



LETTER

Allele-specific protein binding within
the *CD40* locus in human synovial
fibroblasts and immune cellsLarissa Moser,¹ Katerina Laskari,¹ Caroline Ospelt ,¹ Miranda Houtman ,^{1,2}

To cite: Moser L, Laskari K, Ospelt C, *et al.* Allele-specific protein binding within the *CD40* locus in human synovial fibroblasts and immune cells. *RMD Open* 2023;**9**:e003168. doi:10.1136/rmdopen-2023-003168

► Additional supplemental material is published online only. To view, please visit the journal online (<http://dx.doi.org/10.1136/rmdopen-2023-003168>).

LM and KL contributed equally. Accepted 17 September 2023



© Author(s) (or their employer(s)) 2023. Re-use permitted under CC BY. Published by BMJ.

¹Center of Experimental Rheumatology, Department of Rheumatology, University Hospital Zurich, University of Zurich, Zurich, Switzerland

²Division of Rheumatology, Department of Medicine, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden

Correspondence to

Dr Miranda Houtman;
miranda.houtman@usz.ch

The region located upstream of *CD40* is one of the rheumatoid arthritis (RA) risk loci. Genetic variants in this risk locus have been found to affect *CD40* mRNA and protein expression.^{1 2} However, most efforts to elucidate how these non-coding variants affect RA susceptibility were performed in monocytes and B cells. Recently, it has been shown that the *CD40* risk locus is also located within open chromatin in synovial fibroblasts (SF), the resident stromal cells of the joints.^{3 4} Although some effects of genetic variants within the *CD40* risk locus have been reported, it remains to be identified whether these variants act similarly in different cell types. Here, we selected putative causal RA variants within the *CD40* risk locus in SF and assessed cell type specificity of these variants.

The *CD40* risk locus contains 11 single nucleotide polymorphisms (SNPs) in linkage disequilibrium (LD; $r^2 > 0.8$) and is located from ~17Kb of the *CD40* promoter to the first intron of *CD40* (online supplemental file 1A). Based on our previously published data,⁴ the SNPs reside within open chromatin in basal and TNF treated SF. The SNPs rs6074022 (located 6.7Kb upstream of *CD40*), rs1883832 (located at -1 from the start codon of *CD40*), rs4810485 and rs4239702 (both located within the *CD40* transcriptional region) reside within/in the vicinity of regulatory regions (enhancers, promoter) in SF (online supplemental information 1A), highlighting them as putative functional SNPs in SF. Capture HiC analysis suggests that genetic risk variants within the *CD40* locus may influence the regulation of not only *CD40*, but also *NCOA5*, *SLC35C2*, *ELMO2* and/or *ZNF334* (online supplemental file 1B) in SF.

To identify cell-type specific differences within the *CD40* risk locus, we performed electrophoretic mobility shift assays (EMSA) with SF, HT1080, Ramos, THP-1 and Jurkat

nuclear extracts and biotinylated probes containing the SNPs of interest (online supplemental file 2). We detected allele-specific protein binding for rs6074022 (signal for the rs6074022-T (major/risk) allele) in Ramos cells but not in other cell types, suggesting cell-type specific binding at this motif (figure 1A). Several relevant transcription factors, including interferon regulatory factor (IRF) 1, SP1 and ELF1, were found to bind to the regulatory region at rs6074022 in B cells. In contrast, we observed allele-specific binding for rs1883832 and rs4810485 in all tested cell types. For rs1883832, the C (major/risk) allele showed a specific signal, while the T allele showed a stronger signal than the C allele (figure 1B). Again, IRF1 and SP1 as well as TATA-box-binding protein (TBP) are among the proteins that potentially bind to the regulatory region at rs1883832. The rs4810485-G (major/risk) allele showed a stronger signal compared with the T allele and an additional signal in HT1080 and Jurkat nuclear extracts (figure 1C). The regulatory protein RBPJ, which mediates NOTCH signalling, was identified to control *CD40* expression via rs4810485.⁵ Our data suggest that RBPJ can control the expression of *CD40* via rs4810485 in SF and immune cells. No allele-specific binding for rs4239702 was found in any tested cell type (figure 1D).

