Damaging heterozygous mutations in *NFKB1* lead to diverse immunologic phenotypes

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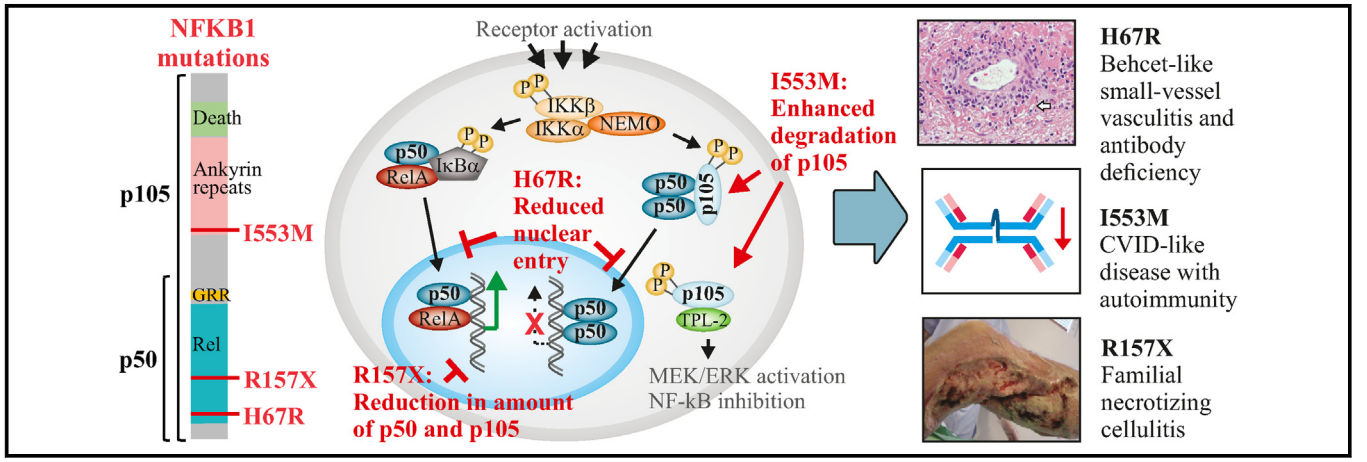
GRAPHICAL ABSTRACT



Background: The nuclear factor κ light-chain enhancer of activated B cells (NF-κB) signaling pathway is a key regulator of immune responses. Accordingly, mutations in several NF-κB pathway genes cause immunodeficiency.

Objective: We sought to identify the cause of disease in 3 unrelated Finnish kindreds with variable symptoms of immunodeficiency and autoinflammation.

Methods: We applied genetic linkage analysis and next-generation sequencing and functional analyses of *NFKB1* and its mutated alleles.

Results: In all affected subjects we detected novel heterozygous variants in *NFKB1*, encoding for p50/p105. Symptoms in variant carriers differed depending on the mutation. Patients harboring a p.I553M variant presented with antibody deficiency, infection susceptibility, and multiorgan autoimmunity. Patients with a p.H67R substitution had antibody deficiency and experienced autoinflammatory episodes, including aphthae, gastrointestinal disease, febrile attacks, and small-vessel vasculitis characteristic of Behçet disease. Patients with a p.R157X stop-gain experienced hyperinflammatory responses to surgery and showed enhanced inflammasome activation. In functional analyses the p.R157X variant caused proteasome-dependent degradation of both the truncated and wild-type proteins, leading to a dramatic loss of p50/p105. The p.H67R variant reduced nuclear entry of p50 and showed decreased transcriptional activity in luciferase reporter assays. The p.I553M mutation in turn showed no change in p50 function but exhibited reduced p105 phosphorylation and stability. Affinity purification mass spectrometry also demonstrated that both missense variants led to altered protein-protein interactions.

Conclusion: Our findings broaden the scope of phenotypes caused by mutations in *NFKB1* and suggest that a subset of

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autoinflammatory diseases, such as Behçet disease, can be caused by rare monogenic variants in genes of the NF-κB pathway. (J Allergy Clin Immunol 2017;140:782-96.)

Key words: *Nuclear factor κ light-chain enhancer of activated B cells, hypogammaglobulinemia, autoinflammation, Behçet disease,* NFKB1*, p50, p105, B cell*

The nuclear factor κ light-chain enhancer of activated B cells (NF-κB) pathway regulates many cellular processes, such as proliferation, apoptosis, stress responses, inflammation, ectodermal development, and immune responses.[1,2] As such, NF-κB signaling plays a key role in inflammatory diseases, and mutations in several NF-κB components cause primary immunodeficiency or ectodermal dysplasia.[3-11]

The NF-κB transcription factor family consists of 5 Rel proteins, p50/p105, p52/p100, RelA, RelB, and c-Rel, which dimerize with each other and drive or inhibit gene expression in the nucleus.[12] The canonical NF-κB pathway is triggered by microbial products or the cytokines IL-1β and TNF and progresses through phosphorylation-dependent degradation of NF-κB inhibitor α (IκBα). IκBα phosphorylation is mediated by the inhibitor of κB kinase (IKK) complex, which includes IKKα and IKKβ and the regulatory protein NF-κB essential modulator (NEMO). This releases RelA- and c-Rel–containing dimers to enter the nucleus and drive transcription of proinflammatory genes.[13]

NFKB1 encodes for p105, which is processed by the proteasome to generate the p50 transcription factor. p50 can heterodimerize with RelA or c-Rel and activate canonical NF-κB signaling or form homodimers that function as repressors of proinflammatory gene expression.[14,15] The full-length p105 inhibits NF-κB signaling by binding to and inhibiting nuclear entry of RelA, c-Rel, and p50 through ankyrin repeats in the C-terminal half of the protein.[16,17]

Recently, haploinsufficiency of p50 was shown to cause antibody deficiency.[3] Common variants in *NFKB1* also associate with inflammatory bowel disease and Behçet disease, a vasculitis of largely unknown cause characterized by recurrent oral and genital aphthous ulcers, uveitis, and skin lesions.[18-22] Here we describe heterozygous *NFKB1* mutations (H67R, p.R157X, and I553M) in 3 Finnish kindreds that variably display dominantly segregating antibody deficiency, recurrent infections, and autoinflammatory features, including Behçet-like disease and hyperinflammatory reactions. Our results show that disease can ensue from dysregulation of NF-κB signaling caused by mutations affecting either p50 or p105.

Abbreviations used
IκBα: NF-κB inhibitor α
IKK: Inhibitor of κB kinase
NEMO: NF-κB essential modulator
NF-κB: Nuclear factor κ light-chain enhancer of activated B cells
NLRP3: NLR family pyrin domain containing 3
WB: Western blotting
WT: Wild-type

METHODS

Study participants

The study was conducted in accordance with the principles of the Helsinki Declaration and was approved by the Helsinki University Central Hospital Ethics Committee. Written informed consent was obtained from patients and healthy control subjects.

DNA extraction and genetic analysis

Genomic DNA was extracted from EDTA blood samples by using the Qiagen FlexiGene DNA kit (Qiagen, Hilden, Germany). HLA-B*51 typing was performed in an accredited (European Federation for Immunogenetics) histocompatibility testing laboratory, the Finnish Red Cross Blood Service.

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