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Supplemental Data

Haploinsufficiency of the NF-κB1 Subunit p50 in Common Variable Immunodeficiency

Manfred Fliegauf, Vanessa Bryant, Natalie Frede, Charlotte Slade, See-Tarn Woon, Klaus Lehnert, Sandra Winzer, Alla Bulashevska, Thomas Scerri, Euphemia Leung, Anthony Jordan, Baerbel Keller, Esther de Vries, Hongzhi Cao, Fang Yang, Alejandro A. Schäffer, Klaus Warnatz, Peter Browett, Jo Douglass, Rohan V. Ameratunga, Jos W.M. van der Meer, and Bodo Grimbacher

Supplemental Data

Affected individuals and clinical histories

This study was conducted under human subjects' protocols and samples were collected with the written consent of all study participants and/or their parental guardians following formal ethical approval by the local ethics committees at the University of Freiburg, the Radboud University, Melbourne Health, the University of Auckland and collaborating institutions. The clinical phenotype of 20 affected family members is summarized in Table 1.

Supplemental Note: Case Reports

The Dutch/Australian CVID family (FamNL1)

FamNL1-16

This woman was born in 1935 and was healthy until she delivered her third child in 1964. She developed cutaneous lesions over the breasts and lower limbs, diagnosed as pyoderma gangrenosum. She was treated with antibiotics and surgery. This resulted in scars and highly vulnerable skin. In 1982 she developed chronic obstructive pulmonary disease (COPD) and abdominal complaints. The tentative diagnosis was inflammatory bowel disease and she was treated with sulfasalazine and prednisone. At that time, her serum immunoglobulins were found to be in decline. The spleen was not enlarged. In 1994 she was referred to the Radboud UMC. She had pansinusitis and Helicobacter- positive atrophic gastritis. CVID was diagnosed (IgG 2.76 g/L, IgA 0.30 g/L and IgM 0.16 g/L) and intravenous immunoglobulin (IVIG) treatment was started. The numbers of B cells and T cells were in the normal adult ranges. In the years following, she suffered from recurrent skin infections, squamous cell carcinoma of the leg (for which plastic surgery was performed) and respiratory tract infections. Treatment with etanercept for the pyoderma in 2008 was not successful. In 2009, she developed pneumonia with sepsis, secondary to an influenza infection. Her general condition worsened and in 2011 she died of pulmonary insufficiency.

FamNL1-18

This female family member was diagnosed with CVID in 2000 at the age of 65 following investigations as part of a family study. Serum immunoglobulins were grossly abnormal: IgG 2.49 g/L (with IgG2 <0.09 g/L and IgG4 <0.01 g/L), IgA 0.13 g/L and IgM 0.53 g/L. Prior to diagnosis she had suffered a prolonged episode of campylobacter enteritis, and frequent sinopulmonary infections. CVID was complicated by splenomegaly and lymphadenopathy, with no evidence of a clonal lymphoproliferative disorder. She had persistent mild thrombocytopenia.

Past history included ischemic heart disease, (onset age 53) with a cigarette smoking history of 20 years. Lung adenocarcinoma was diagnosed in 2012, and she died from cancer-related complications in August 2013. Her daughter (Individual 57) was also diagnosed with CVID, and one granddaughter (Individual 62) is suspected to have CVID as well.

FamNL1-19

This female family member, born in 1938, presented with bronchitis and emphysema in 1977. These were attributed to cigarette smoking. In 1981, she was diagnosed with coronary insufficiency. In 1984, she was investigated for weight loss and diarrhea. Based on the finding of a serum IgG of 3.0 g/L; IgA 0.4 g/L and IgM 0.4 g/l, she was diagnosed with CVID. At that time, her pulmonary function was impaired (vital capacity of 2,500 ml, FEV1 59%). The immunological analysis showed normal B and T cells and normal lymphocyte proliferation tests. In 1985 and 1986, she suffered from pneumonias caused by *Haemophilus influenzae*, and several infective exacerbations of her COPD. In 1988, she was started on immunoglobulin replacement. In the following years, she had several exacerbations of COPD and symptomatic coronary insufficiency leading to bypass surgery. In 1993, she died from pneumonia and septic shock. There were no signs of granulomas, cytopenia, splenomegaly or lymphadenopathy. Permission for genetic testing was not obtained.

