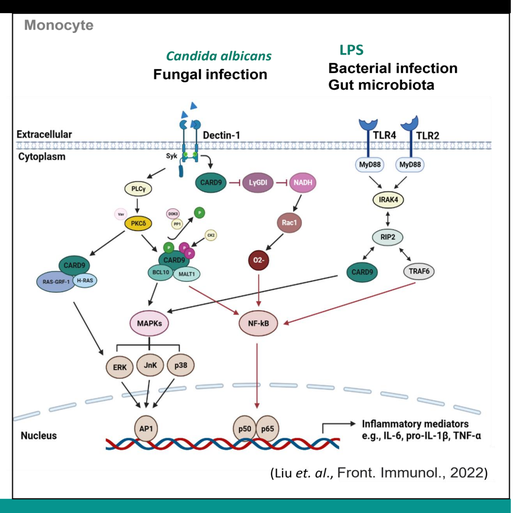
# Dissecting the Genetic and Functional Association of CARD9 with Axial Spondyloarthritis

**Eva Frison[1], Andrew C Brown[3,4], Carla J Cohen[3,4], Manon Jacoutot[1], Gabriele Migliorini[3,4], Roula Said-Nahal[1,2], Giuseppe Scozzafava[3,4], Paul Bowness[4,5], Paul Wordsworth[3,4], Henri-Jean Garchon[1,6], Simon Glatigny[1], Maxime Breban[1,2], Julian C Knight[3,4] , Félicie Costantino[1,2].**

*1 UMR1173, Université Paris Saclay, Université de Versailles St Quentin en Yvelines, Inserm, Infection et Inflammation, Montigny le Btx, France 2 Rheumatology Division, Ambroise Paré Hospital (AP-HP), Boulogne-Billancourt, France*
*3 Centre for Human Genetics, University of Oxford, Oxford, UK 4 NIHR Oxford Biomedical Research Centre, Oxford, UK 5 NDORMS, University of Oxford, Oxford, UK 6 Genetics Division, Ambroise Paré Hospital (AP-HP), Boulogne Billancourt, France*

## INTRODUCTION

- Spondyloarthritis (SpA) is a chronic inflammatory rheumatic disease with a strong genetic component.
- Nearly 50 susceptibility loci have been associated with the disease, most of them containing in non-coding variants whose mechanisms remain largely unknown.
- The CARD9 locus, involved in the activation of pro-inflammatory pathways and in the differentiation of Th17 cells is associated with SpA through genetic variants that remain functionally unexplored.




(Liu *et. al.*, Front. Immunol., 2022)

## AIMS

**To identify the functional basis for the genetic association of *CARD9* variants with axSpA**

**To assess the consequences of CARD9 variants associated with axSpA on proinflammatory cytokines production**

## METHODS

### Genomics

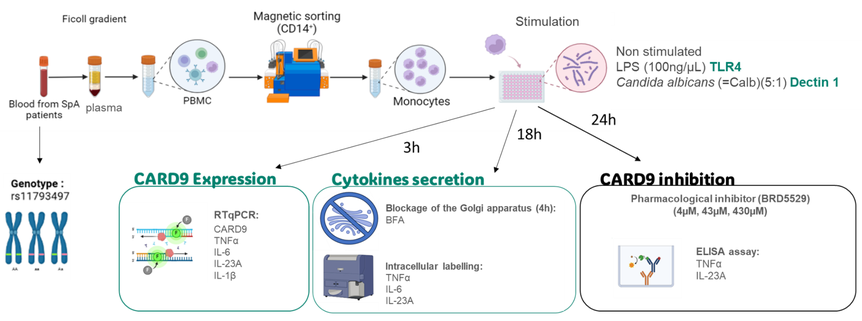
**Fine mapping of the CARD9 locus**

- Bayesian method
- 4,101 axSpA patients and 9,700 HC from United Kingdom

**Regulatory quantitative trait loci (QTL) mapping**

- Expression QTL (eQTL) mapping: RNAseq
- Chromatin accessibility QTL (caQTL) mapping: ATACseq
- Three immune cell types dysregulated during SpA : CD14+ monocytes, CD4+ and CD8+ T cells
- 45 individuals (20 axSpA, 35 healthy controls (HC)

### Ex vivo cytokine production




**Whole blood sampling in 59 axSpA patients**

- DNA genotyping of CARD9 variants using TaqMan assay
- Monocyte isolation through CD14+ magnetic sorting
- Different conditions of stimulation
  - Unstimulated
  - Lipopolysaccharide (100 ng/µl)
  - Candida albicans (5:1)
- RNA extraction after 3 hours of stimulation for RTqPCR
- Flow cytometry after 18 hours of stimulation (IL-6, TNFα and IL-23)

