**Anti-cytokine autoantibodies in a patient with a heterozygous *NFKB2* mutation**

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**Capsule summary**

We report a family with a heterozygous *NFKB2* mutation in which anti-cytokine autoantibodies were identified in one individual. Rituximab therapy for autoantibodies led to a reduction in anti-cytokine autoantibodies and a marked improvement in infectious susceptibility.

**Key Words;** primary immunodeficiency; heterozygous*NFKB2*variant; anti-cytokine autoantibodies.

*To the Editor*

Germline heterozygous *NFKB2* variants which result in an unprocessable NFKB2 p100 subunit have been identified as a cause of primary immunodeficiency (PID) 1. Patients often present with clinical and immunological features consistent with common variable immunodeficiency (CVID), however additional defects in T, and NK cells have been reported widening the immunological phenotype. Multi-system autoimmunity is also frequently present in patients with damaging *NFKB2* variants causing endocrinopathies including central adrenal insufficiency, growth hormone deficiency, and hypothyroidism, as well as cutaneous autoimmune manifestations of alopecia totalis and trachyonychia.

A 2yr-old female (II.2) presented with polyuria, polydipsia, dehydration and dyselectrolytemia. A renal biopsy showed interstitial nephritis with polyclonal B and T lymphocytic infiltrate and she was diagnosed with proximal renal tubular acidosis (RTA). She had a history of a sterile dry cough since birth and chest imaging was consistent with an interstitial pneumonitis. The RTA and cough improved with a prolonged weaning course of oral corticosteroid, but she began to suffer repeated bacterial infections, extensive molluscum contagiosum over the lower half of her body, and developed alopecia totalis and trachyonychia. Imaging confirmed bilateral bronchiectasis and immunological investigation showed panhypogammaglobulinemia, poor response to polysaccharide vaccine, CD4+ T lymphocytosis, poor differentiation of memory T cells, and reduced class-switched memory phenotype **(Table S1).** She was diagnosed with CVID, and replacement therapy with subcutaneous immunoglobulin was commenced. However, she developed recurrent deep seated pyogenic bacterial infections throughout childhood and early teenage years that included; a deep abscess in the left proximal tibia which was complicated by septic arthritis of the hip, septic arthritis of the right knee, osteomyelitis of the left pubic ramus; and pre-patellar bursitis and bacterial cellulitis of the right thigh with associated bacteraemia. She also suffered repeated viral infections, including influenza pneumonitis and respiratory failure that was considered atypically for a predominant antibody deficiency **(Fig S1).**

The index patient’s (II.2) father (I.1) also had a history of recurrent sinopulmonary infections throughout childhood. At 23yrs-old he was diagnosed with EBV negative nasopharyngeal non-Hodgkin’s lymphoma which was successfully treated to complete remission with chemotherapy. He continued to suffer recurrent bacterial respiratory tract infections and immunological investigation led to a diagnosis of CVID, although I.1 has remained well and asymptomatic since his lymphoma treatment with no current therapeutic interventions despite mild hypogammaglobulinemia **(Fig 1 and Table S1).**

Due to the autosomal dominant pattern inheritance of CVID within the family genetic investigation was performed. Whole exome sequencing and Sanger sequencing confirmation identified a heterozygous *NFKB2* NM\_001077494.2 c.2557C>T:p.Arg853Ter variant which resulted in a truncated NFKB2 p100 subunit to be present in the I.1 and II.2 (**Fig 1, Fig S2 and S3**).

Due to disease severity discordance between I.1 and II.2, further immunological investigations were performed. These identified the presence of anti-IFNα, IFNβ, IFNω, IL-12p40 and IL-23 ACAAs in the serum of II.2. No ACAAs were found in serum from I.1 **(Fig 2 and Supplementary Methods)**.

In light of these findings, we treated II.2 with 4 cycles of rituximab 375mg/m2 resulting in a marked reduction in autoantibody titers and clinical improvement with no further invasive infections **(Fig S4)**.

The development and pattern of ACAAs, as well clinical feature of alopecia and trachyonychia, observed in II.2 is reminiscent of that of APECED and thymoma patients in whom there is impairment of thymic medullary function resulting in autoimmunity **(Figure 2b)** 2. Correct function of the NFKB2 pathway is required to establish the thymic medullary compartment and correctly regulate *AIRE* expression 3. *NFKB2Lym1*-/- knockout mice also show significant impairment of medullary thymic epithelial cell (mTEC) maturation and *AIRE* expression leading to the development of autoantibodies 4.Therefore, we hypothesize that errant thymic function in patients with heterozygous *NFKB2* mutations may led to ACAAs and the broad organ specific autoimmunity, including the cutaneous manifestations, which are present in this patient group 1.

