ORIGINAL ARTICLE

A Nonsense N –Terminus NFKB2 Mutation Leading to Haploinsufficiency in a Patient with a Predominantly Antibody Deficiency

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Abstract
The noncanonical NF-κB pathway is implicated in diverse biological and immunological processes. Monoallelic C-terminus loss-of-function and gain-of-function mutations of *NFKB2* have been recently identified as a cause of immunodeficiency manifesting with common variable immunodeficiency (CVID) or combined immunodeficiency (CID) phenotypes. Herein we report a family carrying a heterozygous nonsense mutation in *NFKB2* (c.809G > A, p.W270*). This variant is associated with increased mRNA decay and no mutant NFKB2 protein expression, leading to NFKB2 haploinsufficiency. Our findings demonstrate that bona fide NFKB2 haploinsufficiency, likely caused by mutant mRNA decay and protein instability leading to the transcription and expression of only the wild-type allele, is associated with clinical immunodeficiency, although with incomplete clinical penetrance. Abnormal B cell development, hypogammaglobulinemia, poor antibody response, and abnormal noncanonical (but normal canonical) NF-κB pathway signaling are the immunologic hallmarks of this disease. This adds a third allelic variant to the pathophysiology of NFKB2-mediated immunodeficiency disorders.

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Introduction

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is a major transcription factor which plays an important role in the immune system. The NF-κB family has five members, RelA, RelB, c-Rel, NFKB1-p105/p50, and NFKB2-p100/p52, and they share an N-terminal Rel homology domain (RHD) required for DNA binding and homo/hetero dimerization [1]. NFKB2 is translated as a precursor protein p100, contains an IκB-like-C-terminal portion, and inhibits the nuclear translocation of associated NF-κB members. Proteasome-mediated processing of p100, which is tightly regulated in a signal-induced manner, produces active form of p52 which leads to nuclear translocation of NF-κB complex, predominantly RelB/p52, and regulates target gene expression [2, 3].

Human *NFKB2* gene defects have been shown to be associated with B cell dysregulation in patients with common variable immunodeficiency (CVID) or combined immunodeficiency (CID) [4]. CVID with endocrinopathy and ectodermal

dysplasia and CID phenotypes have been associated with *NFKB2* C-terminus loss-of-function (LOF) mutations, which disrupt the phosphorylation and block the processing of p100 to p52, resulting in cytoplasmic retention of mutant p100 and reduction of p52 expression and nuclear translocation [4, 5]. More recently, our group reported *NFKB2* gain-of-function (GOF) mutations in CID patients with B cell lymphopenia, hypogammaglobulinemia, and multiple severe infections (i.e., *Pneumocystis jirovecii* pneumonitis, EBV, and CVM viremia, among others) with incomplete penetrance [6]. In vitro studies using mutant constructs showed that GOF mutants (E418* and R635*) translocated to the nucleus with RelB in a NF-κB-inducing kinase (NIK)-independent manner, increased CXCL13 gene transcription, and also increased canonical NF-κB activity [6]. Herein, we describe three members of a family carrying a heterozygous nonsense *NFKB2* mutation that causes p100 and p52 haploinsufficiency through mRNA decay and/or protein instability, presenting as predominantly antibody deficiency in one individual (II.2).

Materials and Methods

Human Subjects

All patients or their guardians provided written informed consent in accordance with the Declaration of Helsinki under institutional review board−approved protocols of the National Institute of Allergy and Infectious Diseases, NIH. Blood from healthy donors and patients was obtained under approved protocols, which also allowed for the collection and use of patients' family history and pedigrees for publication. All procedures were based on standard of care, and established clinical guidelines were followed.

Next-Generation Sequencing

For the proband (II.2), whole exome sequencing (WES) was performed using the Ion Torrent AmpliSeq RDY Exome Kit and the Ion Chef and Proton instruments (Life Technologies). For family members (I.1 and II.1), WES was performed using the Nextera DNA Exome Kit and the HiSeq 2500 instrument (Illumina). All WES experiments were performed according to the manufacturer's protocols.

Sanger Sequencing

Sanger sequencing was performed to confirm WES-detected variants and to screen family members as described previously [6]. For *NFKB2* sequencing in cDNA, total RNA was isolated from PBMCs using the RNeasy Plus mini kit (Qiagen) and reverse transcribed by the use of the QuantiTech reverse transcription kit (Qiagen). cDNA was PCR-amplified using GoTaq polymerase (Promega) and exon-specific NFKB2 primers. Forwards: 5'-CTAGACAGAGCCGGGCCTAG-3' or 5'-CTCTAGGCCCCAGGGCCTTA-3'.
; reverse: 5'-CAGGTGGCGCTGTCCCGCCA-3'.

