Manuscript title:

TET2-driver and *NLRC4*-passenger Variants in Adult-Onset Autoinflammation

Authors (printed version):

Ellen De Langhe[§], M.D., Ph.D., Eric Legius, M.D., Ph.D., Rik Schrijvers, M.D., Ph.D.

Affiliation (printed version):

KU Leuven, Leuven, Belgium

[§] Corresponding author: Ellen De Langhe, Division of Rheumatology, University Hospitals Leuven, Herestraat 49, 3000 Leuven, Belgium. Email: ellen.delanghe@uzleuven.be. Telephone: 003216342541. Fax: 003216342543.

Authors (online version):

Ellen De Langhe[§], M.D., Ph.D., Sien Van Loo*, M.Sc., Bert Malengier-Devlies*, Ph.D., Mieke Metzemaekers*, Ph.D., Frederik Staels*, M.D., Jessica Vandenhaute*, Ph.D., Nathalie Berghen, M.D., Ph.D., Raf Sciot, M.D., Ph.D., Anniek Corveleyn, Ph.D., Olga Tšuiko, Ph.D., Mieke Gouwy, Ph.D., Jan Leo Lenaerts, M.D., Patrick Verschueren, M.D., Ph.D., Carine Helena Wouters, M.D., Ph.D., Paul Proost, Ph.D., Patrick Matthys, Ph.D., Eric Legius, M.D., Ph.D., Rik Schrijvers, M.D., Ph.D.

* These authors contributed equally.

Author affiliations (online):

Ellen De Langhe, M.D., Ph.D. Division of Rheumatology, University Hospitals Leuven, Leuven, Belgium. Laboratory of Tissue Homeostasis and Disease, Department of Development and Regeneration, KU Leuven, Leuven, Belgium

Sien Van Loo, MSc, Clinical Department for Human Genetics, University Hospitals Leuven, Leuven, Belgium

Bert Malengier-Devlies, Ph.D. KU Leuven Department of Microbiology Immunology and Transplantation, Laboratory of Immunobiology, Rega Institute for Medical Research, KU Leuven, Leuven, Belgium

Mieke Metzemaekers, Ph.D. KU Leuven Department of Microbiology, Immunology and Transplantation, Laboratory of Molecular Immunology, Rega Institute for Medical Research, KU Leuven, Belgium

Frederik Staels, M.D. KU Leuven Department of Microbiology Immunology and Transplantation, Allergy and Clinical Immunology research group, KU Leuven, Leuven, Belgium

Jessica Vandenhaute, Ph.D. KU Leuven Department of Microbiology Immunology and Transplantation, Laboratory of Immunobiology, Rega Institute for Medical Research, KU Leuven, Leuven, Belgium

Nathalie Berghen, M.D., Ph.D. Division of Rheumatology, University Hospitals Leuven, Leuven, Belgium, Division of Rheumatology, AZ Klina, Brasschaat, Belgium

Raf Sciot, M.D., Ph.D. Department of Pathology, KU Leuven and University Hospitals Leuven, Belgium.

Anniek Corveleyn, PhD, Center for Human Genetics, University Hospitals Leuven, Leuven, Belgium

Olga Tšuiko, PhD, Center for Human Genetics, University Hospitals Leuven, Leuven, Belgium

Mieke Gouwy, Ph.D. KU Leuven Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, Laboratory of Molecular Immunology.

Jan Leo Lenaerts, M.D. Division of Rheumatology, University Hospitals Leuven, Leuven, Belgium.

Patrick Verschueren, M.D. Ph.D. Division of Rheumatology, University Hospitals Leuven, Leuven, Belgium. Skeletal Biology Engineering and Research Center, Department of Development and Regeneration, KU Leuven, Leuven, Belgium.Carine Helena Wouters, M.D. Ph.D. KU Leuven Department of Microbiology Immunology and Transplantation, Laboratory of Immunobiology, Rega Institute for Medical Research, KU Leuven, Leuven, Belgium. Division of Pediatric Rheumatology, Leuven University Hospitals, Leuven, Belgium.

