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Defective natural killer–cell cytotoxic activity in NFKB2-mutated CVID-like disease

To the Editor:

Common variable immunodeficiency (CVID) is the most common symptomatic primary immunodeficiency characterized by low immunoglobulin serum levels, low vaccine responses, and recurrent infections. The clinical presentation of CVID comprises a variable mixture of recurrent infections, autoimmune phenomena, granulomatous disease, and lymphoproliferation. The underlying genetic mechanisms have been elucidated in the last few years in less than 10% to 15% of the cases and involve mutations in *CD19*, *MS4A1* (CD20), *CR2* (CD21), *ICOS*, *TNFRSF13C*, *TNFRSF13B*, *PLCG2* (phospholipase Cg2), *CD81*, *LRBA*, and *PRKCD* (protein kinase Cd).[1-3] Recently, germline heterozygous mutations in NFKB2 were identified in 10 patients to be associated with early-onset CVID with autoimmunity in most cases,[4,5] profound B-cell deficiency,[6] or a CVID-like phenotype.[7] All affected patients had hypogammaglobulinemia with variable association of the following clinical and immunologic features: central adrenal insufficiency (ACTH insufficiency), alopecia totalis or areata, trachyonychia, variable natural killer (NK) cell numbers, and defects in peripheral T and B cells. We report on a male patient with early-onset CVID who carried the heteroyzgous p.Arg853* mutation in NFKB2 and later developed central adrenal insufficiency (ACTH insufficiency), alopecia totalis, and trachyonychia. An extensive immunologic work-up was performed that, besides confirming the presence of T- and B-cell defects, revealed, as a novel finding, impaired NK-cell cytotoxic activity *in vitro*, despite normal NK cell counts.

The index patient was born to Italian nonrelated parents. Routine laboratory investigation was performed at age 15 months for growth delay and showed profound hypogammaglobulinemia —IgG, 95 mg/dL (normal values for age, 264-1509 mg/dL); IgA, less than 5 mg/dL (normal values for age, 17-178 mg/dL); IgM, less than 5 mg/dL (normal values for age, 48-337 mg/dL)—in the presence of normal peripheral B-cell counts ($CD19^+$, 16.5%). Immunoglobulin replacement treatment was initiated. During follow-up, the patient presented with occasional infections of the upper respiratory tract. He developed alopecia totalis at the age of 4 years, followed by the development of trachyonychia. Endocrinological evaluation following a hypoglycemic episode (blood glycemic level, 26 mg/dL) revealed ACTH and cortisol insufficiency for which he was placed on replacement treatment with hydrocortisone. During adolescence, he developed hypothyroidism and required hormone replacement therapy. On the identification of hypogammaglobulinemia, a more extensive immunologic work-up revealed abnormalities of both T and B cells, namely, an accumulation of T cells in the early stages of differentiation with reduction of the terminal stages of T-cell development, for both CD4 and CD8 subsets, and the typical peripheral B-cell block of CVID with reduction in memory B cells, both switched and IgM memory ones, and lack of plasma cells (Table I). Proliferative responses to mitogens were normal (Table I).

