American College of Rheumatology
Empowering Rheumatology Professionals

Arthritis & Rheumatology
Vol. 73, No. 3, March 2021, pp 490–497
DOI 10.1002/art.41531

BRIEF REPORT

A Novel *RELA* Truncating Mutation in a Familial Behçet's Disease–like Mucocutaneous Ulcerative Condition

Fahd Adeeb,[1] Emma R. Dorris,[2] Niamh E. Morgan,[3] Dylan Lawless,[4] Aqeel Maqsood,[1] Wan Lin Ng,[1] Orla Killeen,[5] Eoin P. Cummins,[2] Cormac T. Taylor,[2] Sinisa Savic,[4] Anthony G. Wilson,[2] and Alexander Fraser[6]

Objective. Monogenic Behçet's disease (BD)–like conditions are increasingly recognized and to date have been found to predominantly involve loss-of-function variants in *TNFAIP3.* This study was undertaken to identify genetic and pathobiologic mechanisms associated with a BD-like mucocutaneous ulcerative syndrome and neuromyelitis optica (NMO) occurring in 3 generations of an Irish family (n = 5 cases and 5 familial controls).

Methods. Whole-exome sequencing was used to identify potential pathogenic variants in affected family members and determine segregation between affected and unaffected individuals. Relative v-rel reticuloendotheliosis viral oncogene homolog A (RELA) expression in peripheral blood mononuclear cells was compared by Western blotting. Human epithelial and RelA$^{-/-}$ mouse fibroblast experimental systems were used to determine the molecular impact of the RELA truncation in response to tumor necrosis factor (TNF). NF-κB signaling, transcriptional activation, apoptosis, and cytokine production were compared between wild-type and truncated RELA in experimental systems and patient samples.

Results. A heterozygous cytosine deletion at position c.1459 in RELA was detected in affected family members. This mutation resulted in a frameshift p.His487ThrfsTer7, producing a truncated protein disrupting 2 transactivation domains. The truncated RELA protein lacks a full transactivation domain. The RELA protein variants were expressed at equal levels in peripheral mononuclear cells. RelA$^{-/-}$ mouse embryonic fibroblasts (MEFs) expressing recombinant human RELAp.His487ThrfsTer7 were compared to those expressing wild-type RELA; however, there was no difference in RELA nuclear translocation. In RelA$^{-/-}$ MEFs, expression of RELAp.His487ThrfsTer7 resulted in a 1.98-fold higher ratio of cleaved caspase 3 to caspase 3 induced by TNF compared to wild-type RELA (P = 0.036).

Conclusion. Our data indicate that *RELA* loss-of-function mutations cause BD-like autoinflammation and NMO via impaired NF-κB signaling and increased apoptosis.

INTRODUCTION

Behçet's disease (BD) is an autoinflammatory vasculitic condition characterized by oral and genital ulceration, uveitis, rashes such as erythema nodosum, and arthralgia. The etiology is multifactorial, with a significant genetic component and shared susceptibility loci with Crohn's disease and leprosy, implicating mucosal factors and the innate immune response in BD risk (1).

NF-κB is a family of inducible transcription factors that act as master regulators of immune and apoptotic signaling by controlling the expression of a large array of genes. The NF-κB family is composed of 5 structurally related members: NF-κB1

Supported by a grant from the National Children's Research Centre, Dublin, Ireland (grant C/18/6). The collection of clinical data and samples was supported by Arthritis Ireland and the Arthritis Research Coalition. Dr. Cummins' work was supported by Science Foundation Ireland (grant 15/CDA/3490). Dr. Savic's work was supported by the EU Research and Innovation program Horizon 2020 (ImmunAID grant agreement 779295).

[1]Fahd Adeeb, MB, PhD, Aqeel Maqsood, MB, Wan Lin Ng, MB: University Hospital Limerick, Limerick, Ireland; [2]Emma R. Dorris, PhD, Eoin P. Cummins, PhD, Cormac T. Taylor, PhD, Anthony G. Wilson, MB, PhD: University College Dublin, Dublin, Ireland; [3]Niamh E. Morgan, PhD: University College Dublin, National Children's Research Centre, and Our Lady's Children's Hospital Crumlin, Dublin, Ireland; [4]Dylan Lawless, PhD, Sinisa Savic, MD, PhD: NIHR Leeds Institute of Rheumatic and Musculoskeletal Medicine and St. James's University Hospital, Leeds, UK; [5]Orla Killeen, MB: National Children's Research Centre and Our Lady's Children's Hospital Crumlin, Dublin, Ireland; [6]Alexander Fraser, MD, MB: University Hospital Limerick and University of Limerick School of Medicine, Limerick, Ireland.

Drs. Adeeb, Dorris, and Morgan contributed equally to this work. Drs. Wilson and Fraser contributed equally to this work.

No potential conflicts of interest relevant to this article were reported.

Address correspondence to Emma R. Dorris, PhD, UCD Conway Institute, Belfield, Dublin D04 V1W8, Ireland. Email: emma.dorris@ucd.ie.

Submitted for publication May 11, 2020; accepted in revised form September 17, 2020.

