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RELA haploinsufficiency in CD4 lymphoproliferative disease with autoimmune cytopenias

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To the Editor

The NF-κB family of transcription factors regulates thousands of genes controlling innate and adaptive immunity. The transcription factors comprise hetero- or homo-dimers of five subunits: *NF-κB1*/p50, NF-κB2/p52, *RELA*/p65, *c-REL*, and *REL-B*. Mutations in these subunits generally cause immunologic disorders with variable penetrance and expressivity.[1] For example, *NF-κB1* mutations causing haploinsufficiency can trigger common variable immunodeficiency (CVID), hyper-inflammatory/autoimmune syndromes, combined immunodeficiency, or no disease. The phenotype is influenced by the location and biochemical nature of the causal variant and other genetic and environmental factors.[1] Recently, 4 related patients with *RELA* haploinsufficiency were described with TNF-

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CONFLICT of INTEREST

The authors have no conflicts of interest to report.

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dependent mucocutaneous ulcerations and inflammatory intestinal disease, both treatable with infliximab therapy.[2]

We studied a male born to healthy parents diagnosed at age 5 with Autoimmune Lymphoproliferative Syndrome (ALPS) with refractory immune thrombocytopenic purpura (ITP), anemia, neutropenia and splenomegaly (Figures 1A and 1B), with mild lymphadenopathy. The patient was splenectomized at age 7 and subsequently received corticosteroids, mycophenolate mofetil (MMF), intravenous immunoglobulin (IVIG), eltrombopag, and rituximab for refractory ITP. Platelet counts normalized after MMF and IVIG treatment, suggesting an autoimmune etiology, though the patient did not have elevated autoantibodies (ENA, DNA, RNP, ANA, anti-Cardiolipin, and anti-Thyroid). The patient had episodic aseptic meningitis, with lumbar punctures revealing reactive lymphocytosis in the cerebrospinal fluid consisting of 85% T cells in a 6:1 $CD4^+$ to $CD8^+$ ratio. Additionally, the patient had a systemic reaction to the pneumococcal 23-valent vaccine with leukocytosis and hypertension. Finally, the patient complained of severe recurrent headaches, abdominal pain, vomiting, diarrhea, and weight loss. These complaints were possibly related to MMF therapy and were reduced after switching to eltrombopag. ITP resolved following a third round of rituximab therapy though the patient continues to suffer from recurrent debilitating headaches.

Despite ALPS features, we found no defect in Fas, TCR, or cytokine withdrawal-mediated apoptosis (Figure E1 in the online repository). Immunophenotyping revealed slightly elevated $CD3^+$ double negative T cells (2.4%), increased activated ($HLA\text{-}DR^+$) $CD4^+$ T cells, and increased peripheral blood T cells (Figure 1C and 1D). This was accompanied by a decrease in naïve ($CCR7^+/CD45RA^+$) $CD4^+$ T cells and an increase in terminally differentiated ($CCR7^-/CD45RA^+$) effector T cells (Figure 1E). Furthermore, patient $CD4^+$ T cells had an enhanced Th1-like phenotype ($CXCR3^+/CCR6^-$) (Figure E2A in the Online Repository). Patient naïve $CD4^+$ T cells were intrinsically skewed towards a Th1-effector phenotype with increased production of the NF-κB target cytokine IFNγ (Figure 1F and 1G), though not all NF-κB targets showed increased production, as patient cells made less IL-2 upon re-stimulation. Patient $CD4^+$ T cells were more sensitive to TCR stimulation than control cells (Figure 1H), suggesting that increased antigen sensitivity may account for the elevated effector and diminished naïve T cells. Patient T cells may be further activated due to decreased regulatory responses, as we found decreased iTreg differentiation despite normal levels of $CD25^+/CD127^{low}$ nTreg cells (Figure E2B, E2C, and E2D in the Online Repository); this may be attributable to either increased IFNγ or decreased p65/c-Rel dependent FOXP3 transcription.[3] Finally, B cell populations and immunoglobulins appeared normal (Figure E3 in the Online Repository). Thus, the disease was apparently largely driven by augmented T cell activation and effector function.