TABLE I. Immunologic profile from the index patient mutated in *NFKB2*

Lymphocyte subsets	Index patient (%)	Normal range for age (%)
T cells ($CD3^+$)	89.8	60.5-79.8
$CD3^+CD4^+$	63.8	30.3-48.3
Naive ($CD45RA^+CCR7^+$)	85.8	34.3-74.6
RTE ($CD45RA^+CCR7^+CD31^+$)	66.5	21.1-63.5
Central memory ($CD45RA^-CCR7^+$)	8.5	13.0-43.5
Effector memory ($CD45RA^-CCR7^-$)	4.4	8.5-28.1
Terminally differentiated ($CD45RA^+CCR7^-$)	1.2	0.7-6.6
$CD3^+CD8^+$	22.3	13.8-37.5
Naive ($CD45RA^+CCR7^+$)	92.0	26.7-72.9
Central memory ($CD45RA^-CCR7^+$)	1.8	1.2-11.6
Effector memory ($CD45RA^-CCR7^-$)	4.0	6.0-53.6
Terminally differentiated ($CD45RA^+CCR7^-$)	2.3	3.9-72.0
TCR γ/δ	1.7	0.5-21.5
B cells ($CD19^+$)	4.8	5.7-19.7
RBE ($CD38^{hi}CD21^{dim/lo}CD27^-$)	31.4	15.0-35.3
Naive ($CD38^{dim/lo}CD21^{hi}CD27^-$)	60.5	33.8-79.6
$CD19^{hi}CD21^{lo}$	1.9	1.1-10
Switched memory (IgD^-CD27^+)	0.4	2.7-20.6
IgM memory (IgD^+CD27^+)	2.0	3.5-24.1
Terminally differentiated ($CD38^{hi}CD27^{hi}CD21^{lo}$)	0.17	0.16-8.70
Plasma cells ($CD38^{hi}CD20^-CD138^+$)	0.00	0.04-3.20
NK cells ($CD3^-CD16^+CD56^+$)	4.5	4.6-27.8

Proliferation (cpm)	Index patient	Healthy control
CD3	227,000	77,000
CD3 + IL2	200,000	152,000
PHA	116,000	120,000
PMA + Ionomycin	418,000	285,000
Background	4,000	7,000

PMA, Phorbol 12-myristate 13-acetate; *RBE*, recent bone marrow emigrants; *RTE*, recent thymic emigrants; *TCR*, T-cell receptor.

On the description of *NFKB2* mutations associated with early-onset CVID, ACTH insufficiency, alopecia totalis, and trachyonichia,[4,5] direct gene sequencing for *NFKB2* was performed for the index patient. A c.2557C>T substitution was found, leading to the non-sense mutation p.Arg853* (Fig 1). This is a *de novo* mutation because the patient's parents were wild type for this mutation. Interestingly, all patients described so far to be mutated in NFKB2, with the exception of 3 family members carrying a missense mutation,[6] carry *de novo* mutations.[4,5,7] The p.Arg853* mutation is disease-causing and was identified in the first description of *NFKB2* mutations in CVID.[4] The index patient here described is the 11th patient to be affected with this genetic defect and the second one to carry the p.Arg853* mutation. Interestingly, the index patient is the first patient with germline *NFKB2* mutation to develop clinically relevant hypothyroidism, underlying the clinical heterogeneity of this disorder. By reviewing the clinical data of the reported patients,[4-7] it is evident that some clinical features are common to all patients, whereas others are sporadic (see Table E1 in this article's Online Repository at www.jacionline.org). Furthermore, the clinical and immunologic phenotype appears more severe in the presence of non-sense[4,5,7] rather than missense[6] mutations in NFKB2. Mutations in nuclear factor kappa B (NF-κB) essential