ACAAs have been reported in other PIDs, including patients with biallelic hypofunctional *RAG* variants in whom neutralizing anti-IFNα, IFNω and IL-12 autoantibodies were associated with increased susceptibility to viral infections 5. IFNα, IFNω and IL-12p40 ACAAs were present in II.2, and we may postulate that it is similarly also underlying mTEC impairment in patients with hypofunctional *RAG* genes, *AIRE* deficiency, and thymoma, that leads to the development of this specific ACAA pattern 6. However, the precise role of neutralizing ACAAs in health and disease remains elusive with the presence of neutralizing type 1 interferon ACAAs potentially conveying a beneficial protective counterbalance in those at high risk of, or suffering, autoimmune diseases 7, 8.

Following rituximab treatment we observed a clinical improvement with a reduction in infections despite not all ACAAs measured displaying a significant reduction **(Fig S4).** Given the broad range of ACAAs that have been previously reported, it may be that still unidentified ACAAs or anti-cytokine receptor autoantibody levels were concurrently reduced by rituximab therapy contributing to partial restoration of immunological function and the overall resultant clinical improvement 9. IL-12p40 ACAAs did not significantly decline post-rituximab and therefore we remain vigilant for potential intracellular pathogens although the infectious risks of anti-IL-12 autoantibodies remain unclear 9.

In conclusion, patients with heterozygous *NFKB2* variants may develop ACAAs due to impaired thymic central tolerance, resulting in a more severe clinical phenotype and autoimmunity. Screening of these patients for ACAAs and treatment with rituximab may offer therapeutic benefits.

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**Authorship Contributions**

SNF and APW coordinated the study. WR, SVP, SNF and APW cared for the patients. KAR, RD, YG and GB-M, performed the experiments. KAR, WR, YG, SNF DK, and APW analyzed the laboratory data. RP and SE undertook the genomic analysis and interpretations. KAR, WR, RD, SNF and APW wrote the manuscript. All authors were responsible for the revision and final approval of the manuscript.

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**Figure Legends**

**Figure 1**

**a.)** Family pedigree consistent with a pattern of autosomal dominant inheritance. **b.)** Fluorescent traces showing *NFKB2* c.2557C>T in I.1, II.2, and absent in I.2. **c.)** Western blot demonstrating the presence of a truncated NFKB2 p100 subunit in the patient compared with control. Stimulation with anti-CD3, anti-CD3 and anti-CD70, or PHA shows minimal processing to NFKB2 p52 in the patient. **d.)** Reduced NFKB2 p52 processing in an EBV cell line in response to noncanonical NFKB pathway stimulation with CD40L in patients (I.1, II.2) compared with unaffected relative (I.2). Graph showing relative NFKB2 p52 production normalized to GAPDH from EBV cell line lysates **(Supplementary Methods).**

**Figure 2**

**Fig 2. a.)** *In vitro* culture of whole blood or washed PBMC from II.2 (Patient) and control. TNF-α production in response to TLR ligands lipotechoic acid (LTA) and lipopolysaccharide (LPS) was comparable to control. In whole blood from II.2 IL-12p40 response to LPS was impaired as was IFN-γ response to both mitogen (PHA) and LPS +/- IL-12. PBMC culture under similar conditions led to complete reversal of abnormalities noted in whole blood culture which led to the hypothesis of ACAAs**. b.)** Detection of anti-IL12p40, IL-23, IFNα, IFNβ and IFNω ACAA in the serum of index patient (II.2) at a titration of 1:100. ACAAs were not present in 150 controls or I.1. Those present in II.2 displayed a similar pattern compared with patients with thymoma (n=2) and APECED (n=2). **c.)** Whole blood (WB) showed reduced TNFα production in response to IFNα in II.2 compared with control. Cells from PBMCs were then washed to remove ACAAs and incubated in either fetal calf serum (FCS), complete serum (CS), or phosphate buffered saline (PS) to demonstrate across of range of media that remove of ACAAs restored TNFα production from cells. d.) IL-12p40 ACAA showed biologically active in PBMCs from a control with IL-12 + IL-18 inducing robust production of IFN-γ in FCS or volunteer autologous serum, but inhibition of IFN-γ production in the presence of II.2’s serum due to IL-12p40 ACAA **(Supplementary Methods).**