(p50), NF-κB2 (p52), v-rel reticuloendotheliosis viral oncogene homolog A (RELA; p65), RELB, and c-REL. These proteins mediate transcription of target genes by binding to a κB enhancer DNA element as hetero- or homodimers (2).

Autosomal-dominant forms of BD have been linked to loss-of-function mutations in tumor necrosis factor–induced protein 3–interacting protein 1 (TNFAIP3; A20), a negative regulator of NF-κB (3). Differences in clinical features compared with BD include onset in early childhood, recurrent fevers, severe ocular disease, colitis, and polyarthritis (4). A single family with an autosomal-dominant early-onset mucocutaneous ulcerative syndrome caused by a RELA mutation has been described (5). Those studies implicate distinct genetic and cellular mechanisms involving the NF-κB pathway in familial forms of BD.

Like BD, neuromyelitis optica (NMO; Devic's disease) is a relapsing–remitting inflammatory disorder. NMO is confined to the central nervous system and is associated with specific anti–aquaporin 4 (anti–AQP-4) antibodies. Several studies have demonstrated that both conditions share similar pathophysiology, and NF-κB is key (6). Proteasome and immunoproteasome inhibitors that prevent nuclear accumulation of NF-κB have demonstrated profound effects on astroglial responses to NMO IgG (7).

In addition to being the hallmark feature of NMO, optic neuritis is a recognized rare manifestation of BD. This feature, sometimes described as a posterior segment ocular involvement, is part of the International Study Group for BD (ISGBD) criteria (8) and the revised International Criteria for BD (ICBD) (9).

In this study we determined the genetic and molecular basis of an autosomal-dominant mucocutaneous ulcerative syndrome and NMO in 5 members of a 3-generation Irish family. Whole-exome sequencing identified a mutation in *RELA* resulting in expression of a truncated RELA protein, with suppression of RELA-dependent transactivation activity and increased apoptosis.

MATERIALS AND METHODS

Public and patient involvement. This study has a patient research partner member of the project advisory group who mentors the researchers in lay communication and advises on patient consent forms.

Ethics and sample collection. Written informed consent was obtained from all participants or their guardians. This study was approved by University Hospital Limerick's local ethics committee in accordance with the Declaration of Helsinki. Whole blood or saliva samples were obtained from all family members. Peripheral blood mononuclear cells (PBMCs) were obtained from 3 familial controls and 4 heterozygotes.

Whole-exome sequencing. Libraries were sequenced on an Illumina HiSeq 3000 system. Variants were annotated using Alamut-HT high-throughput annotation software. The variant identified was validated by Sanger sequencing. For additional details, see Supplementary Methods, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41531/abstract.

Cell culture. PBMCs were isolated from whole blood using density-gradient centrifugation. HEK 293T cells (ATCC) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum. Mouse embryonic fibroblasts (MEFs) lacking the NF-κB subunit p65 ($RelA^{-/-}$) were a gift from Alexander Hoffman (University of California, Los Angeles). Recombinant human or mouse tumor necrosis factor (TNF; PeproTech) was used at 10 ng/ml.

Reverse transcriptase–polymerase chain reaction (RT-PCR). Gene expression was measured via TaqMan real-time RT-PCR using ΔC_t relative quantitation. See Supplementary Methods for the assay IDs.

Plasmid transfection. The pRelA-EGFP plasmid (a generous gift from Professor Michael White [University of Manchester, Manchester, UK]) was used as a template to generate RELAp.His487ThrfsTer7 and wild-type RELA plasmids and confirmed by capillary sequencing. Transfection of DNA plasmids was performed using Lipofectamine 3000 transfection reagent (ThermoFisher Scientific) according to the manufacturer's instructions. For additional details see Supplementary Methods, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41531/abstract.

Luciferase reporter assay. An NF-κB–Luciferase PEST reporter vector (Promega) plus a *Renilla* luciferase control reporter vector (pRL-SV40; Promega) were co-transfected into $RelA^{-/-}$ MEFs. Cells were incubated with 10 ng/ml TNF for 5 hours, and luciferase activity was measured using a Dual-Glo Luciferase assay system (Promega). For additional details, see Supplementary Methods, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41531/abstract.

Western blotting. Cells were treated with 10 ng/ml TNF for 24 hours, and whole cell lysates were extracted. Electrophoresis was carried out using 12% Tris–glycine–polyacrylamide gel and wet transfer to PVDF membranes (ThermoFisher Scientific). Membranes were blocked with 5% protein, and antigen–antibody complexes were visualized using chemiluminescence. For additional details, see Supplementary Methods.

Cytokine assays. Enzyme-linked immunosorbent assays were performed to detect human interleukin-6 (IL-6) and TNF, and mouse CXCL10 and IL-6, protein concentrations (R&D Systems), according to the manufacturer's instructions, using 100 µl of supernatant per well.