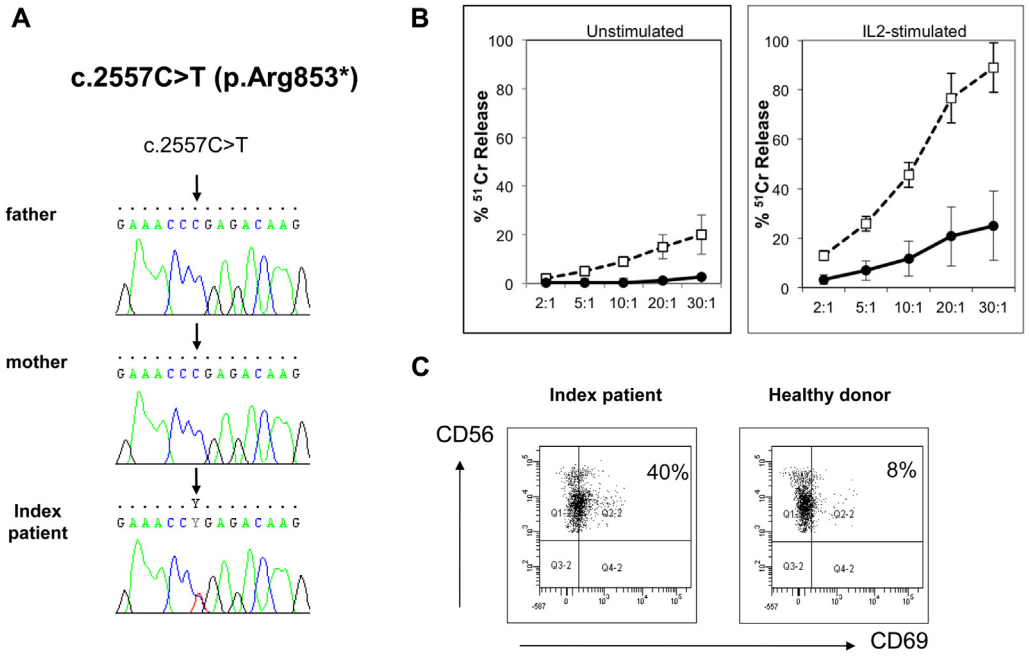

FIG 1. NFKB2 mutation and NK-cell evaluation. **A,** Eletropherograms showing the *de novo* c.2557C>T mutation in NFKB2; the healthy parents resulted wild type for this mutation. **B,** NK-cell cytotoxicity *in vitro* activity from the index patient and a healthy donor. Freshly isolated PBMCs derived from healthy donors (□) and from patients (●) incubated overnight in the presence (IL-2 stimulated) or absence (unstimulated) of hr-IL-2 (100 U/mL) at different E/T ratios were tested against the K562 target cell line. The experiments were performed twice. **C,** Dot plots of CD69 expression on $CD56^{+}CD3^{-}$ gated NK cells from the patient and a representative healthy control.

modifier , a component of the NF-κB cascade, result in a complex form of immunodeficiency with variable clinical and immunologic severity depending on the type of mutations and have been previously reported to result in impaired NK-cell cytotoxicity.[8] Although NF-κB essential modifier is part of the canonical pathway, recent experimental data on NFKB2-mutated mice suggested that there may be overlap in the function of NF-κB members in canonical and noncanonical pathway signaling.[9] Because herpes viral susceptibility and numerical NK-cell abnormalities have been reported in NFKB2-mutated patients,[4,5] we decided to evaluate whether mutations in NFKB2 could also affect NK-cell cytotoxic activity. NK-cell functional evaluation revealed defective NK-cell cytotoxic activity *in vitro*, which could not be restored by the addition of hr-IL-2 (Fig 1, *B*). Then, we further characterized the patient's NK cells by examining the expression pattern of activating and inhibitory NK receptors, including natural cytotoxicity receptors, NKG2D and killer cell immunoglobulin-like receptors molecules, chemokine receptors (CXCR1 and CCR7), and activation marker (CD69) on CD56+ CD3− gated cells by flow cytometry. Patient's NK-cell subsets, both immature ($CD56^{bright}$) and mature ($CD56^{dim}$), showed normal expression of different NK receptors when compared with healthy donors (see Fig E1 in this article's Online Repository at www.jacionline.org). The expression of CD57, an NK-cell maturation marker, and the intracellular levels of perforin in $CD56^{dim}$ $CD16^{+}KIR^{+}$ cells, the more differentiated and cytotoxic NK-cell population, were normal, ruling out a maturational defect (see Fig E1). On the contrary, the expression of CD69 on $CD56^{dim}CD16^{+}$ in patient's NK cells was increased, suggesting an activated steady state of unknown cause (Fig 1, *C*).