FamNL1-21

This male family member, born in 1940, suffered from COPD with many exacerbations since the 1970's. In 1971, an appendectomy was complicated by pneumonia. He developed recurrent sinusitis in the 1980's and had episodes of pneumonia in 1985, 1994, 1995 and 1996. In 1997, he was found to have a serum IgG 6.0 g/L, IgA 1.26 g/L, IgM 0.4 g/L and was diagnosed with CVID. Since then he received IVIG. Three years later his serum IgA was reduced to 0.93 g/L and IgM was

reduced to <0.17 g/L. High-resolution computed tomography (HRCT) of the lung revealed widespread bronchiectasis and some fibrosis.

FamNL1-23

This female family member born in 1947 suffers from COPD and has a history of recurrent lower airway infections. Initially, she was described to have an IgG2 subclass deficiency.[1] In recent years however, she was found to have hypogammaglobulinemia (IgG, IgG1, IgG2, IgM) with absent tetanus response and abnormal polysaccharide response. She is currently on immunoglobulin replacement, but remains in a fragile condition.

FamNL1-24

This female family member born in 1951, also suffered from COPD as well as many lower airway infections. In a previous report, she was described as IgG2 subclass deficient.[1] More recent laboratory findings included decreased IgG and IgG1 levels, an abnormal polysaccharide response, as well as a mannose-binding lectin (MBL) deficiency. Immunoglobulin replacement was commenced late in the disease course. She died of pulmonary insufficiency.

FamNL1-25

This woman was born in 1948. In 2000, she was suffering from asthma and COPD. She was a smoker. At that time, her serum immunoglobulins were moderately abnormal with total IgG of 5.4 g/L, borderline IgA (0.93 g/L) and IgM (0.4 g/L) and low IgG2 0.61, IgG3 0.14 g/L, and undetectable IgG4. In the years following, she developed more pulmonary infections and symptomatic CVID (IgG 4.6 g/L, IgA 1.0 g/L, IgM 0.4 g/L) and was treated with IVIG. Currently, she is suffering from respiratory insufficiency and pulmonary hypertension.

FamNL1-34

This affected male family member was born in 1960. At age 9, he underwent surgery for a ventricular septal defect. In 2004 he was diagnosed with autoimmune hypothyroidism. In 2005 he received surgery for aortic valve insufficiency. The same year he was diagnosed with alopecia areata. Since 2010 he has had recurrent respiratory infections for which he requires frequent antibiotics. He is known to have slightly abnormal serum immunoglobulin concentrations since 2000: IgG 8.0 g/L with IgG1 5.46; IgG2 1.46 (marginally decreased); IgG3 0.32 and IgG4 0.21 g/L, IgA 0.83

g/L and decreased IgM 0.25 g/L. In 2012, he developed pyoderma gangrenosum (like his mother, FamNL1-16), which seemed to respond to IVIG.

FamNL1-36

This is a female born in 1961, who developed recurrent sinusitis and chronic productive cough at age 30. She was found to suffer from CVID (IgG 1.81 g/L, IgA 0.06 g/L, IgM 0.48 g/L). Substitution with IVIG decreased the frequency of infections to approximately 1 - 2 per year, mainly sinusitis. Furthermore, she has had pneumonia, otitis media and severe salmonella enteritis. She is allergic to many antibiotics and has lactase deficiency.

FamNL1-38; FamNL1-39

No clinical data were available for these family members for this study. Both are indicated to have hypogammaglobulinemia (Figure 1A) according to a previous study in which they were described as sIgAD and IgG2 subclass deficient, respectively.[1]

FamNL1-40

This female, born in 1966, has suffered from COPD and recurrent upper airway infections for many years. Initially, in 2000, she was diagnosed with IgA deficiency (IgA < 0.5 g/L), however, in the following years the serum IgG concentrations gradually decreased to 5.1 g/L (IgG1 3.6, IgG2 1.0, IgG3 0.23 and IgG4 < 0.01 g/L); IgM 0.65 g/L. In 2011, she was re-evaluated for her immunodeficiency. Splenomegaly, cytopenias and granulomas were ruled out; a HRCT was normal. Responses to pneumococcal polysaccharide vaccine were absent and she was started on immunoglobulin replacement therapy with a good response. She continues to suffer from sporadic lower respiratory tract infections in the winter despite a normal trough level of IgG 9.5-10.9g/l.

FamNL1-42

This female family member born in 1968 suffers from recurrent paronychia and superficial skin infections but no other infections. Upon work-up she was found to have only minor laboratory abnormalities.

FamNL1-46

This female family member born in 1967 has a history of recurrent airway infections. Laboratory findings include a decreased IgG2 (hypogammaglobulinemia) as well as an insufficient polysaccharide response, whereas tetanus response was

normal. She does not have bronchiectasis and is stable on cotrimoxazole prophylaxis.

