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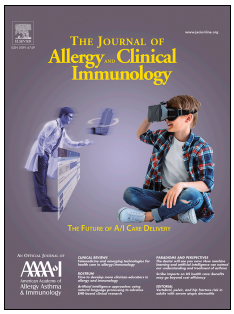

Characterization of the clinical and immunological phenotype and management of 157 individuals with 56 distinct heterozygous *NFKB1* mutations

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PII: S0091-6749(20)30422-X

DOI: https://doi.org/10.1016/j.jaci.2019.11.051

Reference: YMAI 14474

To appear in: *Journal of Allergy and Clinical Immunology*

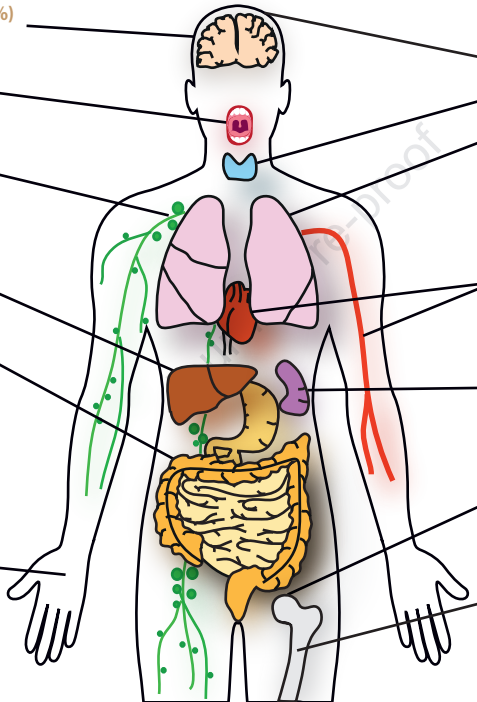
Received Date: 8 July 2019

Revised Date: 4 November 2019

Accepted Date: 11 November 2019

Please cite this article as: Lorenzini T, Fliegauf M, Klammer N, Frede N, Proietti M, Bulashevska A, Camacho-Ordonez N, Varjosalo M, Kinnunen M, de Vries E, van der Meer JWM, Ameratunga R, Roifman CM, Schejter YD, Kobbe R, Hautala T, Atschekzei F, Schmidt RE, Schröder C, Stepensky P, Shadur B, Pedroza LA, van der Flier M, Martínez-Gallo M, Gonzalez-Granado LI, Allende LM, Shcherbina A, Kuzmenko N, Zakharova V, Neves JF, Svec P, Fischer U, Ip W, Bartsch O, Barış S, Klein C, Geha R, Chou J, Alosaimi M, Weintraub L, Boztug K, Hirschmugl T, Dos Santos Vilela MM, Holzinger D, Seidl M, Lougaris V, Plebani A, Alsina L, Piquer-Gibert M, Deyà-Martínez A, Slade CA, Aghamohammadi A, Abolhassani H, Hammarström L, Kuismin O, Helminen M, Allen HL, Thaventhiran JE, Freeman AF, Cook M, Bakhtiar S, Christiansen M, Cunningham-Rundles C, Patel NC, Rae W, Niehues T, Brauer N, Syrjänen J, Seppänen MRJ, Burns SO, Tuijnenburg P, Kuijpers TW, on behalf of the NIHR-BioResource – Rare Diseases Consortium, Warnatz K, Grimbacher B, Characterization of the clinical and immunological phenotype and management of 157 individuals with 56 distinct heterozygous *NFKB1* mutations, *Journal of Allergy and Clinical Immunology* (2020), doi: https://doi.org/10.1016/j.jaci.2019.11.051.

"The phenotype of NFKB1 insufficiency"



Neurological complications (13.9%)
Non-infectious fever (12.0%)

Aphthous ulcerations (17.8%)

Lymphoproliferation:
- Lymphadenopathy (35.3%)

Liver:
- Hepatomegaly (24.7%)
- Liver disease (19.5%)

Gastrointestinal involvement:
- Gastrointestinal infections (28.6%)
  - Autoimmune enteropathy (13.9%)
  - Celiac-like disease (9.3%)
  - IBD-like disease (5.6%)
- Diarrhea of unknown etiology (8.3%)
- Atrophic gastritis (4.6%)

Skin:
- Skin infections (37.7%)
- Rosacea
- Autoimmune (14.9%)
- Psoriasis
- Eczema
- Necrotizing fasciitis

Malignancies (16.8%):
- Lymphoma (11.1%)
- Solid organ cancer (4.6%)

Alopecia

Thyroiditis (6.5%)

Respiratory system:
- Upper respiratory tract infections (83.0%)
- Pneumonia (59.0%)
- Bronchiectasis (25.6%)
- Granulomatous-lymphocytic interstitial lung disease (GLILD) (7.4%)

Cardiovascular system:
- Cardiovascular complications (17.8%)
- Behçet's disease (5.6%)
- Vasculitis (4.6%)

Spleen:
- Splenomegaly (48.5%)
- Splenectomy (11.9%)

Bone/Joints:
- Osteopenia (12.9%)
- Arthritis (10.3%)
- Enthesiopathy

Bone marrow:
- Antibody deficiency (88.9%)
  - Low IgA (87.4%)
  - Low IgG (74.4%)
  - Low IgM (70.9%)
- Cytopenia (43.9%)

Characterization of the clinical and immunological phenotype and management of 157 individuals with 56 distinct heterozygous *NFKB1* mutations

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Acknowledgements and conflict of interest statement

We thank all patients and their families for participation in this study. We are grateful to Mary Buchta, Katrin Hübscher, Pavla Mrovecova and Jessica Rojas-Restrepo for their excellent technical assistance. We are grateful to Francisco Bonilla, Isil B. Barlan, Katharine Halligan, Andrew MacGinnitie, and Anna Simon for their contribution to the patient recruitment process, and Katharina Thoma for helping with the graphical abstract.
This study was supported by the German Research Foundation (DFG) (DFG; SFB1160 – IMPATH) to B.G., and under Germany's Excellence Strategy (CIBSS - EXC-2189 - Project ID 390939984 and RESIST – EXC 2155 – Project ID 39087428), by the E-rare program of the EU, managed by the DFG, grant code GR1617/14-1/iPAD; by the „Netzwerke Seltener Erkrankungen“ of the German Ministry of Education and Research (BMBF), grant code: GAIN_ 01GM1910A, and by research funding from the German Ministry of Education and Research (BMBF, grants # 01E01303 and 01ZX1306F). Some samples have been taken from the CCI-biobank, a partner of the Freeze Biobank Freiburg.
All authors declare that there is no conflict of interest.

Abstract

BACKGROUND: An increasing number of *NFKB1* variants are being identified in patients with heterogeneous immunological phenotypes.

OBJECTIVE: We set out to characterize the clinical and cellular phenotype as well as the management of patients with heterozygous *NFKB1* mutations.

METHODS: In a world-wide collaborative effort, we evaluated 231 individuals harboring 105 distinct heterozygous *NFKB1* variants. To provide evidence for pathogenicity, each variant was assessed *in silico*; additionally, 32 variants were assessed by functional *in vitro* testing of NF-κB signaling.

RESULTS: We classified 56 of the 105 distinct *NFKB1* variants in 157 individuals from 68 unrelated families as pathogenic. Incomplete clinical penetrance (70%) and age-dependent severity of *NFKB1*-related phenotypes were observed. The phenotype included hypogammaglobulinemia (88·9%), reduced switched memory B cells (60·3%), and respiratory (83%) and gastrointestinal (28·6%) infections, thus characterizing the disorder as primary immunodeficiency. However, the high frequency of autoimmunity (57·4%), lymphoproliferation (52·4%), non-infectious enteropathy (23·1%), opportunistic infections (15·7%), autoinflammation (29·6%), and malignancy (16·8%) identified NF-κB1-related disease as an inborn error of immunity with immune dysregulation, rather than a mere primary immunodeficiency. Current treatment includes immunoglobulin replacement and immunosuppressive agents.

CONCLUSION: We present a comprehensive clinical overview of the NF-κB1-related phenotype, which includes immunodeficiency, autoimmunity, autoinflammation, and cancer. Due to its multi-system involvement, clinicians from each and every medical discipline need to be made aware of this autosomal-dominant disease. Hematopoietic stem cell transplantation and NF-κB1 pathway-targeted therapeutic strategies should be considered in the future.

Clinical implications

The aim of this work is to aid diagnosis, management, and treatment of patients with *NFKB1* mutations. Clinical features, complications, current treatment options, and future targeted therapeutic strategies are illustrated.

Capsule Summary

We describe the clinical and immunological features of the to date largest cohort of patients with deleterious heterozygous *NFKB1* mutations. To provide evidence for pathogenicity, we used a combined *in silico* and *in vitro* approach.

Key words

NFKB1 variants and mutations, common variable immunodeficiency, reduced penetrance, variable expressivity, autosomal dominant inheritance.

Abbreviations

ARD, ankyrin repeat domain

Bcl-3, B cell leukemia 3 protein

BAFFR, B-cell activating factor receptor

CMV, cytomegalovirus

CT, computed tomography

CTLA-4, cytotoxic T lymphocyte antigen 4

CVID, common variable immunodeficiency

EBV, Epstein-Barr virus

ESID, European Society for Immunodeficiencies

GFP, green fluorescent protein

GLILD, granulomatous-lymphocytic interstitial lung disease

HAV, hepatitis A virus

HEK293T, human embryonic kidney 293T

HSCT, hematopoietic stem cell transplantation

IBD, inflammatory bowel disease

IκBα, NF-kappa-B inhibitor alpha

JC virus: John Cunningham virus

MAC, Mycobacterium avium complex

NF-κB, nuclear factor of kappa light polypeptide gene enhancer in B cells

NK, natural killer

NLS, nuclear localization signal

PFT, pulmonary function test

RHD, Rel homology domain

PMA, phorbol myristate acetate

PML, progressive multifocal leukoencephalopathy

WT, wild-type

INTRODUCTION

The NF-κB (nuclear factor of kappa light polypeptide gene enhancer in B cells) signaling pathway has been implicated in several biological processes, including cell survival and proliferation, inflammation, and the adaptive immune response[1]. Its activation in lymphocytes is triggered by antigens, molecular patterns, and cytokines. NF-κB transcription factors can form various homo- or heterodimers containing the following five subunits: NF-κB1 (also known as p105 which is processed to p50), NF-κB2 (also known as p100 processed to p52), RelA, RelB, and c-Rel.

In unstimulated cells, p50 predominantly assembles with RelA, and remains inactive in the cytoplasm when complexed with the inhibitor NF-kappa-B alpha (IκBα). Upon stimulation of the canonical (NF-κB1) pathway, the inhibitory IκBα protein is phosphorylated and degraded by the 26S proteasome, thereby releasing the active transcription factor heterodimer p50-RelA, which enters the nucleus and regulates the expression of its target genes. The non-canonical (NF-κB2) pathway is activated following the engagement of a small group of receptors such as the B-cell activating factor receptor (BAFFR) and CD40; this leads to proteasomal processing of p100 to generate p52, which preferentially pairs with RelB. The p52/RelB complex is mainly involved in B-cell survival and activation[2].

The *NFKB1* gene (MIM: 164011) encodes the precursor p105, which is co-translationally processed into the transcriptionally-active p50 subunit[3]. Heterozygous *NFKB1* mutations causing p50 haploinsufficiency have previously been associated with common variable immunodeficiency (CVID12 [MIM: 616576])[4], autoinflammatory, and rheumatologic features such as Behçet's disease[5], EBV-driven lymphoproliferation[6,7], severe gastrointestinal manifestations[8], and susceptibility to opportunistic and viral infections[9,10]. However, these reports only covered a few cases each, and an overview and understanding of the broader clinical spectrum of this NF-κB1-related condition is still lacking.

In a world-wide collaborative effort, we identified 231 individuals harboring 105 distinct heterozygous *NFKB1* variants (Fig. S1). Sequence variants were classified into pathogenicity categories based on genetic and molecular criteria (Table S1). Here we describe the clinical and immunological features of the largest to date cohort of patients (n=157) with 56 distinct *NFKB1* mutations (Table S2 and S3).

METHODS

We analyzed 105 heterozygous *NFKB1* variants, identified in 231 individuals, from 129 unrelated families. For each variant, the following criteria were assessed: the predicted effect on the resulting protein (haploinsufficiency mutations, precursor skipping mutations, missense variants affecting the p105 precursor and the mature p50, and missense variants probably affecting only the functions of the precursor), its localization in a functional domain of NF-κB1, the allele frequency in the Exome Aggregation Consortium data set, supportive functional studies, and the inheritance and segregation data (Table S1). Detailed clinical and laboratory data can be found in Table S3. Multiple *in silico* tools (PolyPhen 2, Sorting Intolerant From Tolerant, Combined Annotation Dependent Depletion, Mutation Taster) have been used to predict the impact of missense changes. Functional assays evaluated the p105 and/or p50 levels in peripheral blood mononuclear leukocytes, neutrophils, or GFP-fused p105 and/or p50 in transfected HEK293T cells by Western blotting. In addition, we determined the nuclear localization and transcriptional activating function in HEK293T cells following transfection of selected GFP-fused p105 and/or p50-like mutant proteins by fluorescence microscopy and by using an NF-κB-responsive fluorescence-based reporter assay or a dual luciferase reporter assay, respectively (Table S4).

RESULTS

Genetic and functional assays

Of 105 variants, our combined *in vitro* and *in silico* assessment identified 56 distinct variants in 157 patients from 68 unrelated kindred as damaging. Of these 56 mutations, 28 have already been described[4,5,7–13], while 28 are novel. Thirty-four variants of the 56 mutations were located in the Rel homology domain (RHD), 17 in the central part of p105, while three affected the ankyrin repeat domain (ARD); in addition, two large deletions were identified (Fig. 1).

Generally, a haploinsufficiency mutation may either cause the lack of expression of the respective allele, or the expression of a severely-truncated protein, that rapidly undergoes decay. As expected, the novel variant p.Ser338Leufs*94 revealed that the mutant p105 and p50 had reduced fluorescence intensity and

aberrant localization, whereas wild-type (WT) p105 localized to the cytoplasm and WT p50 to the nucleus (Fig. 2A and Table S4).

Precursor-skipping *NFKB1* mutations affect the central part of p105. These truncating mutations cause a lack of p105, but lead to the expression of a p50-like protein. Upon transfection of four mutant GFP-fused constructs, p50-like proteins localized to the nucleus and were indistinguishable from WT p50, potentially interfering with target gene transcription (Fig. 2A and Table S4).

Transfection of 12 GFP-fused missense variants of p105 (Fig. 2A and B) revealed normal expression and cytoplasmic localization of the full-length p105, but one of the tested variants (p.Ile87Ser) showed a reduced fluorescence intensity in the cytoplasm and an abnormal accumulation of the signal in high intensity spots. After stimulation with Phorbol myristate acetate (PMA)/Ionomycin, a marginal increase in nuclear fluorescence was observed in cells transfected with WT p105, indicating increased processing to p50 (Fig. 2B). In contrast, the p.Ile87Ser mutant p105 was associated with cytoplasmic clumping upon stimulation, indicating accelerated decay (Fig. 2B). Accordingly, Western blot analysis showed a reduced expression of the mutant p.Ile87Ser in transfected cells (Fig. 2C). In luciferase reporter assays, two of the missense mutations (p.Arg57Cys and p.Ile87Ser, both located in the N-terminal part of the RHD) showed reduced promoter activation (Fig. 2D). In agreement with the ACMG classification, the remaining missense variants might only cause subtle rather than deleterious effects (Table S4).

Patient characteristics

Among the 157 mutation carriers, 121 were classified as affected, while 36 were considered healthy (Table S2). The median age of the whole cohort at the time of evaluation (June 2018) was 38 years (range 6 months-79 years). The median age of healthy subjects (21·5 years) was lower than that of affected patients (39 years) ($P<0.001$) (Fig. 3A). As genetic screening could not be performed in all first-degree relatives of the affected patients, clinical penetrance was estimated to be 70% (Fig. S2). We found an increasing age-dependent penetrance (76·7% in individuals aged ≥10 years, 85·7% in individuals aged ≥30 years and 100%

in individuals aged ≥ 60 years), suggesting that the disease may manifest over time rather than having a *bona fide* reduced penetrance.

The median age at which the first characteristic clinical manifestation occurred (mostly infections, autoimmune manifestations, and inflammatory symptoms), was 12 years (mean 17·2 years; range birth-69 years). The median age at NF-κB1-related disease diagnosis was 23 years (mean 27·1 years; range 1 month-73 years). Primary diagnoses at the time of disease-onset were predominantly antibody deficiency (89·5%), diseases primarily characterized by autoimmunity (57·4%) and immune dysregulation (17·8%), and autoinflammatory disorders including Behçet's disease (5·6%). The median follow-up time was 9 years (mean 11·7 years; range 0 - 50 years).

At the time of clinical data analysis (June 2018), 17·1% of patients of the affected carriers were deceased (Fig. 3B). Mortality rates were higher among males (21·5%) than females (12·9%) (P=0.22). Death occurred at a median age of 52 years (range 35-78). The most frequent causes of death were infections on the background of a chronic illness (12/20), and complications from malignancies (5/20).

Respiratory involvement

Upper respiratory tract infections occurred in 83% of the 106 affected mutation carriers with definite mutations in NFkB1, on whom we had clinical data: 59·8% had sinusitis, 30·4% otitis, and 16·7% pharyngotonsillitis. Chronic sinusitis and nasal polyps led to sinus surgery in 6·9% of patients (Figure 4A and C), while recurrent otitis was treated with tympanostomy tube placement in 5·9% of patients. Lower respiratory tract infections presented as pneumonia in 59% of patients and as bronchitis in 41·7% (Fig. 5A). Recurrent pneumonia (>3 episodes during observation period) occurred in 24·2% of patients with any pneumonia; in 4·9% of patients, lung infection was complicated by pleural empyema. In 39·6% of patients, chronic lung disease was confirmed by pathological pulmonary function test (PFT) and/or the detection of structural abnormalities by radiology or lung biopsy, and was associated with reduced survival (P=0·003) (Fig. 4E-J and R). Patients with a history of pneumonia were at increased risk of developing lung disease (OR 8·9; 95% CI: 3·1-25·9; P<0·001). Bronchiectasis was detected by computed tomography (CT) in 25·6% of

patients (Fig. 4G and 5A). Interstitial lung disease had both granulomatous and interstitial histological patterns (granulomatous-lymphocytic interstitial lung disease, GLILD) in 7·4% of patients (Fig. 4E and 5A), while lung fibrosis and granuloma were detected in 6·4% and 3·2%, respectively. Five patients had pulmonary surgery, three patients with bronchiectasis underwent lobectomy, but one pneumonectomy was complicated by empyema, the remaining two patients with empyema had lung decortication.

Gastrointestinal involvement

Diverse gastrointestinal involvement was observed in 54·2% of patients (Fig. 5A). Gastrointestinal infections occurred in 28·6% (n=30) of patients. The histopathological analysis of gastrointestinal biopsies, obtained in 28% of the 107 patients, revealed Herpes esophagitis (1·9%), eosinophilic esophagitis (0·9%), celiac-like disease (9·3%), chronic enteropathy mimicking inflammatory bowel disease (5·6%), lymphocytic or collagenous colitis (4·6%), and cytomegalovirus (CMV) colitis (0·9%). In 8·3% of patients, no cause for chronic diarrhea was identified, despite extensive fecal examination and normal or non-specific intestinal biopsies. The liver was involved in 24·1% of patients, with cirrhosis (3·7%), nodular regenerative hyperplasia (4·6%), hepatic hemangioma (3·7%) and hepatitis (7·5%) being the major pathologies. Hepatitis was classified as autoimmune in three patients (Fig. 4 K-N), as drug-related in three patients or virally-induced in two patients (hepatitis A virus-related and hepatitis C virus-related in AD.I.1 and C.II.5, respectively).

Autoimmunity and immune dysregulation

Autoimmune conditions affected 57·4% of patients (Fig. 5A). The most common autoimmune conditions were cytopenia (43·9%), enteropathy (13·9%), skin disease (14·9%, Supplementary appendix 2.II), arthritis (10·3%), thyroiditis (6·5%), vasculitis (4·6%), hepatitis (2·8%), pernicious anemia (2·8%), type I diabetes (1·8%) and Addison's disease (0·9%) (Fig. 5A). Autoantibodies against red blood cells or granulocytes were detected in 17·6% of patients. Subjects with autoimmune cytopenia were more likely to have lymphoproliferation (OR 41·2; 95% CI: 12·3-137·6; P<0·001), splenomegaly (OR 36·4; 95% CI: 12-111; P<0·001) or interstitial lung disease (OR 8·3; 95% CI: 0·9-72·2; P=0·05).

Lymphoproliferation and malignancies

Splenomegaly, lymphadenopathy, and hepatomegaly were detected by clinical assessment or ultrasonography in 48·5%, 35·3% and 24·7% of patients respectively (Fig. 4B and D, and 5A). Generalized expansion of the lymphoid compartment was associated with lung, liver and gastrointestinal tract infiltration (OR 9·3; 95% CI: 2·5-34·2; P<0·001). Malignancies occurred in 18 of 107 (16·8%) of patients (Fig. 5A); non-Hodgkin B-cell lymphomas were the most common, 8 patients (7·5%). Solid organ cancer occurred in five patients (4·6% of all patients, including skin, lung and cervical cancer). The median age at diagnosis of cancer was 46 years (range 11-77). Death, primarily cancer-related or secondary to sepsis, occurred in 41·2% of patients with malignancies. Langerhans cell histiocytosis occurred in one child (AF.II.1).

