Lack of Association between the TNFAIP3 Rs2230926 Variant and Rheumatoid Arthritis-Associated Lymphoma.

Joanna Kedra, Raphaele Seror, Philippe Dieudé, Arnaud Constantin, Eric Toussirot, Elias Kfoury, Charles Masson, Divi Cornec, Jean-Jacques Dubost, Laurent Marguerie, et al.

Lack of association between the *TNFAIP3* rs2230926 variant and rheumatoid arthritis-associated lymphoma

Authors: Joanna Kedra, Raphaele Seror, Philippe Dieudé, Arnaud Constantin, Eric Toussirot, Elias Kfoury, Charles Masson, Divi Cornec, Jean-Jacques Dubost, Laurent Marguerie, Sebastien Ottaviani, Franck Grados, Rakiba Belkhir, Olivier Fain, Bruno Fautrel, Peggy Philippe, Muriel Piperno, Bernard Combe, Olivier Lambotte, Christophe Richez, Jérémie Sellam, Thomas Sené, Guillaume Denis, Thierry Lequerre, Thierry Lazure, Xavier Mariette, Gaetane Nocturne

Name	**Highest degree**	**email**	**ORCID**	**Affiliation**
Joanna KEDRA	MD, MSc	jkedra.pro@gmail.com	0000-0003-3535-3183	1. Department of Rheumatology, FHU CARE, AP-HP, Hôpital Bicêtre, Le Kremlin-Bicêtre, France 2. Université Paris-Saclay, INSERM UMR1184, Center for immunology of viral infections and autoimmune diseases, Le Kremlin-Bicêtre France
Raphaele SEROR	MD, PhD	raphaele.seror@aphp.fr	0000-0002-5523-1856	1. Department of Rheumatology, FHU CARE, AP-HP, Hôpital Bicêtre, Le Kremlin-Bicêtre, France 2. Université Paris-Saclay, INSERM UMR1184, Center for immunology of viral infections and autoimmune diseases, Le Kremlin-Bicêtre France
Philippe DIEUDE	MD, PhD	philippe.dieude@aphp.fr	0000-0002-4814-0307	Rheumatology department, Bichat hospital, APHP, Paris, France
Arnaud	MD, PhD	arnaud.constantin@univ-tlse3.fr	0000-0003-	Rheumatology Department, Purpan

CONSTANTIN			2618-2888	Hospital, Toulouse, France & Université Toulouse III – Paul Sabatier, Toulouse, France
Eric TOUSSIROT	MD, PhD	etoussirot@chu-besancon.fr	0000-0002-6228-1464	INSERM CIC-1431 Clinical Investigation Center Biotherapy and Rheumatology, University hospital of Besançon 25000 Besançon France
Elias KFOURY	MD, PhD	elias.kfoury@ch-brive.fr		Hematology department, Dubois hospital, Brive-la-Gaillarde, France
Charles MASSON	MD	chmasson@chu-angers.fr	0000-0003-4928-3788	Department of Rheumatology 4 rue Larrey CHU Angers 49933 Angers cedex 9 France
Divi CORNEC	MD, PhD	divi.cornec@chu-brest.fr	0000-0002-9159-702X	Rheumatology department, Brest university hospital, Brest, France
Jean-Jacques DUBOST	MD	jjdubost@chu-clermontferrand.fr	0000-0001-7415-6962	Department of rheumatology, University Hospital Gabriel Montpied, Clermont-ferrand, France
Laurent MARGUERIE	MD	lmarguerie@hopale.com		Rheumatology department, Fondation Hopale, Berck-sur-Mer, France
Sébastien OTTAVIANI	MD	sebastien.ottaviani@aphp.fr	0000-0003-2250-1503	Department of Rheumatology, Hôpital Bichat- Claude Bernard, AP-HP, 46 rue Henri Huchard, 75018 Paris, France
Franck GRADOS	MD, PhD	Grados.Franck@chu-amiens.fr		Rheumatology department, Amiens University hospital, Amiens, France
Rakiba BELKHIR	MD	rakiba.belkhir@aphp.fr		Department of Rheumatology, FHU CARE, AP-HP, Hôpital

