Novel heterozygous *NFKB1* mutation—variable penetrance in a family cohort

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ABSTRACT

Background: Common variable immunodeficiency (CVID) is a term used to define a heterogeneous group of patients who commonly have hypogammaglobulinemia and variable degrees of modest T cell dysfunction. Recent advances made in next generation sequencing technologies have accelerated the identification of CVID disease-causing genes, including *NFKB1*, a component of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway.

Objective: We sought to identify the genetic defect in a 3-generation family of patients with CVID who presented with cytopenias, eczema, and recurrent sinopulmonary infections.

Methods: Whole exome sequencing and Sanger confirmation was performed, and a combination of molecular and cellular techniques used to assess the variant impact on B and T cell function.

Results: A novel heterozygous frameshift mutation in *NFKB1*, encoding the transcriptional regulator protein p50/p105, was identified. This resulted in c.1584dupG (p.M528fs). We demonstrate that c.1584dupG is a loss-of-function variant, responsible for reduced p105/p50 protein expression in affected individuals as well as variable increased $CD21^{low}$ B cell numbers.

Conclusion: This novel mutation affecting *NFKB1* causes a CVID phenotype with variable clinical manifestations. Given the wide spectrum of age in this kindred, this may reflect diversity of phenotype expression, or more probably, age-related expression of typical features.

Statement of novelty: We report here a novel loss-of-function frameshift mutation in *NFKB1*.

Background

The classical response to invading pathogens involves physiological activation of the inflammatory response. Many of the individual, distinct, and complex immunological host defense pathways that make up this response meet at the level of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation, resulting in DNA binding and transcription (or transcriptional regulation) of several key genes. Thus, mutations in genes involved in the canonical and non-canonical NF-κB transcription factor pathways can broadly affect immune function, and have been shown to cause immunodeficiency disorders (Maffucci et al. 2016; Fliegauf and Grimbacher 2018).

NFKB1 encodes p50/p105 (NFKB1), which upon activation undergoes processing through phosphorylation and polyubiquitination of the Ankyrin repeat domain (ANK), and is cleaved into p50. The p50 transcription factor can homodimerize, leading to suppression of gene expression, or heterodimerize with the

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NF-κB class 2 subunits RelA (p65) or c-Rel, triggering gene expression (Lin et al. 2000; Salmerón et al. 2001; Karin and Lin 2002; Bonizzi and Karin 2004; Vallabhapurapu and Karin 2009).

Activation of the canonical NFKB1 pathway by a variety of receptors, including interleukin (IL)-1R, tumor necrosis factor receptor (TNFR), toll-like receptor (TLR), CD40, CD30, RANK, T cell receptor (TCR) and B cell receptor (BCR), mediated by the IκBα and IKK complex, ultimately results in p50/RelA-dependant gene expression. Given the important role of NFKB1 in the cell regulation, aberrations affecting any component of this cascade are likely to have detrimental effects on host defense.

Recently, mutations affecting *NFKB1* were reported in individuals diagnosed with common variable immunodeficiency (CVID), characterized by hypogammaglobulinemia and modest T cell dysfunction. Results of clinical and immune laboratory presentation varied widely between those affected, however, all resulted from perturbed p50/p105 expression and subsequent dysfunction of NFKB1 pathway activation and gene transcription (Fliegauf et al. 2015; Boztug et al. 2016; Kaustio et al. 2017; Lougaris et al. 2017; Tuijnenburg et al. 2018).

In this case series, we describe a 3-generation Irish-Canadian family with autosomal dominant CVID and include details of clinical and immune laboratory phenotype as well as genetic evaluation. Results of whole exome sequencing (WES) revealed a novel heterozygous frameshift mutation in *NFKB1* resulting in loss-of-function.

Methods

Patients

All patient data and samples were obtained in accordance with the Declaration of Helsinki and the Research Ethics Board at The Hospital for Sick Children. Patient data was compiled prospectively and retrospectively from medical records and entered into the Primary Immunodeficiency Registry and Tissue Bank (REB protocol No. 1000005598). Informed consent was obtained for genetic testing, including WES, as well as extensive investigations to unravel the functional effect of immune abnormalities.

DNA isolation and preparation

Genomic DNA was isolated and extracted from peripheral blood lymphocytes (PBL) using the Geneaid genomic DNA extraction kit (Geneaid Mini Kit, ON, Canada), in accordance with the manufacturer's instructions.