Impaired NK-cell activity has been reported in numerous forms of primary immunodeficiencies.[10] This is the first description of impaired NK-cell activity associated with *NFKB2* mutations. These findings broaden the immunologic defects in NFKB2 deficiency, confirm the heterogeneous and complex immunologic and clinical phenotype in disorders in which NF-κB components are defective, and underline an important role for NF-κB in NK-cell cytotoxic activity.

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Prime role of IL-17A in neutrophilia and airway smooth muscle contraction in a house dust mite–induced allergic asthma model

To the Editor:

Asthma is a bronchial inflammatory disease leading to airway hyperresponsiveness (AHR) and reversible airflow obstruction. The incidence of asthma has increased considerably over the past 50 years, with approximately 300 million people affected worldwide.[1] Most of the animal studies have demonstrated that CD4 T_H2 cells are central in the orchestration and amplification of allergic asthma. However, negative results obtained from T_H2-focused human clinical trials have highlighted the importance of considering the large variety of asthma phenotypes.[2] Indeed, human asthma is highly heterogeneous and is not always only due to T_H2-driven airway inflammation.[3,4] Recently, many studies have suggested that IL-17A, a cytokine mainly produced by T_H17 cells, is involved in certain severe cases of asthma by regulating neutrophilic inflammation and steroid resistance.[5] However, support for this theory remains modest because certain reports have demonstrated (1) that IL-17A or T_H17 cells alone are not sufficient to trigger the disease and (2) that, intriguingly, IL-17A can mediate anti-inflammatory mechanisms. Consequently, we aimed to determine the contribution of IL-17A in a mouse model of acute asthma induced by house dust mites (HDMs).

Asthma was induced in mice by percutaneous sensitization and intranasal challenge with an HDM extract (*Dermatophagoïdes farinae*, or Der f; see Fig E1, *A*, in this article's Online Repository at www.jacionline.org). Exposure to Der f resulted in increased airway resistance, notable production of Der f1–specific IgE, and strong cellular infiltration of the airways (Fig E1, *B-D*). Cell subtype analysis by flow cytometry revealed a mixed influx of eosinophils, neutrophils, and lymphocytes (see Fig E2, *A*, in this article's Online Repository at www.jacionline.org). The recruitment of these inflammatory cells was associated with an upregulation of chemokines involved in eosinophil (RANTES and eotaxin) and neutrophil (CXCL1 and CXCL5) attraction (Fig E2, *B* and *C*). Having found increased levels of eosinophils and neutrophils, we investigated T_H2 and T_H17 responses. Elevated levels of the cytokines IL-4, IL-5, IL-13, and IL-17 in bronchoalveolar lavage associated with an expansion of T_H2 and T_H17 cells in lung tissues were found in Der f–induced asthmatic mice, confirming mixed T_H2-T_H17–driven inflammation in our model (Fig E2, *D* and *E*). The levels of IL-6 and IL-1β, which are known to attract and promote the differentiation of T_H17 cells, were increased in the airways of the asthmatic mice 24 hours after the final challenge (Fig E2, *D*). These molecules have also been recently described as specific biomarkers for neutrophilic and mixed granulocytic inflammation in severe asthma.[5]

To investigate the implication of T_H17 in the development of AHR and granulocytic infiltration, intranasal injections of a blocking anti–IL-17 antibody were given during the challenges. Neutralization of IL-17 in Der f–treated mice significantly reduced AHR (Fig 1, *A*) and dampened neutrophil influx in bronchoalveolar lavage (Fig 1, *B*). Interestingly, neutralization of IL-17 decreased the expression of the proneutrophil chemokines CXCL1 and CXCL5 in the airways of the anti–IL-17 antibody–treated mice (Fig 1, *C* and *D*). In contrast, we found similar levels of eosinophils and T_H2 cytokines in untreated and anti–IL-17 antibody–treated asthmatic mice (Fig 1, *D-F*), suggesting no synergic effect between T_H2 and T_H17 responses. Because IL-13 has been shown to be crucial in the induction of asthma symptoms,[6] we evaluated airway inflammation and AHR after IL-13 neutralization. Our results indicated a partial improvement in AHR (see Fig E3, *A*, in this article's Online Repository at www.jacionline.org) but no modification of bronchial inflammation (Fig E3, *B-D*). These results suggest a prime role for the IL-17–neutrophil axis in our model of allergic asthma.