				Bicêtre, Le Kremlin-Bicêtre, France
Olivier FAIN	MD, PhD	olivier.fain@aphp.fr		Internal Medicine department, Saint-Antoine hospital, APHP, Paris, France
Bruno FAUTREL	MD, PhD	bruno.fautrel@aphp.fr	0000-0001-8845-4274	Sorbonne Université, Institut Pierre Louis d'Epidémiologie et de Santé Publique (iPLESP), UMR S1136, Paris, France and AP-HP, Pitié Salpêtrière hospital, Rheumatology department, Paris, France
Peggy PHILIPPE	MD	peggy.philippe@chru-lille.fr		Rheumatology department, Salengro hospital, Lille, France
Muriel PIPERNO	MD	muriel.piperno@chu-lyon.fr		Rheumatology department, Lyon Sud University hospital, Lyon, France
Bernard COMBE	MD,PhD	Bernard.combe@umontpellier.fr	0000-0003-4002-1861	Montpellier University, Montpellier, France
Olivier LAMBOTTE	MD, PhD	olivier.lambotte@aphp.fr	0000-0003-4425-8516	Internal Medicine department, Bicetre hospital, AP-HP, and Université Paris-Saclay, INSERM UMR1184 AP-HP, 78 rue du Général Leclerc, 94275 Le Kremlin
Christophe RICHEZ	MD, PhD	christophe.richez@chu-bordeaux.fr	0000-0002-3029-8739	Rheumatology department, Bordeaux-Pellegrin University hospital, Bordeaux, France
Jérémie SELLAM	MD, PhD	jeremie.sellam@aphp.fr	0000-0002-	Rheumatology department, Saint-

			1814-6003	Antoine hospital, APHP, Paris, France
Thomas SENE	MD	tsene@for.paris	0000-0002-3162-2537	Department of Internal Medicine, Rothschild Hospital Foundation, 29 Rue Manin 75019 PARIS
Guillaume DENIS	MD	guillaume.denis@ch-rochefort.fr	0000-0003-0434-265X	Hematology department, Rochefort Hospital, Groupe Hospitalier Littoral Atlantique, 1 avenue de Béligon, 17300 Rochefort
Thierry LEQUERRE	MD, PhD	thierry.lequerre@chu-rouen.fr	0000-0001-8126-6596	Rheumatology department, Rouen University hospital, Rouen, France
Thierry LAZURE	MD	thierry.lazure@aphp.fr		Anatomical pathology department, Bicêtre hospital, APHP, Le Kremlin-Bicêtre, France
Xavier MARIETTE	MD, PhD	xavier.mariette@aphp.fr	0000-0002-4244-5417	1. Department of Rheumatology, FHU CARE, AP-HP, Hôpital Bicêtre, Le Kremlin-Bicêtre, France 2. Université Paris-Saclay, INSERM UMR1184, Center for immunology of viral infections and autoimmune diseases, Le Kremlin-Bicêtre France
Gaëtane NOCTURNE	MD, PhD	Gaetane.nocturne@aphp.fr	0000-0001-6809-0733	1. Department of Rheumatology, FHU CARE, AP-HP, Hôpital Bicêtre, Le Kremlin-Bicêtre, France 2. Université Paris-Saclay, INSERM UMR1184, Center for immunology of viral infections and autoimmune diseases, Le Kremlin-Bicêtre

				France

Corresponding author:

Dr Joanna KEDRA, Department of Rheumatology, Hôpital Bicêtre, 78 rue du Général Leclerc, 94275 Le Kremlin Bicêtre, France

Email: jkedra.pro@gmail.com Tel: +33 6 79 75 87 70 Fax: +33145213757

Grants: supported by the French Society of Rheumatology (Société Française de Rhumatologie, SFR).

Word Count: 499/500 words, tables 2/2, references 10/10

Contributorship statement All authors have contributed to this work and have approved the final version of the manuscript.

Abstract (215/250 words)

Objective: Given the association of *TNFAIP3* rs2230926 variant with rheumatoid arthritis (RA) on the one hand, and Sjögren syndrome-related lymphoma on the other hand, the aim of this study was to investigate its contribution to RA-related lymphoma susceptibility.

Methods: In this multicentre case-control study, cases were patients with RA fulfilling ACR-EULAR 2010 criteria, who developed a B-cell non-Hodgkin lymphoma (NHL) or Hodgkin's lymphoma after the diagnosis of RA. Controls were patients with RA and without lymphoma matched on age. Blood or saliva samples were collected for genotyping.

Results: Among the 162 patients included in this study, DNA was available for 145 (89.5%) patients (38 cases and 107 controls), having no difference in demographic and disease characteristics compared to the whole study population. Overall, 100 patients (69.0%) were female, with a mean age of 51.9 years (SD=10.9) at RA diagnosis and a mean disease duration of 10.6 years (SD=5.0). Lymphomas were mostly diffuse large B-cell lymphoma (N=19, 50.0%), follicular lymphoma (N=6, 15.8%) and marginal zone lymphoma (N=4, 10.5%). The genotyping study revealed no significant association between the TNFAIP3 rs2230926 variant and the occurrence of lymphoma (p=0.30)

Conclusion: We did not detect any association between the *TNFAIP3* rs2230926 variant and RA-related lymphoma. This highlights the need for further studies to determine the specific mechanisms driving RA lymphomagenesis.