Paul Proost, Ph.D. KU Leuven Department of Microbiology, Immunology and Transplantation, Laboratory of Molecular Immunology Rega Institute for Medical Research, KU Leuven, Leuven, Belgium.

Patrick Matthys, PhD. KU Leuven Department of Microbiology Immunology and Transplantation, Laboratory of Immunobiology, Rega Institute for Medical Research, KU Leuven, Leuven, Belgium.

Eric Legius, MD, PhD, Department of Human Genetics, KU Leuven and Clinical Department for Human Genetics, University Hospitals Leuven, Leuven, Belgium.

Rik Schrijvers, M.D. Ph.D. KU Leuven Department of Microbiology Immunology and Transplantation, Allergy and Clinical Immunology research group, KU Leuven, Leuven, Belgium.

Ellen De Langhe, Carine Helena Wouters, Rik Schrijvers are members of the European Reference Network for Rare Immunodeficiency, Autoinflammatory and Autoimmune Diseases. Ellen De Langhe is member of the European Reference Network on Rare and Complex Connective Tissue and Musculoskeletal Diseases.

Key words: autoinflammatory disease, periodic fever syndrome, NLRC4, inflammasome, IL-1, IL-18, somatic mosaicism, canakinumab, clonal hematopoiesis, TET2, driver variant, adult-onset

Conflict of interest: EDL, SVL, BMD, MM, FS, JV, NB, RS, AC, OT, MG, JLL, PV, CHW, PP, PM, EL, RS have no conflict of interest related to this work.

Abbreviations: Interleukin (IL), Interleukin-1 receptor antagonist (IL-1RA), NOD-like receptor family CARD containing protein 4 (NLRC4), Tet methylcytosine dioxygenase 2 (TET2), White blood cell (WBC)

To the Editor:

Somatic mosaicism causing autoinflammatory diseases is increasingly recognized[1], but the onset and mechanisms of clonal expansion remain enigmatic. We describe a patient who underwent surgical resection and chemotherapy for Ewing sarcoma at 15 years of age. Five years later, after a primo Epstein-Barr virus infection, persistent mildly elevated C-reactive protein levels without symptoms were noted. Three years later, she developed chronic headache, myalgia, diarrhea, low grade-to-spiking fevers and episodes of urticaria, arthritis, or pleuropericarditis, occasionally requiring hospitalization. Symptoms and inflammatory markers consistently subsided spontaneously after 2 to 7 days, and no infectious triggers were identified except for one *Campylobacter jejuni* episode. Treatment with colchicine, glucocorticoids, or azathioprine improved symptomatology but failed to control biochemical inflammation. To guide treatment choices, a serum cytokine panel was performed, showing strikingly elevated IL-18 levels. Exome-sequencing subsequently confirmed an autoinflammatory disease due to somatic mosaicism in *NLRC4* (p.Val341Leu).[2,3] IL-1 blocking agents (canakinumab) were initiated with rapid resolution of inflammation and symptoms (Figure 1A-C).

Interestingly, the pathogenic *NLRC4* variant was restricted to the hematopoietic, predominantly myeloid, lineage but devoid in pre-chemotherapy bone marrow (see Online Appendix). Therefore, we performed a time-course analysis by droplet digital PCR (ddPCR) and observed a gradual increasing *NLRC4* variant allele fraction (VAF) in white blood cells (WBC), paralleling clinical observations (Figure 1D). However, a clear rationale for the gradual *NLRC4* VAF increase was lacking, as clonal expansion is not associated with activated NLRC4. Exome sequencing of DNA isolated from WBCs obtained pre-chemotherapy and at the age of 29 revealed an additional acquired myeloid driver variant in *TET2* (p.Ile1226Metfs*2)[4] with a similar VAF evolution as the *NLRC4* variant (as assayed by

ddPCR). *TET2* loss-of-function mutations are observed in clonal hematopoiesis of indeterminate potential and hematological malignancies. We found no other relevant acquired variants (see Online Appendix). By genotyping individual WBCs, we confirmed that both pathogenic variants occurred in the same cell (Figure 1E), consistent with their parallel VAFs over time (Figure 1D).