The sites rs6074022 and rs1883832 were shown to be active under stimulatory conditions (online supplemental file 1B) and are correlated with *CD40* gene expression levels in interferon- γ stimulated cells.^{3 6} This is in line with the potential binding of IRF1 to both sites and points towards a role of interferon- γ in regulating this locus.

Overall, we show that rs6074022 is a putative functional SNP in B cells only and that rs1883832 and rs4810485 have an effect in a broad spectrum of RA-relevant cell types. Our

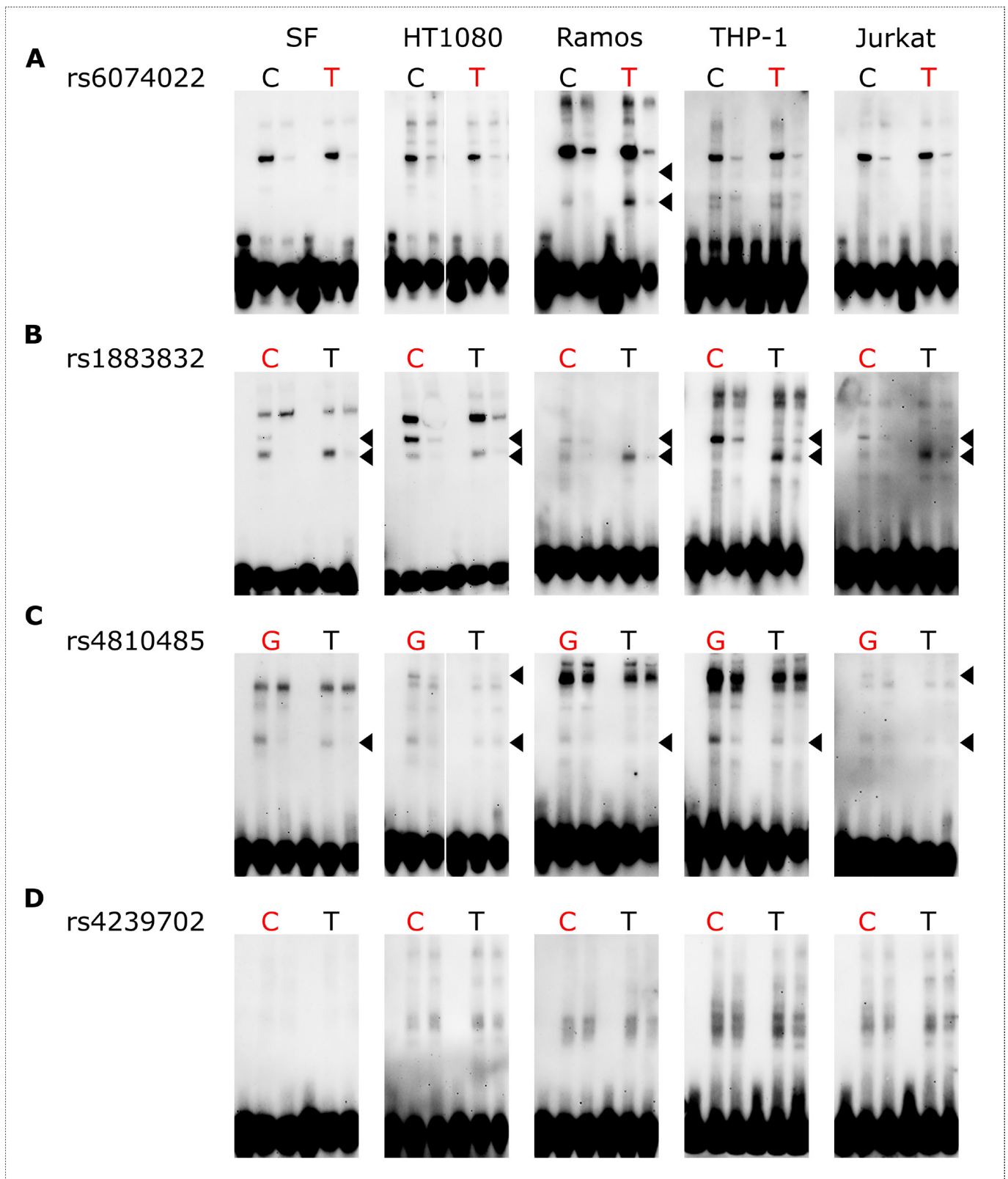


Figure 1 Allele-specific protein binding within the *CD40* risk locus in SF and immune cells. EMSAs were performed with SF, HT1080, Ramos, THP-1 and Jurkat nuclear extracts and biotinylated probes containing rs6074022 (A), rs1883832 (B), rs4810485 (C) and rs4239702 (D). For each allele: lane 1 is probe only, lane 2 is probe with nuclear extract and lane 3 is probe with nuclear extract and excess unlabeled probe. The major/risk allele for each SNP is shown in red. Allele-specific binding is marked with arrowheads. A representative blot of at least three independent experiments is shown (online supplemental file 3). SF, synovial fibroblast.

data stress the importance of cell-type-specific effects of genetic variants and provide the basis for future studies to identify the exact mechanisms by which these genetic risk variants in the *CD40* locus are associated with RA.

Twitter Caroline Ospelt @CarolineOspelt

Acknowledgements We thank Prof. Stephen Eyre for sharing data and Dr. Leonid Padyukov for valuable comments on the manuscript.

Contributors CO and MH were involved with the conception and design of the present study. LM and KL performed the experiments. MH analysed and interpreted the data. LM, KL and CO contributed to the interpretation of the data. MH drafted the manuscript. All authors critically revised the manuscript and approved the final version.

Funding This work was funded by the Swedish Research Council (Vetenskapsrådet; grant number 2020-00342).