Targeted exome sequencing and data analysis

DNA was quantified using the Qubit DNA HS Assay (Life Technologies, Carlsbad, CA, USA) and 100 ng of input DNA subsequently used for library preparation (Ion AmpliSeq Exome Kit; Life Technologies). The exome library was immobilized on Ion PI™ Ion Sphere particles (Ion PI Template OT2 200 Kit v3; Life Technologies). Sequencing was performed utilizing the Ion PI™ Sequencing 200 Kit v3 and Ion PI™ Chip v2 in the Ion Proton semiconductor sequencing system, according to the manufacturer's recommendations (Life Technologies).

Coverage and variant validation

Alignment and variant calling were performed with Torrent Suite (v4.0) on the Ion Proton Server using the Ion Proton AmpliSeq germline low-stringency setting and the hg19 reference genome. The variants were annotated using an in-house annotation pipeline 35 (based on Annovar; November 2014 version), 36 and RefSeq gene models (downloaded from UCSC on 1 August 2015).

Flow cytometry-based immunophenotyping and T cell proliferation assays

B cell phenotypes were determined by flow cytometric analysis using the 4-laser BD analyzer LSR II CFI, BGRV (BD Biosciences, San Jose, CA, USA). Antibodies used included the following: CD19-BV711, CD27-BV421, IgD-PE-Cy7, CD24-BB515, CD38-allophycocyanin, CD21-PE-CF594, CD45RO-PE, CD197-BV421, and CD196-PE-Cy7 (BD Biosciences, San Jose, CA, USA).

Lymphocyte proliferative responses to mitogens, including PHA and the combination of anti-CD3 and anti-CD28 antibodies, and to a panel of recall antigens, including *Candida* species, tetanus toxoid, herpes zoster, and cytomegalovirus, were determined by H-thymidine incorporation (Arpaia et al. 1994). All assays were performed in triplicate and compared with

simultaneously stimulated cells from healthy control subjects.

Western blot analysis

PBL was collected from patients and healthy controls, and T lymphocytes selectively cultured (CD3/CD28 Human T activator Dynabeads; Gibco, Life Technologies, Carlsbad, CA, USA) in the presence of IL-2. Cells were lysed on ice using lysis buffer (1% Triton X-Vanadate) and loaded onto 12% polyacrylamide gel for size fractionation (NuPage 4%–12% Bis-Tris Gel, Invitrogen, Rockford, IL, USA). Proteins were transferred onto polyvinylidene fluoride membrane in accordance with standard Western blotting protocols. p105 and p50 proteins were detected using rabbit anti-NF-κB1 p105/p50 antibody (#3035 Cell Signaling, Danvers, MA, USA; 1:1000 dilution). Anti-rabbit secondary antibody coupled to horseradish peroxidase (7074S, Cell Signaling; Danvers, MA, USA; 1:5000 dilution) was used for signal detection. Immunoreactive bands were visualized with ECL Western Blotting Substrate (ThermoScientific, Life Technologies, Carlsbad, CA, USA).

Serum concentration of immunoglobulins and specific antibodies

Serum concentrations of immunoglobulins (IgG, IgA, and IgM) were measured using nephelometry, and IgE concentrations were measured by using radioimmunoassay with the IgE PRIST kit (Pharmacia Diagnostics, Dorval, QC, Canada). Levels of serum tetanus toxoid and pneumococcus (pneumococcal capsular polysaccharide) were measured by means of ELISA, according to the manufacturer's instructions (Binding Site, Birmingham, UK). Serum antibodies to measles, mumps, and rubella were measured by ELISA with kits available from Euroimmune (Gross-Groenau, Germany). Serum isohemagglutinin titers were determined by means of 2-fold serial dilution with erythrocytes and are reported as the antiglobulin phase, the dilution at which macroscopic agglutination is observed.

Case report

A pedigree of the family members is shown in Figure 1.

Patient 1

Patient 1 was a product of non-consanguineous parents of Irish and French descent. She presented at young adulthood, age 23 years, with recurrent prolonged upper respiratory tract infections (10/year), chest X-ray-proven pneumonias (5/year), chronic sinusitis, and recurrent otitis with purulent otorrhea, fatigue, and weight loss. Her childhood clinical history was unremarkable, with uneventful chickenpox, measles, and mumps infections. She had 1 episode of arthritis at age 2 years and normal echocardiography on examination. A tonsillectomy was performed at 5 years of age.