Immunofluorescence. RelA$^{-/-}$ MEFs (n = 17,500) were seeded into Millicell EZ 8-well chamber slides (Millipore). Cells were fixed in 4% paraformaldehyde (Sigma-Aldrich) for 20 minutes, washed 3 times with phosphate buffered saline (PBS), permeabilized with 0.1% Triton X-100 (Sigma-Aldrich) for 5 minutes, washed 3 times with PBS, and incubated in blocking solution containing 0.5% bovine serum albumin (Sigma-Aldrich) and 50 m*M* ammonium chloride in PBS for 30 minutes. Cells were probed with primary antibody (1:1,000) in blocking solution containing 0.02% sodium azide (Sigma-Aldrich) for 1 hour, washed 3 times with PBS, and probed with goat anti-rabbit Alexa Fluor 568 (1:400; Life Technologies) diluted in blocking solution for 30 minutes. Cells were washed and coverslips were mounted using FluroShield with DAPI mounting media (Sigma-Aldrich).

Confocal microscopy. Confocal images were acquired on a FluoView FV1000 laser scanning microscope (Olympus). Images were acquired with a 60× numerical aperture, 1.35 UPlanSApo oil immersion objective (Olympus) at a resolution of 1,024 × 1,024 pixels in a sequential scanning model.

Statistical analysis. Data are presented as the mean ± SEM. Data were graphed to assess distribution and transformed where required to meet parametric assumptions. Student's *t*-test was used to compare 2 groups, using the unpaired test for independent groups, and type 1 test for paired samples. To compare more than 2 groups, analysis of variance with Tukey's highest significant difference post hoc test was used. Statistical analysis was performed using IBM SPSS Statistics version 24. An alpha value of 0.05 was used.

RESULTS

Clinical phenotype. This study involved an Irish Caucasian family of 10 individuals (Figure 1A). The index case (I.2) reported a transient period of recurrent severe oral ulcers as a teenager; his symptoms, however, spontaneously resolved more than 30 years ago. Two half-sisters (II.2 and II.5) were diagnosed as having early-onset BD satisfying the ISGBD criteria at 10 years old and 15 years old, respectively. The illness was characterized by recurrent oral aphthosis, genital aphthosis, and skin pustulosis. In contrast to cases in the literature with A20 haploinsufficiency (4), affected individuals in this study were negative for antinuclear antibody. A third sister (II.3) developed NMO, characterized by recurrent optic neuritis with AQP-4 antibody positivity. Of note, serial imaging demonstrated no evidence of transverse myelitis or cerebral involvement. A son of the index case declined to be involved in the study but was reported to be well and asymptomatic. Subsequently, a daughter of one of the sisters with BD (III.1) developed recurrent oral aphthosis at the age of 10 years. The 2 half-sisters with BD were successfully treated with anti-TNF medication (etanercept), and the patient with NMO was treated with anti-CD20 (rituximab). The demographic characteristics, clinical manifestations, and HLA–B*51 status of all family members were recorded (Supplementary Table 1, *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41531/abstract).

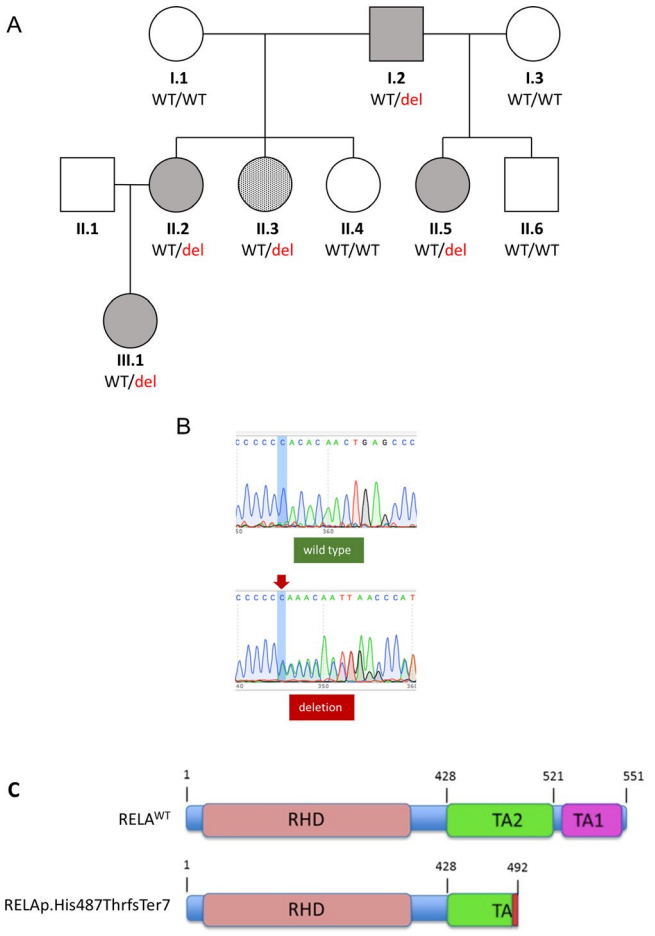

Figure 1. Pedigree of a family with a mucocutaneous ulcerative condition. **A**, Genotype of RELA c.1459delC. Open symbols represent individuals with the homozygous wild-type (WT) genotype; solid symbols represent heterozygotes. Stippled symbol (II.3) represents an individual who was heterozygous for RELA c.1459delC and had neuromyelitis optica. **B**, Sanger sequencing of *RELA* in a wild-type familial control and in a heterozygous affected individual with cytosine base deletion at position c.1459 (**arrow**). **C**, Graphic representation of full-length wild-type RELA with conserved Rel homology domain (RHD) and 2 C-terminal transactivation domains (TA2 and TA1), and of RELAp.His487ThrfsTer7 with partial TA2 but lacking TA1.