Types of infections

Pathogenic bacteria, viruses and fungi were identified in 53·7%, 25%, and 12% of patients, respectively (Fig. 5B). Bacteria were isolated from expectorated sputum samples in 31.5% of patients, with the most common being *Haemophilus influenzae* (23·1%), *Streptococcus* species (17·6%), *Moraxella catarrhalis* (5·6%), or *Pseudomonas* species (4·6%). Stool cultures were positive in 18·5% of patients, with *Clostridium difficile* (6·5%), *Salmonella* species (5·6%) and *Campylobacter jejuni* (3·7%). Ten patients with *NFKB1* mutations developed sepsis (9·3%), four after surgical procedures, three secondary to pneumonia. Bacteria were isolated from blood samples in only three cases (*Escherichia coli*, *Enterococcus faecalis* and *Staphylococcus epidermidis*). Five patients were diagnosed with Mycobacterium avium complex (MAC) infection, affecting the lungs in four patients and the lymph nodes in one child. Disseminated bacillus Calmette-Guérin disease after vaccination and *Mycobacterium genavense* infection occurred in one patient each (W.I.1 and AF.II.1, respectively). In 6·5% of patients with respiratory symptoms, viral pathogens (*influenza virus*, RSV, *rhinovirus* and *adenovirus*) were isolated. In stool samples 9·3% of patients had *norovirus* (5·6%), *rotavirus* (1·9%), *adenovirus* (0·9%), or HAV (0·9%). EBV infection presented as a low-grade/reactivating EBV infection (viral load <500 copies/ml) and EBV-associated lymphoproliferative

disease (viral load >1,000 copies/ml) in seven and three patients, respectively. CMV reactivation caused hepatitis, cytopenia, and retinitis in one patient each. A patient with colitis and diarrhea had a colon biopsy that was positive for the CMV antigen (Table S5). JC virus was detected in the cerebrospinal fluid of three patients with PML. They had normal levels of CD4 and CD8 T cells, but two of them had B-cell depletion therapy (Table S5). PML was the cause of death in one patient (AR.I.4). *Candida* species were isolated from skin swabs, expectorated sputum samples, and stool samples in 5·6% of patients. Dermatophytes accounted for noninvasive skin infections in three patients. Undetectable serum IgE (<2 IU/ml) was found in 54·2% of patients. Respiratory fungal opportunistic infections were caused by Aspergillus species and Pneumocystis jirovecii in three patients each; two of these were under immunosuppressive therapy (Table S5).

Immunological assessment

At the time of diagnosis, the majority (88·9%) of symptomatic patients over age 4 years presented with serum IgG levels below 5 g/l (median 3·6 g/l, range 0-9·9 g/l), and a marked decrease in at least one of the IgA or IgM isotypes (<0·8 g/l and <0·4 g/l, respectively). In 10·7% of patients, all classes of immunoglobulins were found to be normal. A poor response to T-dependent (tetanus and diphtheria toxoid) and T-independent (pneumococcus) antigens was found in 65·2% of individuals. In 50% of patients, the levels of circulating B cells were still within the lower normal range of 6%-19%, or 100-500 cells/µL, respectively (Fig. S3A). In 60·3% of patients, the percentage of IgM-IgD-CD27+ switched memory B cells was ≤ 2% (normal range 6·5%-29·2%) (Fig. S3A). An expansion of $CD21^{low}CD38^{low}CD19^{hi}$ B cells to above 10% was found in 56·1**%** of affected individuals (normal range 1·1%-6·9%) (Fig. S3A). We observed a significant correlation between the expansion of $CD21^{low}$ B cells above 10% and both autoimmune cytopenia (OR 5; 95% CI: 1·1-22·3; P=0·03) and lymphoproliferation (OR 5·7; 95% CI: 1·4-23·5; P=0·01). Overall, opportunistic infections occurred in 15·7% of the patients and were associated with median CD4+ T cell count not as low as expected (588/µL), a profound B-cell defect (median B cell count 46/µL), ongoing immunosuppressive treatments, and a poor outcome (Table S5). Low numbers of circulating NK cells < 100/µL, found in 33·3%

of patients (Fig. S3A), were associated with an increased risk of viral infections (OR 2·8; 95% CI: 1·1-6.9; P=0·02).

Treatment

IgG replacement therapy alone was sufficient to treat 14·5% of the patients who needed medical intervention, while 85·5% of the patients required additional therapy. Antibiotic prophylaxis, antifungal agents and antiviral drugs were added to treat 44·8%, 12·5%, and 12·4% of the patients, respectively (Fig. S4). In addition to IgG replacement, 60·1% of patients with autoimmune cytopenia were treated with systemic corticosteroids. For refractory or recurrent cytopenia, 17·4% of the patients had anti-CD20 monoclonal antibody (rituximab), 15·2% splenectomy, and 8·7% mycophenolate mofetil. GLILD was treated with oral corticosteroids alone in five patients, or in combination with immunosuppressive agents (cyclophosphamide, mycophenolate mofetil, rituximab and cyclosporine), to which there was only a partial response. Non-infectious enteropathy was treated with systemic corticosteroids in 13 of 17 patients, while three patients received azathioprine (M.II.1, AH.I.1, and BB.I.1), eliciting a partial response. In addition to systemic corticosteroids, three patients with inflammatory bowel disease (IBD)-like exacerbations received mesalazine. In patients (84·6%) with oral and genital ulcers, systemic corticosteroids induced a good response. Three individuals with lymphoproliferative disease were treated with anti-CD20 (rituximab), which led to complete remission. Abatacept, a cytotoxic T lymphocyte antigen 4 (CTLA-4) fusion protein, was used to treat one patient with refractory autoimmunity and lymphoproliferation, eliciting a good response (Q.I.1). HSCT is currently planned for four patients with EBV-lymphoproliferative disease, refractory cytopenia with lymphoproliferation, and mycobacterial disease (AB.II.1, S.I.1, AP.I.1, and BL.II.1).

DISCUSSION

Heterozygous *NFKB1* mutations causing p50 haploinsufficiency have previously been reported to be associated with various phenotypes ranging from mere antibody deficiency to multi-organ autoinflammatory conditions[4-10]. However, a comprehensive clinical description of the extended phenotype

of the NF-κB1-related phenotype has been lacking. Here, we show that antibody deficiency was the main finding in patients with *NFKB1* mutations (88·9%). However, this may well present as an ascertainment bias, as this survey was initiated by clinical immunology centers of the ESID (European Society for Immunodeficiencies). Only 76·9% of patients fulfilled the revised ESID registry criteria for CVID, indicating that also patients with normal or only mildly-affected humoral immunity may have an impaired canonical NF-κB signaling. The median age was lower in healthy mutation carriers than in affected patients, and progressive development of humoral immunodeficiency was observed in some individuals, suggesting an age-dependent manifestation and expressivity of *NFKB1*-related phenotypes. Hypogammaglobulinemia is the reason for the high incidence of bacterial infections in our cohort, especially those affecting the upper (83%) and lower respiratory tract (59%), the skin (37·7%) and the gastrointestinal tract (28·6%). Notably, 15·7% of patients developed opportunistic infections, a much higher percentage than expected in CVID[14,15]. The observed difference can be explained by our definition of CVID (https://esid.org/Working-Parties/Clinical-Working-Party/Resources/Diagnostic-criteria-for-PID2#Q3), which did not exclude patients with a T-cell defect[16]. However, a measurable CD4 T cell defect was observed in some of our patients (15·1%), but was not necessarily associated with opportunistic infections (P=0·57)[17]. Our observations suggest that in addition to the *NFKB1* mutation, an immunosuppressive treatment may impair the T-cell response and, in combination with the lack of B cells, contribute to the pathogenesis of opportunistic infections. The clinical phenotype was also dominated by lymphoproliferation, particularly splenomegaly (48·5%) and lymphadenopathy (35·3%), and by autoimmunity (mainly cytopenia) (43·9%). Low serum IgA and IgM levels, which reflect the loss of switched memory B cells, were associated with an increased risk of developing autoimmunity and splenomegaly. Thus, defective isotype switching and somatic hypermutation may each account for the increased presence of autoreactive B cells[18]. Autoimmune cytopenia and lymphoproliferation were also associated with an elevated proportion of $CD21^{low}$ B cells (>10%). $CD21^{low}$ B cells develop after chronic stimulation and have been found to be enriched in autoreactive clones[19]. Impaired canonical NF-κB signaling has been observed not only in *NFKB1*-haploinsufficient patients, but also in CVID-patients with the CVID 21^{low} phenotype, thus potentially contributing to the accumulation of

CD21^{low} B cells[20]. Conversely, a Th1-skewed profile in peripheral blood T cells, combined with the overexpression of proinflammatory cytokines such as IL-1β and tumor necrosis factor (TNF-α), may contribute to the autoinflammatory symptoms[6,8].

Therapeutic strategies for individuals with antibody deficiency include immunoglobulin replacement therapy Patients with autoimmunity and immune dysregulation shall be treated with steroids and rituximab. However, the beneficial effect has to be weighed against the infectious risk of immunosuppression. The CTLA-4 fusion protein abatacept was used to treat one patient with good response. Additional therapeutic options that still require evaluation include hematopoietic stem cell transplantation (HSCT), and targeted therapeutic strategies such as proteasome inhibitors. The potential therapeutic effect of anti-TNF, which inhibits TNF-mediated NF-κB activation, suggests that NF-κB1-related diseases result in dysregulated, rather than defective, NF-κB signaling.

However, immune dysregulation may result not only from a defective, but also from an increased NF-κB activation[21,22]. Indeed, p50 homodimers, stabilized by B cell leukemia 3 protein (Bcl-3), function as inhibitory factors for NF-κB1 transcriptional activity because they do not contain the transcriptional activation domain that is otherwise exclusively present in RelA (and RelB and c-Rel); however, they do compete with p50/RelA heterodimers for binding to DNA[23].

While studying this cohort, it became clear that the development of drugs specifically interfering with the NF-κB signaling pathway will be an important step forward not only for the personalized treatment of patients with NF-κB-related disease, but also for patients with more common autoimmune or inflammatory conditions.

Author contributions

Study design: TL, MF, BG

Writing of manuscript: TL, MF, BG, NK

Clinical data analysis: TL, BG

Genetic data analysis: MF, BG, NF, MP, AB, NCO

Pathology results: MS, NK

Production of immunological and functional data: TL, MF, NK, NF, MV, MK, FA, CS

Collection of genetic, clinical and immunological data: BG, TL, MF, NK, NF, EDV, JVDM, RA, CMR, YDS, RK, TH, FA, RES, PS, BS, LAP, MVDF, MMG, LIGG, LMA, AS, NKu, VZ, JFN, PS, UF, WI, OB, SB, CK, RG, JC, MA, LW, KB, TH, MMDSV, DH, VL, AP, LA, MPG, ADM, CAS, AA, HA, LH, OK, MH, HLA, JET, AF, MC, SBa, MC, CCR, NCP, WR, TN, NB, JS, MRJS, SOB, PT, TWK, KW

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Figure legends

Figure 1. Localization of *NFKB1* mutations. Numbers indicate amino acid positions. Horizontal black bars delineate the location of four different groups of damaging *NFKB1* mutations. For each mutation, the number of carriers is indicated.

Figure 2. Subcellular localization, expression and activity of distinct types of *NFKB1* variants. HEK293T cells were transiently transfected with N-terminal GFP-fused constructs, as indicated. Nuclei were stained with Hoechst 33342 (blue). (A) Haploinsufficiency mutations caused aberrant signals, whereas p50-like proteins (precursor-skipping variants) were localized to the nucleus. Missense variants (introduced into the full-length p105) produced signals that were indistinguishable from WT p105. (B) PMA/ionomycin treatment caused clumping of the p.Ile87Ser mutant. (C) Western Blot analysis (i) confirmed that transfected WT and transfected mutant p105 each underwent processing to p50, and (ii) revealed the limited expression of the p.Ile87Ser variant. GAPDH was used as loading control. (D) Loss of luciferase reporter activity with p.Arg57Cys and p.Ile87Ser mutants. Relative light units were normalized to co-transfected Renilla luciferase. Mock not shown. DNA amounts were compensated with non-related plasmid DNA. Depicted data represent the results from 2 to 4 experimental repeats, additional data can be found in Figure S6.

Figure 3. Clinical course and survival rate of *NFKB1* cohort. (A) Cumulative percentage of symptomatic patients who developed infections, autoimmunity, lung disease and cancer. (B) Kaplan-Meier survival curve with 95% confidence interval (dotted lines).

Figure 4. Exemplary CT and MRI findings and histopathology in patients with damaging heterozygous *NFKB1* mutations. (A) and (C) from the same patient polypoid shifting of the ethmoidal cells as well as both sinus maxillares. Lower displacement of the frontal sinus and the sphenoid sinus. (B) and (D) from the same

patient. Hepatosplenomegaly. Multiple liver hemangiomas and small liver cysts. Additional signs of focal nodular hyperplasia. Individual cystic lesions of the spleen. Widening of the portal vein due to possible portal venous hypertension. (E-J) Several CT scans from different patients showing multiple pulmonary nodules, bronchiectasis with inflammatory changes and interstitial lung disease. (K) and (L) Hepatitis with T cell dominant lymphocytic inflammation. K) Portal (asterisk) and intralobular (arrowhead) inflammation. L) Higher magnification image showing intralobular lymphocytes and epithelioid cells, reminiscent of microgranulomas, with apoptosis of hepatocytes (nuclear remnants highlighted by arrowhead). (M) CD3-positive T-cells encircling an apoptotic hepatocyte, suggestive of T-cell driven damage. (N) Corresponding area to (M), showing CD4-positive T cells, few monocytes and intrasinusoidal macrophages (Kupffer cells). (O), (P) and (Q) Slightly chronic gastritis with patchy lymphocytic inflammation of the antrum (O, P highlighted by arrowhead) and corpus (Q highlighted by arrowhead). (R) Chronic lymphocytic peribronchitis. Magnifications indicated by bars.

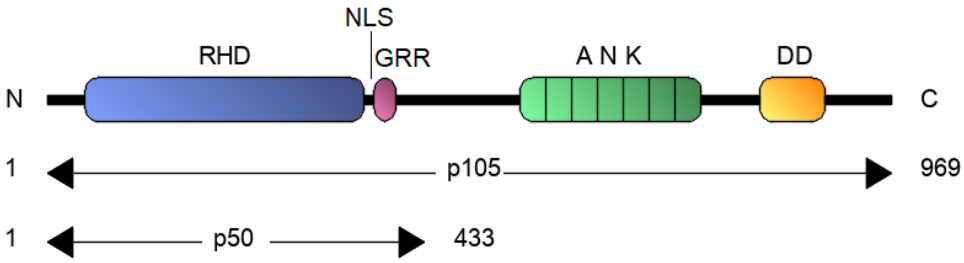
Figure 5. Main clinical findings in patients with damaging *NFKB1* mutations. Percentage distribution of clinical manifestations (A), and infection types (B).

Described	Novel
del 103370996-103528207 (1)	p.M14Qfs*9 (2)
del 103436974-103652655 (1)	p.Q84* (1)
p.I47Yfs*2 (1)	p.K95Vfs*25 (2)
p.R54_K86del (6)	p.E120* (2)
p.E63fs*64 (2)	p.L176* (1)
p.I87Lfs*16 (2)	p.203* (1)
p.Q99* (2)	p.L215Afs*11 (1)
p.A156fs*12 (5)	p.D279Vfs*11 (1)
p.R157* (11)	p.N291Mfs*141 (10)
p.G165Afs*32 (2)	p.G292Vfs*140 (1)
p.D191_K244delinsQ (17)	p.P317_I322 delinsL+ p.N323Y (1)
p.K244_D279delinsN (7)	p.Q333* (2)
p.K278Efs*3 (1)	p.S338Lfs*94 (4)
p.R284* (6)	p.F310Ifs*76 (5)
p.S302Ffs*7 (8)	p.E358Kfs*73 (1)
p.Y319*(1)	p.G395fs* (1)
p.R336Gfs*96 (1)	p.D356_P403del (1)
p.G434_Q498del (3)	p.Y405* (1)

Haploinsufficiency mutations

Described	Novel
p.G384Efs*48 (2)	p.Y415* (1)
p.V456* (3)	p.K441* (1)
p.F459Lfs*26 (1)	p.L529Afs*14 (5)
p.A475Pfs*10 (1)	p.S546Rfs*8 (1)
p.A506Vfs*17 (1)	p.I567Nfs*6 (1)
p.H513Qfs*28 (2)	
p.D541* (2)	

Precursor skipping mutations

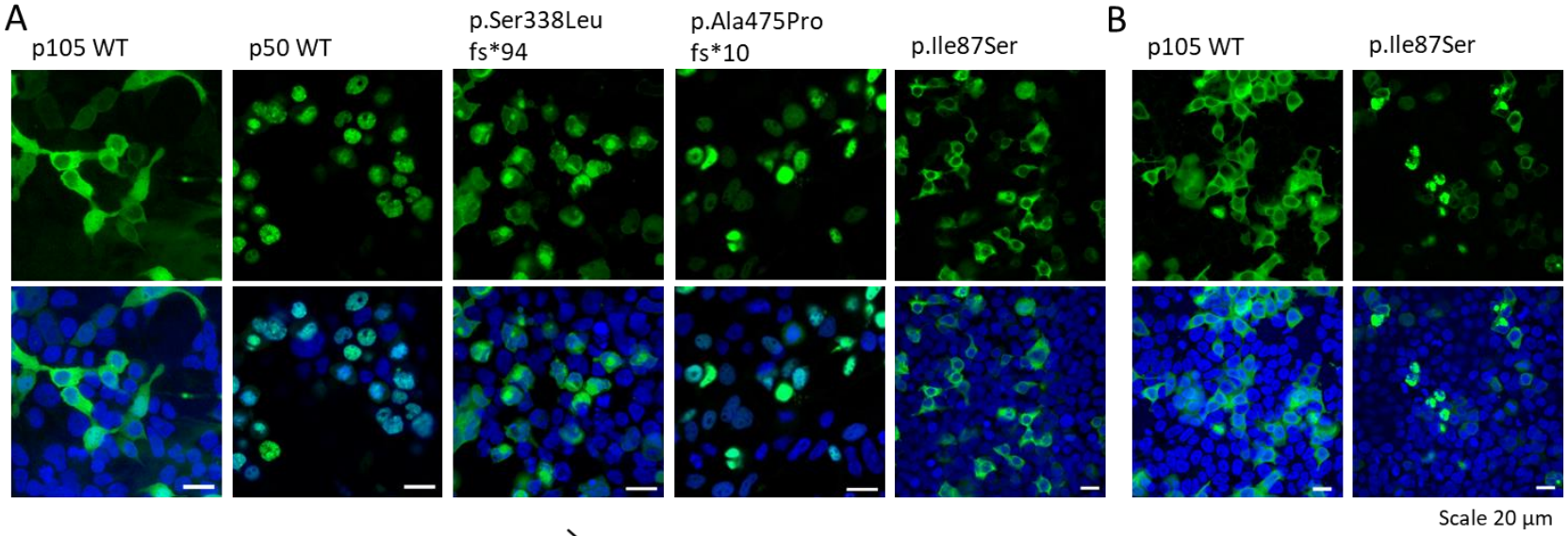


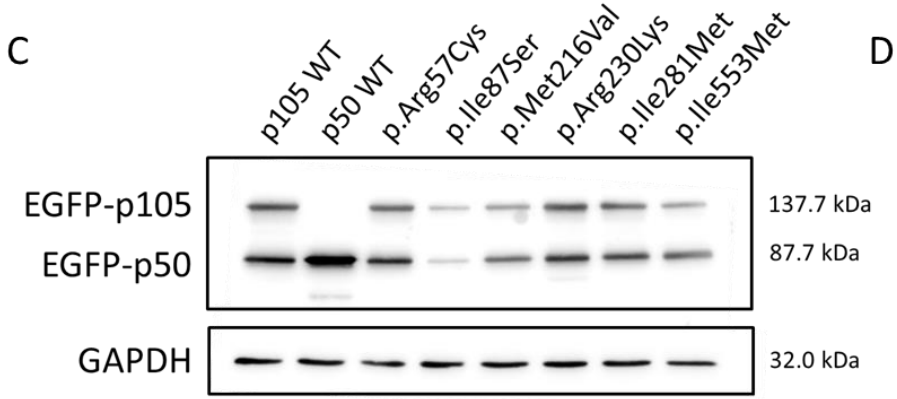
Missense variants affecting p105 and p50

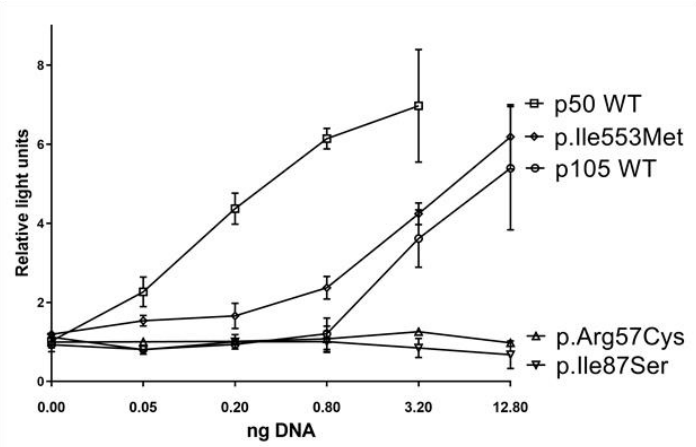
Described	Novel
p.H67R (9)	p.R57C (2)
p.I87S (1)	p. H67Y (2)

Missense mutation affecting only the p105 precursor

Described
p.I553M (3)







Haploinsufficiency mutation	p.Ser338Leufs*94
Precursor skipping mutation	p.Ala475Profs*10
Missense variants	p.Arg57Cys p.Ile87Ser p.Met216Val p.Arg230Lys p.Ile281Met p.Ile553Met

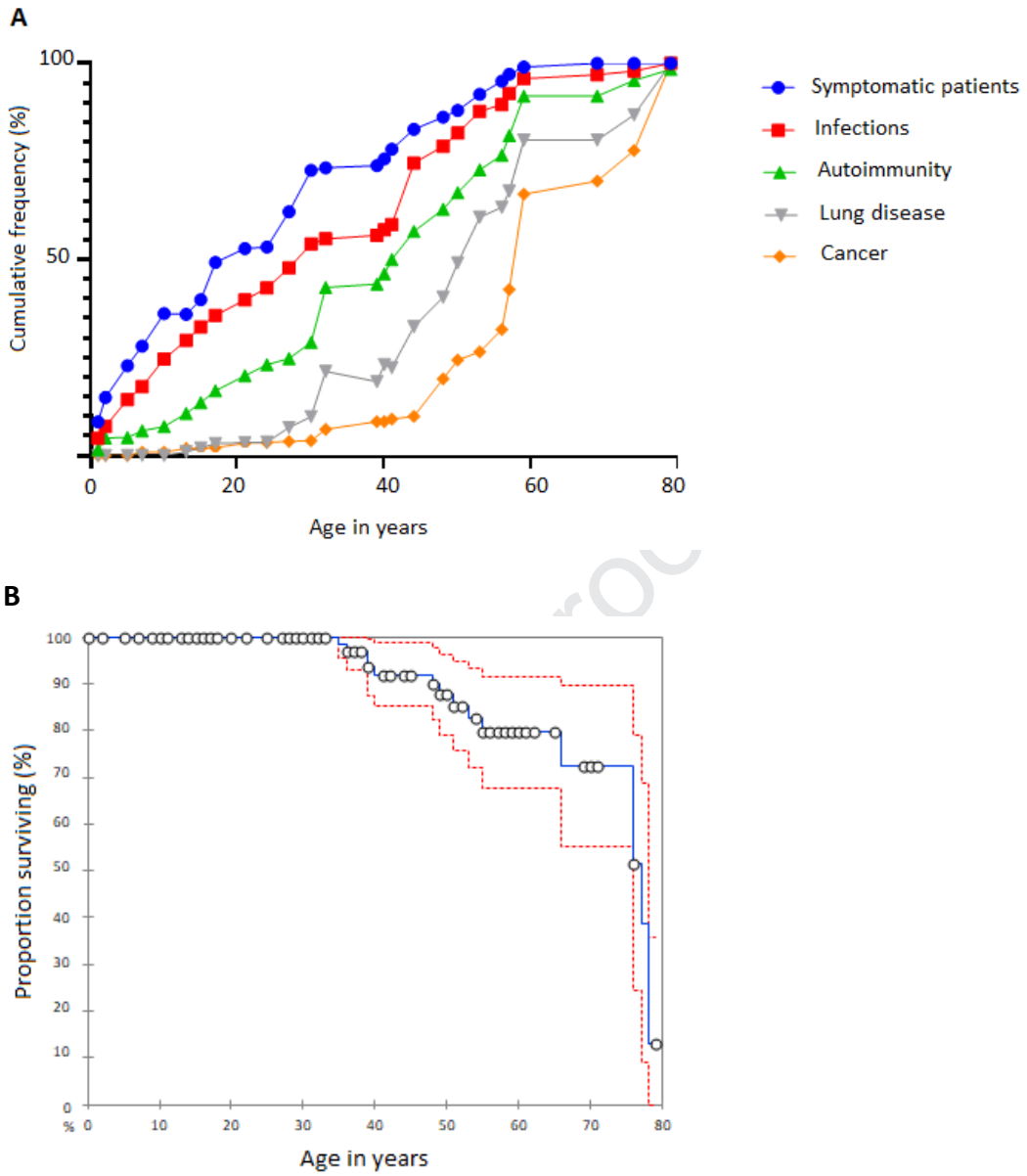


Figure 3. Cumulative frequency of clinical manifestations and survival rate in *NFKB1* cohort.