FamNL1-48

This female family member, born in 1985, was diagnosed with aortic stenosis at age 15, for which she was treated with balloon valvuloplasty twice and surgical valvotomy twice between 2001 and 2004. In her early youth, she had transient IgG2 and IgG4 deficiencies. There were no respiratory infections, but occasional furunculosis and folliculitis. During pregnancy (2006), there was again a transient hypogammaglobulinemia (IgG 3.72 g/L) with low IgG1 (2.47 g/L) and IgG2 (0.72 g/L). *Postpartum* she developed autoimmune thyroiditis, for which she received levothyroxine. In recent years her IgG and IgM concentrations normalized (IgG 10.2 g/L; IgM 0.74 g/L) with a slightly decreased IgA (0.59 g/L).

FamNL1-49

Since the age of 9 months, this female, born in 1986, has suffered from enteritis and frequently recurring otitis media. When she was 18 months old, hypogammaglobulinemia was diagnosed and she was treated with IVIG. In 1990, the diagnosis of transient hypogammaglobulinemia of childhood was considered, and therefore, the immunoglobulin substitution was interrupted. In 1993, she was diagnosed with giardiasis. When it was found, in 1994, that the response to both protein and polysaccharide vaccines was absent, immunoglobulin substitution was restarted. Because of intolerance of subcutaneous substitution and difficult intravenous access, a vascular port device was inserted and was replaced several times due to local infections and thrombosis. Apart from recurrent respiratory infections and a *Trichophyton* skin infection, there have been no other clinical manifestations of CVID. In 2012, her serum immunoglobulin concentrations were: IgG 9.1 g/L (partly due to IVIG), IgA 1.41 g/L and a decreased IgM of 0.11 g/L.

FamNL1-54

This female family member born in 1988 was found to have decreased IgG1 levels (hypogammaglobulinemia) as well as an insufficient polysaccharide response, while tetanus response was normal. She currently does not have any clinical problems.

FamNL1-55

This female, born in 1990, presented with decreased levels of IgG, IgG1, IgG2 (hypogammaglobulinemia). Tetanus and polysaccharide responses were sufficient. She suffers from recurrent respiratory tract infections and has consequently developed brochiectasis. Immunoglobulin substitution was started a few years ago.

FamNL1-57

This is a 54 year old female with CVID complicated by lymphocytic interstitial pneumonitis (LIP), and chronic liver disease due to nodular regenerative hyperplasia (NRH). She was diagnosed with CVID in 2000 at 39 years of age after investigations were initiated on the basis of recurrent sinopulmonary infections and chronic diarrhea. Despite replacement immunoglobulin with trough IgG levels consistently >10 g/L, she continued to have frequent lower respiratory tract infections requiring intravenous antibiotics (causative organisms include *Moraxella catarrhalis* and *Haemophilus influenzae*).

No infectious cause for ongoing diarrhea has been found after extensive investigation by standard and Harada culture, specific antigen testing for *Giardia*, *Cryptosporidium* and *Clostridium difficile*, and PCR for *Norovirus*. Colonic biopsies revealed a mild lymphocytic infiltrate. Gastric and duodenal biopsies were normal. There was no biochemical evidence of pancreatitis and MRI of the pancreas was normal.

Lymphocytic interstitial pneumonia (LIP) was diagnosed 2 years after the diagnosis of CVID: Widespread reticulonodular infiltrates were documented on HRCT. Bronchoscopic biopsies demonstrated a lymphocytic infiltrate, without evidence of an infective cause. NRH presented 5 years after the diagnosis of CVID when she was found to have persistently elevated liver enzymes in a mixed obstructive and hepatitic pattern. A liver biopsy revealed an eosinophilic and lymphocytic infiltrate surrounding the portal tracts, characterized by a mixture of regeneration and atrophy. MRI revealed the presence of multiple regenerative nodules. She has been treated with ursodeoxycholic acid with some improvement.

FamNL1-62

This affected family member is a 29 year-old female who suffers from mild, recurrent sinus infections, which respond to oral antibiotics. Her IgG levels have fluctuated, but are decreasing over time however; her most recent levels met criteria for a CVID diagnosis (IgG, 4.7 g/L; IgA 0.4 g/L; IgM 0.3 g/L). She has not

commenced IVIG therapy. She is monitored for signs of infections and autoimmunity. She was diagnosed with CVID in 2015 following genetic analysis.

The German CVID family (Fam089)

Fam089-I1

This affected father of the index proband Fam089-II2 was diagnosed with CVID in 2010 following a severe pneumonia accompanied by a pleural empyema (initial immunoglobulins: IgG 1.42 g/L, IgA 0.08 g/L, IgM 0.40 g/L). Immunoglobulin replacement therapy (IVIG) was initiated, but initially only irregularly administered, later in six-weekly intervals. He remains well with only rare infections and no non-infectious complications. In addition to CVID, he has multiple liver hemangiomas.