Growing evidence supports an intimate link between neutrophils and severe asthma. However, the contribution of neutrophils to the development of AHR in T_H17-dependent allergic airway disease remains to be elucidated. Consequently, to discover to what extent the complete disappearance of AHR after IL-17A neutralization was due to the decreased neutrophil influx, neutrophils were depleted by administration of anti-Ly6G (Fig 2, *A*). Neutrophil depletion partially decreased AHR in Der f–treated mice (Fig 2, *B*), with no impact on eosinophil levels (Fig 2, *C*), demonstrating that the effect of IL-17A was definitely not mediated only by neutrophils and suggesting a direct role for IL-17 in bronchial contraction. To confirm this hypothesis, we incubated isolated bronchi from control and asthmatic mice with IL-17A for 12 hours and evaluated the contractile force generated in response to methacholine (MCh) or potassium

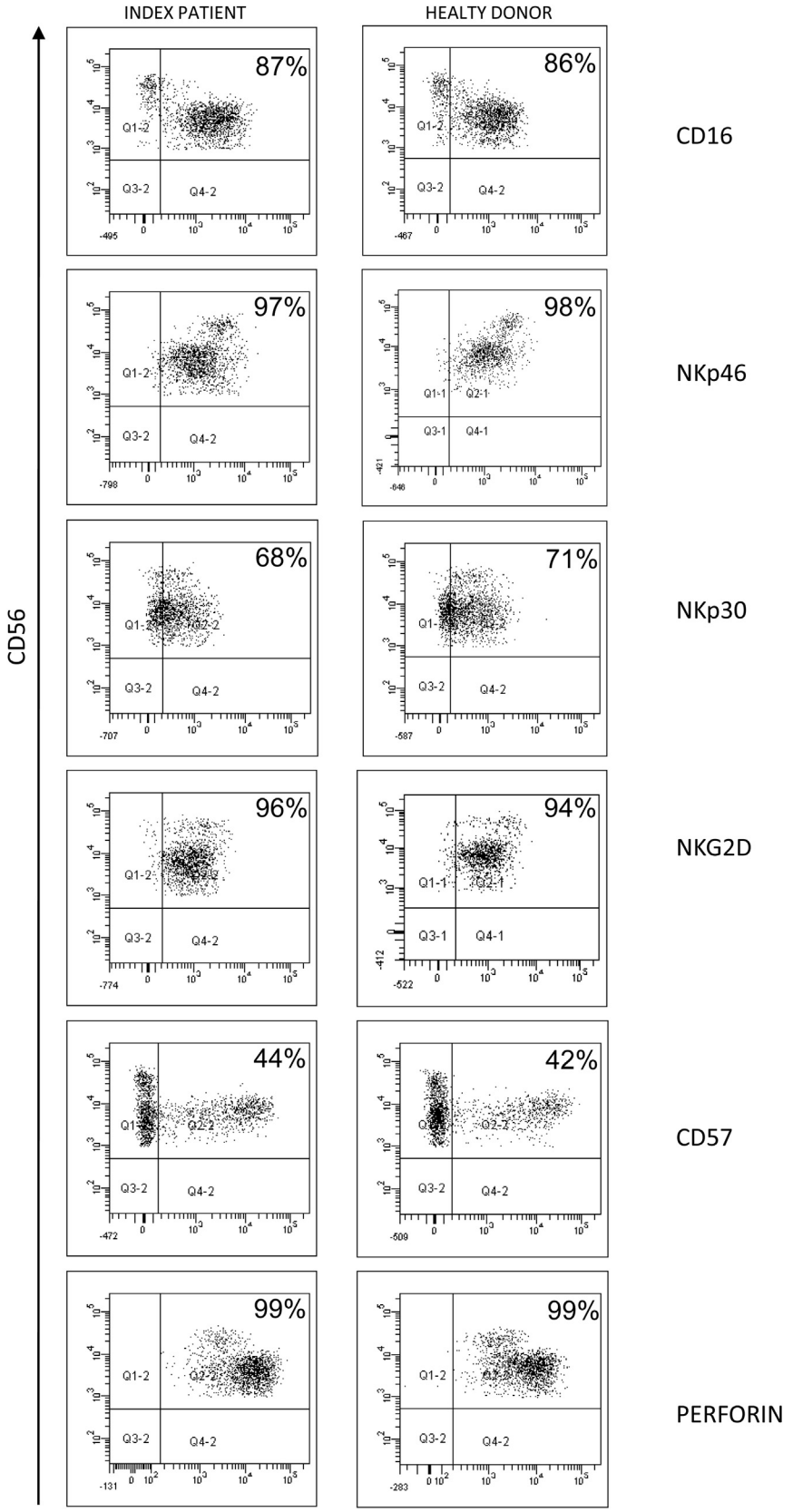

FIG E1. Flow cytometry analysis was performed on CD56+CD3- gated cells derived from patient *(left columns)* and from healthy donor *(right columns)*. Activating NK cell receptors (CD16, NKp46, NKP30, NKG2D), maturation markers of CD56dim NK cell population (CD57 and perforin content), HLA class I specific NK cell receptors (NKG2A, KIR molecules), CD56dim and CD56bright NK cell chemokine receptors (CXCR1 and CCR7 respectively) are shown. The slight differences regarding KIRs molecule expression between the index patient and the healthy donor fall within the physiologic variability.