We conclude that a hematopoietic stem and progenitor cell acquired both the passenger *NLRC4* and driver *TET2* pathogenic variant, resulting in clonal hematopoiesis, skewed myelomonocytic differentiation, and progressive NLRC4-inflammasome-driven autoinflammation. Pathogenic *TET2* loss-of-function variants not only drive clonal hematopoiesis but can also lead to elevated proinflammatory cytokine levels and are linked with other inflammatory conditions such as atherosclerosis, chronic obstructive pulmonary disease, or gout in humans and mouse models.[4,5] Our data support a type of driver–passenger model, in which variant *TET2* drives expansion and variant *NLRC4* is a pathogenic "passenger" in the expanding clone that explains this late-onset autoinflammatory disease. These data are also consistent with a synergy between inflammation and myeloid-predominant clonal expansion and observations in Tet2-deficient mouse models and humans with hematoinflammatory disorders.[1]

Ellen De Langhe, M.D., Ph.D.
Eric Legius, M.D., Ph.D.
University Hospitals Leuven Leuven, Belgium
Rik Schrijvers, M.D., Ph.D
KU Leuven Leuven, Belgium

A complete list of authors is available with the full text of this letter at NEJM.org.

Research of CHW, PP and PM is supported by grants from the FWO (Fonds Wetenschappelijk Onderzoek – Vlaanderen) (G0A3218N and G080818N) and by C1 grant (C16/17/010) of KU Leuven. JV received a PhD-SB-fellowship from FWO (1S10618N). MM was supported by a 'For Women in Science' PhD fellowship from L'Oréal, UNESCO and FWO, FS (11B5520N) is a fellow of the Research Foundation-Flanders (FWO). RS is funded by an FWO senior clinical investigator fellowship (1805518N). EDL, PV, CHW, RS are members of the European Reference Network for Rare Immunodeficiency, Autoinflammatory and Autoimmune Disorders (Project ID No 739543). Disclosure forms provided by the authors are available with the full text of this letter at NEJM.org.

REFERENCES

1. Beck DB, Ferrada MA, Sikora KA, et al. Somatic Mutations in UBA1 and Severe Adult-Onset Autoinflammatory Disease. N Engl J Med 2020;383(27):2628–38.

2. Canna SW, de Jesus AA, Gouni S, et al. An activating NLRC4 inflammasome mutation causes autoinflammation with recurrent macrophage activation syndrome. Nat Genet 2014;46(10):1140–6.

3. Romberg N, Al Moussawi K, Nelson-Williams C, et al. Mutation of NLRC4 causes a syndrome of enterocolitis and autoinflammation. Nat Genet 2014;46(10):1135–9.

4. Bick AG, Weinstock JS, Nandakumar SK, et al. Inherited causes of clonal haematopoiesis in 97,691 whole genomes. Nature 2020;586(7831):763–8.

5. Agrawal M, Niroula A, Cunin P, et al. TET2-mutant clonal hematopoiesis and risk of gout. Blood 2022;140(10):1094–103.

Figure 1.

(A) Selected clinical (urticaria and pleuropericarditis on a combined fluorodeoxyglucose positron emission and computer tomography image at timepoint *e*) and histological findings (*left*, skin biopsy with prominent neutrophils in the dermis (arrows) and *right*, a bone marrow biopsy with a hypercellular myeloid compartment, immature mononuclear cells and mature polynuclear cells (arrow indicates mitosis), both at timepoint *d*). **(B)** C-reactive protein, total white blood cell (WBC), neutrophil, interleukin (IL)-18, and IL-1RA evolution over time with indicated time points (*a-f*). Dotted lines represent cut-off values, asterix represents the mean of healthy controls. **(C)** Pedigree and Sanger sequencing results. **(D)** Variant allele fraction over time in different cell types, expressed in years of age for *NLRC4* (upper panel) and *TET2* (lower panel) (ClinVar accession numbers SCV003803079, SCV003803080). **(E)** Results of genotyping of 19 out of 20 selected single T-cells obtained at age 30.6 years.