Immunoblotting

Total PBMCs were stimulated with anti-CD3 (OKT3, 1μg/mL; eBioscience; 16–0037-85) for 48 h. Cells were washed with PBS once and lysed in lysis buffer (50 mM Tris pH 7.4, 150 mM NaCl, 2 mM EDTA, 0.5% Triton X-100 and 0.5% NP40 and halt protease and phosphatase inhibitor cocktail [Sigma]), and samples were adjusted to have equal concentration of total protein and subjected to immunoblotting. Cell lysates were prepared and analyzed with anti-phospho-NFKB2 (S866/S870, Cell signaling #4810) and anti-NFKB2 (Cell Signaling Technology #3017) as previously described [6]. For the T cells blasts, total PBMCs were stimulated with anti-CD3 and anti-CD28 (1μg/mL; eBioscience) in the presence of IL-2 (10 ng/ml, Cell Signaling Technology) and cultured for 6–10 days. IL-2 was added every 2–3 days.

Evaluation of Cell Proliferation

PBMCs, either freshly isolated or thawed from liquid nitrogen-stored samples, were incubated with CellTrace Violet Cell Proliferation Kit (1 μM; Invitrogen). After 20 min, 10 volumes of RPMI/10% fetal bovine serum (FBS) were added, and the cells were washed twice with RPMI/10% FBS. 1×10^5 cells were seeded into 96-well plates and stimulated with anti-CD3 antibody and anti-CD28 antibody (1 μg/mL each, eBioscience; 16–0037-85 and 16–0289-85). For B cell proliferation, cells were stimulated with anti-IgM (10 μg/ml, Jackson Lab; 109–006-129), CD40L (100 ng/ml, Enzo; ALX522–110-C010), CpG (0.5 μM, Enzo; ALX 746–006-C100), IL-4 (50 ng/ml, Peprotech; 200–04), and IL-21 (50 ng/ml, Peprotech; 200–21) as indicated in the figure. After incubation for 3–5 days (3 days for T cells and 4–5 days for B cells), cells were stained with fluorochrome-conjugated CD4, CD8, or CD19 (BD Biosciences, Clone RPA-T4, RPA-T8, HIB19 respectively) for 30 min at 4 °C (dark). Cells were washed with PBS twice, and cells were acquired and analyzed by flow cytometry (Becton Dickinson FACSCanto II) and FlowJo software (FlowJo 10.5.2, TreeStar).

Results

Index patient II.2 is a 14-year-old Hispanic boy diagnosed with CVID at the age of 7 years. He was born at term to a young, non-consanguineous family. At the age of 1 month, he presented with bronchiolitis, his first infectious episode. He

Table 1 Clinical and laboratory features in patients with heterozygous *NFKB2* mutation

ID	I.1	II.1	II.2	Reference Value
Age and gender	46y M	17y F	14y M	14-18y / Adults
Lymphocyte phenotype				
CD3%	*86*	*91.6*	66	62–81/60–83.7
CD3/uL	1668	2308	**818**	1090–2600/714–2266
CD4/CD3%	55.1	*62.3*	31	31–53/31.9–62.2
CD4/CD3/uL	1069	*1570*	**384**	560–1450/359–1565
CD4 Naïve %	**4.6**	56.5	44.9	31–57/14–67.3
CD4 Central memory%	63.9	*37.3*	*31.8*	10–27/25.9–64.1
CD4 Effector memory %	*30.9*	**6.1**	19.5	12–44/4.5–29.9
CD4 TEMRA %	0.6	**0.3**	**3.7**	4–12/0.3–7
CD8/CD3%	27	25.8	*31*	19–30/11.2–34.8
CD8/CD3/uL	524	650	384	320–960/178–853
CD8 Naïve %	26.8	*69.1*	50.1	18–61/25.4–72.5
CD8 Central memory%	26.6	7.4	*12.3*	3–12/5.9–39.7
CD8 Effector memory %	30.1	**12.4**	**18.7**	25–58/5.5–33.8
CD8 TEMRA %	16.4	11	18.8	5–20/4.8–32.9
CD19%	7.9	**5.4**	13	6–21/3.3–19.3
CD19/uL	153	**136**	161	140–560/61–321
switched memory B %	19.5	12	**2**	4–16/4.3–19.9
NK cells%	**5.8**	**3**	16	6–23/6.2–34.6
NK cells/uL	**113**	**76**	198	150–700/126–729
Immunoglobulins (mg/dL)				
IgG	1287	n.a	870*	700–1600
IgA	183	n.a	**< 7**	70–400
IgM	80	n.a	**16**	40–230
Vaccine responses				
	n.a	n.a	Protective titers to tetanus toxoid; negative to measles, mumps, rubella, hepatitis A and hepatitis B	
Clinical manifestations				
	Asymptomatic	Asymptomatic	Bronchiolitis, pneumonias, bronchiectasis, and segmental atelectasis	