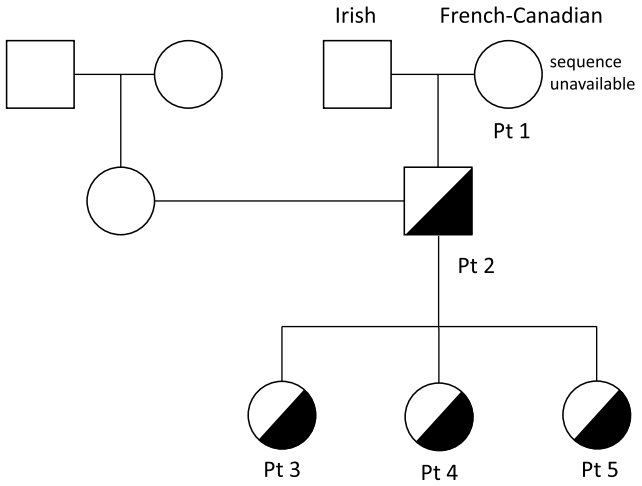

Figure 1: Pedigree of kindred with heterozygous *NFKB1* mutations. DNA sample from patient 1 (Pt 1) was unavailable for sequencing. Patient 2 (Pt 2), son of patient 1, as well as patients 3, 4, and 5 (Pt 3, 4, 5) carry the heterozygous *NFKB1* c.1584dupG, p.M528fs mutation.

During her initial evaluation with our Immunology team at age 26 years, she presented with cylindrical bronchiectasis on bronchoscopy of the left lower lobe and the lingula. She had normal tuberculin test results and sweat chloride levels (7 mEq/L). Immunological evaluation (Table 1) showed pan-hypogammaglobulinemia, with low IgG, IgM, and IgA levels, as well as low anti-tetanus toxoid titer (0.03 ng/mL). IgE levels were normal, as were protein and albumin levels. Her CBC and differential was unremarkable. Lymphocyte counts were normal, however, B cell counts were almost absent with only 46 cells/μL of CD19+ cells alongside decreased NK cell numbers of 53 cells/μL. Assay for spontaneous suppressive cell activity assay was positive, and E-rosette and AET rosette assessment of T lymphocytes were normal at 58% and 70%, respectively. Results of mixed lymphocyte reaction were also normal. Lymphocyte proliferation responses to all mitogens except STA were normal, however, antigen stimulation

Table 1: **Immune work-up of patients.**

	Patient 1 (26 y)	Patient 2 (37 y)	Reference range/control (adult)	Patient 3 (10 y)	Patient 4 (7 y)	Patient 5 (3 y)	Reference range/control (pediatric)
WBC ($\times 10^9$/L)	8325	9730	4370–9600	10 260	10 550	12 900	4000–13 000
Neutrophils ($\times 10^9$/L)	6225	6300	2000–7150	5900	5850	6300	1600–8000
Lymphocytes ($\times 10^9$/L)	1660	2080	1160–3180	2840	3370	4510	1200–5770
Eosinophils ($\times 10^9$/L)	168	410	30–270	80	470	370	30–406
Markers (cells/μL)							
CD3+	1477	1318	700–2100	1755	2644	2927	2000–6900
CD19+	**46**	465	100–500	469	550	1038	700–2500
CD3+/CD4+	856	866	300–1400	1034	1876	1787	1400–5100
CD3+/CD8+	457	432	200–900	629	674	943	600–2200
NK (CD15/56+)	**53**	124	90–600	628	278	487	100–1000
Mitogenic response							
PHA (stim. index)	>450	1076	>450	790	2165	580	>450
Immunoglobulins (g/dL)							
IgG	**0.17**	**2.5**	>7	9	5.9	4.2	>3.2
IgA	**0.34**	<**0.1**	>0.5	**0.3**	0.7	<**0.1**	>0.5
IgM	**0.16**	<**0.1**	>0.5	**0.4**	0.5	**0.4**	>0.5
Isohemagglutinins	NA	A: NA, **B: 4**	—	NA (AB)	NA (AB)	**A: 16**, B: NA	—

Note: Patients 1 and 2 received IVIG for persistent, profound hypogammaglobulinemia. Patients 3, 4, and 5 have protective specific antibodies for non-live vaccines, normal protein, and albumin levels. C, control; NA, not available.

responses to *Candida* and Tuberculin PPD were decreased.

Due to her clinical presentation and diminished immune responses, she was started on fresh frozen plasma (FFP), the only IgG replacement option that was available at the time she was diagnosed. She was eventually transferred to intramuscular and then intravenous immunoglobulin therapy. Laboratory assessments showed adequate IgG replacement. Unfortunately, the patient contracted blood product-related Hepatitis C infection resulting in liver cirrhosis, portal hypertension, massive ascites and varicose veins requiring multiply ligation and sclerotherapy due to bleeding, and eventually liver failure. Other immunodeficiency related clinical manifestations include chronic cough, yellow sputum, Mycoplasma colonization, chronic *Salmonella* gastroenteritis which resolved after 6 months of antibiotic therapy, chronic non-infectious diarrhea, and hepatosplenomegaly.