Fam089-II2

This affected daughter was born in 1979 and was diagnosed with CVID in May 1995 after initially presenting with idiopathic thrombocytopenic purpura (ITP) in April 1995, which was treated by splenectomy. She has a history of frequent respiratory tract infections since childhood including two bouts of pneumonia in 2000 and 2005 despite immunoglobulin replacement therapy, which was started following diagnosis of CVID in 1995.

During the course of the disease she developed an autoimmune hemolytic anemia with incomplete warm autoantibodies as well as an infection-induced neutropenia requiring recombinant human granulocyte-colony stimulating factor (rhG-CSF) treatment for several years. In 2008, she presented with pronounced hepatomegaly and generalized lymphadenopathy. A malignancy was ruled out through multiple biopsies and steroid treatment was initiated, leading to clinical improvement.

Furthermore, she has distinct pulmonary changes suggestive of interstitial lung disease, however, no signs of granulomatous lung disease or lymphocytic infiltrate have been found on biopsy to date. Additionally, she has a history of intermittent diarrhea and recurrent arthralgias.

The affected mother has four daughters (Fam089-III1; -III2; -III3 and –III4), aged 8, 6, 4 and 2 years respectively. The second and the forth daughter both harbor the mutation. The second daughter (Fam089-III2) was found to have (a transient) hypogammaglobulinemia when investigated at the age of 14 months. No complications have been reported for the youngest sister (Fam089-III4).

The CVID family from New Zealand (FamNZ)

FamNZ-I2

This family is of European origin. The mother (FamNZ-I2; 73 years), suffered from alopecia and had an episode of immune thrombocytopenia requiring prednisone treatment. No recurrent infections have been documented and immunoglobulins are normal (IgG 9.9 g/L, IgA 0.58 g/L, IgM 0.8 g/L).

FamNZ-II1

The proband (FamNZ-II1) is a 48 year old male. He was identified as having thrombocytopenia at the age of 2 years. Subsequently he developed persistent neutropenia and autoimmune hemolytic anemia. He underwent a splenectomy and was found to have hypogammaglobulinemia (IgG 5.17 g/L, (7-14) IgA <0.07 g/L, IgM of 0.5 g/L). He has been on long-term IVIG.

FamNZ-II-2

The sister (FamNZ-II-2; aged 46 years) of the index family member suffered from recurrent infections, and was diagnosed with CVID at age 20. Pre-treatment IgG levels are not conclusively documented in her clinical records. She developed alopecia in early adulthood and had mild bronchiectasis as well as NRH of the liver with portal hypertension. She was diagnosed with a marginal zone non-Hodgkin lymphoma and received chlorambucil and Rituximab treatment.

FamNZ-II3

The brother (FamNZ-II3; 42 years) of the index person is unaffected, and had no pneumonias, (antinuclear antibody (ANA) negative; weakly positive thyroid antibodies (47); normal thyroid-stimulating hormone (TSH)) or other complications. Liver and renal functions were normal. Immunoglobulin levels were within the normal range: IgG 7.78 (reference values: 7-16); IgG1, 4.79 g/L (3.8-9.3); IgG2, 2.22 g/L (2.4-7.0); IgG3, 0.473 g/L (0.2-1.8); IgG4, 0.178 g/L (0.04-0.9); IgA 1.5 (0.4-2.5); IgM 0.56 (0.8-4). His three children (FamNZ-III1, -III2 and -III3) are also healthy and have not been examined by genetic testing.