Numbers in italic/bold indicate values above/below the normal range (respectively) compared with age-matched controls. The patient was placed on IgG replacement therapy without anti-polysaccharide antibody response testing. * on IgG replacement therapy; n.a, data not available. Ranges in pediatric and adult controls are listed on the right, based on published report [15] and NIH (adult) respectively. The T cell subsets are expressed as the percentage among CD4 or CD8; naïve (CD45RA^{+}/CD62L^{+}), central memory (CD45RA^{−}/CD62L^{+}), effector memory (CD45RA^{−}/CD62L^{−}), and TEMRA cells (CD45RA^{+}/CD62L^{−}). Switched memory B cells (CD20+/CD27+/IgM- or IgD-) are expressed as the percentage among CD19 cells

was diagnosed with a left lobar pneumonia with effusion at the age of 2 years and a right lobar pneumonia at age 6 (no organisms identified). He received all scheduled vaccines without complications. Clinical and laboratory immune evaluation at age 7 was relevant for clubbing, difficulty breathing, and a

chest CT scan showing bilateral bronchiectasis and segmental atelectasis. His immunologic laboratory evaluation showed hypogammaglobulinemia involving the 3 major isotypes with IgG 472 mg/dL, IgA < 7 mg/dL, and IgM 21 mg/dL; protective titers to tetanus toxoid, but negative to measles, mumps,

a

c.809G>A
p.W270*
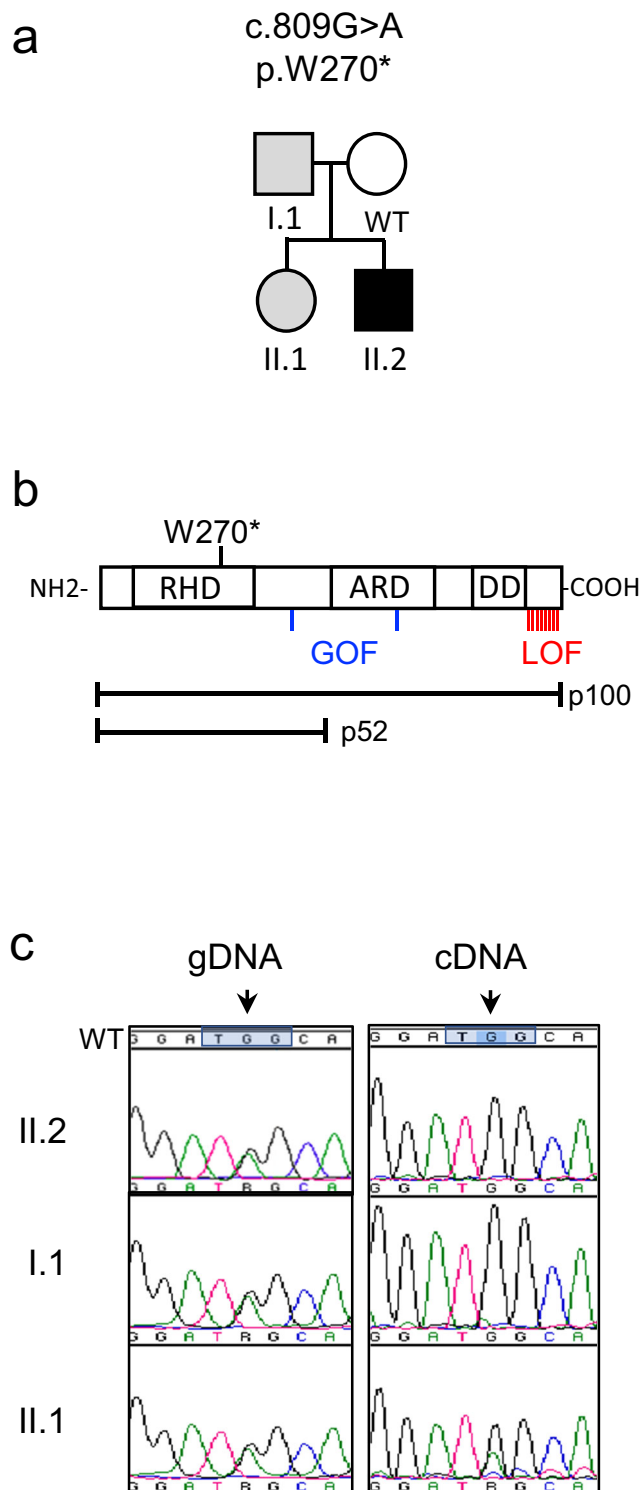
◄ **Fig. 1** Genetic and pedigree of family with *NFKB2* mutation. (**a**) Pedigree showing family members with a germline mutation in *NFKB2*. Affected or asymptomatic mutation positive individuals indicated by filled black color or gray color respectively. (**b**) Schematic representation of the domains of NFKB2-p100 showing Rel homology domain (RHD), ankyrin repeats (ARD), death domain (DD), and location of mutations. Previously reported germline NFKB2 mutations in CVID or CID patients are indicated below the domain. Blue color indicates gain-of-function (GOF; E418* and R635*); C-terminus loss-of-function mutations are shown in red (LOF; R853fs, R853X, D854fs, K855fs, K855X, D865fs, D865G, S866R, S866fs, A867fs, A867V, Y868C, and Q871X). (**c**) Sequence chromatogram of genomic DNA (from whole blood) or complementary DNA (from total PBMCs) from indicated individuals with *NFKB2* mutation. Arrows indicate the position of the mutation

respiratory infection rate. Immune evaluation at age 14 showed similar laboratory results (Table 1). His father (I.1) and sister (II.1) are healthy and clinically asymptomatic.