Competing interests CO is associate editor of RMD Open. The other authors have no conflict of interest to declare.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by Cantonal Ethics Committee Zurich (approval numbers 2019-00115 and 2019-00674). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines,

terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution 4.0 Unported (CC BY 4.0) license, which permits others to copy, redistribute, remix, transform and build upon this work for any purpose, provided the original work is properly cited, a link to the licence is given, and indication of whether changes were made. See: <https://creativecommons.org/licenses/by/4.0/>.

ORCID iDs

Caroline Ospelt <http://orcid.org/0000-0002-9151-4650>

Miranda Houtman <http://orcid.org/0000-0001-5317-0327>

REFERENCES

- 1 Jacobson EM, Concepcion E, Oashi T, *et al*. A graves' disease-associated Kozak sequence single-nucleotide polymorphism enhances the efficiency of CD40 gene translation: a case for translational pathophysiology. *Endocrinology* 2005;146:2684–91.
- 2 Vazgiourakis VM, Zervou MI, Choulaki C, *et al*. A common SNP in the CD40 region is associated with systemic lupus erythematosus and correlates with altered CD40 expression: implications for the pathogenesis. *Ann Rheum Dis* 2011;70:2184–90.
- 3 Tsuchiya H, Ota M, Sumitomo S, *et al*. Parsing multiomics landscape of activated synovial fibroblasts highlights drug targets linked to genetic risk of rheumatoid arthritis. *Ann Rheum Dis* 2021;80:440–50.
- 4 Ge X, Frank-Bertoncelj M, Klein K, *et al*. Functional genomics atlas of synovial fibroblasts defining rheumatoid arthritis heritability. *Genome Biol* 2021;22:247.
- 5 Li G, Martínez-Bonet M, Wu D, *et al*. High-throughput identification of noncoding functional SNPs via type IIS enzyme restriction. *Nat Genet* 2018;50:1180–8.
- 6 Fairfax BP, Humburg P, Makino S, *et al*. Innate immune activity conditions the effect of regulatory variants upon monocyte gene expression. *Science* 2014;343:1246949.