Supplemental Figure S1

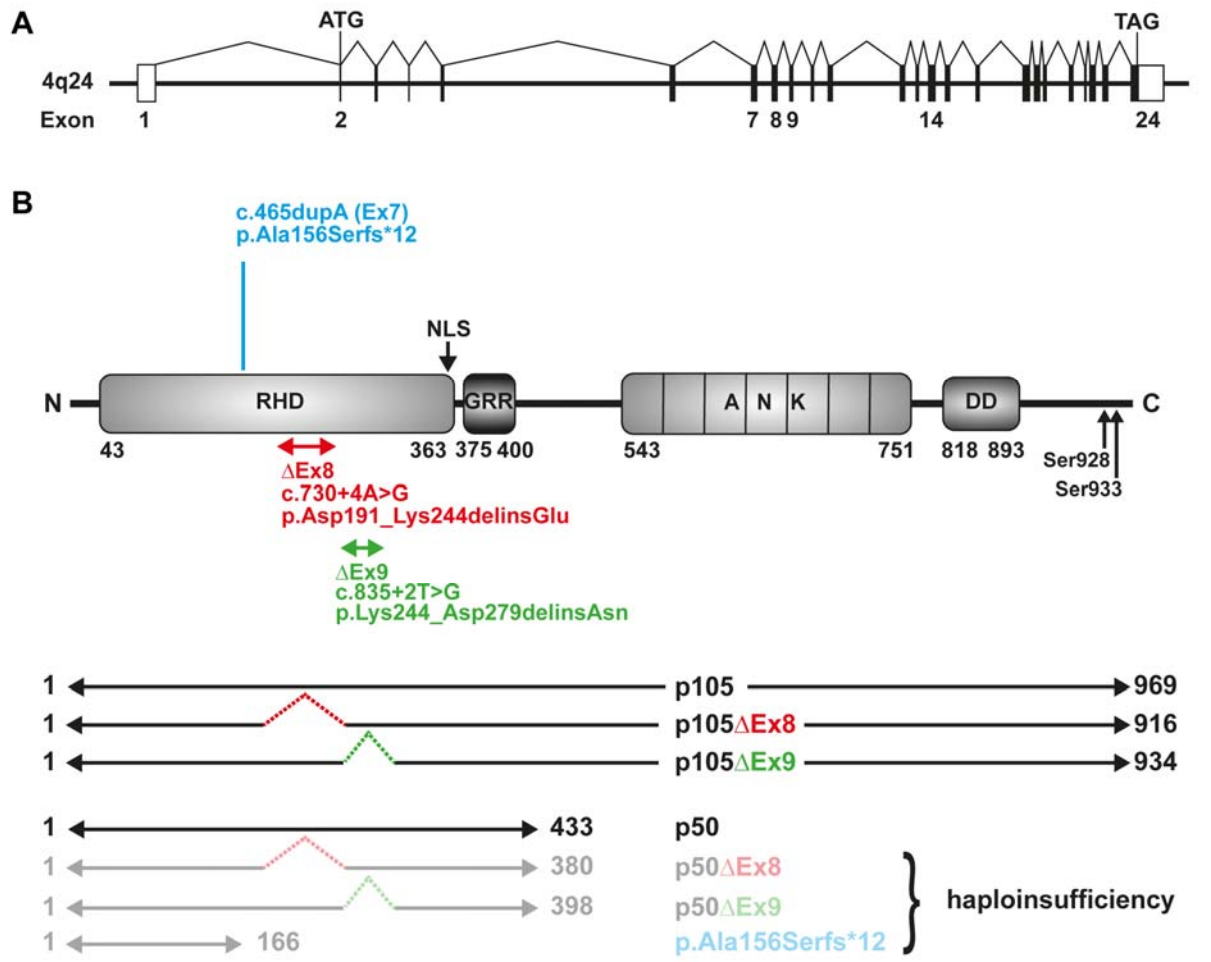

Figure S1. Three mutations in *NFKB1* cause haploinsufficiency of the p50 subunit.

(A) *NFKB1* spans 116 kb and comprises 24 exons (GenBank annotation NC_000004.11; 103422486 - 103538459) and encodes for the 969 amino acid p105 and its shorter isoform 2 (968 amino acids), which uses an alternate in-frame splice acceptor at the 5' end of exon 4.

(B) In-frame skipping of exon 8 or exon 9 deletes 53 or 35 amino acids respectively, from the Rel homology domain (RHD) of NF-κB1. The deletion affects both, the p105 precursor and the active p50 subunit. The frame-shift mutation in exon 7 predicts a non-functional severely truncated protein. The RHD mediates DNA-binding, nuclear translocation and dimerization. The active subunit p50 (amino acids 1-433) is released upon phosphorylation at serines 928 and 933 (in variant 1; NM_003998.3 or

serines 927 and 932 in variant 2; NM_001165412.1) and leads to poly-ubiquitination and subsequent degradation by the 26S proteasome. Amino acid positions and the two C-terminal phosphorylation sites are indicated. NLS, nuclear localization sequence; GRR, glycine-rich region, ANK, ankyrin repeat domain; DD, death domain.

Supplemental Reference

1. Finck, A., Van der Meer, J.W., Schäffer, A.A., Pfannstiel, J., Fieschi, C., Plebani, A., Webster, A.D., Hammarström, L., and Grimbacher, B. (2006). Linkage of autosomal-dominant common variable immunodeficiency to chromosome 4q. Eur. J. Hum. Genet. *14*, 867-875.