Whole exome sequence (WES) and/or Sanger confirmation was performed in all family members. A heterozygous nonsense *NFKB2* mutation creating a premature stop codon p.W270* (NM_001077494, c.809G > A, p.W270*) was detected in the proband II.2, his sister II.1, and their father I.1 (Fig. 1a–c, Table 2). This variant was predicted to undergo nonsense-mediated decay by MutationTaster and to be deleterious by the Combined Annotation Dependent Depletion algorithm (CADD GRCh37-v1.4), with CADD phred scores of 37, where CADD_phred scores ≥ 20 predict that a variant is among the 1% most deleterious of all possible substitutions. Variant c.809G > A was not reported in the Genome Aggregation Database (gnomAD; gnomad.broadinstitute.org), a database that generally excludes individuals with severe pediatric diseases.The sequencing data have been deposited with links to BioProject accession number PRJNA655454 in the NCBI BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/).

While NF-κB canonical activation is rapid and independent of protein synthesis, the noncanonical NF-κB pathway is slow and relies on the inducible processing of NFKB2 precursor. Previous reports demonstrated that TCR signaling can stimulate the NF-κB noncanonical pathway, as other well-known agonists (e.g., CD40L, lymphotoxin, RANKL, and BAFF) also do [9]. To induce the activation of the noncanonical NF-κB pathway, PBMCs of all mutation-positive family members, as well as healthy donors (HD), were stimulated with anti-CD3 for 48 h, and NFKB2 protein expression was tested. Immunoblot analysis revealed that PBMCs expressed markedly reduced levels of p100 and p52 as well as phosphorylated NFKB2/p100 upon stimulation, when compared to healthy donor controls. Truncated mutant NFKB2 p.W270* (predicted at 30 kDa) was not detected in the three mutation carriers (Fig. 2a). Due to the different lymphocyte numbers and subsets between the three mutation carriers, T cells blasts were generated to test NFKB2 expression in TCR-expressing/responsive cells. The mutant protein was not detected either in the T cell blasts, similar as in the PBMC experiment.

b

c

rubella, hepatitis A, and hepatitis B; low CD3 and CD4 T cells, with normal CD8 T cell, NK cell, and B cell enumeration; the patient also presented abnormal B cell subsets with low memory B cells (patient 8) [7] and reduced somatic hypermutation (patient 14) [8] as previously reported. Since his diagnosis the patient has been receiving IgG replacement and antibiotic prophylaxis with partial reduction in his

Table 2 Novel or rare variants in immune-related genes in the index patient (II.2) and corresponding carrier status in family members (I.1 and II.1)

dbSNP ID	Gene	Variant	Index (II.2)	ExAC MAF	SIFT	Polyphen2_HDIV	Polyphen2_HVAR	Mutation Taster	CADD	GERP++	SiPhy	SPIDEX	Father (I.1)	Sister (II.1)
	NFKB2	NM_001077494 c.809G>A, p.W270*	het	0.000000	.	.	.	A	37	5.15	18.606	−3.197	het	het
rs758893991	HAVCR1	NM_012206 c.329G>A, p.R110H	het	0.000059	T	P	B	N	15.79	−1.42	7.299	−0.0698	ND	ND
rs770929943	SERPINB9	NM_004155 c.989C>T, p.A330V	het	0.000058	T	B	B	D	17.68	2.87	10.449	.	ND	ND
rs779859900	TSPAN32	NM_139022 c.951_952del, p.G317fs	het	0.000600	.	.	.	P	.	.	.	.	ND	ND
	KMT2A	NM_001197104 c.8120G>A, p.R2707Q	het	0.000000	D	P	P	D	23	5.51	19.614	.	ND	het
	ADCY7	NM_001114 c.1115G>A, p.R372H	het	0.000000	D	D	D	D	34	4.83	17.906	.	ND	ND
rs774425599	NOD2	NM_022162 c.1999G>A, p.E667K	het	0.000033	T	D	D	D	24.1	5.52	14.922	0.011	ND	ND
rs755784920	DSG4	NM_001134453 c.127A>G, p.T43A	het	0.000025	T	D	D	D	21.1	5.32	14.135	0.82	ND	ND
rs772907803	LIG1	NM_000234 c.1340G>A, p.S447N	het	0.000000	T	B	B	D	22.1	4.99	16.218	0.4755	het	het
	LBP	NM_004139 c.588+1G>T	het	0.000000	.	.	.	D	23.7	5.55	14.877	−3.862	ND	ND
	PLCG1	NM_002660 c.2536G>A, p.V846M	het	0.000000	D	D	P	D	26.1	5.82	20.105	.	het	het
rs756470214	DROSHA	NM_013235 c.1883C>T, p.T628M	het	0.000017	D	D	D	D	31	5.88	19.833	−0.959	ND	ND
rs201623844	NFKB2	NM_001077494 c.1962C>T, p.V654V	het	0.0029	.	.	.	D	12.49	4.43	.	0.582	het	het