At the age of 36 years, the patient developed ophthalmic melanoma resulting in loss of vision in the affected eye. She suffered severe bronchiectasis with pyocele 2 years later for which she underwent left lower lobe lobectomy. Concurrently, she slowly developed progressing cerebellar symptoms with abnormal Romberg and heel/toe gait, however, CT imaging of the head was unremarkable.

Following a prolonged course of weight loss, intermittent fevers, and 1 episode of septicaemia, worsening productive cough, failed empiric antibiotic therapy, and increased fatigue, the patient was subsequently evaluated for malignancy. She was diagnosed with lymphoma and later died at age 45 years.

Patient 2

Patient 2, the son of patient 1, was evaluated at 1 year of age and found to have low IgG, IgM, and IgA levels (Table 1). At the age of 10 years he developed chronic immune thrombocytopenia and has been on regular monthly intravenous IgG replacement therapy since age 11 years. He was diagnosed with

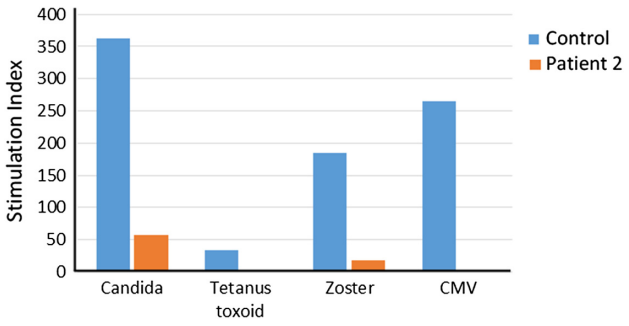

Figure 2: Reduced lymphocyte proliferation responses to antigens. T lymphocyte proliferation responses in patient 2 were reduced when assayed against a panel of recall antigens including Candida, tetanus toxoid, herpes zoster, and cytomegalovirus (CMV).

persistent abdominal lymphadenomegaly, and percutaneous needle aspiration biopsy showed follicular hyperplasia. His CBC and differential were unremarkable, with normal numbers of circulating CD3+, CD4+, CD8+, CD19+ and NK cells. Stimulation of T cells with anti-CD3 and anti-CD28 was markedly reduced, implying a T cell defect. In vitro lymphocyte responses to a series of antigens, including *Candida* species, tetanus toxoid, zoster, and cytomegalovirus, were also extremely low indicating the antigen specific memory T cells were either lacking or present at low frequencies (Figure 2). Assessment of B cell phenotype by flow cytometry showed increased number of IgD+ memory cells, but diminished class switching (CD38+/CD24+) (Figure 3). No transitional B cells and elevated number in CD21^{low} B cells were detected.

Patient 3

Patient 3, daughter of patient 2, is a 10 year old with a history of 3 episodes of chickenpox at ages 3, 5, and 7 years. Given the clinical history of her grandmother and father, live viral vaccines were avoided. Her CBC