Whole genome sequencing revealed a *de-novo* heterozygous nonsense mutation (NM_021975.3:c.736C>T, p.Arg246*) in *RELA* (Figure 1I) leading to reduced p65 protein and mRNA, consistent with nonsense-mediated decay of the variant transcript (Figure 1J). By contrast, c-Rel, NF-κB1, and NF-κB2 protein levels were normal (Figure E4A and E4B in the online repository). Interestingly, while significantly less p65 was immunoprecipitated from patient cells, c-Rel, p50, and p52 were co-immunoprecipitated at higher relative levels

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in patient cells compared to controls, suggesting possible loss of the p65 homodimer (Figure E4A, E4C, and E4D in the online repository). While patient cells had lower baseline IκBα expression and maximum phospho-p65 after stimulation, NF-κB activation kinetics were comparable to healthy donor cells (Figure 1K and 1L). Hence, the patient's disease likely results from insufficient p65 dimers and altered regulation of their specific targets.[4]

Given the differences between our case and those previously reported, we conclude that *RELA* haploinsufficiency, like *NF-κB1* haploinsufficiency, can cause widely different clinical manifestations.[2,5] While the variant type and overall genetic/environmental variability are key modifying factors, variants in other genes also determine the observed clinical phenotype in an apparently "monogenic" disease. To wit, our patient has multiple rare and potentially deleterious variants inherited from asymptomatic parents in genes affecting immune responses (Table E1 in the Online Repository). In particular, the variants in NF-κB regulators *IKBIP* and *IKBKE* may potentially alter the patient's phenotype. IKBIP encodes IKK-β-interacting protein (IKIP) that is induced by p53 and whose overexpression causes apoptosis.[6] The patient's L363S IKBIP variant could possibly attenuate TNF-induced apoptosis via the TNF-p53 pathway. IKBKE encodes for IKK-E, a homologue of IKK-A and IKK-B, which was originally identified as an IKK-A/B independent IκBα kinase and can phosphorylate STAT1 during viral responses.[7,8] The patient's E50K IKBK variant eliminates a critical hydrogen bond with the catalytic lysine (K38 in IKK-E) that likely inactivates the kinase.[9] This mutation may further depress NF-κB activation under certain circumstances, though it should be noted that overexpression of kinase dead IKK-E (K38A) attenuates PMA-induced NF-κB activation, a feature that we did not observe. Though further investigation is needed to determine whether these variants modify the patient's condition, it is unlikely that they cause disease by themselves since they were inherited from the unaffected father.