Identification of a novel *RELA* variant. Whole-exome sequencing identified a heterozygous deletion c.1459delC in exon 11 of *RELA* (ENST00000406246). This was validated by Sanger sequencing and was detected in all affected individuals and the individual with NMO (Figure 1B). The mutation resulted in a frameshift encoding RELA amino acid 487 followed by 5

amino acids and a stop codon (QLSPC*) encoding a truncated RELA protein of 492 amino acids, ~6 kd smaller than wild-type RELA (Figure 1C). This mutation has not been identified in publicly available genetic data sets (https://gnomad.broadinstitute.org).

Expression of RELAp.His487ThrfsTer7 protein in individuals heterozygous for RELA c.1459delC. Individuals heterozygous for *RELA* c.1459delC had 2 bands when probed with an anti-RELA antibody targeted to an N-terminus epitope, with an ~6-kd difference in protein size, as predicted (Figure 2A).

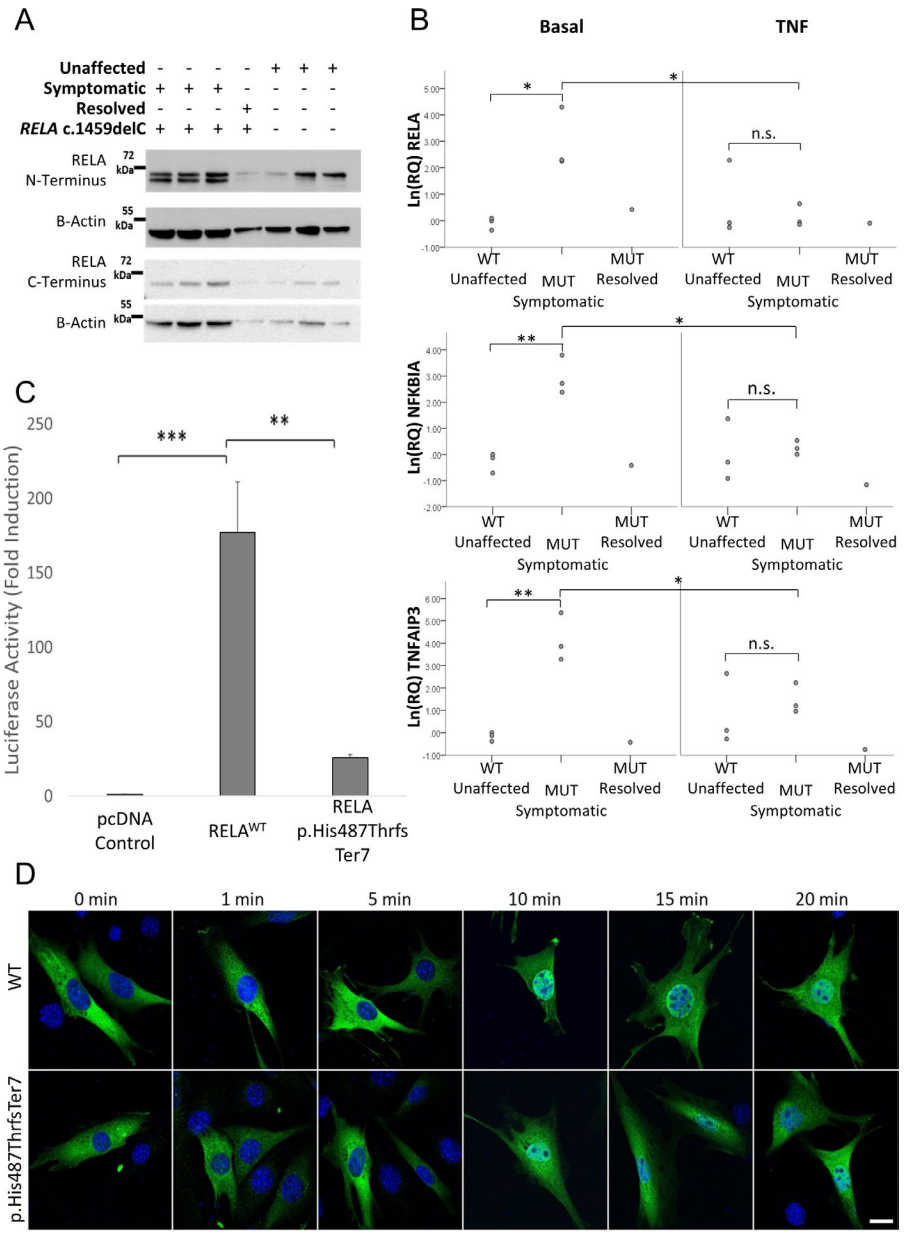

Figure 2. RELAp.His487ThrfsTer7 is an expressed truncated protein and is associated with altered NF-κB signaling. **A**, Western blot analysis of peripheral blood mononuclear cells (PBMCs) from unaffected individuals, symptomatic individuals, and the individual in whom symptoms had resolved. Individuals heterozygous for *RELA*c.1459delC had 2 detectable RELA bands when probed with an RELA N-terminus, but not C-terminus, antibody. The RELA bands correspond to full-length RELA and the predicted RELAp.His487ThrfsTer7. **B**, Up-regulation of mRNA for basal RELA, NFKBIA, and TNFAIP3 in RELAp.His487ThrfsTer7 patient cells (MUT) compared to wild-type (WT) familial control PBMCs. After stimulation with tumor necrosis factor (TNF) for 4 hours, there was no significant difference (NS) between mRNA levels in unaffected and symptomatic individuals. Values are the mean ln(relative quantitation [RQ]). Symbols represent individual subjects. **C**, Luciferase activity in the mouse embryonic fibroblast (MEF) RelA$^{-/-}$ transfection model, demonstrating the impaired ability of RELAp.His487ThrfsTer7 to transactivate NF-κB response elements. Values are the mean ± SEM fold increase in NF-κB activity in TNF-stimulated cells compared to unstimulated pcDNA, plotted as the ratio of luciferase to *Renilla* (n = 3 independent experiments). **D**, Representative confocal images of MEF RelA$^{-/-}$ transfection models treated with TNF for the indicated times. Green indicates RELA; blue indicates the nucleus. Bar = 10 μm. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