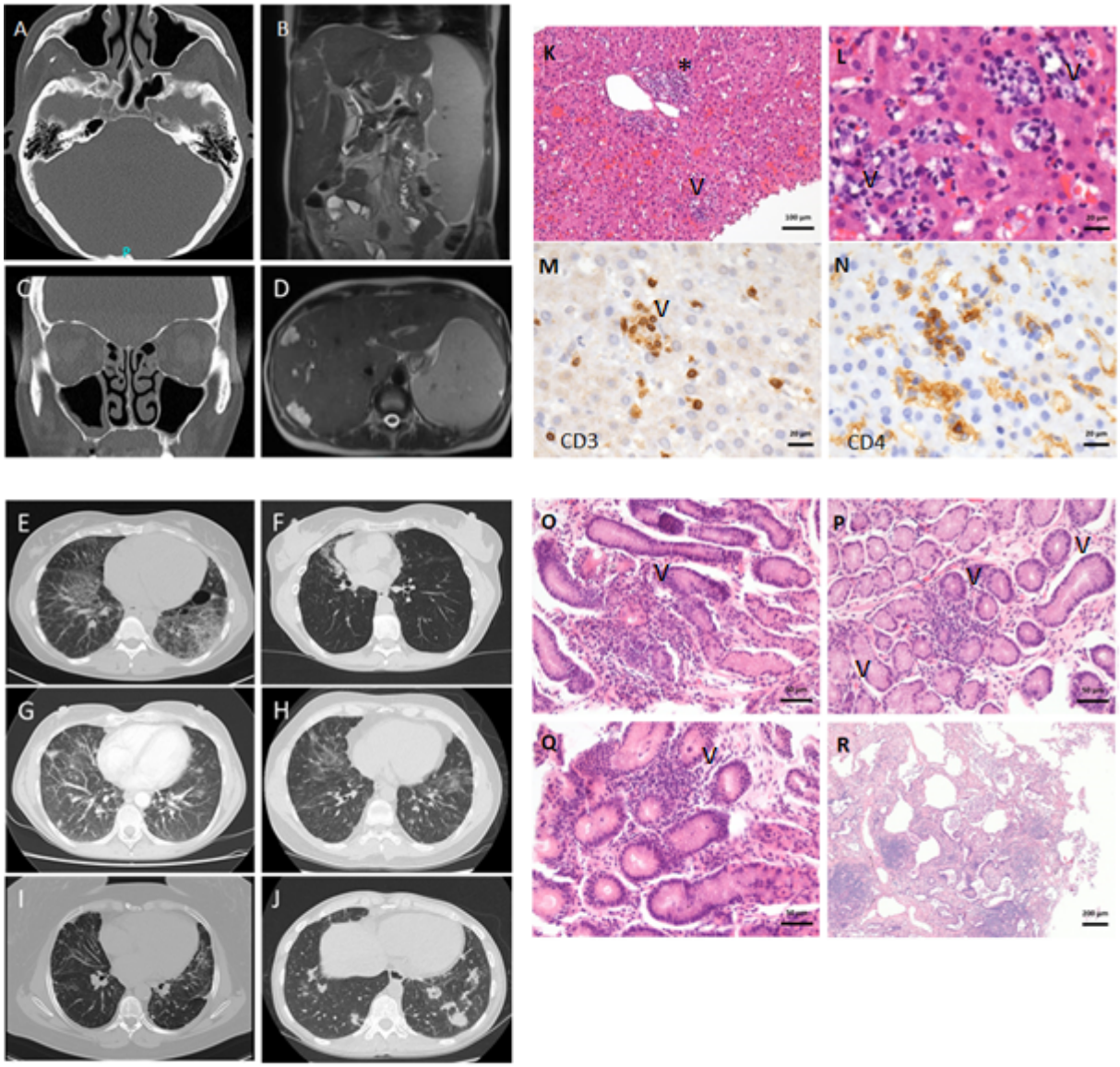
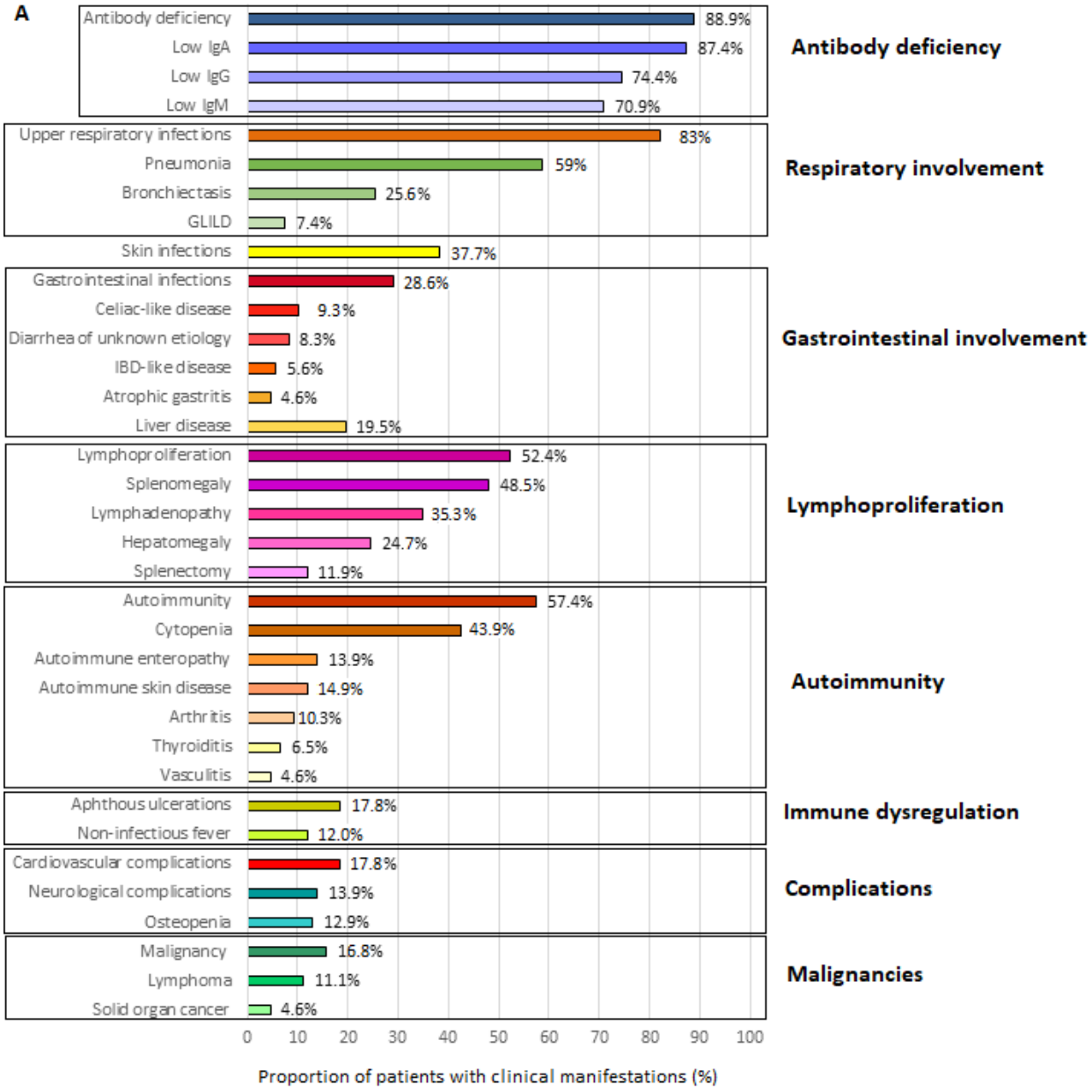


Figure 4. Exemplary CT and MRI findings and histopathology in patients with damaging heterozygous *NFKB1* mutations.

A



B

Bacterial infections 53.7%	
H. influenzae 23.1%	*Pseudomonas spp.* 4.6%
Streptococcus spp. 17.6%	*Staphylococcus spp.* 4.6%
C. difficile 6.5%	*C. jejuni* 3.7%
Mycobacterium spp. 6.5%	*E. coli* 3.7%
Moraxella catarrhalis 5.6%	*Enterococcus spp.* 2.8%
Salmonella spp. 5.6%	
Viral infections 25.0%	
EBV 9.3%	RSV 2.8%
CMV 6.5%	*Adenovirus* 1.9%
Norovirus 5.6%	*Rotavirus* 1.9%
JC virus 2.8%	*Rhinovirus* 0.9%
Influenza virus 2.8%	HAV 0.9%
Fungal infections 12.0%	
Candida spp. 5.6%	*Dermatophytes* 2.8%
Aspergillus spp. 2.8%	*Pneumocystis spp.* 2.8%
Parasitic infections 4.6%	
Giardia lamblia 3.7%	
Cryptosporidium spp. 0.9%	

Figure 5. Main clinical findings in patients with damaging *NFKB1* mutations.

SUPPLEMENTARY APPENDIX

Clinical and immunological phenotype and management of 157 individuals with 56 heterozygous *NFKB1* mutations

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1. Supplementary Methods

1.I. Genetic analysis and sequence variant interpretation

We identified 231 individuals harboring 105 distinct heterozygous *NFKB1* variants. The inclusion criteria were: clinical diagnosis of CVID according to the European Society for Immunodeficiencies diagnostic criteria for CVID (https://esid.org/Working-Parties/Clinical-Working-Party/Resources/Diagnostic-criteria-for-PID2#Q3)[1], predominantly antibody deficiency, autoimmunity and immune dysregulation, autoinflammatory phenotype, and family history for primary immunodeficiency (PID). While 30 variants had previously been described, 75 were novel. *NFKB1* variants were detected by targeted next generation, whole-exome- or whole-genome sequencing (Fig. S1). Sequence variants were classified according to the American College of Medical Genetics and Genomics guidelines[3] as pathogenic (39), likely pathogenic (17), benign (1), likely benign (4) and of uncertain significance (44) (Fig. S1 and Table S1). Finally, 157 individuals with the 56 pathogenic or likely pathogenic heterozygous *NFKB1* mutations were included into the main analysis cohort. Out of 94 subjects with non-pathogenic variants, twenty-nine individuals, carrying variants of uncertain significance and with available clinical information, were separately documented and showed a "NF-κB1-related" phenotype (Table S2 and S3).

1.II. Cohort

The study cohort included 157 mutation carriers, including 121 affected individuals, and 36 unaffected subjects, i.e. relatives without manifestations of PID (Fig. S2). Thirteen relatives of mutation carriers had no available gDNA/genotyping but fulfilled the criteria for PID (Tab S2). Three of them, all previously described (NA.II.19, F1.II.1, AU.I.2), were added to the cohort of affected individuals, the other 10 were allocated to the group of 14 individuals lacking adequate clinical information and were hence only included in the epidemiologic analysis. Ninety-two mutation carriers have previously been published[4–14]. Hence, the present work represents a meta-analysis, spiked with detailed clinical information on fourty-four out of 107 novel *NFKB1* affected mutation carriers (Table S2 and S3).

The research was conducted in accordance to the principles of the Helsinki Declaration and following approved protocols of the Albert-Ludwigs-Universität Freiburg, Germany. Samples were collected with the written informed consent of all study participants, or their parents in the case of minors, under local ethics board-approved protocol 295/13 version 140782. For all individuals, data were collected by the attending physicians using a detailed questionnaire including genetics, clinical history, laboratory values, and treatment.

The following autoimmune phenomena were detected: autoimmune cytopenia, pernicious anemia, thyroiditis, vitiligo, psoriasis, alopecia, enteropathy, arthritis, hepatitis, Addison's disease and diabetes mellitus. The following infections

were defined as opportunistic: invasive mycoses (aspergillosis, candidiasis and pneumocystosis), cytomegalovirus – and Epstein-Barr virus (EBV)-associated disease, John Cunningham virus (JC) virus infection, *Pseudomonas* species pneumonia, cryptosporidiosis and atypical mycobacterial infection.

Laboratory tests included whole blood cell count, serum immunoglobulin levels, vaccine response to tetanus, diphtheria toxoid (T-dependent response), and pneumococcus (T-independent response), and flow cytometry analysis of peripheral circulating lymphocytes (with T- and B-cell subtypes, if available). Serum immunoglobulin levels under immunoglobulin replacement therapy were excluded from analysis. Antibody responses to vaccination were classified as impaired if the response to at least one type of antigen was defective. When autoantibodies against platelets, red blood cells, or granulocytes were not available, the diagnosis of autoimmune cytopenia was established based on the clinical history, physical examination, blood cell analysis, and therapeutic response[15]. Infections were confirmed by direct microscopic examination of the specimen, DNA or RNA identification, culture, or serological screening from representative specimens. Complement analysis, lymphocyte mitogen proliferation, bone marrow examination, biopsies and radiologic investigations were performed in selected individuals according to clinical indications.

1.III. Statistics

Statistical analysis was processed using GraphPad Prism software (version 7; GraphPad Software, La Jolla, California) and P values of less than 0.05 were considered significant. Log-rank Mantel Cox test was used to compare survival curves.

1.IV. Generation of mutation constructs

The wild-type and mutant *NFKB1* full-length coding sequences were subcloned into the expression vector pEGFP-C1 (Clontech/Takara, Saint-Germain-en-Laye, France) or pTO-GFP-N[10] to generate GFP-fusion constructs.

1.V. Cell culture and transfection

HEK293T cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 1% penicillin-streptomycin (all from ThermoScientific, Germany) and seeded in 24-well plates (Greiner, Frickenhausen, Germany). Cells were transfected with jetPEI transfection reagent (Polyplus, Illkirch, France), according to the suppliers´ recommendations. All cell lines were routinely tested for Mycoplasma.

1.VI. Analysis of nuclear localization

Plates with HEK293T cells, seeded onto collagen-coated (Collagen A, Biochrom AG, Berlin) cover slides and transfected with wild-type or mutant p105 or p50 were rinsed with phosphate buffered saline (PBS) and cells were fixed with 4.0% formaldehyde solution; nuclei were stained with Hoechst33342 (Sigma, Taufkirchen Germany). After 48 hours, cells have been stimulated with PMA/Ionomycin (100ng/ml and 2µg/ml, respectively) for 30 minutes before fixation and staining. Images were taken on Zeiss laser scanning microscope LSM710 equipped with a 63x oil immersion objective (Carl Zeiss, Jena, Germany) and evaluated with the Zeiss ZEN black software.

1.VII. Fluorescence-based promoter reporter assay

To assess the NF-κB1 transcriptional activating function of wildtype and mutant p105/p50, a fluorescence-based promoter reporter assay in transfected cells was performed. An expression vector for the red fluorescent protein tdTomato under the control of a NF-κB1 responsive promoter, composed of 5xNF-κB binding sites $[TGGGGACTTTCCAC]_5$) fused to the CMV minimal promoter, was used as reporter. A vector in which the tdTomato sequences were fused to the CMV minimal promoter (lacking specific transcription factor binding sites) was used as negative control, while the expression vector in which the full-length CMV promoter drives tdTomato expression was used as positive control. Vector constructs for wildtype and/or mutant p100/p50 were transfected together with the reporter and a non-fused p65 (providing a transactivation domain) into HEK293T cells. Reporter activity was determined with or without activation of NF-κB signaling with TNF-α (25 ng/ml, Abcam, Germany) or PMA/ionomycin (50 ng/ml and 1 µg/ml, respectively, Sigma, Taufkirchen Germany). The fluorescence intensity was examined as an indicator of reporter activity by the FluoroSpot Analyzer (CTL Immunospot, Bonn, Germany).

1.VIII. Dual luciferase reporter assay

HEK293T cells were co-transfected with GFP-WT and missense mutant p105, in addition to the NF-κB luciferase and the Renilla luciferase control reporter, using JetPEI (Polyplus, Illkirch, France), according to the suppliers´ protocol. Increasing amounts of construct DNA were used. Equal expression of constructs was verified by co-transfecting with GFP vector. Transfection efficiency (GFP) was controlled in fluorescent microscopy. Cells were lysed with passive lysis buffer after 48 hours and transferred to black 96-well plates (Thermo Fisher Scientific, Denmark). The light emission was examined as an indicator of reporter activity. After luciferase measurement, the signal was quenched and Renilla was measured as internal control. The signal was normalized to mock. This assay was performed on the EnVision Multilabel Plate Reader (Perkin Elmer, Bonn, Germany).

1.IX. Western blotting

Transfected HEK293T cells (2.4 x 10^6), unstimulated or treated with TNF (30 ng/ml for 30 minutes), were washed in PBS and lysed on ice (lysis buffer 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 2mM EDTA, 1 µM Na_3VO_4, 50 mM NaF, 1.0% Triton X-100; Protease Inhibitor Cocktail). Supernatants were collected and total protein concentrations were determined using BCA assay. Twenty µg per sample were loaded and protein size fractionated using a 12% polyacrylamide gel. Proteins were transferred onto a PVDF membrane for 90 minutes at 45 V. Membranes were incubated in Tris Buffered Saline, with Tween (TBST) with 5% milk overnight and p105/p50 was detected using a rabbit primary antibody raised against the N-terminus (#13586; Cell Signaling; Frankfurt, Germany) after two hours of incubation. Signals were detected with horseradish-peroxidase-coupled anti-rabbit secondary antibodies using enhanced chemiluminescence (LumiGlo; Cell Signaling, and SignalFire Plus; Cell Signaling) after two hours of incubation. GAPDH antibody (#G9295; Sigma/Merck; Darmstadt, Germany) was used as a loading control.

2. Supplementary Results

2.I. Supplementary patients' characteristics

One-hundred-nineteen cases were familial, following an autosomal dominant mode of inheritance, and 38 cases were sporadic. The penetrance was not significantly higher in male individuals (72·2%) when compared to females (67·2%) (P=0·55). The origin of the mutation carriers in our cohort was as follows: 65·1% European, 13·4% mixed ethnicity, 11·4% Asian, 6% North American, 2% South American, 1·4% Australian and 0·7% African, most likely representing an ascertainment bias (Table S2). In a large proportion of patients (39·3%) the disease onset was before 10 years of age; 29·2% of patients developed symptoms between 10 to 20 years of age. Among patients with CVID, 24·4% had a CVID-infection only phenotype while 52·5% had a complex phenotype characterized by infections, autoimmunity, inflammation and lymphoproliferation. Among 6% of patients with autoinflammatory disorder, 3·4% were classified as having Behçet's disease according to the point score system defined in the New International Criteria for Behçet's Disease (ICBD)[16].

2.III. Skin and mucocutaneous involvement

Skin disease occurred in 54·6% of patients. Skin infections were observed in 37·7% of patients (Fig. 5A). Viral infections occurred in 26·4% and included shingles (14·2%), *Herpes simplex* virus infections (5·6%), and warts (5·7%). Mucocutaneous infections were also common (18·9%). Skin abscesses and cellulitis were more common (16·2%) than

folliculitis and furunculosis (3·8%). Autoimmune skin manifestations were found in 14·9% of patients and consisted of vitiligo (6·5%), alopecia (6·5%) and psoriasis (0·9%). Oral aphthous ulcerations occurred in 17·8% of patients and were associated with genital aphthous ulcers in 5·6% of patients (Fig.5A). Pyoderma gangrenosum and erythema nodosum were reported in two patients each (NA.II.16, NA.III.34, and AS.II.1, F1.II.4, respectively). One patient developed morphea (BA.II.2).

2.V. Other complications

Neurological complications occurred in 13·9% of patients and included peripheral neuropathy (4·6%), bacterial and idiopathic meningitis (2·8% and 1·8%, respectively) and progressive multifocal leukoencephalopathy (PML) (1·8%). Cerebral vasculitis was detected in three patients and resulted in brain ischemia in two. Cardiovascular complications were observed in 17·8% of patients and included arteriosclerotic vasculopathy (11·1%), congenital heart defect (ventricular septal defect, mitral valve defect and patent ductus arteriosus, 2·8%), atrial arrhythmia (2·8%) and myocarditis (1·8%). Osteopenia and osteoporosis were diagnosed in 12·9% of patients by dual-energy X-ray absorptiometry (DEXA) measurement of bone mineral density. All but one patient with osteopenia or osteoporosis had been treated with corticosteroids. Non-infectious episodes of fever and systemic inflammation were observed in 12% of patients (Fig. 5A). Bone marrow analysis was not performed in all the patients, but a significant increase of diffuse and nodular CD3+ T-cellular infiltrates, absent plasma cells, but no major abnormalities in the hematopoietic compartment was found in five patients; aplastic bone marrow was detected in one case. Early-onset Langerhans cell histiocytosis occurred in one child (AF.II.1). Three patients developed portal hypertension following nodular regenerative hyperplasia (NRH) of the liver (NZ.II.2 and Z.I.1) and liver cirrhosis (AH.I.1), respectively. One patient was affected by idiopathic chronic pancreatitis resulting in an exocrine pancreatic insufficiency (AU.I.2); another patient developed a post-operative pancreatitis (F1.II.1).