Key words: rheumatoid arthritis, lymphoma, TNFAIP3, A20, single nucleotide polymorphism

There is an increased risk of lymphoma in patients with auto-immune diseases (AID) especially rheumatoid arthritis (RA) and primary Sjögren Syndrome (pSS) with a respective relative risk of 2 and 15 [1]. The mechanisms promoting lymphomagenesis in AID might be diverse [2]. Disease activity and chronic antigenic stimulation of autoimmune B cells play a key role in lymphomagenesis associated with pSS [3]. In this context, a complete control of the NF-kB pathway activation is required to avoid B-cell clonal proliferation. *TNFAIP3* encodes for the protein A20, a key gatekeeper of NF-kB activation and the *TNFAIP3* rs2230926 variant was previously found to be associated with lymphoma in pSS [4,5]. This variant is responsible for a slight decreased control of NF-kB activation. In patients with RA, mechanisms leading to lymphoma occurrence, mainly diffuse large B-cell lymphoma (DLBCL), is less understood. From an epidemiologic point of view, it has been demonstrated that high cumulative RA disease activity increase odds for lymphoma [6]. Given the association of *TNFAIP3* rs2230926 with the overall RA [7], and pSS-related lymphoma [4,5], we decided to investigate its contribution to RA-related lymphoma susceptibility.

To this end, a multicentre case-control study was performed. Cases were patients with RA fulfilling ACR-EULAR 2010 criteria, who developed a B-cell NHL or Hodgkin's lymphoma after the diagnosis of RA. Controls were patients with RA and without lymphoma matched on age. Fifty-four cases of RA-related lymphoma were included and matched to 108 controls (1:2) [8]. DNA was available for 145/162 (89.5%) patients (38 cases and 107 controls), having no difference in demographic and disease characteristics compared to the whole study population (table 1). Overall, 101 patients (69.2%) were female, with a mean age of 51.9 years (SD=10.9) at RA diagnosis and a mean disease duration of 10.5 years (SD=4.9). Lymphomas were mostly DLBCL (N=19, 50.0%), follicular lymphoma (N=6, 15.8%) and marginal zone lymphoma (N=4, 10.5%). The genotyping study revealed no significant association between the *TNFAIP3* rs2230926 variant and the occurrence of lymphoma (p=0.30) (table 2).

Even if this study could suffer from a relatively low power of detection (estimated power of 80%), this lack of association suggests distinct mechanisms of lymphomagenesis in RA and pSS. Lymphoma histology and localization differ in pSS and RA associated lymphoma: MALT lymphoma of salivary glands in pSS versus nodal DLBCL in RA. RF positivity and/or cryoglobulinemia are associated with lymphoma occurrence in pSS but not in RA [3]. In RA, instead of a direct transformation of autoimmune B cells, lymphoma might derive from associated subsets of B cells such as memory B cells. Interestingly, memory B cells involved in RA are increased in longstanding disease [9]. Recently, *TBL1XR1* rare variants were found to induce ABC DLBCL by promoting aberrant memory B cells, which are prone to avoid plasmocytic differentiation on recall and to undergo systemic dissemination [10].

In aggregates, we did not detect any association between the *TNFAIP3* rs2230926 variant and RA-related lymphoma. This highlights the need for further studies to determine the specific mechanisms driving RA lymphomagenesis.

Acknowledgements:

The authors wish to acknowledge the French Society of Rheumatology (Société Française de Rhumatologie – SFR) for the grant provided to support the genetic analysis.

Statements and declarations:

Competing interests:

The authors have no disclosure to declare in relationship to the present study.

Funding details:

The French Society of Rheumatology (Société Française de Rhumatologie – SFR) provided a grant to support the genetic analysis.

ESPOIR cohort: An unrestricted grant from Merck Sharp and Dohme (MSD) was allocated for the first 5 years. Two additional grants from INSERM were obtained to support part of the biological database. The French Society of Rheumatology, Abbvie, Pfizer, Lilly, and more recently Fresenius and Galapagos also supported the ESPOIR cohort study. We also thank Nathalie Rincheval for expert monitoring and data management and all the investigators who recruited and followed the patients (F. BERENBAUM, Paris- Saint Antoine, MC. BOISSIER, Paris-Bobigny, A .CANTAGREL, A CONSTANTIN, Toulouse, B. COMBE , Montpellier, M.DOUGADOS, Paris-Cochin, P BOUMIER, Amiens, B. FAUTREL, Paris-La Pitié, RM. FLIPO, Lille , Ph. GOUPILLE, Tours, F. LIOTE, Paris- Lariboisière, O VITTECOQ, Rouen, X MARIETTE, Paris Bicetre, Ph DIEUDE, Paris Bichat, A.SARAUX, Brest, Th SCHAEVERBEKE, Bordeaux, J. SIBILIA, Strasbourg). One biological resources centre (Paris-Bichat, S Tubiana) was in charge of centralising and managing biological data collection.