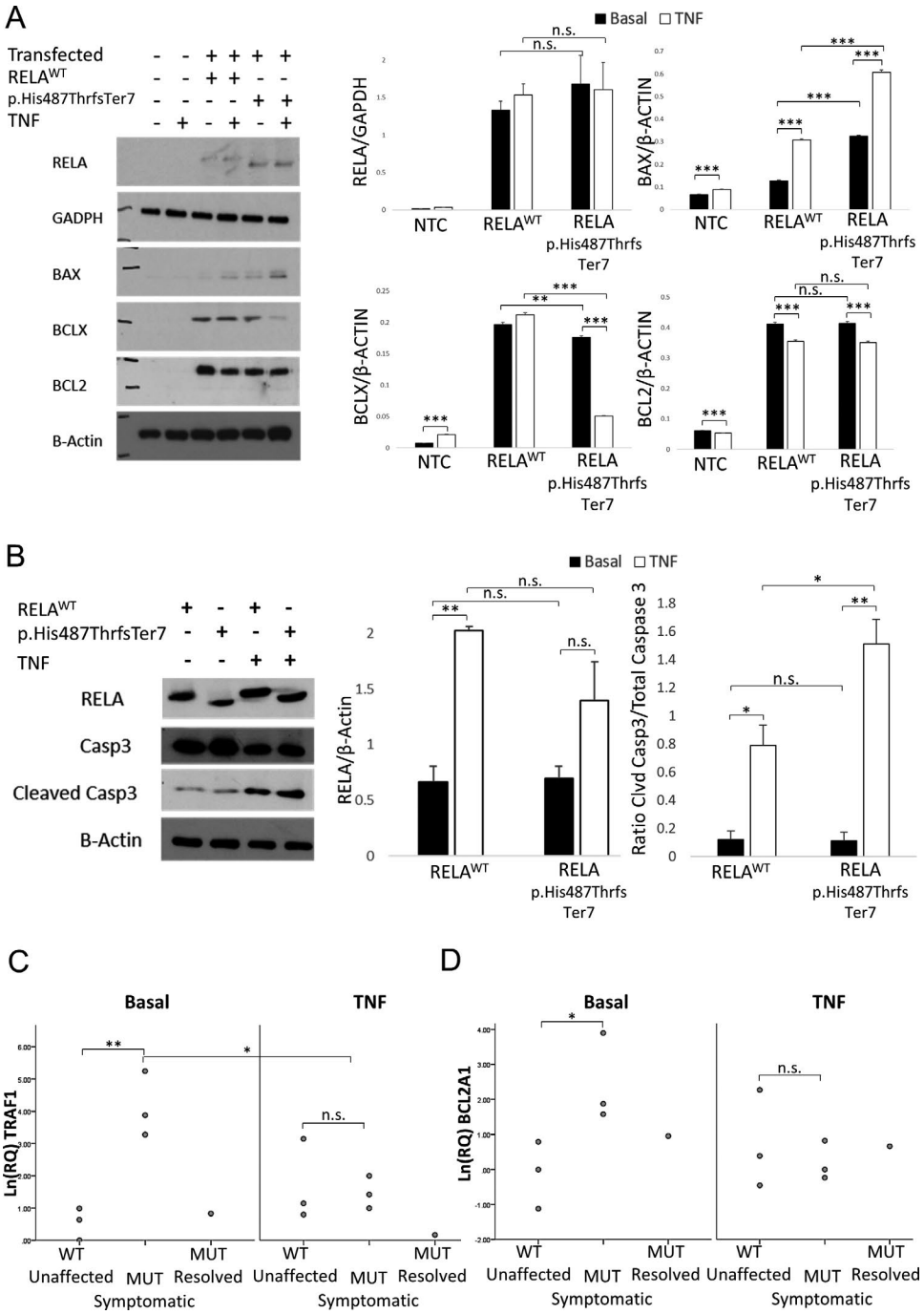

Figure 3. RELAp.His487ThrfsTer7 increases apoptosis. **A**, Western blotting and densitometry of HEK 293T cells in the transfection model, showing no difference in expression levels of wild-type (WT) RELA and RELAp.His487ThrfsTer7. Cells expressing RELAp.His487ThrfsTer7 showed increased Bax and decreased Bcl-x, both basally and in response to tumor necrosis factor (TNF), compared to wild-type RELA. Bcl-2 was down-regulated by TNF but was not significantly different (NS) between RELAp.His487ThrfsTer7- and wild-type RELA-expressing cells. NTC = nontransfected cells. **B**, Increased ratio of cleaved caspase 3 (compared to pro–caspase 3) after TNF stimulation in cells expressing RELAp.His487ThrfsTer7 compared to wild-type RELA. **C** and **D**, Up-regulation of mRNA for *TRAF1* and *BLC2A1* in RELAp.His487ThrfsTer7-expressing patient peripheral blood mononuclear cells (PBMCs) (MUT) compared to wild-type familial control PBMCs. TNF induced down-regulation of *TRAF1* in RELAp.His487ThrfsTer7-expressing patient PBMCs. In **A** and **B**, bars show the mean ± SEM. In **C** and **D**, symbols represent individual subjects. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

Heterozygotes and familial homozygous wild-type controls were also probed with a C-terminus antibody targeted to transactivation domain 1, which was predicted to be missing from RELAp.His487ThrfsTer7. As expected, only a single band (65 kd) was detected in all samples (Figure 2A).

Altered expression and sensitivity to TNF of the NF-κB pathway in RELAp.His487ThrfsTer7 individuals. Compared to wild-type familial PBMC controls, RELAp.His487ThrfsTer7 heterozygotes had higher basal gene expression of RELA (32.6-fold up-regulation; $P = 0.011$), NFKBIA (10.5-fold up-regulation; $P = 0.002$), and TNFAIP3/A20 (24.8-fold up-regulation; $P = 0.011$).

However, RELAp.His487ThrfsTer7 patient PBMCs exhibited reduced TNF-induced induction compared with wild-type PBMCs, with RELA down-regulated 2.8-fold ($P = 0.037$), NFKBIA down-regulated 2.7-fold ($P = 0.012$), and TNFAIP3 down-regulated 2.705-fold ($P = 0.035$) compared to basal (unstimulated) cells (Figure 2B).