2.VI. Supplementary treatment

Nearly all patients with chronic lung disease received IgG replacement therapy (94·9%). Antibiotic prophylaxis was used in 78·9% of the patients with bronchiectasis. Pyoderma gangrenosum was treated in one patient with antibiotics, surgery and etanercept, with a poor response (NA.II.16), another patient seemed to have responded to IVIG (NA.III.34). Patients with arthritis were treated with systemic corticosteroids (n=3) and disease-modifying antirheumatic drugs (n=5), including methotrexate, cyclosporine, azathioprine, hydroxychloroquine and sulfasalazine. All patients with autoimmune hepatitis (n=3) received systemic steroids. One patient with liver failure but no evidence of autoantibodies was treated with steroids and azathioprine and subsequently with an unsuccessful liver transplantation (C.II.3). In

11·9% of the patients, splenectomy was necessary due to an enlarged spleen and hypersplenism (n=3), uncontrolled autoimmunity (n=7) or malignancy (n=2), with good success.

2.VII. Comparison of the *NFKB1* phenotype to the one of general CVID

Generally, the phenotype of thus far described patients with *NFKB1* mutations matches the one of CVID. The frequencies of pneumonia, sinusitis and gastrointestinal infections, mostly caused by bacteria and viruses, were very similar in *NFKB1* patients when compared to CVID cohorts. Pulmonary manifestations, such as bronchitis, bronchiectasis and GLILD, showed a lower frequency, but had the same features in *NFKB1* patients, in comparison to CVID patients. In contrast, non-infectious gastrointestinal disease, including liver disease, was more common than documented in CVID, but had the same characteristics of CVID-related enteropathy (sprue-like villous atrophy, enteropathy reminiscent of IBD, chronic diarrhea of unknown etiology, NHR). Autoimmunity was more common in *NFKB1* patients than in CVID cohorts (Table S6). Patients with *NFKB1* mutations were twice as likely as CVID affected patients to be diagnosed with autoimmune cytopenia, the most common autoimmune manifestation in both groups[17]. In addition, splenomegaly and lymphadenopathy were more common in patients with *NFKB1* mutations in comparison to CVID cohorts. The incidence of malignancies was similar to that observed in the New York CVID cohort study[18], but higher than reported in the European cohort of 2,212 patients[19]. B cell subsets distribution was similar in *NFKB1* and in CVID patients. However, a stronger expansion of $CD21^{low}$ B cells and transitional B cells was found in *NFKB1* patients compared to a general CVID cohort [20] (Table S6).

The comparison of categories of *NFKB1* mutations and their clinical presentation was limited because of the unequal sample sizes. However, an apparent genotype-phenotype correlation was found (Fig. S5): haploinsufficiency and precursor skipping mutations were associated with a higher incidence of infections, lung disease, autoimmunity and lymphoproliferation, in comparison to the missense variants in the N-terminal half of p105. Malignancies were more common in the cohort of the patients with haploinsufficiency mutations. Conversely, missense variants in the N-terminal half of p105 were associated with a higher incidence of autoinflammatory manifestations. Missense variants affecting the precursor p105 and the mature p50 might not lead to an overall loss of NF-κB function but might variably affect downstream events, thus explaining the milder associated phenotype.

3. How to diagnose disease-causing *NFKB1* mutations?

Taking all the above into account, *NFKB1* mutations should be suspected in any patient with a CVID phenotype, as *NFKB1* mutations may manifest as infection-only hypogammaglobulinemia as well as CVID with any autoimmune or autoinflammatory complication. However, a normal or mildly affected humoral immunity does not rule out the presence

of an *NFKB1* mutation. Cases with an autosomal dominant inheritance and rheumatologic features such as seronegative arthritis, panniculitis, vasculitis including but not limited to Behçet's disease, clearly increases the suspicion of disease-causing mutations in *NFKB1*. The diagnostic procedure involves two steps: first, identification of a variant affecting *NFKB1*, and second, evaluation of the effect of the observed genetic variant, according to probability predictions and functional *in vitro* tests, if indicated. To gain further insight into the biological effect of *NFKB1* variants, we ectopically (over-)expressed selected mutant NF-κB1 proteins in a standard cell culture system, such as transiently transfected HEK293T cells. Western blotting and fluorescence microscopy have been used to test for integrity and subcellular localization of the GFP-fused mutant protein. Reporter-based assays have been adopted to evaluate the NF-κB transcriptional activation.

4. Differential diagnosis to the *NFKB1* phenotype

Autoinflammatory symptoms, i.e. oral and genital aphthous ulcerations (18·5%), non-infectious episodes of fever and systemic inflammation (12%), and vasculitis (4·6%), occurred not infrequently in our cohort, also reported in CVID patients due to biallelic loss-of-function mutations in adenosine deaminase 2 (*ADA2*, formerly cat eye syndrome chromosome region, candidate 1, *CECR1*)[21] (MIM: 607575). Like the *NFKB1* phenotype, the ADA2 deficiency of can also manifest with humoral immunodeficiency due to a deficiency in the B cell compartment, increased susceptibility to human *Herpesviridae* infections, lymphoproliferation and autoimmunity, especially enteropathy and cytopenia[22]. In patients with hypogammaglobulinemia, lymphoproliferation, respiratory and gastrointestinal involvement and cytopenia, *CTLA-4* (MIM: 123890) and lipopolysaccharide-responsive, beige-like anchor protein (*LRBA*) (MIM: 606453) deficiency should also be entertained as differential diagnoses[23]. Both conditions result in defective CTLA-4 expression, thus impairing the regulatory T-cell (Treg) function. In our cohort, Treg frequencies were not extensively tested, but were found decreased in about one-third of the patients (Fig. S3). Furthermore, Treg functions have previously been reported as normal in a small cohort of the patients with NFKB1 mutations[10]. CTLA-4 insufficiency, like NF-κB1-related disease, is an autosomal dominant trait, whereas LRBA and ADA2 deficiencies are autosomal recessive. In addition, autosomal dominant *STAT3* gain-of-function mutations may cause autoimmune cytopenia and multi-organ autoimmunity, lymphoproliferation, hypogammaglobulinemia, infections and short stature. Likewise, activated phosphoinositide 3-kinase δ syndromes (APDS) present with infections, lymphoproliferation, hypogammaglobulinemia, autoimmunity and malignancies.

5. Selected case vignettes of *NFKB1* mutations

5.I. Q.I.1 (c.118+1G>A; IVS3+1G>A, if exon 3 is skipped the consequence is c.40_118del which leads to p. Met14Glnfs*9) predicted haploinsufficiency

This patient is a 17 year-old female born to non-consanguineous parents. She initially came to medical attention at age 11 months with aphthous stomatitis. From age 22 months, she had a recurrent hemorrhagic rash and nose bleeding, despite normal platelets count, regarded as hemorrhagic vasculitis. When she was two years old, she presented with idiopathic thrombocytopenic purpura (ITP) and hemolytic anemia, treated with corticosteroids, until remission. However, multi-lineage autoimmune cytopenia re-occurred after puberty. She also suffered from recurrent respiratory tract infections, including pneumonia and bronchitis. *Streptococcus* species, *Haemophilus influenzae*, and *Candida* species were isolated from respiratory specimens. Since her immunoglobulin levels were low (IgG 3·6 g/L, IgA 1·37 g/L, IgM 0·36 g/L), CVID was diagnosed and immunoglobulin replacement therapy was initiated. Additionally, high-dose IVIG therapy was used. Splenomegaly and lymphadenopathy were evident by physical examination, while lung biopsy showed a non-specific lymphoid hyperplasia. Sirolimus was used for a short period without effect. Rituximab and mycophenolate mofetil were then started to treat the refractory autoimmune cytopenia with good effect and concomitant B cell lymphopenia. During follow-up, she developed polyarthritis, not responsive to non-steroidal anti-inflammatory drugs, but to sulfasalazine. At age 16, the CTLA-4 fusion protein abatacept was used, replacing rituximab and mycophenolate mofetil, with a good response.

5.II. AJ.III.1 (c.872delA; p.Asn291Metfs*141) predicted haploinsufficiency

This affected female presented aged 11 with severe pancytopenia. Immunological investigations showed low immunoglobulins levels (IgG 2·1 g/L, IgA 0·18 g/L, IgM 0·61 g/L), together with a negative response to diphtheria and tetanus vaccinations, reduced class-switched memory B cells (1·3%), and a slightly decreased NK cells (73/μL). Treatment with steroids and intravenous immunoglobulins was effective. Autoimmune neutropenia improved under granulocyte colony stimulating factor (G-CSF) therapy. Apart from an *Influenza* virus type B infection and a vulvovaginitis caused by *Citrobacter* and *Candida albicans*, she did not suffer from significant infections and lung disease. She experienced several episodes of abdominal discomfort, but gastrointestinal magnetic resonance (MR) imaging and lower endoscopy were normal. Physical examination documented hepatosplenomegaly, and MR imaging showed para-aortic and iliac lymphadenopathy, leading to suspected aortic vasculitis. She suffered from recurrent genital and mouth ulcerations, with accompanying elevated inflammatory markers but without fever. The patient also reported arthralgias and enthesopathy, without swelling. A diagnosis of Behçet's-like disease was established and treatment with colchicine was started, however without any effect. Following, azathioprine had to be suspended due to hepatopathy, and treatment with anti-TNF adalimumab is currently planned. The same variant was then identified in

additional family members: the mother and the brother had hypogammaglobulinemia, but five asymptomatic carriers were observed in the same family; the uncle had succumbed to Hodgkin's B-cell lymphoma prior to immunological and genetic testing.

5.III. BF.II.1 (c.1365delT; p.Val456*) predicted precursor skipping (previously described by Lougaris et al. and Keller et al.)[7,24]

The proband is a 54 year old male. During childhood, two cervical lymph nodes were excised and an episode of thrombocytopenia occurred. He did not exhibit other disease manifestations until the age of 38 year, when he started to suffer from recurrent respiratory tract infections (pharyngotonsillitis, sinusitis, otitis and bronchitis), which poorly responded to repeated antibiotic courses and to nasal septum surgery. At age 43, he had right basal pneumonia lasting 4 weeks with fever at 39°C but unknown microbiological etiology. On high-resolution chest CT (HRCT) granulomas accompanied by lymphoid infiltration appeared, leading to the diagnosis of GLILD. Following pneumonia, the detection of hypogammaglobulinemia (IgG 0·08 g/L, IgA 0·05 g/L, IgM 0·05 g/L) hinted at the diagnosis of CVID, and immunoglobulin replacement therapy was initiated. Furthermore, he developed autoimmune manifestations including vitiligo, seronegative arthritis, and keratoconjunctivitis sicca. He had multiple herpes zoster reactivations and an acute *Salmonella enteritidis* gastroenteritis. *Haemophilus influenzae* was detected by sputum analysis. At physical examination, lymphadenopathy, splenomegaly and hepatomegaly were evident. Nodular regenerative hyperplasia (NRH) was identified on hepatic sonography. Bone marrow biopsy showed a lack of plasma cells and nodular lymphocytic infiltrates. The patient was treated with steroids and cyclosporine. Lymphocyte immunophenotyping displayed reduced frequency of class-switched memory B cells (1·4%) with relatively high frequency of CD21low B cells (18·9%) and transitional B cells (27·9%) (EUROclass: B+ smB- 21 lo Tr high). The sister of the index case (BF.II.2) presented with ITP, chronic sinusitis, necrotizing tonsillitis, recurrent bronchitis, severe chronic periodontitis and pneumonia at the age of 33 years. She was diagnosed with GLILD with bronchiectasis. She had two herpes zoster reactivations and CMV viremia with increased liver enzymes and cytopenia requiring systemic antiviral therapy. Multiple hepatic hemangiomas were detected by ultrasonography and liver histology revealed a T-cell infiltration resulting in cholangitis. Lymphoid hyperplasia, splenomegaly and hepatomegaly were documented. During follow-up, an aphthous stomatitis and intermittent arthralgia have been also reported.

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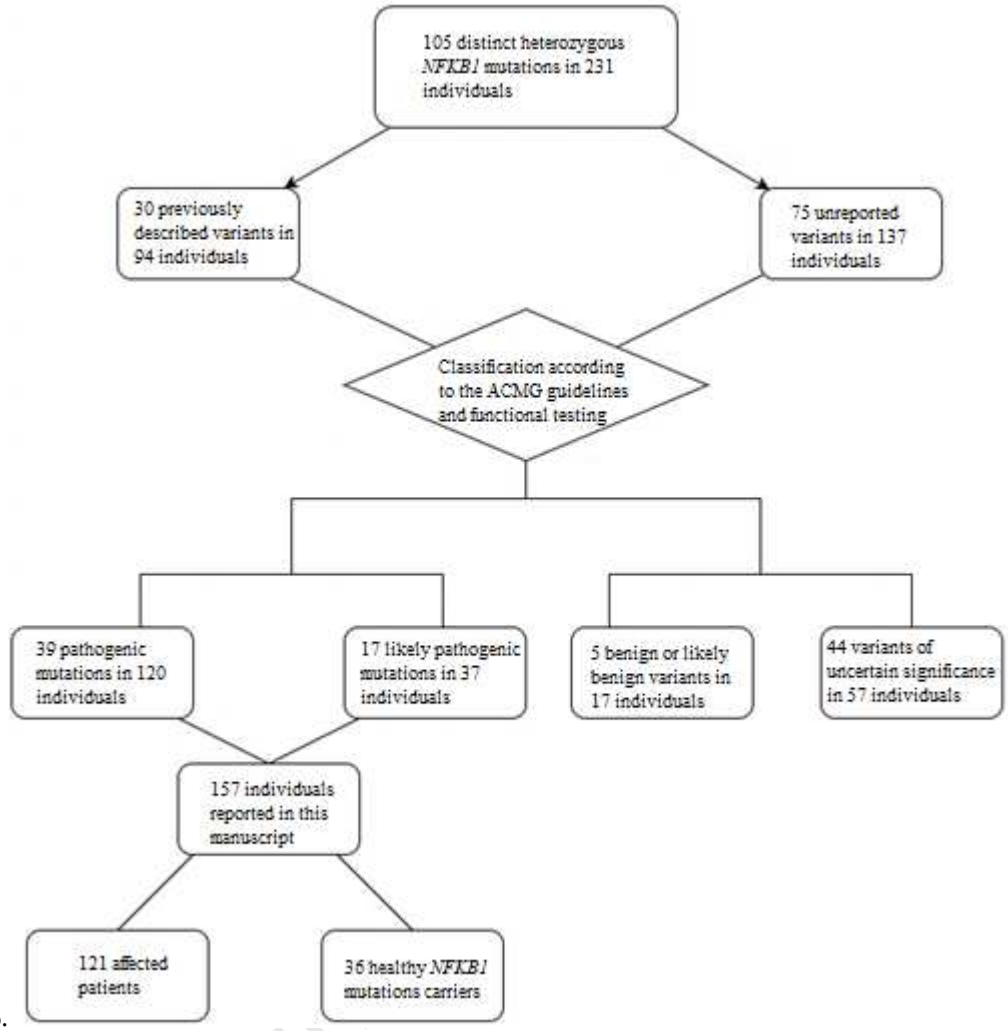
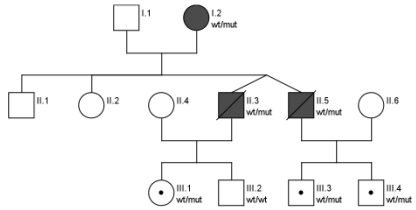


316.

Figure S1. Diagnostic flow-chart leading to the identification of 56 damaging *NFKB1* mutations in 157 mutation carriers, of which 121 considered affected. After the identification and characterization of 105 distinct heterozygous *NFKB1* variants, 56 were classified as pathogenic according to the American College of Medical Genetics and Genomics (ACMG) guidelines.

Fam C (p.Arg54_Lys86del)



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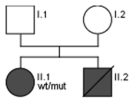
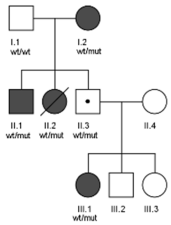
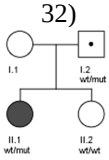


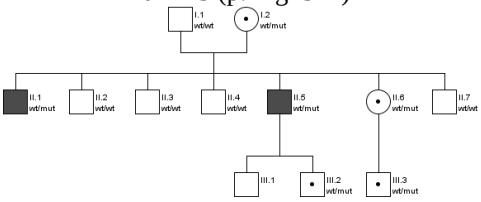
Fam NZ (p.Ala156Serfs*12)



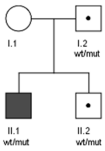
Fam AB (p.Gly165Alafs*32)



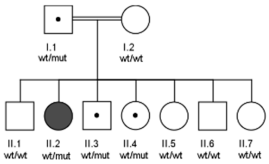
Fam F3 (p.Arg157*)



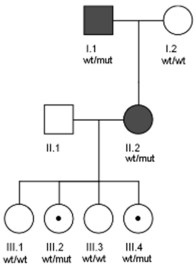
Fam AF (p.Asp191_Lys244delinsGlu)



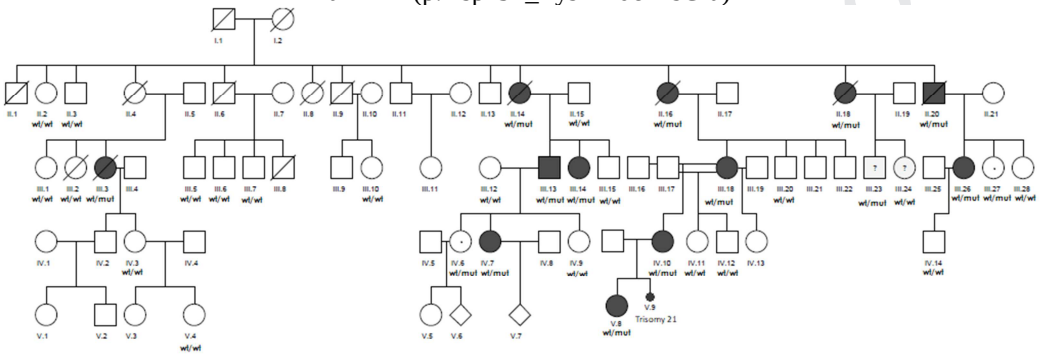
Fam AA (p.Arg157*)



Fam 089 (p.Lys244_Asp279delinsAsn)



Fam NA (p.Asp191_Lys244delinsGlu)



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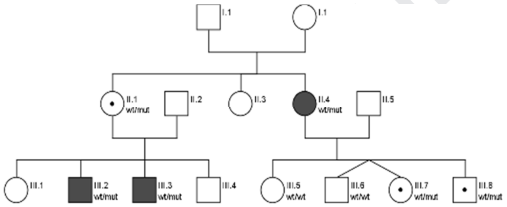
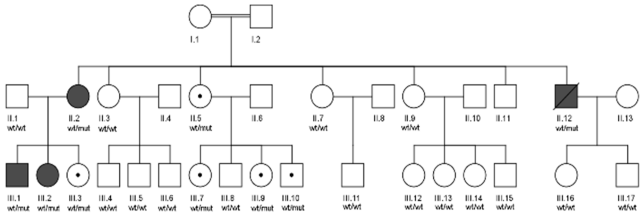
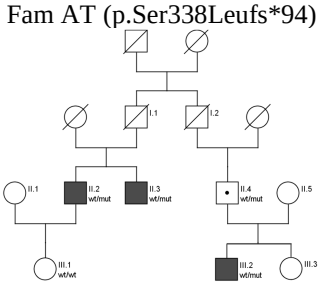


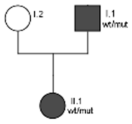
Fam AJ (p.Asn291Metfs*141)



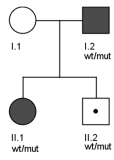
Fam AT (p.Ser338Leufs*94)



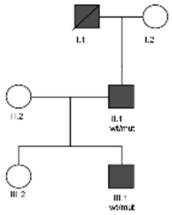
Fam AM (p.Ser302Phefs*7



Fam AV (p.Phe310Ilefs*76)



Fam AN (p.Ser302Phefs*7)



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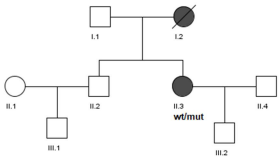
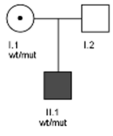
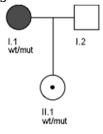


Fam AS (p.Gln333*)



Fam BB (p.Gly384Glufs*48)



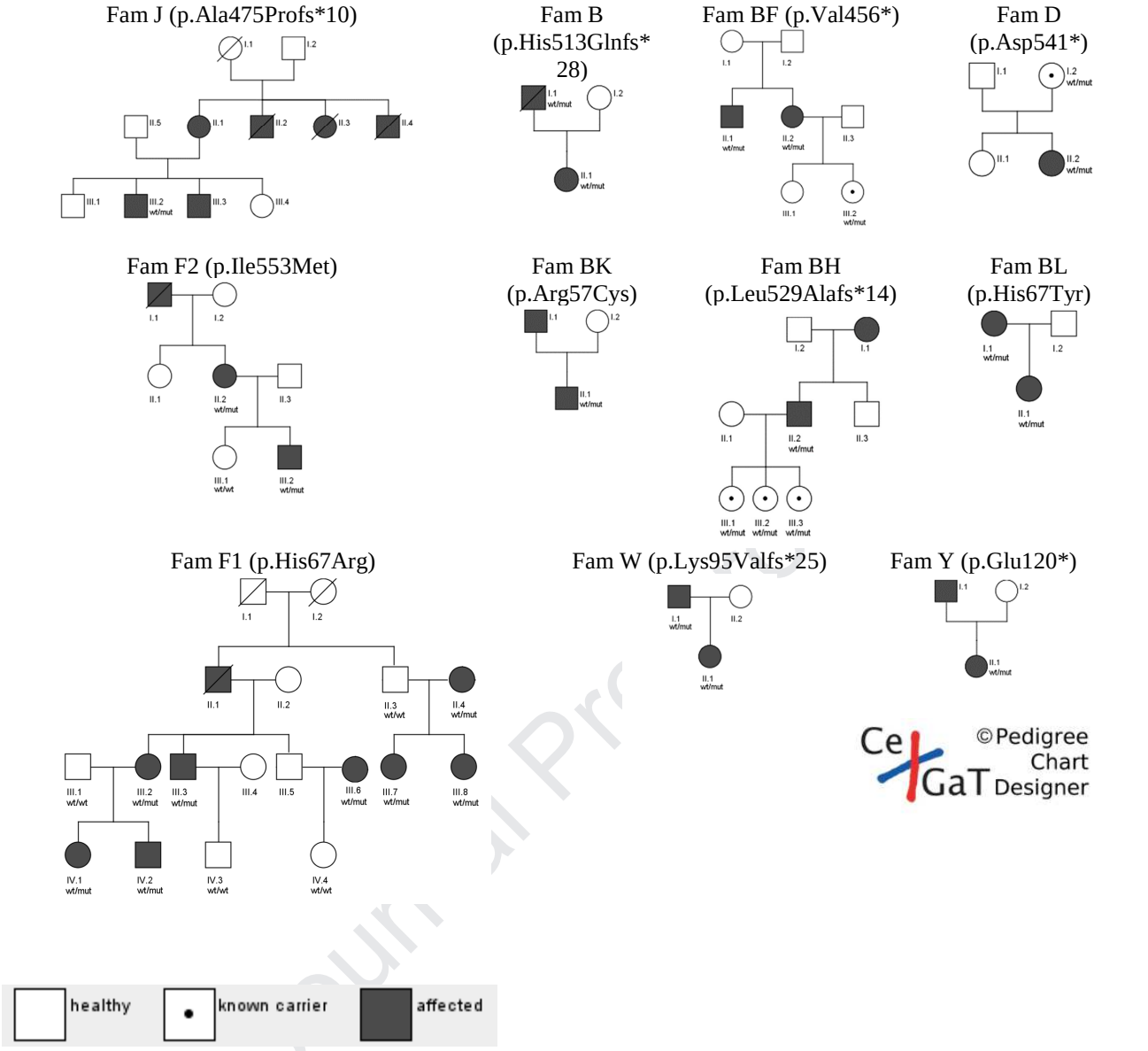


Figure S2. Autosomal dominant inheritance of heterozygous *NFKB1* mutations in 30 affected families. The pedigrees of 116 out of 119 familial cases were available; 38 cases were sporadic. Circles represent females, squares represent males; filled symbols correspond to affected individuals, healthy carriers are designated placing a spot in an open symbol. Clear symbols correspond to healthy members with wild-type *NFKB1*, symbols with a diagonal line are used to represent deceased individuals. Genetic analysis has been performed in the indicated family members.