In summary, we expand the known clinical phenotype of *RELA* haploinsufficiency by linking it to an ALPS-like disease associated with refractory autoimmune cytopenias, increased T cell activation and proliferation, and enhanced Th1 effector responses in the absence of mucocutaneous ulcerations and inflammatory intestinal disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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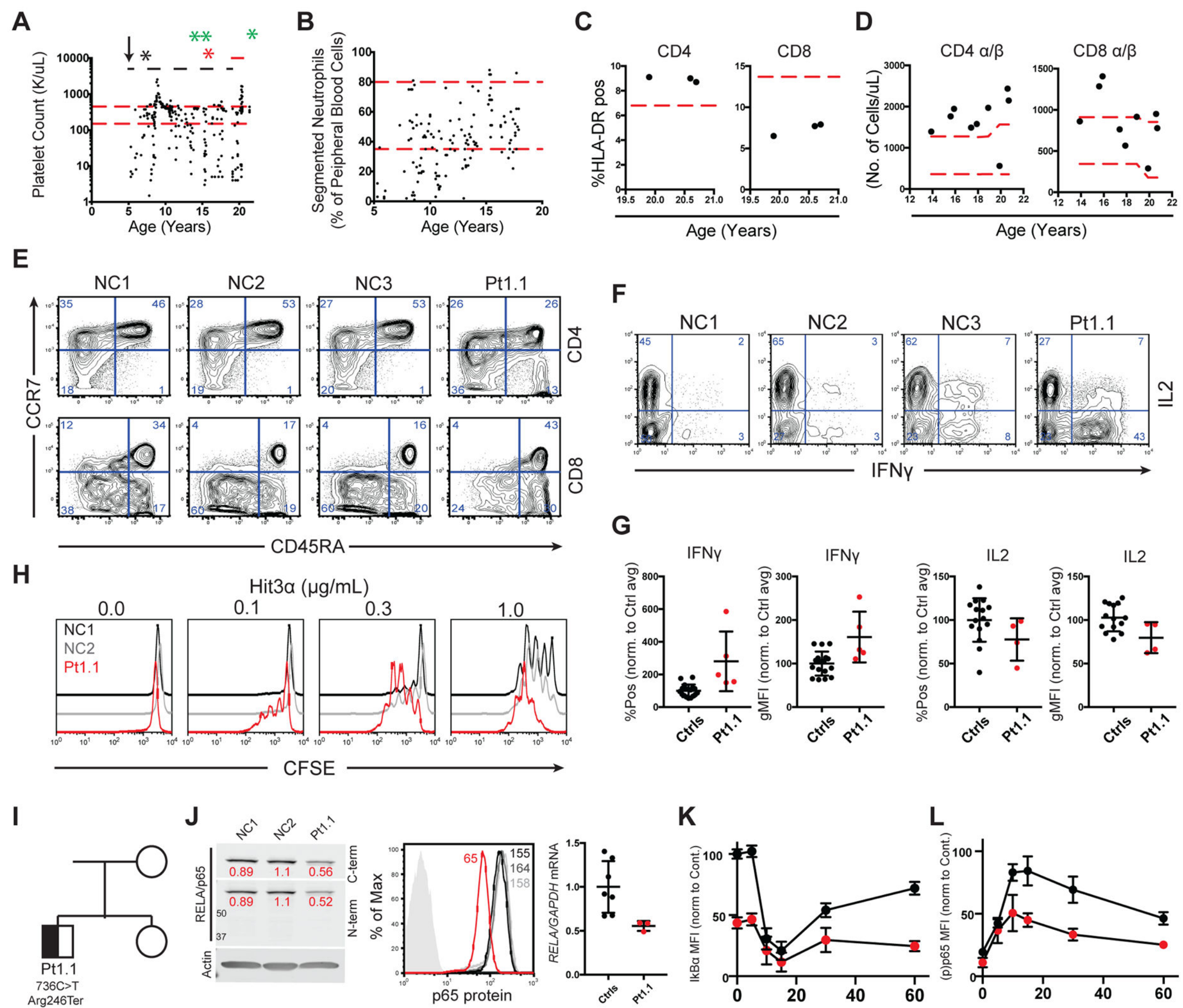

Figure 1.

RELA haploinsufficiency leads to a T cell mediated autoimmune lymphoproliferative disease

Patient platelet and neutrophil counts (red dashed lines, arrow, black, green, and red asterisks represent the normal range, age of onset, splenectomy, rituximab administration, and systemic immune response to pneumococcal vaccine, respectively. Black dashed and solid red lines represent MMF and eltrombopag administration, respectively (A/B). $CD4^{+}$ and $CD8^{+}$ cells expressing HLA-DR, red line indicates high end of normal range (C). $CD4^{+}$ and $CD8^{+}$ α/β T cells/uL of patient blood, red lines indicate normal range (D). CCR7 and CD45RA expression on $CD3/CD4^{+/+}$ lymphocytes (E). Representative IFNγ and IL2 production in phorbol 12-myristate 13-acetate and ionomycin (PMA/I) restimulated $CD4^{+}$ T cells (F). IFNγ and IL2 production in cells stimulated as in F, pooled from 4 separate experiments (G). Proliferation of $CD4^{+}$ blasts following re-stimulation with 1 μg/mL anti-CD28 and the indicated dose of anti-CD3 (n=3) (H). Family pedigree and de-novo RELA variant (I). Protein expression in $CD4^{+}$ T cell blasts by western blot (RELA/Actin ratios in red) (left), flow cytometry (center), and RELA mRNA levels determined by qRT-PCR (right)

(J). IkBα degradation and p65 phosphorylation following PMA/I stimulation, pooled from 3 separate experiments (K and L, respectively).

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