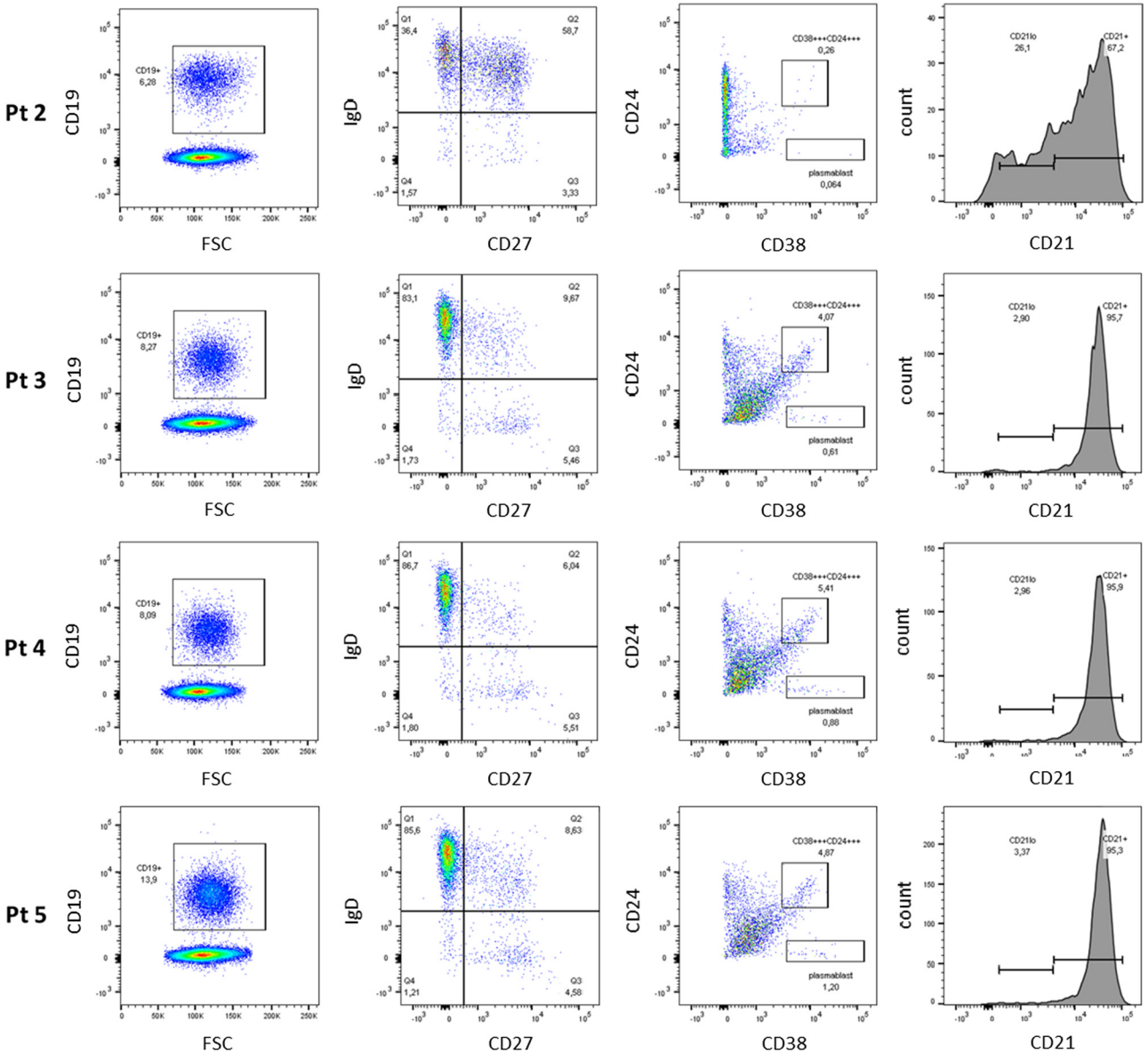

Figure 3: Assessment of class-switched memory B cells. Flow cytometry analysis shows increased number of IgD+ memory B cells, but diminished class-switching (CD38+ CD24+) in Patient 2 (Pt 2). No transitional B cells and increased CD21^{low} and CD27+ IgD+ B cell counts were found. Assessment of B cell phenotype reveals normal age appropriate populations in patients 3, 4, and 5.

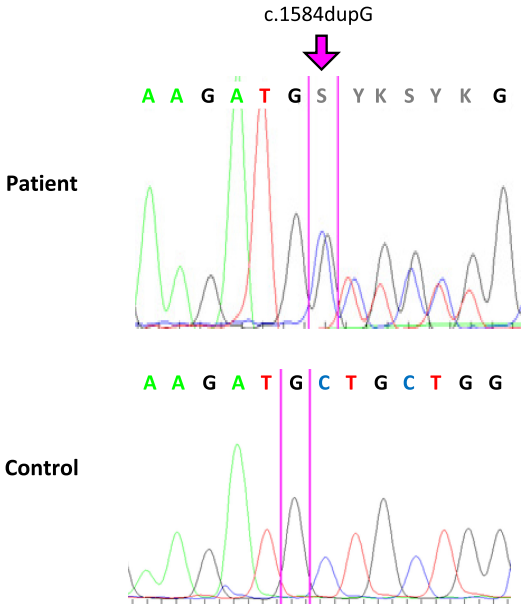

Figure 4: Heterozygous mutation in *NFKB1* in patients with CVID. Electropherogram of the patient sequence (upper panel) shows the heterozygous mutation c.1584dupG, resulting in the frameshift p.M528fs. The wild-type control sequence is shown in the lower panel.