Novel and rare variant (nonsynonymous, exonic, and splicing mutations) in immune-related genes were filtered with MAF <=0.001 and CADD score >15

A rare NFKB2 variant (MAF <0.01) was also included

ExAC Exome Aggregation Consortium minor allele frequency (MAF) for ALL populations

SIFT: D[eleterious]; T[olerated]

PolyPhen2_HVAR [Mendalian disease] and PolyPhen2_HDIV [complex phenotype]: D probably_damaging; P possibly_damaging; B benign

MutationTaster: D disease_causing per algorithm; A disease_causing per external info; N polymorphism (per algorithm); P polymorphism [per external info]

GERP [Genomic Evolutionary Rate Profiling] score threshold of 2 or greater indicates truly constrained sites

SiPhy [SIte-specific PHYlogenetic analysis] higher scores indicate higher conservation

CADD Whole-genome Combined Annotation-Dependent Depletion [CADD] scores

SPIDEX (delta PSI splicing index Z-score)(Xiong et al., Science 2015) Z score of −2 it is interpreted as −2.5 standard deviations away from the mean

ND indicates not detected

Fig. 2 NFKB2 protein expression in PBMCs and T cell blasts. (a) Total PBMCs from healthy donors (HD) and individuals with NFKB2 mutation were stimulated with anti-CD3 (OKT3, 1ug/ml) for 48h. Cell lysates were prepared and analyzed for the phosphorylation of p100 (S866/870, Cell signaling #4810) and expression of full-length (p100) and processed form (p52) by western blotting (Cell signaling #3017) as previously described [6]. b-actin was used as a loading control. Data shown are representative of two independent experiments. (b) To generate T cell blasts, total PBMCs were stimulated with anti-CD3 and anti-CD28 (1 ug/mL) in the presence of IL-2 (10ng/ml) for 6-10 days. IL-2 was added every 2-3 days. Cell lysates were prepared and analyzed for the full-length (p100) and processed form (p52) of NF-kB by western blotting. b-actin was used as a loading control. Data shown are representative of two independent experiments. The graphs show the relative expression levels of p100 and p52, determined with densitometry and normalized to b-actin protein expression using Image Studio
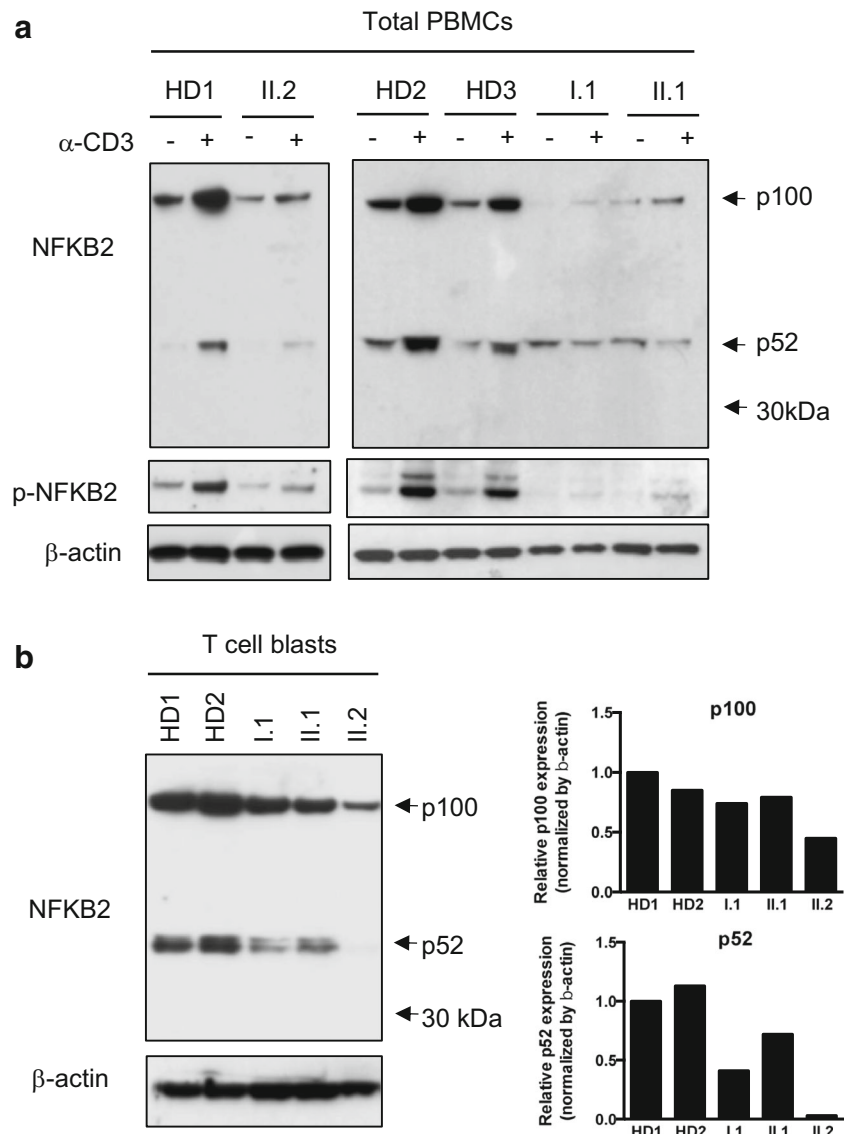
Interestingly, the clinically affected family member (II.2) showed a markedly reduced NFKB2 p100/p52 expression when compared with the asymptomatic mutation carriers or the healthy donor controls (Fig. 2b). Based on the above-mentioned results, we speculated the mutant protein could be unstable, its mRNA subject to decay, or both mechanisms be involved. *NFKB2* mRNA to cDNA conversion and further sequencing revealed that while the affected patient (II.2) and his father's (I.1) cells only transcribed the wild type allele; the sister's (II.1) cells transcribed low/residual amounts of the mutant allele when compared with the wild-type allele (Fig. 1c). To further test the effect of reduced NFKB2 expression on downstream signaling, CXCL13 expression, a chemokine associated with noncanonical NF-κB activation, was measured by real-time PCR assay. Both, patient II.2 and his asymptomatic father I.1 showed markedly reduced CXCL13 expression, suggesting that NFKB2 haploinsufficiency also affects downstream signaling, although it does not correlate with clinical