Impaired transcriptional activity in RELAp.His487ThrfsTer7. To determine if the transcriptional activity of the 2 RELA isoforms differed in response to TNF, we performed an NF-κB luciferase experiment. Transfection efficiency was comparable between wild-type RELA and RELAp.His487ThrfsTer7 transfections (Supplementary Figure 1, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41531/abstract). $RelA^{-/-}$ MEFs expressing wild-type RELA had increased promoter activity (mean ± SEM 6.78 ± 0.78-fold) compared to those expressing RELAp.His487ThrfsTer7 ($P = 0.003$) (Figure 2C).

Nfkbia gene expression was measured in these cells (Supplementary Figure 2, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41531/abstract). RELAp.His487ThrfsTer7 had reduced expression compared to wild-type RELA basally ($P < 0.001$) and in response to TNF ($P = 0.009$).

RELAp.His487ThrfsTer7 does not impede RELA translocation to the nucleus. To determine if the differential NF-κB–responsive promoter activity was related to translocation to the nucleus of the RELA variants, we used the $RelA^{-/-}$ MEF transfection model as described above. There was no significant difference in translocation (Figure 2D), with both wild-type RELA and RELAp.His487ThrfsTer7 demonstrating nuclear translocation 10 minutes after TNF exposure.

RELAp.His487ThrfsTer7 and TNF-induced cytokine production. Of the 3 symptomatic RELAp.His487ThrfsTer7 individuals, only 1 had detectable basal levels of messenger RNA (mRNA) for IL-6, whereas samples from all 3 wild-type familial controls had detectable IL-6 (Supplementary Figure 3A, *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41531/abstract). Gene expression of the chemokine CCL5 was up-regulated 15.2-fold in RELAp.His487ThrfsTer7 patient PBMCs compared to wild-type familial controls; however, TNF-induced down-regulation of CCL5 was not statistically significant (Supplementary Figure 3B). There was no significant difference in TNF secretion between patient and familial control PBMCs ($P = 0.778$) (Supplementary Figure 3C). In $RelA^{-/-}$ MEFs, we observed no difference in the secretion of IL-6 or CXCL10 between wild-type RELA- and RELAp.His487ThrfsTer7-transfected cells (Supplementary Figures 3D and E).