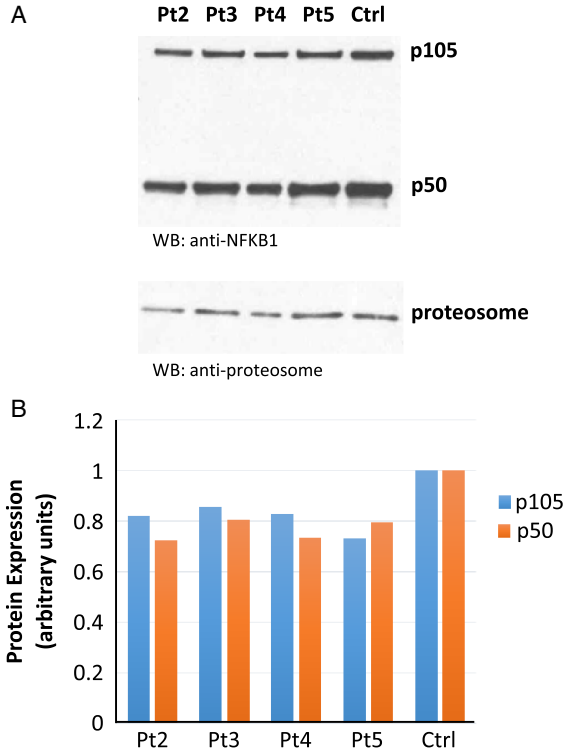

Figure 6: Western blot analysis of NFKB1 expression. (A) Detection of p50/p105 protein levels in patients with the heterozygous p.M528fs mutation compared to control. (B) Densitometric analysis reveals reduced levels of the precursor protein p105 as well as the p50 subunit in patients 2–5. Results are normalized against α-proteosome expression to ensure equal loading.

and differential were unremarkable, with normal B and T cell lymphocyte function. She had normal IgG levels with adequate specific antibody titers, but decreased levels of IgA and IgM. Her varicella zoster virus specific IgG antibody titers were negative. Despite 3 episodes of chickenpox, she was unable to mount an appropriate immune response.

Patient 4

Patient 4, daughter of patient 2, is a 7 year old female. She too suffered 3 episodes of chickenpox. Her immunological evaluations to date have been unremarkable. Like her sister, her varicella zoster virus specific antibody titers were negative.

Patient 5

Patient 5, the youngest sibling of patients 3 and 4, is a 3 year old female with eczema and repeated upper

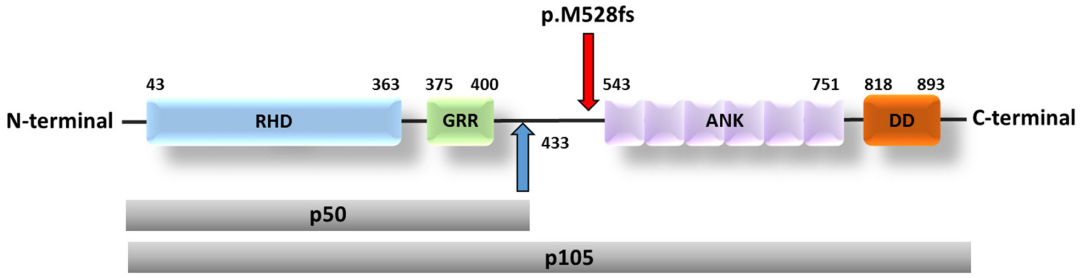

Figure 5: Mutation in NFKB1 is localized upstream of the ankyrin repeat domain. NFKB1 is composed of 4 distinct domains; Rel homology domain (RHD), glycine rich region (GRR), ankyrin repeat domain (ANK), and death domain (DD). Following phosphorylation and polyubiquitination, the p105 protein is cleaved at amino acid 433 to generate p50. The frameshift mutation identified in our patients, p.M528fs, likely leads to protein instability and increased degradation.