disease penetrance (Fig. 3; sister II.1 was not tested due to limited sample availability). The canonical NF-κB signaling, which showed increased cytokine generation upon lipopolysaccharide (LPS) stimulation in patients carrying *NFKB2* GOF mutations [6], was comparable with the HD in I.1 and II.2 (Fig. 3b–c).

When T and B cell proliferation were tested in the index patient and his father, normal TCR-induced T cell proliferation and normal B cell proliferation upon T cell-dependent and independent activation were observed, despite the changes observed in their immune phenotype (Table 1) (Fig. 4a–b).

Altogether these results suggest that (a) the mutant mRNA is fully (i.e., I.1 and II.2) or partially (i.e., II.1) unstable and degraded through mechanisms like nonsense-mediated mRNA-decay (NMD) (Fig. 1a) and (b) the transcribed mutant protein, if any, is unstable and undetectable (Fig. 2). Moreover, low NFKB2 expression in the symptomatic patient's T cell blasts (~50% reduction in p100 and > 90% reduction in p52
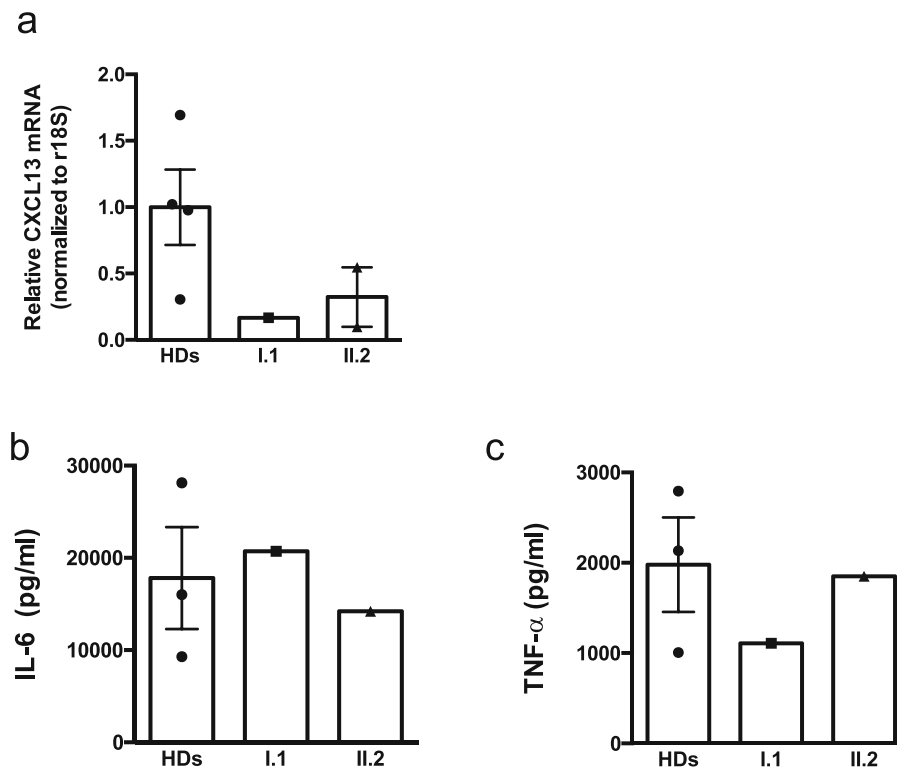
Fig. 3 CXCL13 expression and cytokine generation in patients with *NFKB2* mutation. (**a**) Enriched CD4 T cells were stimulated with Dynabeads-CD3/28 for 48 h. Levels of *CXCL13* mRNA in activated CD4 T cells were measured by real-time PCR using the probe for *CXCL13* and normalized to 18S rRNA as previously described [6]. Data obtained from two independent experiments with two normal controls in each experiment. All data were performed in triplicate and the expression of *CXCL13* in patient samples was relatively quantified compared with the normal controls) compared with the asymptomatic mutation carriers (15–20% reduction in p100 and 30–60% in p52) may help to explain the clinical penetrance correlation, but further research is needed to support this finding. by comparing with the average value of each set of normal controls, with expression levels normalized as 1. Data are shown as means +SEM of four healthy donor controls (HD), I.1 ($n = 1$) and II.2 ($n = 2$). 'n' represents the number of replicates. (**b-c**) Total PBMCs were stimulated with LPS (100 ng/ml) for 24 h. Cytokine expression in supernatants was analyzed by using multiplex bead assay (R&D systems) and Luminex 100/200 according to the manufacture's instruction. Data are shown as means +SEM of three healthy donor controls (HD)