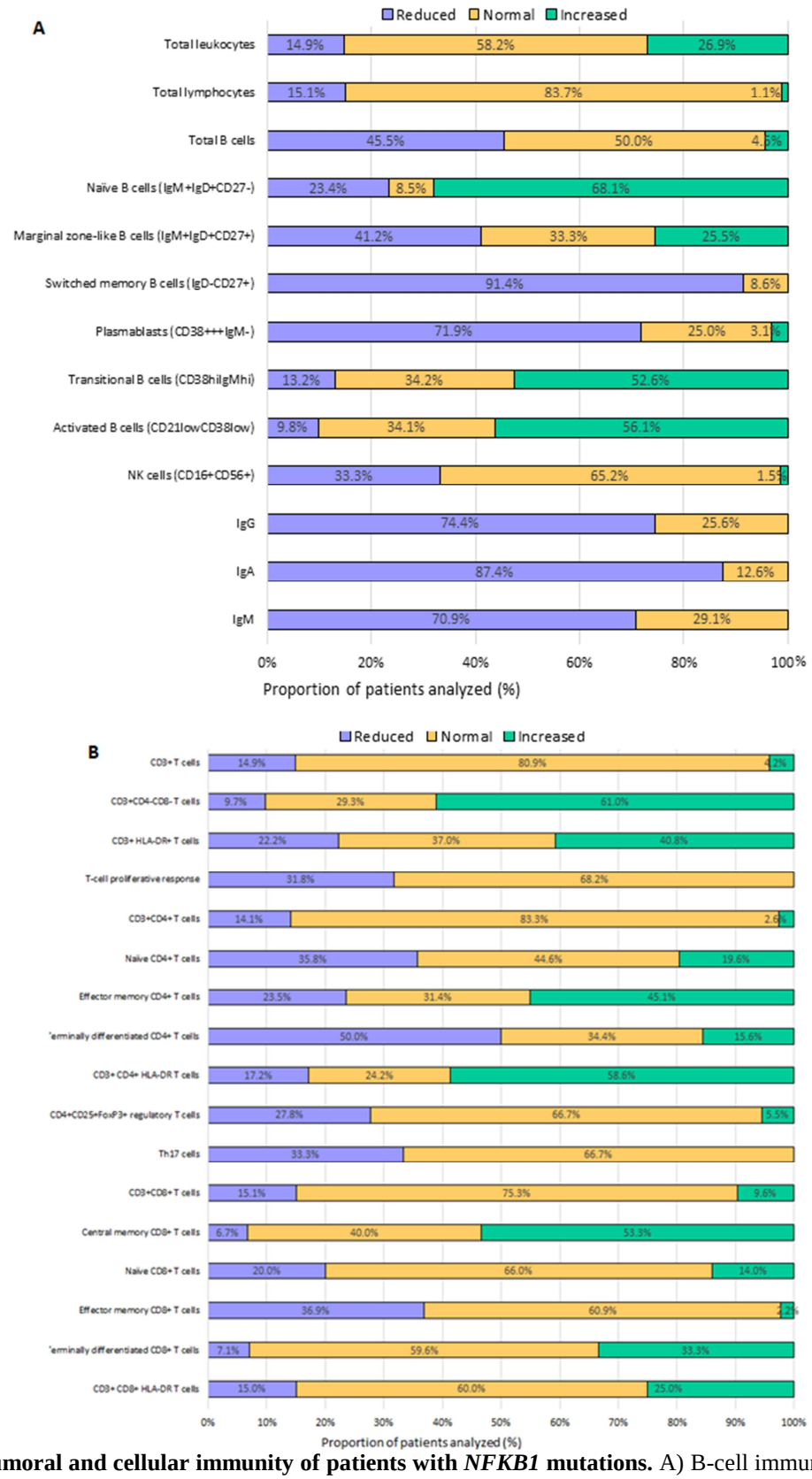


Figure S3. Humoral and cellular immunity of patients with *NFKB1* mutations. A) B-cell immunophenotyping, NK cell values and immunoglobulin levels in patients with *NFKB1* mutations are shown. B) T-cell immunophenotyping is shown. Reduced absolute numbers or relative percentages are represented in blue, normal values in yellow, and increased values in green. Normal ranges are age-related.

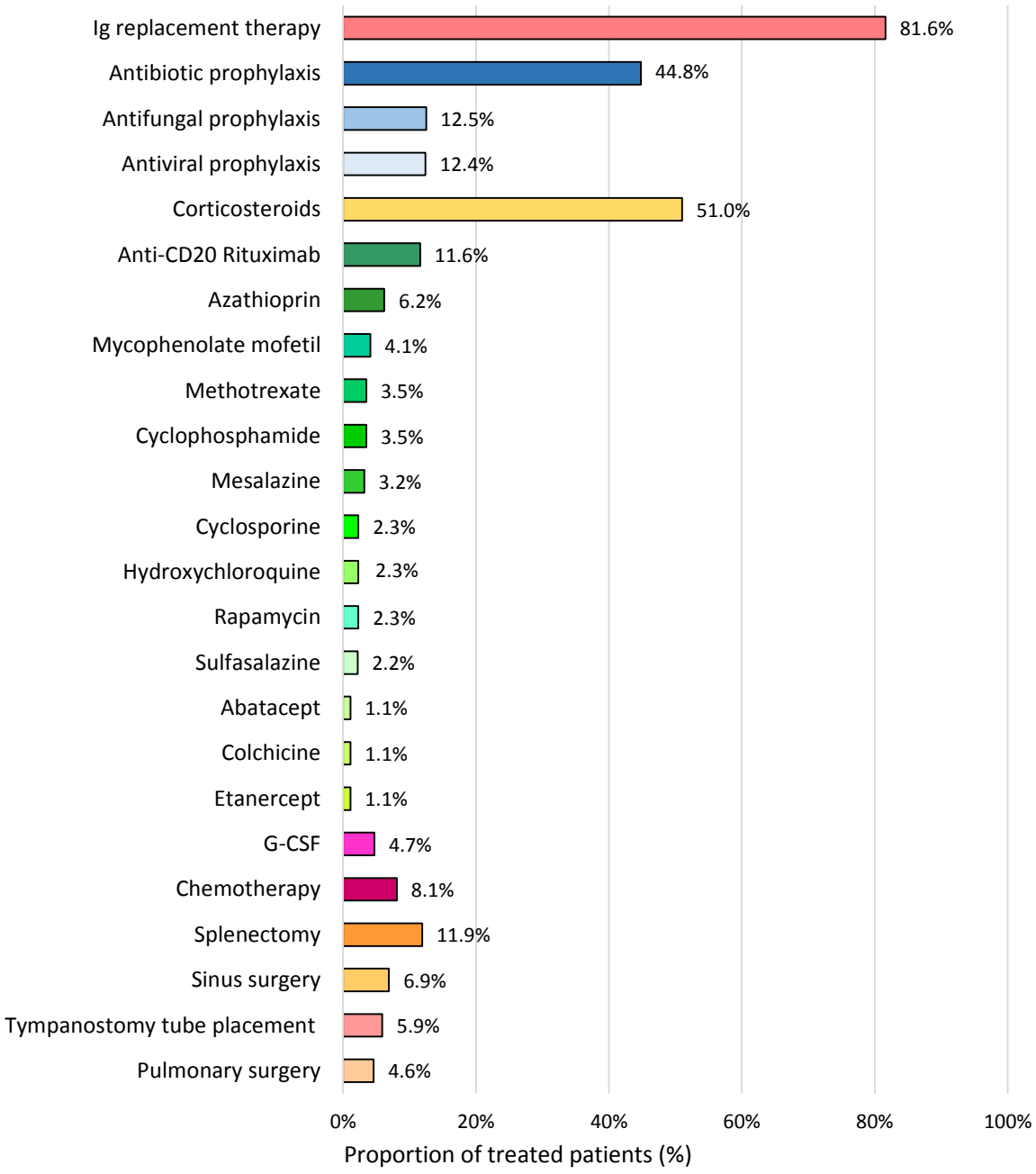


Figure S4. Treatment of patients with damaging heterozygous *NFKB1* mutations. Percentage distribution of type of treatments within the cohort of affected *NFKB1* mutation carriers. Blue bars indicate antimicrobial prophylaxis, green bars indicate immunosuppressive treatment, orange bars indicate surgical treatment.

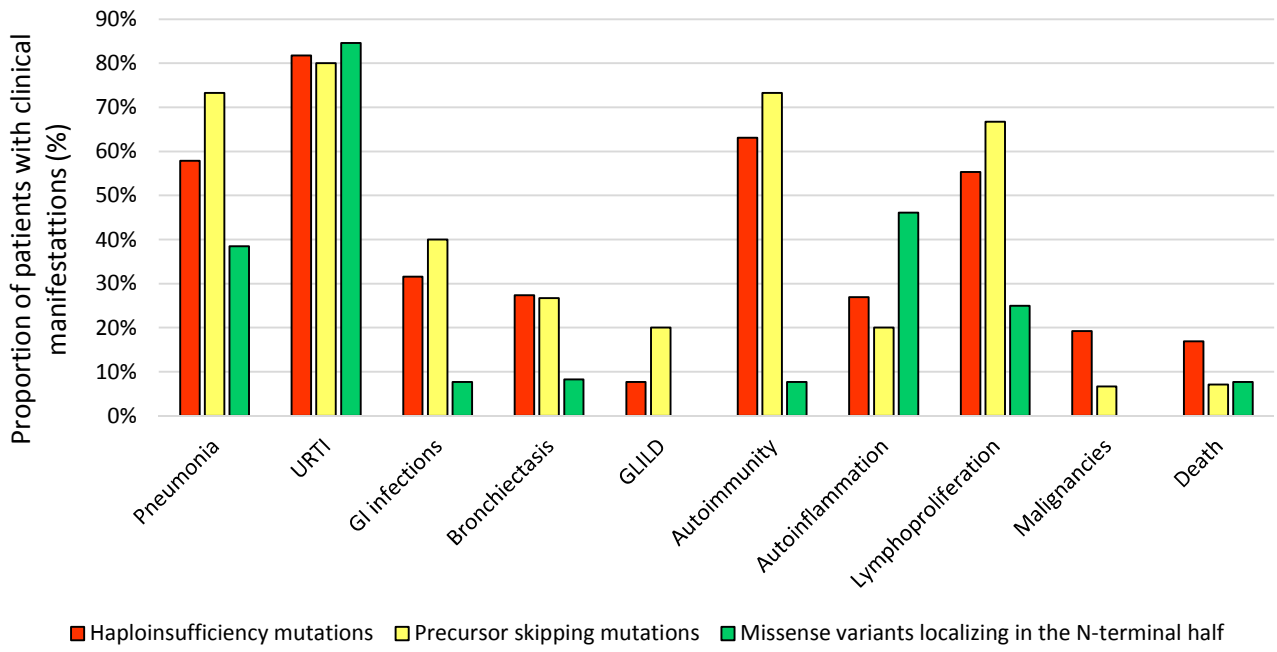


Figure S5. Genotype-phenotype correlation in patients with *NFKB1* mutations. Percentage distribution of clinical manifestations within the cohort of patients with haploinsufficiency *NFKB1* mutations (red), in comparison to the cohort of patients with precursor skipping *NFKB1* mutations (yellow), and to the cohort of patients with missense *NFKB1* mutations affecting the p105 precursor and the mature p50 (green).

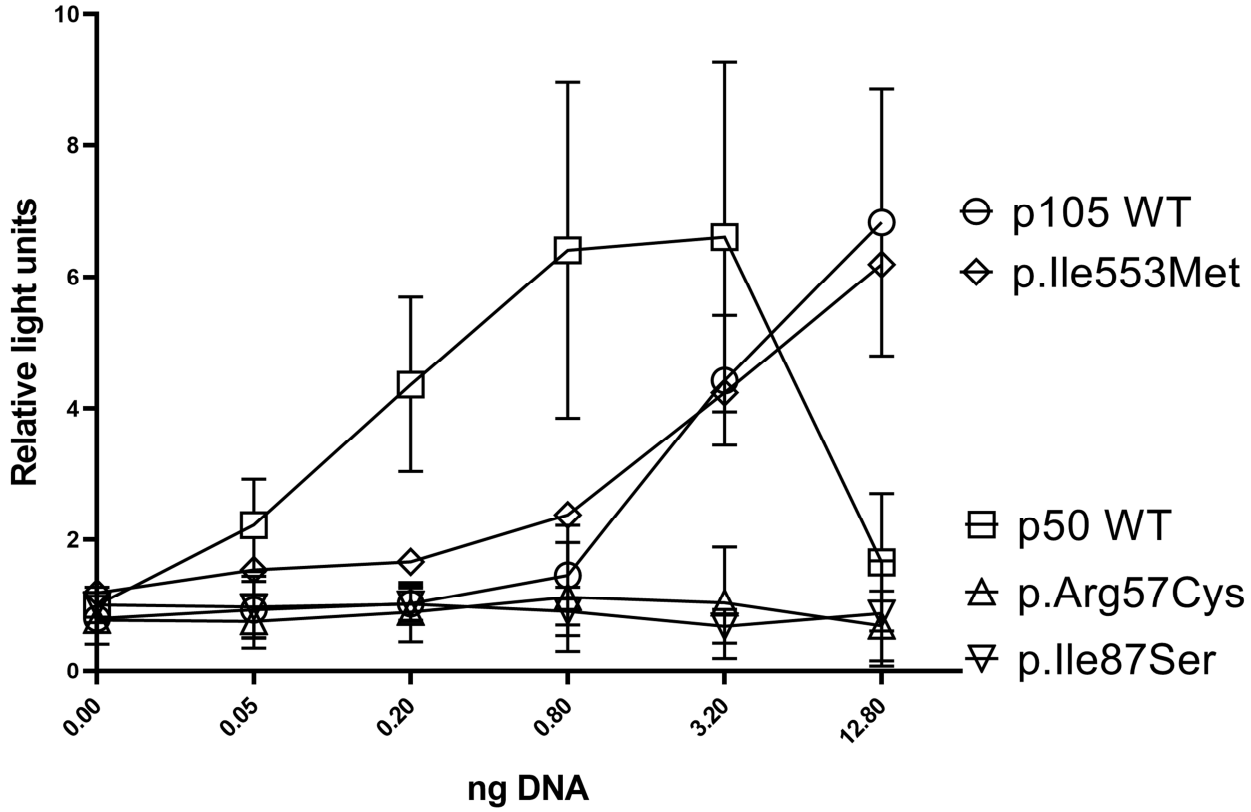


Figure S6. HEK293T cells were transiently transfected with N-terminal GFP-fused constructs in duplicates, as indicated. Mutants p.Arg57Cys and p.Ile87Ser showed loss of luciferase reporter activity. WT p105 and variant p.Ile553Met showed comparable increase in emitted light. In contrast to WT p105, WT p50 construct light signal increased rapidly, emitting even higher signal, to fall down at an amount of DNA between 3.20ng and 12.80ng. Consumption of endogenous RelA and inhibitory effect of p50 homodimers might contribute to these results. Relative light units were normalized to co-transfected Renilla luciferase. Mock is not shown. DNA amounts were compensated with non-related plasmid DNA. Data represent the results from 3 to 4 experimental repeats; p.Ile553Met was done once in duplicates.

Variant	cDNA	Protein	Type of variant	Predicted type of change	Affected/healthy	SIFT	Polyphen2	CADD	MutationTaster	ExAC freq	ACMG classification	Described
HAPLOINSUFFICIENCY MUTATIONS (typically truncations in the N-terminal "p50" half of p105)												
1	del 103370996-103528207		Large deletion	Truncation	1						P(Ia) (PVS1, PS3, PM2)	Y[12]
2	del 103436974-103652655		Large deletion	Truncation	1						LP(I) (PVS1, PM2)	Y[12]
3	c.118+1G>A	p.Met14Glnfs*9	Splice-site	Exon skipping	2			27.2	D		P(Ib) (PVS1, PM1, PM2)	N
4	c.139delA	p.Ile47Tyrfs*2	Deletion	Frameshift	1						P(Ia) (PVS1, PS3, PM1, PM2, PM6)	Y[5]
5	c.160-1G>A	p.Arg54_Lys86del	Splice-site	Exon skipping	3/3			27.3			LP(II) (PS3, PM1, PM2)	Y[12]
6	c.187delG	p.Glu63Lysfs*64	Deletion	Frameshift	2			35			P(Ib) (PVS1, PM1, PM2, PP1)	Y[12]
7	c.250C>T	p.Gln84*	Nonsense	Truncation	1						P (Ib) (PVS1, PM1, PM2)	N
8	c.259-4A>G	p.Ile87Leufs*16	Splice-site	Exon skipping?	1			9.635		8.3E-06	LP(II) (PS1, PM1)	Y[9]
9	c.259-2A>G	p.Ile87Leufs*16	Splice-site	Exon skipping?	1						P(Ib) (PVS1, PM1, PM2)	N
10	c.285_286delGG	p.Lys95Valfs*25	Deletion	Frameshift	2						P(Ib) (PVS1, PM1, PM2, PP1)	N
11	c.295C>T	p.Gln99*	Nonsense	Truncation	2			22.4	D		P(Ib) (PVS1, PM1, PM2)	Y[12]
12	c.358G>T	p.Glu120*	Nonsense	Truncation	2			22.3	D		P(Ib) (PVS1, PM1, PM2, PP1)	N
13	c.465dupA	p.Ala156Serfs*12	Insertion	Frameshift	4/1						P(Ia) (PVS1, PS3, PM1, PM2)	Y[4]
14	c.469C>T	p.Arg157*	Nonsense	Truncation	4/7						P(Ia) (PVS1, PS3, PM1, PM2, PP1)	Y[5]
15	c.494delG	p.Gly165Alafs*32	Deletion	Frameshift	1/1						P(Ia) (PVS1, PS3, PM1, PM2)	Y[32]
16	c.522_525dupTGAC	p.Leu176*	Nonsense	Truncation	1						P(Ib) (PVS1, PM1, PM2)	N
17	c.607C>T	p.Gln203*	Nonsense	Truncation	1						P(Ib) (PVS1, PM1, PM2, PP1)	N
18	c.638_641dupTGCG	p.Leu215Alafs*11	Insertion	Frameshift	1						LP(I) (PVS1, PM2)	N
19	c.730+4A>G	p.Asp191_Lys244delinsGlu	Splice-site	Exon skipping	13/4						P(III) (PS3, PM1, PM2, PM4, PP1)	Y[4]
20	c.731-13_733del	p.Lys244_Asp279delinsAsn or p.Lys244Serfs*27	Deletion	Exon skipping or retained intron	1						P(III) (PM1, PM2, PM4)	N
21	c.830dupA	p.Lys278Glufs*3	Insertion	Frameshift	1			35			P(Ib) (PVS1, PM1, PM2)	Y[12]
22	c.835+2T>G	p.Lys244_Asp279delinsAsn	Splice-site	Exon skipping	4/2			25.4			P(III) (PS3, PM1, PM2, PM4)	Y[4]
23	c.836-3C>T	p.Asp279Valfs*11	Splice-site	Exon skipping?	1						P(Ib) (PVS1, PM1, PM2)	N
24	c.850C>T	p.Arg284*	Nonsense	Truncation	3/3			44			P(Ib) (PVS1, PM1, PM2)	Y[12]
25	c.872delA	p.Asn291Metfs*141	Deletion	Frameshift	5/5						P(Ib) (PVS1, PM1, PM2)	N
26	c.875delG	p.Gly292Valfs*140	Deletion	Frameshift	1						P(Ib) (PVS1, PM1, PM2)	N
27	c.904dupT	p.Ser302Phefs*7	Insertion	Frameshift	8			34		8.2E-06	P(Ic) (PVS1, PM1, PP1)	Y[11]
28	c.950_964delCAAAGTATAAAGATA (15bp del)+c.967A>T	p.Pro317_Ile322delinsLeu+p.Asn323Tyr	In-frame deletion	Truncation	1						LP(IV) (PM1, PM2, PM4, PM6)	N
29	c.957T>A	p.Tyr319*	Nonsense	Truncation	1			36			P(Ib) (PVS1, PM1, PM2)	Y[9]
30	c.997C>T	p.Gln333*	Nonsense	Truncation	1/1						P(Ib) (PVS1, PM1, PM2)	N
31	c.1005delG	p.Arg336Glyfs*96	Deletion	Frameshift	1			35			P(Ib) (PVS1, PM1, PM2)	Y[12]
32	c.1012delT	p.Ser338Leufs*94	Deletion	Frameshift	3/1						P(Ib) (PVS1, PM1, PM2)	N
33	c.1066+1G>C	p.Phe310Ilefs*76	Splice-site	Exon skipping?	2						P(Ib) (PVS1, PM1, PM2, PP1)	N
34	c.1066+1G>T	p.Phe310Ilefs*76	Splice-site	Exon skipping?	2/1						P(Ib) (PVS1, PM1, PM2)	N
35	c.1071_1074delAGAA	p.Glu358Lysfs*73	Deletion	Frameshift	1						P(Ib) (PVS1, PM1, PM2)	N
36	c.1183delG	p.Gly395fs*	Deletion	Frameshift	1						LP(I) (PVS1, PM2)	N