Table 2: **Characteristics of patients with reported mutations in NFKB1.**

Domain affected	RHD	RHD	RHD	RHD	RHD	RHD	GRR	HD	ANK
Reference	Kaustio et al. 2017	Fliegauf et al. 2015	Fliegauf et al. 2015	Fliegauf et al. 2015	Kaustio et al. 2017	Boztug et al. 2016	Dieli-Crimi et al. 2018	Lougaris et al. 2017	Kaustio et al. 2017
Heterozygous NFKB1 mutation	H67R	p.Ala156Serfs*12	p.Asp191_Lys244 delinsGlu	p.Lys244_Asp279 delinsAsn	R157X	G165A*31	Gly384Glu*48 (NOD2 His352Arg)	Ala506Valfs*17	I553M
Age of diagnosis	3–44 y	2 y, 20 y—CVID phenotype, 74 y autoimmunity, 44 y—healthy carrier	2–73 y	16 and 64 y, 5 y carrier with transient hypogamaglo-bulinemia, and an additional, 5 y old healthy carrier	56 and 62 y	15 y/NA	34 y	7 y	18 and 36 y
Presenting phenotype	Behcet like phenotype	CVID (2/4)	CVID (10/12)	CVID (2/4)	Necrotising cellulitis—familiar (2/2)	CVID (1/2)	CVID with severe gastro-intestinal manifes-tations	CVID (1/1)	CVID (2/2)
Hypogamma-globulinemia	+ (5/9)	+ (2/4)	+ (12/12)	+ (2/4)	–	2/2	+ (1/1)	+ (1/1)	+ (1/2)
Specific antibody deficiency	+ (5/9)	+ (2/4)	+ (12/12)	+ (2/4)	–	NA	+ (1/1)	+ (1/1)	+ (1/2)
Necrotizing cellulitis	–	–	–	–	+ (2/2)	–	–	–	–
Mouth ulcers	+ (6/9)	–	–	–	–	–	–	–	–
Recurrent arthritis	+ (2/9)	–	–	–	–	+ (1/2)	–	–	+ (2/2)
Recurrent abdominal pain/ colitis	+ (4/9)	–	+ (1/12)	–	–	–	+ (1/1)	+ (1/1)	+ (2/2)
Autoimmunity	–	+ (3/4)	+ (3/12)	2/4	–	+ (1/2)	+ (1/1)	+	+ (2/2)
ITP/AHIA/ neutropenia	–	+ (3/4)	+ (1/12)	1/4	–	+ (1/2)	+ (1/1)	–	–
Alopecia	–	+ (2/4)	+ (1/12)	–	–	–	–	–	–
Asthma	–	–	+ (1/12)	–	–	–	–	–	+ (2/2)
Healthy carrier	–	+ (1/4)	–	1/4	NA	–	–	–	NA
Malignancy	+ (1/9)	+ (1/4)	+ (2/12)	–	–	–	–	+ (1/1)	–

(*continued*)

***Table 2:* (Continued)**

Domain affected	RHD	RHD	RHD	RHD	RHD	RHD	GRR	HD	ANK
Recurrent sinopulmonary infections	+	NA	+ (10/12)	2/4	–	+ (2/2)	+ (1/1)	+ (1/1)	+
Bronchiectasis	NA	+ (1/4)	+ (3/12)	–	–	NA	+ (1/1)	NA	+ (1/2)
Lymphadenopathy	NA	+ (1/4)	+ (1/12)	1/4	–	+	NA	NA	–
Hepatomegaly	NA	–	+ (1/12)	1/4	–	+	–	NA	NA
Effect on NFKB1/ NF-κB pathway	Reduced nuclear localization, increased binding between p50/ NEMO results in impaired nuclear entry	Premature termination, altered, rapidly degrading protein	Rapid degradation p50, haploinsufficiency	p50 haploinsufficiency	Hyperinflammatory reactions, no antibody deficiency, NLRP3-inflammosome mediated macrophage hyperactivation (↑IL-1β)	Decreased p50 levels, impaired p105 phosphorylation	Premature termination, altered protein	Premature termination of translation, aberrant p50	Decrease in S893 and S907 phosphorylation, altered posttranslational processing

Note: Review of literature reported patients with heterozygous NFKB1 mutations (not including Tuijnenburg et al. (2018)). ITP, immune thrombocytopenic purpura; AIHA, autoimmune hemolytic anemia

respiratory tract infection. While her T cell response to phytohemagglutinin was normal, the patient's response to mitogen stimulation with anti-CD3 were decreased. She also had decreased IgA and IgM but normal IgG levels with adequate antibody responses to vaccination.

Identification of NFKB1 mutation

WES was performed on patient 2. We identified a novel heterozygous variant in the gene *NFKB1* (NM_03998.3), c.1584dupG, resulting in p.M528fs (Figures 4 and 5). This was confirmed by Sanger sequencing of patients 2–5 and segregated with disease. The M528fs mutation in *NFKB1* has not been previously reported in dbSNP, ClinVar, or HGMD. Due to unavailability of genetic material, presence of the *NFKB1* variant in patient 1 could not be confirmed.

Signalling evaluation

Western blot evaluation of samples obtained from our patients carrying the novel heterozygous *NFKB1* mutation showed a marked decrease in p50 and p105 protein levels, indicating that this variant is a loss-of-function mutation (Figure 6).