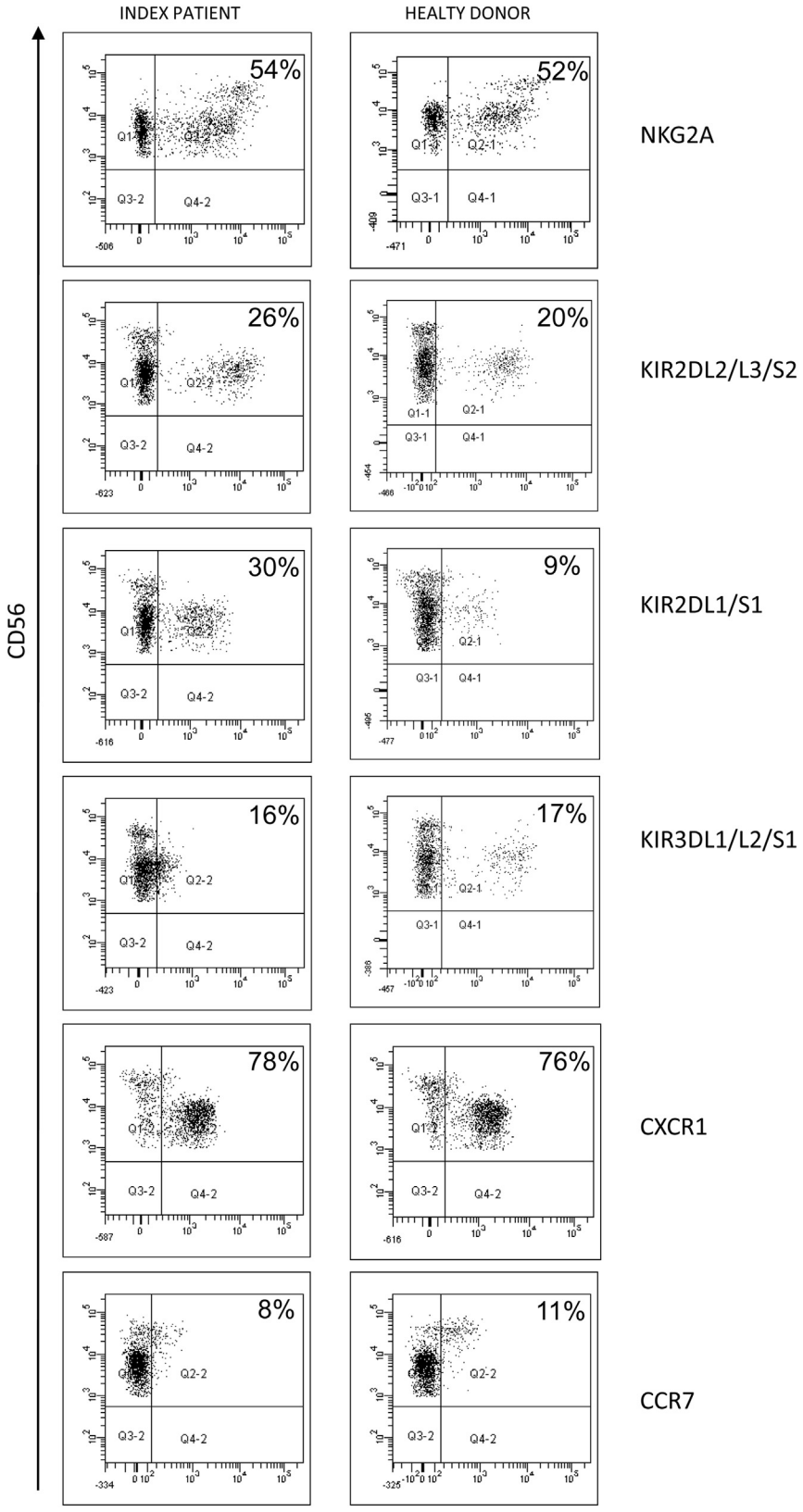

FIG E1. (*Continued*).

TABLE E1. Clinical features of patients with germline *NFKB2* mutations*

Feature	Patient 1*	Patient 2*	Patient 3*	Patient 4*	Patient 5†	Patient 6†	Patient 7‡	Patient 8§	Patient 9§	Patient 10§	Patient 11‖
Sex	Female	Female	Male	Male	Female	Male	Male	Female	Male	Male	Male
Mutation	p.Lys855Serfs*7	p.Lys855Serfs*7	p.Lys855Serfs*7	p.Arg853*	p.Asp865Valfs*17	p.Asp865Valfs*17	p.A867Cfs*19	p.Asp865Gly	p.Asp865Gly	p.Asp865Gly	p.Arg853*
Age at diagnosis	30 y	6 y	3 y	10 y	9 y	7 y	2.5 y	40 y	20 y	Infancy	4 y
URTI	+	+	+	+	+	+	+	+	+	+	+
LRTI	+	−	+	+	+	−	+	+	−	−	−
Recurrent herpes infections	+	+	+	−	−	−	−	−	−	−	−
Alopecia universalis	−	+	−	+	−	−	+	+ (Alopecia areata)	+ (Alopecia areata)	+ (Alopecia areata)	+
ACTH deficiency	+	+	+	+	−	−	−	−	−	−	+
Hypothyroidism	−	−	−	−	−	−		−	−	−	+
Trachyonychia	−	+	−	+	−	−	+	−	−	−	+
Onychomycosis	−	+	−	−	−	−	−	−	−	−	−
Asthma	+	+	+	+	−	−	−	−	−	−	−

In all cases, except for patients 8, 9, and 10, the mutations are *de novo* mutations.
LRTI, Lower respiratory tract infection; *URTI*, upper respiratory tract infection.
*Chen et al.[4]
†Liu et al.[5]
‡Lindsley et al.[6]
§Lee et al.[7]
‖Index patient.