37	c.1210+1G>A	p.Asp356_Pro403del	Splice-site	Exon skipping?	1						LP(II) (PS3, PM2, PM4)	N
38	c.1211_1214dupGGTA	p.Tyr405*	Nonsense	Truncation	1			28.1			P(Ib) (PVS1, PM2, PM6)	N
39	c.1301-1G>A	p.Gly434_Gln498del	Splice-site	Exon skipping?	2/1			24			LP(IV) (PM1, PM2, PM4)	Y[9]
PRECURSOR SKIPPING MUTATIONS (typically in the central part of p105 producing p50-like proteins)												
40	c.1149delT	p.Gly384Glufs*48	Deletion	Frameshift	1/1	D	P		D		LP(I) (PVS1, PM2)	Y[13]
41	c.1245_1246delTG	p.Tyr415*	Nonsense	Truncation	1						LP(I) (PVS1, PM2)	N
42	c.1321A>T	p.Lys441*	Nonsense	Truncation	1						P(Ib) (PVS1, PM1, PM2)	N
43	c.1365delT	p.Val456*	Nonsense	Truncation	2/1						P(Ia) (PVS1, PS3, PM1, PM2, PP1)	Y[7]
44	c.1377delT	p.Phe459Leufs*26	Deletion	Frameshift	1			23.2			P(Ib) (PVS1, PM1, PM2)	Y[9]
45	c.1423delG	p.Ala475Profs*10	Deletion	Frameshift	5			15.2			P(Ia) (PVS1, PS3, PM1, PM2, PP1)	Y[12]
46	c.1517delC	p.Ala506Valfs*17	Deletion	Frameshift	1						P(Ia) (PVS1, PS3, PM1, PM2)	Y[7]
47	c.1537_1541delCATGC	p.His513Glnfs*28	Deletion	Frameshift	2			35		1.7E-05	P(Ic) (PVS1, PM1, PP1)	Y[12]
48	c.1584dupG	p.Leu529Alafs*14	Insertion	Frameshift	2/3						P(Ib) (PVS1, PM1, PM2)	N
49	c.1621_1622delGA	p.Asp541*	Nonsense	Truncation	1/1			35		3.3E-05	P(Ia) (PVS1, PS3, PM1)	Y[12]
50	c.1726dupA	p.Ile567Asnfs*6	Insertion	Frameshift	1						LP(I) (PVS1, PM2)	N
51	c.1752+1G>A	p.Ser546Argfs*8	Splice-site	Exon skipping?	1						LP(I) (PVS1, PM2)	N
MISSENSE VARIANTS AFFECTING BOTH, THE p105 PRECURSOR AND THE MATURE p50 (localizing in the N-terminal "p50" half of p105)												
52	c.169C>T	p.Arg57Cys	Substitution	Missense	2	D	D	35	D		LP(V) (PS3, PM2, PP1, PP3)	N
53	c.199C>T	p.His67Tyr	Substitution	Missense	2	D	D	27.7	D		LP(V) (PM2, PM5, PP1, PP3)	N
54	c.200A>G	p.His67Arg	Substitution	Missense	9	D	D	25.8	D		LP(II) (PS3, PM2, PP1, PP3)	Y[10]
55	c.260T>G	p.Ile87Ser	Substitution	Missense	1	D	D	31	D		LP(II) (PS3, PM2, PP3)	Y[12]
56	c.293T>A	p.Val98Asp	Substitution	Missense	1	D	D	29.3	D		U(PM2, PP3)	Y[12]
57	c.843C>G	p.Ile281Met	Substitution	Missense	1	D	D	25.2	D		U(PM2, PP3)	Y[12]
58	c.106G>A	p.Ala36Thr	Substitution	Missense	1	T	B	20.8	N		U(PM2, PM6)	N
59	c.191G>T	p.Gly64Val	Substitution	Missense	2	D	D	29	D		U(PM2, PP3)	N
60	c.269A>C	p.Tyr90Ser	Substitution	Missense	1		D	27.8	D		U(PM2, PP3)	N
61	c.470G>C	p.Arg157Pro	Substitution	Missense	2	D	D	33	D		U(PM2, PP1, PP3)	N
62	c.508G>A	p.Gly170Ser	Substitution	Missense	1	T	P	23.2	D		U(PM2)	N
63	c.556G>T	p.Asp186Tyr	Substitution	Missense	2	D	D	20.6	D	8.2E-06	U(PP1, PP3)	N
64	c.592C>T	p.Arg198Cys	Substitution	Missense	1	D	D	26.8	D		U(PM2, PP3)	N
65	c.641G>A	p.Arg214Gln	Substitution	Missense	1				D		U(PM2)	N
66	c.646A>G	p.Met216Val	Substitution	Missense	1/1	T	D	20.2	D		U(PM2)	N
67	c.689G>A	p.Arg230Lys	Substitution	Missense	4	T	P	23	D		U(PM2, PP1)	N
68	c.734C>T	p.Ala245Val	Substitution	Missense	1	D	D	34	D		U(PM2, PP3)	N
69	c.736C>A	p.Pro246Thr	Substitution	Missense	2	D	D	28.9	D		U(PM2, PP3)	N
70	c.856T>A	p.Tyr286Asn	Substitution	Missense	1	D	D	28.8	D		U(PM2, PP3)	N
71	c.885G>C	p.Trp295Cys	Substitution	Missense	1	D	D	29.9	D		U(PM2, PP3)	N
72	c.978A>C	p.Lys326Asp	Substitution	Missense	2	D	D	23.7	D	0.02413	U(PM6, PP3, BS1)	N
73	c.1004G>A	p.Arg335Gln	Substitution	Missense	1					0.000157	U	N
74	c.1049A>G	p.Tyr350Cys	Substitution	Missense	4	D	D	24.6	D		U(PM2, PP1, PP3)	N
75	c.1115C>T	p.Ser372Leu	Substitution	Missense	1	T	P	22.9	D	8.24E-06	U	N
76	c.1126G>A	p.Gly376Ser	Substitution	Missense	1	D	P	23.6	D	4.12E-05	U(PP3)	N
77	c.1147G>T	p.Ala383Ser	Substitution	Missense	1	T	B	14.1	D		U(PM2, BP4)	N
78	c.1156G>A	p.Gly386Arg	Substitution	Missense	1	D	P	25.4	D	1.65E-05	U(PP3)	N
79	c.1177G>A	p.Gly393Ser	Substitution	Missense	1	T	P	17.8	D	1.65E-05	U(BP4)	N
MISSENSE VARIANTS PROBABLY AFFECTING ONLY THE FUNCTIONS OF THE PRECURSOR (localizing to the C-terminal half of p105 precursor)												
80	c.1659C>G	p.Ile553Met	Substitution	Missense	3	D	D	26.3	D		LP(II) (PS3, PM2, PP1, PP3)	Y[10]
81	c.1307T>C	p.Met436Thr	Substitution	Missense	1	T	B	5.7	N	4.12E-05	U(BP4)	N
82	c.1388T>C	p.Ile463Thr	Substitution	Missense	3	T	B	0.002	N	0.0002553	U(BS4)	N
83	c.1424C>G	p.Ala475Gly	Substitution	Missense	2	T	B	2.5	N	0.0001565	U(BP4)	N
84	c.1427C>T	p.Thr476Ile	Substitution	Missense	2	T	B	0.058	N	9.06E-05	U(BP4)	N
85	c.1480A>C	p.Ser494Arg	Substitution	Missense	1	T	B	0.461	N	1.65E-05	U(BP4)	N
86	c.1519A>G	p.Met507Val	Substitution	Missense	1	T	B	4.273	N	0.009727	LB(I) (BS1, BP4)	N

87	c.1736G>A	p.Arg579Lys	Substitution	Missense	4/3	T	D	18.74	D	0.00183	B (BS1, BS4)	N
88	c.1845G>T	p.Leu615Phe	Substitution	Missense	6	T	B	11.05	N	0.001944	LB(I) (BS1, BP4)	N
89	c.1985G>A	p.Ser662Asn	Substitution	Missense	1	T	P	23.7	N		U(PM2)	N
90	c.2136T>G	p.His712Gln	Substitution	Missense	2	D	B	11.3	N	0.002126	LB(I) (BS1, BP4)	N
91	c.2251A>G	p.Thr751Ala	Substitution	Missense	1		P	23.2	D		U(PM2)	N
92	c.2326C>G	p.Pro776Ala	Substitution	Missense	1	T	D	22.9	D		U(PM2, PP3)	N
93	c.2378C>G	p.Pro793Arg	Substitution	Missense	1	T	D	23.9	D	2.47E-05	U(PP3)	N
94	c.2440G>A	p.Glu814Lys	Substitution	Missense	1	T	B	18.27	N		U(PM2, BP4)	N
95	c.2457G>C	p.Gln819His	Substitution	Missense	1						U(PM2)	N
96	c.2462A>G	p.Tyr821Cys	Substitution	Missense	1	T	B	11.99	D	1.65E-05	U(BP4)	N
97	c.2650G>A	p.Glu884Lys	Substitution	Missense	1	D	D	24.4	D	1.65E-05	U(PP3)	N
98	c.2831C>A	P.Thr944Asn	Substitution	Missense	1	T	B	12.3	N	0.000362	LB(BS1,BP4)	N
VARIANTS WITH UNKNOWN EFFECT												
99	c.160-4G>C	p.Arg54_Lys86del	Splice-site	Exon skipping?	1						U(PM2)	N
100	c.590-8C>T		Splice-site	Exon skipping?	2			8.9			U(PM2)	N
101	c.1750-10C>G		Splice-site	Exon skipping?	1						U(PM2)	N
102	c.2348G>A	p.Trp783*	Nonsense	Truncation	2						U(PM2)	N
103	c.2592+3A>G	p.Asp808Leufs*22	Splice-site	Frameshift	1						U(PM2)	N
104	c.2593-4A>G	p.Val865Thrfs*27	Splice-site	Exon skipping?	2			5.587		0.001921	U(BS1)	N
105	c.2671delG	p.Ala891Glnfs*6	Deletion	Frameshift	1						U(PM2)	N

Table S1. Variants classification. SIFT (Sorting Intolerant From Tolerant) score: the amino acid substitution is predicted damaging (D) or tolerated (T). Poliphen2 score: a mutation is classified as benign (B), possibly damaging (P), or probably damaging (D). MutationTaster score: a variant is defined as a disease mutation (D) or a harmless polymorphism (N). CADD (combined annotation dependent depletion) score ranks genetic variants according to diverse genomic features. American College of Medical Genetics and Genomics (ACMG) classification: according to the evidence of pathogenicity, a variant is classified as pathogenic (P), likely pathogenic (LP), of uncertain significance (U), benign (B), and likely benign (LB). The evidence of pathogenicity or of benign impact is defined as very strong (VS), strong (S), moderate (M), and supporting (P).

Case No.	c.DNA	Reference	Affected	Healthy	Sex	Age at evaluation /death Δ	Country of origin	Age at onset	Age at diagnosis	First manifestation	Diagnosis
I.II.1	del 103370996-103528207	*Tuijnenburg et al.*	Yes	No	NA	26	NA	12	18	Infections	CVID
K.II.1	del 103436974-103652655	*Tuijnenburg et al.*	Yes	No	NA	65	NA	NA	44	Infections	CVID
Q.I.1	c.118+1G>A	Unpublished	Yes	No	F	16	RUS	1	7	Inflammation	CVID
R.I.1	c.118+1G>A	Unpublished	Yes	No	NA	NA	DEU	NA	NA	NA	NA
S.I.1	c.139delA	*Schipp et al.*	Yes	No	F	27	DEU	14	26	Autoimmunity	CVID/ALPS
C.I.2	c.160-1G>A	*Tuijnenburg et al.*	Yes	No	F	79	NA	40	52	Infections	CVID
C.II.3	c.160-1G>A	*Tuijnenburg et al.*	Yes	No	M	36 Δ	NA	NA	16	Infections	CVID
C.II.5	c.160-1G>A	*Tuijnenburg et al.*	Yes	No	M	39 Δ	NA	NA	13	Infections	CVID
C.III.1	c.160-1G>A	*Tuijnenburg et al.*	No	Yes	F	18	NA	-	-	-	Healthy
C.III.3	c.160-1G>A	*Tuijnenburg et al.*	No	Yes	M	16	NA	-	-	-	Healthy
C.III.4	c.160-1G>A	*Tuijnenburg et al.*	No	Yes	M	13	NA	-	-	-	Healthy
L.II.1	c.187delG	*Tuijnenburg et al.*	Yes	No	F	48	NA	NA	22	Infections	CVID
L.II.2*	c.187delG	*Tuijnenburg et al.*	Yes	No	M	NA Δ	NA	NA	NA	NA	Antibody deficiency
T.I.1	c.250C>T	Unpublished	Yes	No	F	14	GBR/IND	11	13	Infections	CVID
U.I.3	c.259-4A>G	*Maffucci et al.*	Yes	No	M	48	NA	21	NA	NA	CVID
V.I.1	c.259-2A>G	Unpublished	Yes	No	F	41	ESP	2,5	20	Infections	CVID
W.I.1	c.285_286delGG	Unpublished	Yes	No	M	49	AUS	12	15	NA	CVID
W.II.1	c.285_286delGG	Unpublished	Yes	No	F	18	AUS	16	16	Autoimmunity	Autoimmunity and immune dysregulation
X.I.1	c.295C>T	Unpublished	Yes	No	F	60	DEU	30	30	Infections	CVID
O.II.1	c.295C>T	*Tuijnenburg et al.*	Yes	No	F	39	GBR/IND	NA	23	Infections	CVID
Y.II.1	c.358G>T	Unpublished	Yes	No	F	38	DEU	6	6	Family history	CVID
Y.I.1*	c.358G>T	Unpublished	Yes	No	M	NA	DEU	NA	NA	NA	NA
NZ.I.2	c.465dupA	*Fliegauf et al.*	Yes	No	F	76	NZL/EU	12	73	Autoimmunity	CVID
NZ.II.1	c.465dupA	*Fliegauf et al.*	Yes	No	M	51	NZL/EU	2	7	Autoimmunity	CVID
NZ.II.2	c.465dupA	*Fliegauf et al.*	Yes	No	F	49 Δ	NZL/EU	10	15	Infections	CVID
NZ.II.3	c.465dupA	*Fliegauf et al.*	No	Yes	M	46	NZL/EU	-	-	-	Healthy
NZ.III.1	c.465dupA	Unpublished	Yes	No	F	15	NZL/EU	NA	NA	Infections	CVID
Z.I.1	c.469C>T	Unpublished	Yes	No	M	61	DEU	10	47	Infections	CVID
AA.II.2	c.469C>T	*Schipp et al.*	Yes	No	F	20	ISR	11	19	Lymphoproliferation	CVID
AA.I.1	c.469C>T	*Schipp et al.*	No	Yes	M	53	ISR	-	-	-	Healthy
AA.II.3	c.469C>T	*Schipp et al.*	No	Yes	M	16	ISR	-	-	-	Healthy
AA.II.4	c.469C>T	*Schipp et al.*	No	Yes	F	23	ISR	-	-	-	Healthy
F3.II.1	c.469C>T	*Kaustio et al.*	Yes	No	M	62	FIN	48	NA	Infections	Autoinflammatory disorder
F3.II.5	c.469C>T	*Kaustio et al.*	Yes	No	M	56	FIN	28	NA	Infections	Autoinflammatory disorder
F3.I.2	c.469C>T	*Kaustio et al.*	No	Yes	F	NA	FIN	-	-	-	Healthy
F3.II.6	c.469C>T	*Kaustio et al.*	No	Yes	F	NA	FIN	-	-	-	Healthy
F3.II.4	c.469C>T	*Kaustio et al.*	No	Yes	M	NA	FIN	-	-	-	Healthy
AB.II.1	c.494delG	*Boztug et al.*	Yes	No	F	18	AUT	2	15	Infections	CVID
AB.I.2	c.494delG	*Boztug et al.*	No	Yes	M	NA	AUT	-	-	-	Healthy
AC.I.1	c.522_525dupTGAC	Unpublished	Yes	No	F	37	DEU	2,5	19	Infections	CVID
AD.I.1	c.607C>T	Unpublished	Yes	No	F	29	BRA	16	16	NA	CVID/ALPS
AE.I.1	c.638_641dupTGCG	Unpublished	Yes	No	NA	0.1	DEU	0,1	0,1	Inflammation	Autoinflammatory disorder
NA.II.16	c.730+4A>G	*Fliegauf et al.*	Yes	No	F	76 Δ	NLD/AUS	29	59	Inflammation	CVID
NA.II.18	c.730+4A>G	*Fliegauf et al.*	Yes	No	F	77 Δ	NLD/AUS	NA	64	Family history	CVID
NA.II.19*	c.730+4A>G	*Fliegauf et al.*	Yes	No	F	55 Δ	NLD/AUS	39	46	Infections	CVID
NA.II.21	c.730+4A>G	*Fliegauf et al.*	Yes	No	M	76 Δ	NLD/AUS	30	57	Lung disease	CVID
NA.III.25	c.730+4A>G	*Fliegauf et al.*	Yes	No	F	66 Δ	NLD/AUS	52	NA	Lung disease	CVID
NA.III.34	c.730+4A>G	*Fliegauf et al.*	Yes	No	M	57	NLD/AUS	44	52	Autoimmunity	CVID
NA.III.36	c.730+4A>G	*Fliegauf et al.*	Yes	No	F	56	NLD/AUS	30	30	Infections	CVID
NA.III.40	c.730+4A>G	*Fliegauf et al.*	Yes	No	F	51	NLD/AUS	34	45	Infections	CVID
NA.III.42	c.730+4A>G	*Fliegauf et al.*	No	Yes	F	49	NLD/AUS	-	-	-	Healthy
NA.IV.48	c.730+4A>G	*Fliegauf et al.*	No	Yes	F	32	NLD/AUS	-	-	-	Healthy
NA.IV.49	c.730+4A>G	*Fliegauf et al.*	Yes	No	F	31	NLD/AUS	0	1	Infections	CVID
NA.V.57	c.730+4A>G	*Fliegauf et al.*	Yes	No	F	56	NLD/AUS	NA	39	Infections	CVID
NA.V.62	c.730+4A>G	*Fliegauf et al.*	Yes	No	F	32	NLD/AUS	NA	30	NA	CVID
AF.II.1	c.730+4A>G	Unpublished	Yes	No	M	NA	ESP	0,7	6	Autoimmunity	CVID
AF.I.1	c.730+4A>G	Unpublished	No	Yes	M	NA	ESP	-	-	-	Healthy
AF.II.2	c.730+4A>G	Unpublished	No	Yes	M	NA	ESP	-	-	-	Healthy
AG.I.1	c.731-13_733del	Unpublished	Yes	No	M	7	DEU	6,75	7	Autoimmunity	CVID
M.II.1	c.830dupA	*Tuijnenburg et al.*	Yes	No	NA	32	NA	24	27	Autoimmunity	CVID
F089.I.1	c.835+2T>G	*Fliegauf et al.*	Yes	No	M	71	DEU	15	64	Infections	CVID
F089.II.2	c.835+2T>G	*Fliegauf et al.*	Yes	No	F	38	DEU	2,5	16	Infections	CVID
F089.III.2	c.835+2T>G	*Fliegauf et al.*	No	Yes	F	8	DEU	-	-	-	Healthy
F089.III.4	c.835+2T>G	*Fliegauf et al.*	No	Yes	F	4	DEU	-	-	-	Healthy
AH.I.1	c.835+2T>G	Unpublished	Yes	No	F	54	DEU	39	39	NA	CVID
H.II.1	c.835+2T>G	*Tuijnenburg et al.*	Yes	No	NA	35 Δ	NA	NA	24	Autoimmunity	CVID
AI.I.1	c.836-3C>T	Unpublished	Yes	No	M	9	CZE	2	2,5	Autoimmunity	Autoimmunity and immune dysregulation
A.II.1	c.850C>T	*Tuijnenburg et al.*	No	Yes	F	56	NA	-	-	-	Healthy
A.II.4	c.850C>T	*Tuijnenburg et al.*	Yes	No	F	54	NA	49	52	Infections	CVID
A.III.2	c.850C>T	*Tuijnenburg et al.*	Yes	No	M	28	NA	NA	3	NA	Antibody deficiency
A.III.3	c.850C>T	*Tuijnenburg et al.*	Yes	No	M	22	NA	7	10	Infections	Antibody deficiency
A.III.7	c.850C>T	*Tuijnenburg et al.*	No	Yes	F	26	NA	-	-	-	Healthy
A.III.8	c.850C>T	*Tuijnenburg et al.*	No	Yes	M	24	NA	-	-	-	Healthy
AJ.III.1	c.872delA	Unpublished	Yes	No	F	13	DEU/TUR	11	12	Inflammation	CVID/Behçet's disease