Discussion

In this study we demonstrate NFKB2 haploinsufficiency in a patient with a pediatric onset CVID phenotype. The product of the mutated allele is deficient either because of mRNA decay and/or protein degradation, resulting in reduction of both p100 and p52 NFKB2 protein expression and NFKB2/p100 phosphorylation upon stimulation. This is a different mechanism than *NFKB2* C-terminus LOF mutations originally reported by Chen et al., (so-called p52 haploinsufficiency) [5] or GOF mutations originally reported by our group [6]. While *NFKB2* C-terminus LOF mutations are associated with reduced p52 expression and nuclear translocation due to the failure of phosphorylation and processing of C-terminus-mutated p100; GOF mutations show decreased levels of p100 due to truncated mutant expression leading to increased nuclear translocation of RelB/GOF mutant complexes that in its turn increase the activation of downstream pathways [6]. Moreover, while C-terminal LOF mutations are almost fully penetrant (1/43 asymptomatic) and endocrine defects are common (21/43), GOF and haploinsufficiency mutations are not fully penetrant (1 (or 2)/5 asymptomatic for GOF and 2/3 for haploinsufficiency) and endocrine defects have not been described in neither of these allelic variants [4–6]. It is likely that the C-terminus NFKB2 mutants exert a dominant negative effect on NF-kB signaling by preventing nuclear translocation of associated NF-κB members, that in its turn results in an almost fully penetrant disease.