Discussion

The *NFKB1* gene encodes p50/p105, which upon receptor activation undergoes proteosomal processing of the C-terminal to produce p50, a transcription factor that forms, among others, a p50/RelA heterodimer to regulate gene transcription. Heterozygous *NFKB1* mutations are described in the literature with variable clinical and laboratory manifestations (summarized in Tables 2 and 3). The features in these patients vary even within families. The most common features associated with heterozygous *NFKB1* mutations are hypogammaglobulinemia associated with recurrent sinopulmonary infections, as well as autoimmune disorders including immune mediated cytopenias and enteropathy. Some patients suffered benign or malignant lymphoproliferation (Tuijnenburg et al. 2018). In addition, other phenotypes lacking infections, such as necrotizing cellulitis, were associated with distinct mutations in *NFKB1* (Kaustio et al. 2017).

Within our kindred, analysis of B cell populations demonstrated a reduction in class-switched memory B cells and accumulation of $CD21^{low}$ B cells, with a broad range of non-switched memory B cells, and an inability to generate plasma blasts (CD38+/CD27++). The only

Table 3: **Characteristics of patients with reported mutations in NFKB1.**

Domain	RHD	RHD	RHD	RHD	RHD	RHD	RHD	RHD	RHD	RHD	RHD	RHD	RHD + ANK	Connecting loop	ANK	ANK
Heterozygous NFKB1 mutation	c.160-1G>A	c.187 del	c.293 T>A	c.295 C>T	c.260 T>G	c.843 C>G	c.850 C>T	c.835+2T>C	c.830 dup	c.904 dup	c.1005 del	Large del.103370996-103528207	Large del.103436974-103652655	c.1423del	c.1539_1543del	c.1621_1622del
Infections	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+
Autoimmunity	+	+	–	–	–	+	–	+	+	+	+	–	+	–	+	+
Malignancy	+	–	+	–	–	–	–	–	–	+	–	–	+	–	–	–
Hypogammaglobulinemia	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+/–
Necrotizing cellulitis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Mouth ulcers	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Recurrent arthritis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Gastrointestinal involvement	–	–	–	–	–	–	–	+	–	–	+	–	–	+	–	–
Cytopenias	–	+	–	–	–	+	–	–	–	–	–	–	+	–	–	–
Sinopulmonary infections	+	+	+	+	+	+	–	–	–	+	+	+	+	+	–	–
Bronchiectasis	+	–	+	–	+	+	–	–	+	+	+	+	+	+	–	–
Lymphadenopathy	+	–	–	–	–	+	+	–	–	+	–	+	+	–	–	+
Hepatomegaly	–	–	–	–	–	–	+	–	–	–	+	+	–	–	+	–
Splenomegaly	+	+	–	–	–	+	+	+	+	+	+	+	–	–	+	+
CD19+	Normal	Low	Low	Normal	Low	Low	Normal-Low	Low	Low	Low	Normal	Normal	Low	Normal	Normal	Normal-Low

Note: Heterozygous NFKB1 mutations in the European population. PID/CVID patient cohort by Tuijnenburg et al. (2018).

difference between asymptomatic and clinically affected *NFKB1* variant carriers was the increased numbers of $CD21^{low}$ B cell population. However, it is plausible to speculate that changes in B cell subpopulations, specific antibody titres, as well as clinical susceptibility to infections and autoimmunity are age-related.

p50/p105 protein levels were consistently reduced by approximately 40% in symptomatic individuals with CVID (Tuijnenburg et al. 2018). The T cell phenotypic presentation and function was generally unaffected, however decreased NK cell numbers were observed (Dieli-Crimi et al. 2018; Tuijnenburg et al. 2018). T cell proliferation against anti-CD3/anti-CD28 in patients of a European cohort were previously shown to be intact (Tuijnenburg et al. 2018). In our cohort, patient 1 and patient 4 had aberrant T cell proliferation responses to the mitogen CD3.

Conclusion

Mutations that disrupt the function of p105/p50 lead to defects in the canonical NF-κB pathway, resulting in autosomal dominant CVID with highly variable features, even within 1 family. Our kindred represents a perfect example of this diversity, from complete antibody deficiency and malignancy to subtle immune aberrations and clinical manifestations which are predominately age-related. As CVID is known to be predominately an immunodeficiency affecting adults, it is reasonable to speculate that the children in our kindred display, at this stage, only the early signs of CVID.

Abbreviations used

NFKB1	nuclear factor kappa-light-chain-enhancer 1 of activated B cells
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
CVID	common variable immunodeficiency
ANK	ankyrin repeat domain
AD-EDA-ID/ NEMO	autosomal dominant ectodermal dysplasia with immune deficiency
DD	death domain
NLRP3	NLR family pyrin domain containing 3
RHD	Rel homology domain
PMA	Phorbol 12-myristate 13-acetate
SCID	severe combined immunodeficiency
TCR	T cell receptor
TCRvB	T cell receptor v Beta
WES	whole exome sequencing

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