AJ.II.2	c.872delA	Unpublished	Yes	No	F	41	DEU/TUR	NA	NA	NA	Antibody deficiency
AJ.III.3	c.872delA	Unpublished	Yes	No	M	9	DEU/TUR	NA	NA	NA	Antibody deficiency
AJ.II.4	c.872delA	Unpublished	Yes	No	M	40 Δ	DEU/TUR	NA	NA	NA	Autoimmunity and immune dysregulation
AJ.III.5	c.872delA	Unpublished	No	Yes	F	21	DEU/TUR	-	-	-	Healthy
AJ.III.6	c.872delA	Unpublished	No	Yes	F	22	DEU/TUR	-	-	-	Healthy
AJ.II.9	c.872delA	Unpublished	No	Yes	F	47	DEU/TUR	-	-	-	Healthy
AJ.III.8	c.872delA	Unpublished	No	Yes	M	23	DEU/TUR	-	-	-	Healthy
AJ.III.12	c.872delA	Unpublished	No	Yes	F	15	DEU/TUR	-	-	-	Healthy
AK.I.1	c.872delA	Unpublished	Yes	No	F	69	DEU/TUR	69	69	Infections	CVID
AL.I.1	c.875delG	Unpublished	NA	NA	NA	NA	DEU/TUR	NA	NA	NA	NA
AM.II.1	c.904dupT	Unpublished	Yes	No	F	5	NLD	2	2	Infections	CVID
AM.I.1	c.904dupT	Unpublished	Yes	No	M	50	NLD	18	49	Infections	CVID
AN.II.1	c.904dupT	*Rae et al.*	Yes	No	M	52	GBR	48	50	NA	CVID
AN.III.1	c.904dupT	*Rae et al.*	Yes	No	M	15	GBR	10	10	NA	CVID
AN.I.1*	c.904dupT	*Rae et al.*	Yes	No	M	NA Δ	GBR	NA	NA	Infections	PID
AO.I.1	c.904dupT	Unpublished	Yes	No	M	59	RUS	16	37	NA	CVID
AP.I.1	c.904dupT	Unpublished	Yes	No	M	18	USA	15	16	NA	CVID/ALPS
N.II.1	c.904dupT	*Tuijnenburg et al.*	Yes	No	F	58	uk	NA	56	Lymphoproliferation	CVID
AQ.I.1	c.950_964delCAAAGTATAAAGATA (15bp del)+c.967A>T	Unpublished	Yes	No	F	48	DNK	43	44	Infections	CVID
AR.I.4	c.957T>A	*Maffucci et al.*	Yes	No	F	48 Δ	USA	19	NA	NA	CVID
AS.II.1	c.997C>T	Unpublished	Yes	No	M	25	ECU	14	21	Autoimmunity	CVID
AS.I.1	c.997C>T	Unpublished	No	Yes	F	57	ECU	-	-	-	Healthy
P.II.1	c.1005delG	*Tuijnenburg et al.*	Yes	No	NA	56	NA	NA	43	Autoimmunity	CVID
AT.III.2	c.1012delT	Unpublished	Yes	No	M	16	DEU	9	13	Infections	CVID
AT.II.4	c.1012delT	Unpublished	No	Yes	M	50	DEU	-	-	-	Healthy
AT.II.2	c.1012delT	Unpublished	Yes	No	M	48	DEU	27	40	NA	CVID
AT.II.3	c.1012delT	Unpublished	Yes	No	M	54	DEU	51	52	NA	CVID
AU.II.3	c.1066+1G>C	Unpublished	Yes	No	F	42	DEU	0	27	Infections	CVID
AU.I.2*	c.1066+1G>C	Unpublished	Yes	No	F	53 Δ	DEU	41	42	Infections	CVID
AV.II.1	c.1066+1G>T	Unpublished	Yes	No	F	11	ESP	3	8,3	Infections	CVID
AV.I.2	c.1066+1G>T	Unpublished	Yes	No	M	45	ESP	18	40	Infections	Antibody deficiency
AV.II.2	c.1066+1G>T	Unpublished	No	Yes	M	8	ESP	-	-	-	Healthy
AW.I.1	c.1071_1074delAGAA	Unpublished	Yes	No	M	30	DEU	2,5	15	Infections	CVID
AX.I.1	c.1183delG	Unpublished	Yes	No	M	10	RUS	1,7	10	Autoimmunity	CVID
AY.I.1	c.1210+1G>A	Unpublished	Yes	No	F	70	DEU	57	57	Infections	CVID
AZ.I.1	c.1211_1214dupGGTA	Unpublished	Yes	No	F	38	IRN	26	37	Infections	CVID
BA.II.1	c.1301-1G>A	*Maffucci et al.*	Yes	No	M	51 Δ	USA	42	NA	NA	CVID
BA.II.2	c.1301-1G>A	*Maffucci et al.*	Yes	No	F	33	USA	19	NA	NA	CVID
BA.II.3	c.1301-1G>A	*Maffucci et al.*	No	Yes	NA	NA	USA	-	-	-	Healthy
BB.I.1	c.1149delT	*Dieli-Crimi et al.*	Yes	No	F	33	ESP	7,5	11	Infections	CVID
BB.II.1	c.1149delT	*Dieli-Crimi et al.*	No	Yes	F	6	ESP	-	-	-	Healthy
BC.I.1	c.1245_1246delTG	Unpublished	Yes	No	F	2	RUS	1	1,3	Autoimmunity	Antibody deficiency
BD.I.1	c.1321A>T	Unpublished	Yes	No	M	13	PRT	10	13	Autoimmunity	Autoimmunity and immune dysregulation
BF.II.1	c.1365delT	*Lougaris et al.*	Yes	No	M	54	DEU	6	43	Autoimmunity	CVID
BF.II.2	c.1365delT	*Lougaris et al.*	Yes	No	F	56	DEU	33	47	Autoimmunity	CVID
BF.III.2	c.1365delT	*Lougaris et al.*	No	Yes	F	uk	DEU	-	-	-	Healthy
BM.I.5	c.1377delT	*Maffucci et al.*	Yes	No	F	25	USA	7	NA	NA	CVID
J.III.2	c.1423delG	*Tuijnenburg et al.*	Yes	No	M	48	GBR	NA	35	Infections	CVID
J.III.3*	c.1423delG	*Tuijnenburg et al.*	Yes	No	M	NA	GBR	NA	NA	NA	CVID/PID
J.II.1*	c.1423delG	*Tuijnenburg et al.*	Yes	No	F	NA	GBR	NA	NA	NA	CVID/PID
J.II.3*	c.1423delG	*Tuijnenburg et al.*	Yes	No	F	NA Δ	GBR	NA	NA	NA	CVID/PID
J.II.4*	c.1423delG	*Tuijnenburg et al.*	Yes	No	M	NA Δ	GBR	NA	NA	NA	CVID/PID
BG.I.1	c.1517delC	*Lougaris et al.*	Yes	No	M	41	ITA	7	7	Infections	CVID
B.I.1	c.1537_1541delCATGC	*Tuijnenburg et al.*	Yes	No	M	78 Δ	NA	26	28	Infections	CVID
B.II.1	c.1537_1541delCATGC	*Tuijnenburg et al.*	Yes	No	F	49	NA	20	43	Autoimmunity	CVID
BH.II.2	c.1584dupG	Unpublished	Yes	No	M	36	CAN	2	NA	Infections	CVID
BH.I.1*	c.1584dupG	Unpublished	Yes	No	F	NA	CAN	NA	NA	NA	CVID
BH.III.1	c.1584dupG	Unpublished	No	Yes	F	9	CAN	-	-	-	Healthy
BH.III.2	c.1584dupG	Unpublished	No	Yes	F	6	CAN	-	-	-	Healthy
BH.III.3	c.1584dupG	Unpublished	No	Yes	F	3	CAN	-	-	-	Healthy
D.I.2	c.1621_1622delGA	*Tuijnenburg et al.*	No	Yes	F	59	NA	-	-	-	Healthy
D.II.2	c.1621_1622delGA	*Tuijnenburg et al.*	Yes	No	F	36	NA	NA	NA	Autoimmunity	CVID
BI.I.1	c.1726dupA	Unpublished	Yes	No	F	59	DEU	42	53	NA	CVID
BJ.I.1	c.1752+1G>A	Unpublished	NA	NA	NA	NA	NA	NA	NA	NA	NA
F2.III.2	c.1659C>G	*Kaustio et al.*	Yes	No	M	32	FIN	1	18	Infections	Antibody deficiency
F2.II.3	c.1659C>G	*Kaustio et al.*	Yes	No	F	61	FIN	2,5	36	Infections	Antibody deficiency
F2.I.1*	c.1659C>G	*Kaustio et al.*	Yes	No	M	78 Δ	FIN	NA	NA	Infections	Antibody deficiency
BK.II.1	c.169C>T	Unpublished	Yes	No	M	17	DEU	12	12	NA	CVID
BK.I.1*	c.169C>T	Unpublished	Yes	No	M	uk	DEU	NA	NA	NA	NA
BL.I.1	c.199C>T	Unpublished	Yes	No	F	36	USA	18	35	Autoimmunity	Autoimmunity and immune dysregulation
BL.II.1	c.199C>T	Unpublished	Yes	No	F	9	USA	6	7	Infections	Antibody deficiency
F1.II.1*	c.200A>G	*Kaustio et al.*	Yes	No	M	39 Δ	FIN	5	39	Infections	Autoinflammatory disorder
F1.II.4	c.200A>G	*Kaustio et al.*	Yes	No	F	55	FIN	10	44	Infections	CVID
F1.III.2	c.200A>G	*Kaustio et al.*	Yes	No	F	49	FIN	28	29	Infections	Antibody deficiency
F1.III.3	c.200A>G	*Kaustio et al.*	Yes	No	M	38	FIN	15	25	Infections	CVID

F1.III.6	c.200A>G	*Kaustio et al.*	Yes	No	F	30	FIN	5	NA	Infections	CVID
F1.III.7	c.200A>G	*Kaustio et al.*	Yes	No	F	29	FIN	2,5	6	Infections	Autoinflammatory disorder
F1.III.8	c.200A>G	*Kaustio et al.*	Yes	No	F	25	FIN	1	15	Infections	CVID/Behçet's disease
F1.IV.1	c.200A>G	*Kaustio et al.*	Yes	No	M	10	FIN	0	10	Infections	Antibody deficiency
F1.IV.2	c.200A>G	*Kaustio et al.*	Yes	No	F	7	FIN	0,1	1,7	Infections	Antibody deficiency
G.II.1	c.260T>G	*Tuijnenburg et al.*	Yes	No	F	37	GBR	NA	21	Infections	CVID
Possible *NFKB1* patients											
F.II.1	c.293T>A	*Tuijnenburg et al.*	Yes	No	F	71	GBR	NA	54	Infections	CVID
E.II.1	c.843C>G	*Tuijnenburg et al.*	Yes	No	NA	25	GBR	NA	7	Autoimmunity	Antibody deficiency
BM.I.1	c.106G>A	Unpublished	Yes	No	M	12	IRN	2,5	3	Infections	CVID
BN.I.1	c.269A>C	Unpublished	Yes	No	M	61	DEU	NA	NA	uk	CVID
BO.I.1	c.470G>C	Unpublished	Yes	No	M	40	DEU	14	39	Infections	CVID
BO.I.2	c.470G>C	Unpublished	Yes	No	M	NA	DEU	NA	NA	NA	CVID
BP.I.1	c.508G>A	Unpublished	Yes	No	F	28	IRN	14	NA	Infections	CVID
BQ.I.1	c.556G>T	Unpublished	Yes	No	F	NA	NA	NA	NA	Inflammation	Autoinflammatory disorder
BQ.II.1	c.556G>T	Unpublished	Yes	No	F	NA	NA	NA	NA	Inflammation	Autoinflammatory disorder
BR.I.1	c.641G>A	Unpublished	Yes	No	M	25	DEU	18	23	Infections	CVID
BS.I.1	c.646A>G	Unpublished	No	Yes	M	NA	DEU	-	-	-	Healthy
BS.II.1	c.646A>G	Unpublished	Yes	No	M	6	DEU	1	1	NA	Antibody deficiency
BT.II.1	c.689G>A	Unpublished	Yes	No	M	11	DEU/TUR	0,5	4	Infections	CVID
BT.I.1	c.689G>A	Unpublished	Yes	No	F	NA	DEU/TUR	NA	NA	NA	Antibody deficiency
BT.II.2	c.689G>A	Unpublished	Yes	No	F	5	DEU/TUR	NA	NA	NA	Antibody deficiency
BT.II.3	c.689G>A	Unpublished	Yes	No	F	7	DEU/TUR	NA	NA	NA	Antibody deficiency
BU.I.1	c.734C>T	Unpublished	Yes	No	M	67	DEU	NA	63	Infections	CVID
BV.I.1	c.736C>A	Unpublished	Yes	No	F	57	DEU	NA	NA	NA	CVID
BW.I.1	c.736C>A	Unpublished	Yes	No	M	41	DEU	NA	39	NA	CVID
BX.I.1	c.856T>A	Unpublished	Yes	No	F	42	DEU	NA	26	NA	CVID
BY.I.1	c.885G>C	Unpublished	Yes	No	M	61	DEU	28	35	NA	CVID
BZ.I.1	c.978A>C	Unpublished	Yes	No	M	18	IRN	2	8	Infections	CVID
CA.I.1	c.978A>C	Unpublished	Yes	No	M	13 Δ	IRN	0,5	2	Infections	Antibody deficiency
CB.I.1	c.1156G>A	Unpublished	Yes	No	M	8	ESP	NA	NA	NA	Antibody deficiency
CC.I.1	c.2326C>G	Unpublished	Yes	No	F	59	TUR	47	NA	Infections	CVID
CD.I.1	c.2650G>A	Unpublished	Yes	No	F	43	DEU	17	22	Autoimmunity	CVID
CE.I.1	c.160-4G>C	Unpublished	Yes	No	F	11	USA	NA	NA	Inflammation	Autoinflammatory disorder
CF.I.1	c.1750-10C>G	Unpublished	Yes	No	M	NA	NA	5	NA	Lymphoproliferation	Autoimmunity and immune dysregulation
CG.I.1	c.2592+3A>G	Unpublished	Yes	No	M	51	DEU	41	43	Infections	CVID

Table S2. Baseline description of heterozygous *NFKB1* mutations carriers. *: mutation deferred by family segregational analysis, NA: not available, ALPS: autoimmune lymphoproliferative syndrome.

Case No.	Abscesses	Skin infections	Sepsis	Pneumonia	URTI	GI infections	Bronchiectasis	Other lung abnormalities	Autoimmune thyroiditis	Atrophic gastritis	Celiac-like disease	IBD	Diarrhea of unknown etiology
I.II.1	0	1	0	1	1	0	1	0	0	0	0	0	0
K.II.1	0	0	0	1	1	0	1	0	0	0	0	0	0
Q.I.1	0	1	0	1	1	0	0	1	0	0	0	0	0
S.I.1	0	1	1	1	1	1	1	1	0	0	0	1	0
C.I.2	0	0	0	1	1	0	1	1	0	0	0	0	0
C.II.3	0	0	0	1	1	0	1	0	0	0	0	0	0
C.II.5	0	1	0	0	1	0	1	0	0	0	1	0	0
L.II.1	0	0	0	NA	1	0	0	0	0	0	0	0	0
T.I.1	0	0	0	1	0	0	1	0	0	0	0	0	0
U.I.3	0	0	0	1	1	0	1	0	1	0	0	0	0
V.I.1	1	1	0	0	1	1	0	0	0	0	0	0	0
W.I.1	0	0	0	1	1	0	1	0	0	0	0	0	0
W.II.1	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0
X.I.1	0	1	0	0	1	1	0	0	0	0	1	0	0
O.II.1	0	0	0	0	1	1	0	0	0	0	0	0	0
Y.II.1	0	0	0	1	1	0	NA	NA	0	0	0	0	0
NZ.I.2	0	0	0	0	0	0	0	0	0	0	0	0	0
NZ.II.1	0	0	0	0	0	0	0	0	0	0	0	0	0
NZ.II.2	0	1	1	1	1	1	1	0	0	0	0	0	0
NZ.III.1	0	0	0	1	1	0	0	0	0	0	0	0	0
Z.I.1	1	1	0	1	1	1	NA	NA	0	1	0	1	0
AA.II.2	0	0	0	1	1	1	1	0	0	0	0	0	0
F3.II.1	1	0	1	1	0	0	0	0	0	0	0	0	0
F3.II.5	1	1	1	0	0	0	0	0	0	0	0	0	0
AB.II.1	1	0	0	0	1	0	0	0	0	0	0	0	0
AC.I.1	1	1	0	1	1	0	NA	NA	0	0	1	0	0
AD.I.1	1	1	0	1	1	0	0	0	0	0	0	0	0
NA.II.16	NA	1	1	1	1	1	NA	0	0	1	1	1	0
NA.II.18	0	0	0	1	1	1	NA	0	0	0	0	0	0
NA.II.19	0	0	1	1	1	0	NA	0	0	0	0	0	1
NA.II.21	0	0	0	1	1	0	1	1	0	0	0	0	0
NA.III.25	0	0	0	1	1	0	0	0	0	0	0	0	0
NA.III.34	0	1	0	1	1	0	0	0	1	0	0	0	0
NA.III.36	0	0	0	1	1	1	NA	NA	0	0	0	0	0
NA.III.40	0	0	0	1	1	0	0	0	0	0	0	0	0
NA.IV.49	0	1	0	0	1	1	NA	NA	0	0	0	0	0
NA.V.57	0	0	0	1	1	0	0	1	0	0	0	0	0
NA.V.62	0	0	0	0	1	NA	NA	NA	0	0	0	0	0
NA.V.8	0	0	0	0	1	0	0	0	0	0	0	0	0
AF.II.1	0	0	0	1	1	1	0	0	0	0	1	0	0
AG.I.1	0	0	0	0	0	0	0	0	0	0	0	0	0
M.II.1	0	1	0	0	1	1	1	1	0	1	1	0	0
F089.I.1	1	1	0	1	1	0	0	0	0	0	0	0	1
F089.II.2	1	1	0	1	1	1	0	1	0	0	0	0	0
AH.I.1	0	1	0	0	1	0	0	1	0	0	0	0	0
H.II.1	0	0	0	1	1	0	0	0	0	0	1	0	0
AI.I.1	0	0	0	0	1	0	NA	NA	0	0	0	0	0
A.II.4	0	0	0	1	1	0	0	0	0	0	0	0	0
A.III.2	0	0	0	1	1	0	0	0	0	0	0	0	0
A.III.3	0	0	0	1	0	0	0	0	0	0	0	0	0
AJ.III.1	0	1	0	0	0	0	NA	NA	0	0	0	0	1
AJ.II.2	0	0	0	0	0	0	NA	NA	0	0	0	0	0
AJ.III.3	0	0	0	0	0	0	NA	NA	0	0	0	0	0
AJ.II.4	0	0	0	0	0	0	NA	NA	0	0	0	0	0
AK.I.1	0	1	0	0	0	1	0	0	0	0	0	0	0
AM.II.1	0	0	0	1	1	0	0	0	0	0	0	0	0
AM.I.1	0	1	0	0	1	0	0	0	0	0	0	0	0
AN.II.1	0	0	0	0	1	0	0	1	0	0	0	0	0
AN.III.1	0	1	0	0	1	0	0	0	1	0	0	0	0
AO.I.1	0	0	0	1	1	1	0	0	0	0	0	0	0
AP.I.1	0	1	0	1	1	0	0	0	0	0	0	0	0
N.II.1	0	0	1	0	0	0	0	0	0	0	0	0	0
AQ.I.1	1	0	0	0	1	1	0	0	0	0	0	0	0
AR.I.4	1	0	0	1	0	0	0	0	0	0	0	0	0
AS.II.1	0	1	0	1	1	1	0	0	0	0	0	0	0
P.II.1	0	0	0	1	1	1	1	1	0	0	0	0	0
AT.III.2	1	1	0	0	1	0	0	0	0	0	0	1	0
AT.II.2	0	0	0	0	1	0	0	0	0	0	0	0	0
AT.II.3	0	0	0	0	1	0	0	0	0	0	0	0	0
AU.II.3	0	1	0	0	1	1	NA	NA	0	0	0	0	0

Case No.	Abscesses	Skin infections	Sepsis	Pneumonia	URTI	GI infections	Bronchiectasis	Other lung abnormalities	Autoimmune thyroiditis	Atrophic gastritis	Celiac-like disease	IBD	Diarrhea of unknown etiology
AU.I.2	0	0	1	1	1	1	1	1	0	0	0	0	0
AV.II.1	0	0	0	0	1	0	0	0	0	0	0	0	0
AV.I.2	0	0	0	1	1	0	1	0	0	1	0	0	1
AW.I.1	0	0	0	1	1	1	1	0	0	0	0	1	0
AX.I.1	0	0	0	0	1	0	0	0	0	0	0	0	0
AY.I.1	0	0	0	0	1	0	0	0	0	0	0	0	0
AZ.I.1	0	0	0	0	1	1	NA	NA	0	0	1	0	0
BA.II.1	0	1	0	1	1	0	0	1	0	0	0	0	0
BA.II.2	0	1	0	1	1	1	0	0	0	0	0	0	0
BB.I.1	1	1	0	1	1	1	1	0	0	0	0	1	0
BC.I.1	0	0	0	0	1	0	0	0	0	0	0	0	0
BD.I.1	0	0	0	1	1	0	0	0	0	0	0	0	0
BF.II.1	1	1	0	1	1	1	0	1	0	0	0	0	0
BF.II.2	0	1	0	1	1	1	1	1	0	0	0	0	0
BM.I.5	0	1	0	1	1	1	1	0	0	0	0	0	0
J.III.2	0	0	0	1	1	0	1	0	0	0	0	0	1
BG.I.1	0	0	0	1	1	1	0	0	1	0	1	0	0
B.I.1	1	0	0	1	0	0	0	0	0	0	0	0	0
B.II.1	0	0	0	1	0	0	0	0	1	0	0	0	0
BH.II.2	0	1	0	0	1	0	0	0	0	0	0	0	0
D.II.2	0	0	0	1	1	0	0	1	0	0	0	0	0
BI.I.1	0	1	0	1	1	0	0	0	0	0	0	0	0
F2.III.2	0	1	0	1	1	0	0	0	0	0	1	0	0
F2.II.3	0	0	0	1	1	0	1	0	1	0	0	0	1
BK.II.1	0	1	0	1	1	0	0	0	0	0	0	0	0
BL.I.1	0	1	0	1	1	0	0	0	1	1	0	0	0
BL.II.1	1	0	0	0	1	0	0	0	0	0	0	0	0
F1.II.1	1	1	1	1	1	0	NA	NA	0	0	0	0	0
F1.II.4	0	1	0	0	1	0	0	0	0	0	0	0	1
F1.III.2	0	0	0	0	1	0	0	0	0	0	0	0	0
F1.III.3	0	0	0	0	1	1	0	0	0	0	0	0	0
F1.III.6	0	0	0	1	1	0	0	0	0	0	0	0	0
F1.III.7	0	0	0	0	1	0	0	0	0	0	0	0	1
F1.III.8	0	1	0	0	1	0	0	0	0	0	0	0	0
F1.IV.1	0	0	1	0	0	0	0	0	0	0	0	0	0
F1.IV.2	0	0	0	0	1	0	0	0	0	0	0	0	0
G.II.1	0	0	0	1	0	0	1	1	0	0	0	0	1
	17/105	40/106	10/107	62/105	88/106	30/105	23/90	16/93	7/107	5/107	10/107	6/107	9/107
Possible NFKB1 patients													
F.II.1	0	1	0	1	0	0	1	1	1	0	0	0	0
E.II.1	0	0	0	1	1	0	1	0	0	0	0	0	0
BM.I.1	0	0	0	1	1	0	1	0	0	0	1	0	0
BO.I.1	1	0	0	1	1	1	0	0	0	0	0	0	0
BP.I.1	0	1	0	1	1	1	0	0	0	0	0	0	0
BQ.I.1	0	1	0	1	1	0	1	0	0	0	0	0	0
BT.I.1	1	0	0	0	1	1	0	0	0	0	0	0	0
BS.II.1	0	0	0	0	1	0	0	0	0	0	0	0	0
BT.II.1	0	0	0	1	1	uk	0	0	0	0	0	0	0
BU.I.1	0	0	0	1	0	1	0	0	0	0	1	0	0
BV.I.1	0	0	0	1	0	0	1	0	0	1	0	0	0
BW.I.1	0	1	0	0	1	0	0	0	0	0	0	0	0
BX.I.1	1	0	0	0	1	1	0	0	0	1	0	1	0
BY.I.1	0	0	0	0	1	0	1	0	0	1	1	0	0
BZ.I.1	0	0	0	1	1	1	0	0	0	0	1	0	0
CA.I.1	0	0	0	1	1	0	1	0	0	0	0	0	1
CC.I.1	1	1	0	1	0	0	0	0	0	0	0	0	1
CD.I.1	1	1	0	0	1	1	1	0	1	0	0	1	0
CD.I.1	0	0	0	0	0	0	0	0	0	0	0	0	0
CG.I.1	0	1	0	1	1	0	0	1	0	0	0	0	1