References

1 Zintzaras E, Voulgarelis M, Moutsopoulos HM. The risk of lymphoma development in autoimmune diseases: a meta-analysis. *Arch Intern Med* 2005;**165**:2337–44.

2 Kedra J, Nocturne G, Mariette X, Seror R. Inflammation-targeted therapies and cancer. Joint Bone Spine. 2021;88(4):105176.

3 Nocturne G, Pontarini E, Bombardieri M, *et al.* Lymphomas complicating primary Sjögren's syndrome: from autoimmunity to lymphoma. *Rheumatology (Oxford)* 2019;60:3513-3521.

4 Nocturne G, Boudaoud S, Miceli-Richard C, *et al.* Germline and somatic genetic variations of TNFAIP3 in lymphoma complicating primary Sjogren's syndrome. *Blood* 2013;**122**:4068–76.

5 Nocturne G, Tarn J, Boudaoud S, *et al.* Germline variation of TNFAIP3 in primary Sjögren's syndrome-associated lymphoma. *Ann Rheum Dis* 2016;**75**:780–3.

6 Baecklund E, Iliadou A, Askling J, *et al.* Association of chronic inflammation, not its treatment, with increased lymphoma risk in rheumatoid arthritis. *Arthritis Rheum* 2006;**54**:692–701.

7 Musone SL, Taylor KE, Nititham J, *et al.* Sequencing of TNFAIP3 and association of variants with multiple autoimmune diseases. *Genes Immun* 2011;**12**:176–82.

8 Kedra J, Seror R, Dieudé P et al. Lymphoma complicating rheumatoid arthritis: results from a French case–control study. RMD Open 2021;7:e001698.

9 Fedele AL, Tolusso B, Gremese E, *et al.* Memory B cell subsets and plasmablasts are lower in early than in long-standing rheumatoid arthritis. *BMC Immunol* 2014;**15**:28.

10 Venturutti L, Teater M, Zhai A, *et al.* TBL1XR1 Mutations Drive Extranodal Lymphoma by Inducing a Pro-tumorigenic Memory Fate. *Cell* 2020;**182**:297-316.e27.

Table 1: characteristics of the genotyping study population

	Whole genotyping study population (N=145)	**Cases (N=38)**	**Controls (N=107)**
Male gender, N (%)	45 (31.0)	20 (52.6)	25 (23.4)
Age at RA diagnosis, mean (SD)	51.9 (10.9)	49.8 (12.6)	52.7 (10. 2)
Age at the time of matching, mean (SD)	62.0 (10.1)	61.2 (10.2)	62.2 (10.1)
ACPA positivity, N (%)	106 (73.1)	35 (92.1)	71 (66.4)
RF positivity, N (%)	111 (76.6)	35 (92.1)	76 (71.0)
Erosions on X-rays at the time of matching, N (%)	55 (37.9)	30 (78.9)	25 (23.4)
DAS28 at the time of matching, mean (SD)	2.9 (1.5)	3.9 (1.7)	2.6 (1.4)
RA duration at the time of matching, mean (SD)	10.6 (5.0)	11.9 (9.7)	10.0 (0.3)
Year of RA diagnosis, median (IQR)	2002 (1978-2016)	2000 (1978-2016)	2004 (2002-2005)
Year of lymphoma diagnosis/10-year ESPOIR visit, median (IQR)	2013 (1996-2018)	2012 (1996-2018)	2014 (2012-2015)

Footnote: IQR: interquartile range; ACPA: anti-citrullinated peptide antibodies; DAS28: disease activity score in 28 joints; RA: rheumatoid arthritis; RF: rheumatoid factor; the time of matching corresponded to the diagnosis of lymphoma for cases and the 10-year ESPOIR visit for controls

Table 2: Testing for association of *TNFAIP3* rs2230926 with RA-related lymphoma

***TNFAIP3* rs2230926**	**Cases (N=38)**	**Controls (N=107)**	**OR (95% CI)**	**p-value***
MAF	2.6%	5.6%	0.45 (0.10-2.08)	0.30
TT	36 (94.7%)	96 (89.7%)	0.49 (0.10-2.30)	0.35
TG	2 (5.3%)	10 (9.3%)	0.53 (0.11-2.55)	0.42
GG	0 (0.0%)	1 (0.9%)	-	0.54

MAF: minor allele frequency

*chi-square test