Increased apoptosis upon transfection with RELAp.His487ThrfsTer7. Exposure to TNF increased expression of the proapoptotic protein Bax under all conditions in HEK 293T cells ($P < 0.001$) (Figure 3A). Compared to those transfected with RELAp.His487ThrfsTer7, HEK 293T cells transfected with wild-type RELA had reduced expression of Bax ($P < 0.001$ for both basal and TNF-induced expression) and increased expression of the antiapoptotic protein Bcl-x (BCL2L1) ($P = 0.011$ for basal expression and $P < 0.001$ for TNF-induced expression). There was no difference in Bcl-2 expression between wild-type RELA– and RELAp.His487ThrfsTer7-transfected cells (Figure 3A).

In $RelA^{-/-}$ MEFs the ratio of cleaved caspase 3 to caspase 3 induced by TNF exposure was 1.98-fold higher in RELAp.His487ThrfsTer7 compared to wild-type RELA ($P = 0.036$) (Figure 3B). In PBMCs, we compared the expression of 2 antiapoptotic markers that had previously been associated with RELA haploinsufficiency: TRAF1 and BCL2A1 (5). TRAF1 gene expression was up-regulated 7.62-fold in symptomatic RELAp.His487ThrfsTer7 heterozygotes compared to wild-type familial controls ($P = 0.005$) (Figure 3C). Exposure to TNF down-regulated TRAF1 (mean ± SD 2.7 ± 0.591-fold) compared to unstimulated controls ($P = 0.045$). BLC2A1 was up-regulated 22.2-fold in RELAp.His487ThrfsTer7 patient cells compared to their wild-type familial controls ($P = 0.049$) (Figure 3D).

DISCUSSION

Loss-of-function variants in 2 NF-κB pathway genes, *TNFAIP3* and *RELA*, have been implicated in monogenic forms of Behçet's-like autoinflammatory syndromes (5,10). Here we describe the first reported case associated with RELA truncation rather than haploinsufficiency. Both protein variants were expressed at approximately equal levels in PBMCs. Mutant proteins have the potential to modulate biologic systems in ways distinct from RELA loss. The mutation causes deficiencies in NF-κB signaling leading to enhanced TNF-induced apoptosis.

Heterozygous individuals had increased levels of RELA and TNFAIP3, perhaps related to disease activity; however, there was loss of TNF-induced up-regulation of these genes. NF-κB modulation of gene expression is determined by a range of factors, including graded dose discrimination and NF-κB oscillations (11). We demonstrated in a fibroblast model that the mutant allele is a much weaker transcriptional activator compared to wild-type RELA, unrelated to the kinetics of translocation of RELA variants to the nucleus. This is likely to be due to loss of DNA binding activities or cofactor binding that are known to reside in this particular

C-terminal region (12). Although the mutation does not directly involve the DNA binding domain of RELA, distal residues that do not directly contact DNA can affect DNA–RELA proximity and electrostatic surface potential, which can alter DNA–RELA recognition and binding affinity (13). Thus, the RELAp.His487ThrfsTer7 mutant appears to behave as a hypomorph, maintaining certain key elements of RELA function, e.g., the ability to translocate to the nucleus in response to TNF, but losing key features associated with its C-terminal transactivation domain. A similar observation was made in a single individual with a de novo missense RELA mutation (c.1534_1535delinsAG, p.Asp512Ser) who had impaired NF-κB responses in lipopolysaccharide-stimulated fibroblasts (14).

Previously, a 2-generation US family with an autosomal-dominant BD-like condition with a reported *RELA* variant in the canonical spice donor site was described (5). In that family, the mutant transcript was not detectable, with consequent RELA haploinsufficiency. The associated illness included mucocutaneous ulceration and early-onset colitis responsive to anti-TNF therapy. The pathogenic mechanism is explained by increased sensitivity of mutant fibroblasts to TNF-induced apoptosis, which we replicated in our study using transfected MEFs. A recently described autosomal-dominant autoinflammatory condition due to non-cleavable RIPK1 is also explained by hypersensitivity to apoptosis and necroptosis in myeloid cells, while fibroblasts of these patients appear to be resistant to cell death (15). The later onset of ulceration and lack of colitis in our RELAp.His487ThrfsTer7 family may be related to a less severe reduction in protein function compared with the 50% reduction in the US family. RELA is a key regulator of homeostasis at the surface of the mucosa, promoting epithelial barrier integrity. Once this barrier is compromised, a genetic impairment in RELA-dependent signaling may lead to abnormal sensitivity to TNF.

It is noteworthy that a family member with the mutation had an autoimmune condition, NMO, without features of the BD-like syndrome. Both BD and NMO are relapsing–remitting inflammatory disorders and may share similar pathophysiology (6). Furthermore, optic neuritis is a recognized, if uncommon, clinical feature of BD. The individual with NMO had no clinical or imaging evidence of other nervous system involvement such as transverse myelitis or cerebral lesions. The co-occurrence of autoimmune and autoinflammatory conditions has not been widely reported; however, a review of TNFAIP3-related autosomal-dominant BD found a high prevalence of autoantibodies associated with common autoimmune conditions, including systemic lupus erythematosus, type 1 diabetes mellitus, and Hashimoto thyroiditis. Notably, common variants in *TNFAIP3* have been associated with the risk for most of these conditions (16).