Case No.	Autoimmune cytopenia	Autoimmune skin disease	Hepatopathy	Aphthous ulcers	Vasculitis	Arthritis	Neurological manifestations	Cardiovascular abnormalities	Hepatomegaly	Splenomegaly	Lymphadenopathy	Malignancies
I.II.1	1	0	0	0	0	0	0	0	0	1	1	0
K.II.1	1	0	0	0	0	0	1	0	0	1	1	1
Q.I.1	1	0	0	1	1	1	0	0	1	1	1	0
S.I.1	1	0	1	1	0	0	1	0	1	1	1	0
C.I.2	0	0	0	0	0	0	0	0	0	0	0	1
C.II.3	0	0	1	0	0	0	0	0	0	1	1	0
C.II.5	0	0	1	1	0	0	1	0	0	0	0	1
L.II.1	1	0	0	0	0	0	0	0	0	0	0	0
T.I.1	0	1	1	0	0	0	0	0	1	1	0	0
U.I.3	0	1	0	0	0	0	0	0	0	0	0	0
V.I.1	0	0	0	1	0	0	1	1	0	1	0	0
W.I.1	1	0	0	0	0	0	0	0	0	1	0	1
W.II.1	1	0	0	0	0	0	0	0	0	0	0	0
X.I.1	1	0	0	0	0	0	0	0	1	0	0	1
O.II.1	0	0	0	0	0	0	0	0	0	0	0	0
Y.II.1	0	1	0	0	0	1	1	0	0	0	0	0
NZ.I.2	1	1	0	0	0	0	0	1	0	0	0	0
NZ.II.1	1	0	0	0	0	0	0	0	0	0	0	0
NZ.II.2	1	1	1	0	0	0	0	0	1	1	1	1
NZ.III.1	0	0	0	0	0	0	0	0	0	0	0	0
Z.I.1	1	0	1	0	0	0	1	1	1	1	1	0
AA.II.2	1	0	0	0	0	0	0	0	1	1	1	0
F3.II.1	0	0	0	0	0	0	0	0	0	0	0	0
F3.II.5	0	0	0	0	0	0	0	0	0	0	0	0
AB.II.1	1	0	0	0	0	0	0	0	1	1	1	1
AC.I.1	1	0	0	0	0	0	0	1	0	1	1	0
AD.I.1	1	0	1	0	0	0	1	0	1	1	0	0
NA.II.16	0	1	0	0	0	0	0	0	0	0	0	1
NA.II.18	1	0	0	0	0	0	0	1	NA	1	1	1
NA.II.19	0	0	0	0	0	0	0	1	0	0	0	0
NA.II.21	0	0	0	0	0	0	0	1	0	0	0	0
NA.III.25	0	0	0	0	0	0	1	1	0	0	0	0
NA.III.34	0	1	0	0	0	0	0	1	0	0	0	0
NA.III.36	0	0	0	0	0	1	0	0	NA	NA	NA	0
NA.III.40	0	0	0	0	0	0	0	0	0	0	0	0
NA.IV.49	0	0	0	0	0	0	0	1	0	0	0	0
NA.V.57	0	0	0	0	0	0	0	0	0	0	0	0
NA.V.62	0	0	0	0	0	0	0	0	0	0	0	0
NA.V.8	0	0	0	0	0	0	0	0	0	0	0	0
AF.II.1	0	0	0	0	0	0	0	0	1	1	0	1
AG.I.1	1	0	0	0	0	0	0	0	1	1	1	0
M.II.1	1	0	0	0	0	0	0	0	0	1	0	0
F089.I.1	0	0	0	0	0	0	0	0	0	0	0	0
F089.II.2	1	1	1	1	0	0	1	0	1	1	1	0
AH.I.1	1	0	1	0	1	1	1	0	1	1	0	0
H.II.1	1	0	0	0	0	0	0	0	0	1	0	0
AI.I.1	1	0	0	1	0	0	0	0	1	1	1	0
A.II.4	0	0	0	0	0	0	0	0	0	0	0	0
A.III.2	0	0	0	0	0	0	0	0	0	0	0	0
A.III.3	0	0	0	0	0	0	0	0	0	1	1	0
AJ.III.1	1	0	1	1	1	0	1	1	1	1	1	0
AJ.II.2	0	1	0	0	0	0	0	0	0	0	0	0
AJ.III.3	0	0	0	1	0	0	0	0	0	0	0	0
AJ.II.4	0	0	0	0	0	0	1	0	NA	NA	NA	1
AK.I.1	0	0	0	0	0	0	0	0	0	1	1	0
AM.II.1	1	0	1	1	0	0	0	1	1	1	1	1
AM.I.1	0	0	0	0	0	0	0	0	0	0	0	0
AN.II.1	1	0	0	0	0	1	0	0	0	1	1	0
AN.III.1	1	0	0	0	0	0	0	0	0	0	0	0
AO.I.1	0	0	0	0	0	0	0	1	0	0	1	1
AP.I.1	1	0	0	0	0	0	1	1	1	1	1	0
N.II.1	1	1	0	0	0	0	0	0	0	1	1	1
AQ.I.1	0	0	0	0	0	0	1	0	0	0	0	0
AR.I.4	1	0	0	0	0	0	1	0	NA	1	NA	0
AS.II.1	1	0	0	0	0	0	0	0	0	1	1	0
P.II.1	1	0	1	0	0	1	0	0	0	1	0	0
AT.III.2	1	0	0	1	0	0	0	0	0	1	1	0
AT.II.2	0	0	0	0	0	0	0	0	0	0	0	0
AT.II.3	0	0	0	0	0	0	0	0	0	0	0	0
AU.II.3	1	0	0	0	0	0	1	0	0	1	1	0

Case No.	Autoimmune cytopenia	Autoimmune skin disease	Hepatopathy	Aphthous ulcers	Vasculitis	Arthritis	Neurological manifestations	Cardiovascular abnormalities	Hepatomegaly	Splenomegaly	Lymphadenopathy	Malignancies
AU.I.2	1	0	1	0	0	0	0	0	1	1	0	1
AV.II.1	1	0	0	0	0	0	1	0	0	1	1	1
AV.I.2	0	1	0	0	0	0	1	0	0	0	0	0
AW.I.1	0	0	0	0	0	0	0	1	0	1	1	0
AX.I.1	1	0	0	0	0	0	0	0	1	1	1	0
AY.I.1	0	0	0	0	0	0	0	0	0	0	0	0
AZ.I.1	0	1	0	0	0	0	0	0	NA	NA	NA	0
BA.II.1	1	0	0	1	0	0	0	0	0	1	1	0
BA.II.2	0	1	0	0	0	0	0	0	0	0	0	0
BB.I.1	1	0	1	1	0	0	0	0	1	1	0	0
BC.I.1	1	0	0	0	0	0	1	1	1	1	0	0
BD.I.1	1	1	0	0	0	0	0	0	1	1	0	0
BF.II.1	1	1	0	0	0	1	1	0	1	1	1	0
BF.II.2	1	0	1	1	0	0	1	0	1	1	1	0
BM.I.5	0	0	0	0	0	0	0	0	0	0	0	0
J.III.2	0	0	0	0	0	0	0	0	0	0	0	0
BG.I.1	0	0	0	0	0	0	0	0	0	1	0	0
B.I.1	1	0	0	0	0	0	1	0	1	1	0	1
B.II.1	0	1	0	0	0	0	0	0	0	0	0	0
BH.II.2	1	0	0	0	0	0	0	0	0	1	1	0
D.II.2	1	0	0	0	0	0	0	0	0	1	0	0
BI.I.1	1	0	0	0	0	1	0	0	0	1	1	0
F2.III.2	0	0	0	0	0	0	0	1	0	0	0	0
F2.II.3	0	0	0	0	1	1	0	0	0	0	0	1
BK.II.1	0	0	0	0	0	0	0	0	0	0	0	0
BL.I.1	0	0	0	0	0	0	1	1	0	0	1	0
BL.II.1	0	0	0	0	0	0	0	0	0	0	1	0
F1.II.1	0	0	0	0	0	0	0	0	NA	NA	NA	0
F1.II.4	0	0	1	1	0	1	0	0	0	0	0	0
F1.III.2	0	0	0	0	0	0	0	0	0	0	0	0
F1.III.3	0	0	0	1	0	0	0	0	0	0	0	0
F1.III.6	0	0	0	1	0	0	0	0	0	0	0	0
F1.III.7	0	0	0	1	0	1	1	1	0	0	0	0
F1.III.8	0	0	0	1	1	0	1	0	0	0	1	0
F1.IV.1	0	0	0	0	0	0	0	0	0	0	0	0
F1.IV.2	0	0	0	1	0	0	0	0	0	0	0	0
G.II.1	0	0	0	0	0	0	0	0	0	1	0	0
	47/107	16/107	16/107	19/107	5/107	11/107	25/107	19/107	25/101	50/103	36/102	18/107
Possible NFKB1 patients												
F.II.1	0	0	0	0	0	0	0	0	0	0	0	1
E.II.1	1	0	0	0	0	0	0	0	0	1	1	0
BM.I.1	1	0	0	0	0	0	0	0	1	1	1	0
BO.I.1	1	1	1	0	0	0	1	0	0	1	0	0
BP.I.1	0	0	0	0	0	0	0	0	1	1	0	0
BQ.I.1	0	0	0	0	0	0	0	0	0	0	0	0
BT.I.1	0	0	0	0	0	0	0	1	0	0	0	0
BS.II.1	0	0	0	0	0	0	0	0	0	0	1	0
BT.II.1	0	0	0	0	0	0	0	0	0	0	0	0
BU.I.1	0	0	1	0	0	0	0	0	0	0	1	0
BV.I.1	1	0	0	0	0	0	0	1	0	1	0	0
BW.I.1	1	0	0	0	0	0	0	0	0	0	0	0
BX.I.1	0	0	0	0	0	1	0	0	0	1	0	0
BY.I.1	1	1	1	0	0	0	0	0	0	1	1	1
BZ.I.1	0	0	0	0	0	1	1	0	1	1	0	0
CA.I.1	0	0	0	0	0	0	0	0	0	0	1	0
CC.I.1	0	0	0	1	0	0	0	0	0	0	0	0
CD.I.1	1	0	1	0	0	0	0	0	1	1	1	0
CD.I.1	0	0	0	0	0	0	0	0	0	0	0	0
CG.I.1	0	1	1	0	0	1	1	1	1	1	1	0

Table S3. Clinical spectrum of patients with damaging heterozygous *NFKB1* mutations. NA: not available.

cDNA	Protein	Previously described functional tests	Undescribed functional tests
HAPLOINSUFFICIENCY MUTATIONS			
del103370996-103528207		WB: reduced NF-κB1 protein levels in patient's cells[12].	
c.139delA	**p.Ile47 Tyrfs*2**	WB: reduced NF-κB1 protein levels in patient's cells[5].	
c.160-1G>A	**p.Arg54_ Lys86del**	WB: reduced NF-κB1 protein levels in patient's cells[12].	
c.465dupA	**p.Ala156 Serfs*12**	WB: reduced NF-κB1 protein levels in patient's cells[4].	
c.469C>T	**p.Arg157***	WB: reduced NF-κB1 protein levels in patient's cells[5]. Dual luciferase reporter assay: reduced NF-κB activation[10].	
c.494delG	**p.Gly165 Alafs*32**	WB: detectable but severely decreased levels of p50[6].	
c.730+4A>G	**p.Asp191_ Lys244 delinsGlu**	WB: reduced NF-κB1 protein levels in patient's cells. Fluorescence microscopy: reduced florescence intensity and altered subcellular localization of GFP-fused mutant proteins[4].	Fluorescence based promoter reporter assay: reduced NF-κB activation (data not shown).
c.835+2T>G	**p.Lys244_ Asp279 delinsAsn**	WB: reduced NF-κB1 protein levels in patient's cells[4].	
c.850C>T	**p.Arg284***	WB: reduced NF-κB1 protein levels in patient's cells[12].	
c.1012delT	**p.Ser338 Leufs*94**		FM: reduced florescence intensity and altered subcellular localization of GFP-fused mutant proteins (Figure 2A).
c.1210+1 G>A	**p.Asp356_ Pro403del**	WB: reduced NF-κB1 protein levels in patient's cells[12].	
PRECURSOR SKIPPING MUTATIONS			
c.1365delT	**p.Val456***		FM: p50-like protein localizes to the nucleus (data not shown).
c.1423delG	**p.Ala475 Profs*10**	WB: reduced NF-κB1 protein levels in patient's cells[12].	FM: p50-like protein localizes to the nucleus (Figure 2A).
c.1517delC	**p.Ala506 Valfs*17**		FM: p50-like protein localizes to the nucleus (data not shown).
c.1537_1541 delCATGC	**p.His513 Glnfs*28**	WB: reduced NF-κB1 protein levels in patient's cells[12].	
c.1621_1622 delGA	**p.Asp541***	WB: reduced NF-κB1 protein levels in patient's cells. Presence of mutant p50-like protein (with increased molecular weight)[12].	FM: p50-like protein localizes to the nucleus (data not shown).
MISSENSE VARIANTS AFFECTING THE p105 PRECURSOR AND THE MATURE p50			
c.169C>T	**p.Arg57Cys**		WB: presence, expected size, and increased ratio of mutant p105/p50 (Figure 2C). FM: predominant p105 localization in the cytoplasm and clumping of fluorescent signal after stimulation (Figure 2A and B). Dual luciferase reporter assay: reduced NF-κB activation (Figure 2D).
c.200A>G	**p.His67Arg**	WB: normal NF-κB1 protein levels in patient's cells. Dual luciferase reporter assay: reduced NF-κB activation.	

		Immunofluorescence microscopy: reduction in efficiency of p50 nuclear localization[10].	
c.260T>G	**p.Ile87Ser**	WB: reduced NF-κB1 protein levels in patient's cells[12].	WB: presence and expected size of the p105 protein (Figure 2C). FM: predominant p105 localization in the cytoplasm (data not shown). Dual luciferase reporter assay: reduced NF-κB activation (Figure 2D).
c.293T>A	p.Val98Asp	WB: reduced NF-κB1 protein levels in patient's cells[12].	
c.470G>C	p.Arg157 Pro		WB: presence and expected size of the p105 and p5 proteins.
c.592C>T	p.Arg198 Cys		WB: presence and expected size of the p105 protein (data not shown). FM: predominant p105 localization in the cytoplasm (data not shown). Dual luciferase reporter assay: normal NF-κB activation (data not shown).
c.641G>A	p.Arg214 Gln		WB: presence and expected size of the p105 protein (data not shown). FM: predominant p105 localization in the cytoplasm (data not shown). Dual luciferase reporter assay: normal NF-κB activation (data not shown).
c.646A>G	p.Met216 Val		WB: presence and expected size of the p105 protein (Figure 2C). FM: predominant p105 localization in the cytoplasm (data not shown). Dual luciferase reporter assay: normal NF-κB activation (data not shown).
c.689G>G	p.Arg230 Lys		WB: presence and expected size of the p105 protein (Figure 2C). FM: predominant p105 localization in the cytoplasm (data not shown). Dual luciferase reporter assay: normal NF-κB activation (data not shown).
c.843C>G	p.Ile281 Met	WB: reduced NF-κB1 protein levels in patient's cells[12].	WB: presence and expected size of the p105 protein (Figure 2C). FM: predominant p105 localization in the cytoplasm (data not shown). Dual luciferase reporter assay: normal NF-κB activation (data not shown).
MISSENSE VARIANTS PROBABLY AFFECTING ONLY THE FUNCTIONS OF THE PRECURSOR			
c.1519A>G	p.Met507 Val		WB: presence and expected size of the p105 protein (data not shown). FM: predominant p105 localization in the cytoplasm (data not shown). Dual luciferase reporter assay: normal NF-κB activation (data not shown).
c.1659C>G	**p.Ile553 Met**	WB: normal NF-κB1 protein levels in patient's cells. But increased p105 degradation, with rising TNF concentrations. Dual luciferase reporter assay: normal NF-κB activation. Immunofluorescence microscopy: normal p50 nuclear localization. Mass spectrometric analyses:	WB: presence and expected size of the p105 protein (Figure 2C). FM: predominant p105 localization in the cytoplasm (data not shown). Dual luciferase reporter assay: normal NF-κB activation (data not shown).

Patient	Opportunistic infection	CD4+ T cells (absolute number/%)	Naive CD4 T cells	B cells (absolute number/%)	Immunosuppressive treatment	Survival

		decreased phosphorylation status[10].	
c.1736G>A	p.Arg579 Lys		WB: presence and expected size of the p105 protein (data not shown). FM: predominant p105 localization in the cytoplasm (data not shown). Dual luciferase reporter assay: normal NF-κB activation (data not shown).
c.1845G>T	p.Leu615 Phe		WB: presence and expected size of the p105 protein (data not shown). FM: predominant p105 localization in the cytoplasm (data not shown). Dual luciferase reporter assay: normal NF-κB activation (data not shown).
c.2831C>A	p.Thr944 Asn		WB: presence and expected size of the p105 protein (data not shown). FM: predominant p105 localization in the cytoplasm (data not shown). Dual luciferase reporter assay: normal NF-κB activation (data not shown).
VARIANT WITH UNKNOWN EFFECT			
c.2650G>A	p.Glu884 Lys		WB: presence and expected size of the p105 protein (data not shown). FM: predominant p105 localization in the cytoplasm (data not shown). Dual luciferase reporter assay: normal NF-κB activation (data not shown).

Table S4. Assessment of selected *NFKB1* variants with four different assays. Mutations assumed to be pathogenic are marked in bold. WB: Western blot, FM: fluorescence microscopy.

NA.II.16	*Aspergillus* (lung) *Candida* (lung)	<100	NA	200	Intermittent steroids	Died at 76 y
Z.I.1	*Aspergillus* (lung) JC virus (CNS)	810 (49·3)	350 (42·8)	371 (22·6)	Intermittent steroids	Alive
C.II.5	*Aspergillus* (lung)	650 (56·5)	NA	42 (3·7)	Steroids Azathioprin	Died at 39 y
AZ.I.1	*Candida* (GI tract) *Cryptosporidium* (GI tract)	1234 (64)	NA	41(2)	Intermittent steroids	Alive
AR.I.4	*Pneumocystis* (lung), MAC (lung) JC virus (SCN)	1177	NA	0 (0)	NA	Died at 48 y
H.II.1	CMV (invasive)	2129 (39)	NA	132 (3)	No	Died at 35 y
AO.I.1	CMV (GI tract)	105 (17)	NA	6 (1)	Intermittent sterois, CHOP	Alive
BF.II.2	CMV (hepatitis and cytopenia)	1015 (52·1)	90 (9)	98 (6·5)	Intermittent steroids	Alive
AB.II.1	EBV (lymphoproliferation)	390-660	140	20-80	Rituximab (to treat EBV-related lymphoproliferation)	Alive
S.I.1	EBV (lymphoproliferation) Adenovirus (GI tract)	(40-65)	(7-9)	(4-11)	Steroids, MMF	Alive
A.III.3	EBV (lymphoproliferation) JC virus (CNS)	735 (29·1)	110 (15·5)	0,5 (0)	No	Alive
BM.I.5	MAC (lung)	518	NA	0 (0)	NA	Alive
BL.II.1	MAC (lymphadenitis)	486 (25·6)	274 (14·4)	509 (26·8)	No	Alive
W.I.1	Disseminated BCG	340 (17·8)	200 (59)	130	No	Alive
AF.II.1	*Mycobacterium genavense* (lung)	860 (26)	160 (19)	480 (11·7)	MTX, mercaptopurine, vinblastin, steroids	Alive
G.II.1	MAC (lung)	449 (43·9)	200 (44)	0 (0)	NA	Alive
AU.I.2	*Stenotrophomonas maltophilia* (lung) Adenovirus (lung)	42 (9)	NA	1 (0·6)	Intermittent steroids, CHOP	Died at 53 y

Table S5. Clinical spectrum of opportunistic infections in patients with damaging heterozygous *NFKB1* mutations. NA: not available, CHOP: cyclophosphamide, hydroxydaunorubicin, oncovin, prednisone, MMF: mycophenolate mofetil, MTX: methotrexate.

	Several cohorts of CVID patients	Cohort of *NFKB1* patients
Gender	51·1% female, 48·9% male[19]	56·1% female, 43·9% male
Onset	Two peaks (before age 10 and in the second to third decade of life[19,25,26])	Median age 12 years
Mortality	19·6% (median age of death: 43 years)[18]	17·1% (median age of death: 52 years)
Pneumonia	58%[27]	59%
Sinusitis	63%[27]	59·8%
Gastrointestinal infections	27%[27]	28·6%
Bronchitis	69%[27]	41·7%
Bronchiectasis	37%[27]	25·6%
GLILD	10-20%[26]	7·4%
Viral infections	23%[27,28]	25·0%
Opportunistic infections	5-6%[27,28]	15·7%
Non-infectious enteropathy	9-15·4%[18,25,29]	23·1%
Liver disease	9·1% in the New York CVID cohort[18], but abnormal liver function and NRH in 44% and 12% of 108 CVID patients, respectively[30]	19·5% (4·6% with NRH)
Autoimmunity	30%[18,19,31,32]	57·4%
Autoimmune cytopenia	21%[31]	43·9%
Splenomegaly	25-40·5%[17,19,20]	48·5%
Lymphadenopathy	26%[17,19,20]	35·3%
Malignancy	15%[18]	16·8%
Lymphoma	7·4%[18]	11·1%
Solid organ cancer	5·6%[18]	4·6%
Low levels of switched memory B cells	58%[20]	60·3%
Expansion of CD21^{low} B cells	42·8%[20]	56·1%
Expansion of transitional B cells	15%[20]	36·8%

Table S6. Comparison of the *NFKB1* phenotype to the one of general CVID. NRH: nodular regenerative hyperplasia.