Of note, somatic NFKB2 gene arrangements producing partially deleted C-terminal domains which lost transcriptional repression activity and facilitated its constitutive transcriptional activity have been shown to be associated with human malignant hematologic diseases [10]. Four cases of malignancies were also reported in patients with germline NFKB2 mutations, one patient with GOF mutation and three in patients with C-terminus LOF mutations, providing evidence that aberrant or constitutive NFKB2 activation disrupts the normal cell function and could lead to cancer development as well as immune dysregulation [4].
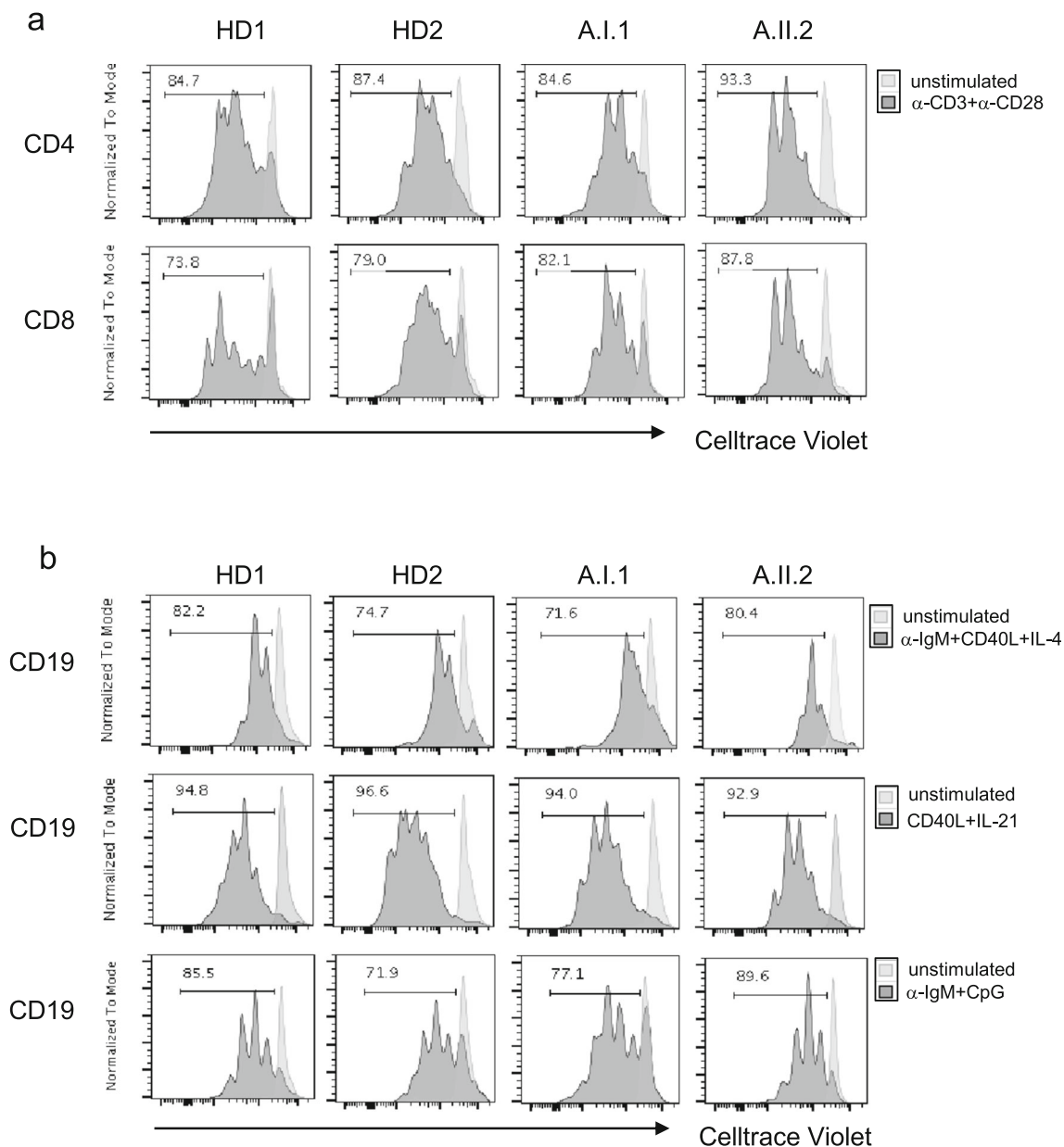
Fig. 4 Proliferation of T and B cells from patients and healthy donor controls. (**a**) Total PBMCs were stained with CellTrace Violet and stimulated with anti-CD3 and anti-CD28 for 3 days. Data are representative of three experiments. (**b**) For B cell proliferation, cells were stimulated with indicated agonists (anti-IgM, CD40L, IL-4, CpG, IL-21) for 4–5 days. Numbers indicate the percentage of cells having undergone at least one cellular division assessed by CellTrace Violet dye dilution. Data are representative of two experiments

CVID is the most common symptomatic phenotype among primary immunodeficiency patients, and monogenic cases account for ~10–20% of them [11]. Several reports demonstrated that the noncanonical NF-κB pathway has a critical role in B cell differentiation and isotype class switching [3, 12] and several variants in noncanonical pathway have been reported in CVID patients such as *BAFFR*, *TACI*, *TRAF3*, *NIK*, and *NFKB2* [4, 11]. Of note, in 2012 patient II.2 was reported to have 2 synonymous *TACI* variants, homozygous p.T27T (rs8072293) and heterozygous p.S277S (rs11078355) [7]. These variants are now considered to be likely benign and

present in high proportions in different healthy control populations (allele frequency 0.81 and 0.49, respectively; gnomAD). Moreover, the asymptomatic father I.1 and sister II.1 in this report carry the same TACI synonymous variants in the same zygosity status as the index patient II.2 and therefore are unlikely associated with disease clinical penetrance or expressivity by themselves. Most of previously reported *NFKB2*-mutated patients have abnormal B cell differentiation with reduced switched memory B cells and some with low NK cell number [4]. Similar as previous findings, low-switched memory B cells and hypogammaglobulinemia were observed in patient II.2

(Table 1), suggesting that B cell development was dysregulated in the patient with NFKB2 haploinsufficiency.

Interestingly, a female patient with a novel heterozygous interstitial 5.54 Mb deletion at 10q24.31-q25.1 was previously reported [13]. This defect involved 90+ genes, including *NFKB2*, and the patient presented at birth with lobar holoprosencephaly, cleft lip and palate, and hypoplastic kidneys, among multiple congenital anomalies. As the clinical and laboratory immunologic phenotype was not part of the original description, and the patient could not be evaluated for our study, the role of *NFKB2* haploinsufficiency in her phenotype remains to be determined. Noteworthy, patients with chromosomal aberrations could provide important clues for the study of primary immunodeficiencies [14].

In summary, NFKB2 haploinsufficiency appears as a novel pathophysiologic mechanism underlying primarily antibody deficiency as CVID, with incomplete clinical penetrance. Our findings extend the genetic, immunologic, and pathophysiology underlying NFKB2-mediated disease, adding a new allelic variant (haploinsufficiency) to the previously reported mechanisms (i.e., C-terminus LOF, and GOF) and contributing to the penetrance-expressivity conundrum associated with these defects.

Compliance with Ethical Standards

Conflict of Interest   The authors have no conflict of interest to disclose.

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