share that the underlying abnormality of NK cell dysfunction is the main clinically relevant immunodeficiency.

NK cells have long been recognized for their critical role in cancer surveillance,[2,3] now with clinical trials supporting their potential as effective cancer immunotherapeutics.[45] Individual case descriptions of NKD have suggested an inherent role for NK cells in control of cancer.[46] Our cohort provides more concrete evidence of increased susceptibility to malignancy, although we could not identify specific cancer risks. Whereas the observed malignancies were predominately hematological, only a few patients had a cancer history at initial presentation; it was generally something that developed later. Given the relatively short follow-up for some individuals (0-16 years, depending on when they were enrolled), we anticipate an increasing cancer incidence. This supports the hypothesis that NK cells play a critical role in cancer cell surveillance. Thus, our findings underscore the importance of vigilant cancer screening and monitoring in patients with NKD (Fig E6).

NK cells are increasingly recognized for their roles in immune regulation and autoimmunity, with extensive support in experimental animal models and correlative human associations.[47] One-third of this cohort had some type of autoimmune or autoinflammatory condition, suggesting a potential role for NK cells in maintaining immune homeostasis. Cytopenias were the most frequent, but numerous other diagnoses were present, and multivariable analyses (not shown) did not identify specific risks. The relatively high incidence in this cohort, however, makes a case for the role of human NK cells in immune regulation. Interestingly, only a few patients had HLH and almost all had NK-IEI genes, emphasizing that deficiency of NK cell cytotoxicity is only 1 component of HLH, and that concomitant deficiency of cytotoxic T-cell killing or inflammatory control mechanisms is likely central to that pathology.

Genetic investigations in phenotypically defined immunodeficiency cohorts have led to revised disease classifications and an increased understanding of new immune mechanisms that could be derived only from laboratory investigation of novel gene candidates. Importantly, the knowledge of these mechanisms has given rise to precision medicine interventions that have been transformative therapies for certain IEIs, which would not have been possible without genetic and mechanistic insights. The association of an immune disease with a causative gene relies on specific criteria for causality.[48] By applying these criteria, we have identified several novel genetic explanations for NKD. Some of these represent new disease associations for the gene, whereas others represent phenotypic expansions of known immunodeficiencies or are known NKD genes. Some of the novel genes identified in individuals from our NKD cohort are NK cell–forward initial presentations for what ultimately were identified in other individuals as broader IEIs. We have listed 3 genes as potentially novel causative NKD genes, which will be reported after peer review of the biological validation. Importantly, these discoveries have expanded our knowledge of NK cell biology and revealed unexpected connections, such as the involvement of multiple genes of the CDC45-MCM-GINS helicase complex in NKD, leading to an understanding of nuances in DNA replication and replication stress in NK cell development.[49] Overall, we identified genetic etiologies in 29% of the cohort, which is comparable to ES solve rates published for other phenotypically defined immunodeficiency cohorts.[35,50] Our ongoing research is focused on the remaining unsolved cases and applying discovery techniques that go beyond ES, as well as identifying additional mechanistic insights.

Collectively, our cohort defines the relevance of NK cells to, and nonredundant roles for NK cells in, human immunity. As established in animal models,[51] there is a specific need for NK cells in some individuals in optimal defense against herpesviruses. The increased incidence of malignancy in the cohort also emphasizes the long-held role that NK cells serve in helping to control the emergence of cancer, and emphasizes early detection in patients with NKD. Although we have made progress in identifying genetic causes of NKD in our cohort, much remains to be uncovered and still learned, and we look forward to further mechanistic discoveries of relevance to these patients and to NK cell biology. We hope our findings will enhance clinical recognition of NKD, leading to improved diagnosis and the development of targeted therapies.

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Clinical implications: NKD should be considered in patients with unusual susceptibility to herpesviruses or papillomaviruses alongside persistently reduced NK cell function; genetic testing should be obtained to identify one of the known causes.

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