Autosomal-dominant forms of BD-like autoinflammation related to *TNFAIP3* loss-of-function mutations are increasingly recognized. The pathogenic mechanism is believed to involve increased inflammasome activation and production of proinflammatory cytokines by leukocytes. Our data demonstrate a mechanism of increased sensitivity to TNF-induced apoptosis caused by loss-of-function mutations in RELA. The emerging mechanistic and clinical studies suggest identification of the underlying genetic causes of Mendelian forms of BD could inform a personalized medicine approach using either IL-1 or TNF inhibitors.

ACKNOWLEDGMENT

We thank Stephanie Skeffington for her insight and advice.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Dorris had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Adeeb, Dorris, Morgan, Lawless, Maqsood, Ng, Killeen, Cummins, Taylor, Savic, Wilson, Fraser.

Acquisition of data. Adeeb, Dorris, Morgan, Lawless, Maqsood, Ng, Killeen, Cummins, Taylor, Savic, Wilson, Fraser.

Analysis and interpretation of data. Adeeb, Dorris, Morgan, Lawless, Maqsood, Ng, Killeen, Cummins, Taylor, Savic, Wilson, Fraser.

REFERENCES

1. Takeuchi M, Mizuki N, Meguro A, Ombrello MJ, Kirino Y, Satorius C, et al. Dense genotyping of immune-related loci implicates host responses to microbial exposure in Behcet's disease susceptibility. Nat Genet 2017;49:438–43.
2. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation [review]. Signal Transduct Target Ther 2017;2:17023.
3. Zhou Q, Wang H, Schwartz DM, Stoffels M, Park YH, Zhang Y, et al. Loss-of-function mutations in TNFAIP3 leading to A20 haploinsufficiency cause an early-onset autoinflammatory disease. Nat Genet 2016;48:67–73.
4. Aeschlimann FA, Batu ED, Canna SW, Go E, Gul A, Hoffmann P, et al. A20 haploinsufficiency (HA20): clinical phenotypes and disease course of patients with a newly recognised NF-κB-mediated autoinflammatory disease. Ann Rheum Dis 2018;77:728–35.
5. Badran YR, Dedeoglu F, Castillo JM, Bainter W, Ohsumi TK, Bousvaros A, et al. Human RELA haploinsufficiency results in autosomal-dominant chronic mucocutaneous ulceration. J Exp Med 2017;214:1937–47.
6. Walker-Caulfield ME, Guo Y, Johnson RK, McCarthy CB, Fitz-Gibbon PD, Lucchinetti CF, et al. NFκB signaling drives pro-granulocytic astroglial responses to neuromyelitis optica patient IgG. J Neuroinflammation 2015;12:185.
7. Ichikawa HT, Conley T, Muchamuel T, Jiang J, Lee S, Owen T, et al. Beneficial effect of novel proteasome inhibitors in murine lupus via dual inhibition of type I interferon and autoantibody-secreting cells. Arthritis Rheum 2012;64:493–503.
8. International Study Group for Behçet's Disease. Evaluation of diagnostic ('classification') criteria in Behçet's disease—towards internationally agreed criteria. Br J Rheumatol 1992;31:299–308.
9. International Team for the Revision of the International Criteria for Behçet's Disease, Davatchi F, Assaad-Khalil S, Calamia KT, Crook JE, Sadeghi-Abdollahi B, et al. The International Criteria for Behçet's Disease (ICBD): a collaborative study of 27 countries on the sensitivity and specificity of the new criteria. J Eur Acad Dermatol Venereol 2014;28:338–47.

10. Berteau F, Rouviere B, Delluc A, Nau A, le Berre R, Sarrabay G, et al. Autosomic dominant familial Behcet disease and haploinsufficiency A20: a review of the literature. Autoimmun Rev 2018;17:809–15.
11. Tian B, Nowak DE, Brasier AR. A TNF-induced gene expression program under oscillatory NF-κB control. BMC Genomics 2005;6:137.
12. Feldman I, Feldman GM, Mobarak C, Dunkelberg JC, Leslie KK. Identification of proteins within the nuclear factor-κB transcriptional complex including estrogen receptor-α. Am J Obstet Gynecol 2007;196:394.
13. Mulero MC, Wang VY, Huxford T, Ghosh G. Genome reading by the NF-κB transcription factors. Nucleic Acids Res 2019;47:9967–89.
14. Frederiksen AL, Larsen MJ, Brusgaard K, Novack DV, Knudsen PJ, Schrøder HD, et al. Neonatal high bone mass with first mutation of the NF-κB complex: heterozygous de novo missense (p.Asp512Ser) RELA (Rela/p65). J Bone Miner Res 2016;31:163–72.
15. Tao P, Sun J, Wu Z, Wang S, Wang J, Li W, et al. A dominant autoinflammatory disease caused by non-cleavable variants of RIPK1. Nature 2020;577:109–14.
16. Eyre S, Hinks A, Bowes J, Flynn E, Martin P, Wilson AG, et al. Overlapping genetic susceptibility variants between three autoimmune disorders: rheumatoid arthritis, type 1 diabetes and coeliac disease. Arthritis Res Ther 2010;12:R175.