### Test of a pharmacological inhibitor of CARD9

- The pharmacological inhibitor of CARD9 BRD5529 was tested on monocytes from SpA patients
- Cells were stimulated after 1h of preincubation with BRD5529 at three different concentrations
- We then examined the levels of TNFα, IL-23A and IL-6 using ELISA assays

## RESULTS

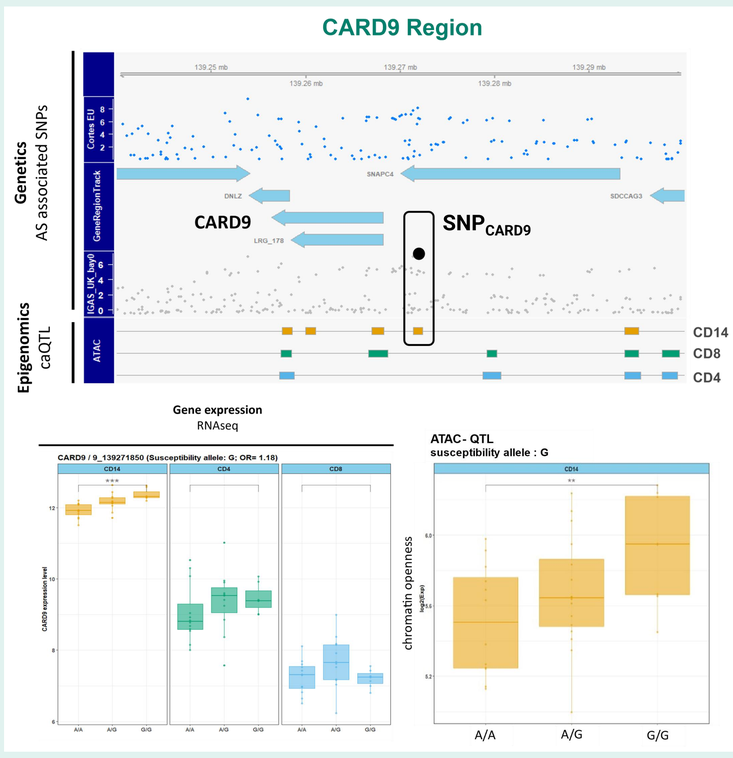
### Genomics

**Fine mapping of CARD9 locus**

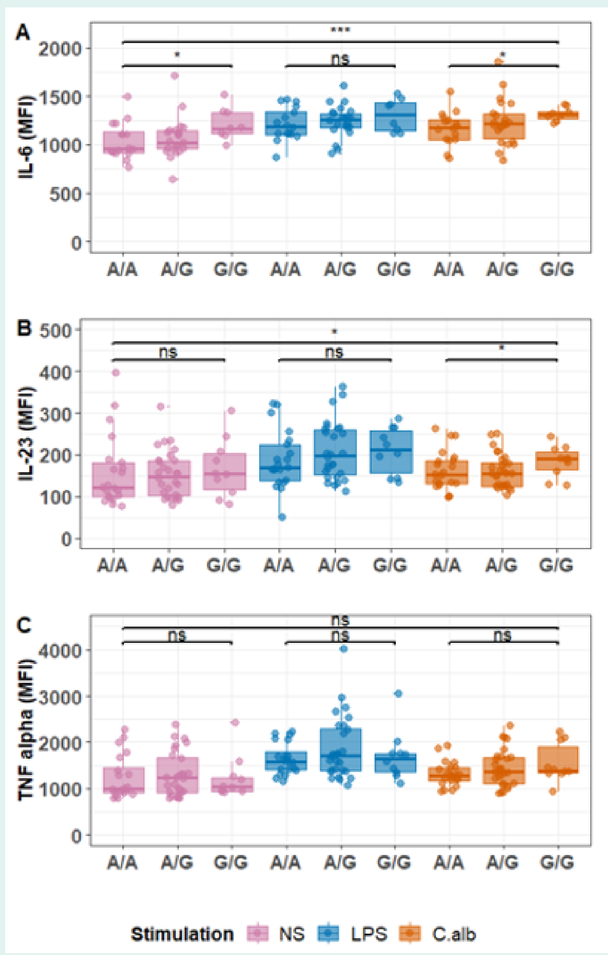
- One candidate SNP in the 95% credible set ($SNP_{CARD9}$)

**Regulatory quantitative trait loci mapping**

- $SNP_{CARD9}$ overlaps a CD14-specific chromatin opening site upstream of CARD9 promoter
- axSpA-susceptibility allele of $SNP_{CARD9}$ is associated with increased CARD9 mRNA expression in CD14+ monocytes (eQTL)
- axSpA-susceptibility allele of $SNP_{CARD9}$ is associated with enhanced chromatin accessibility in its surrounding region (caQTL)

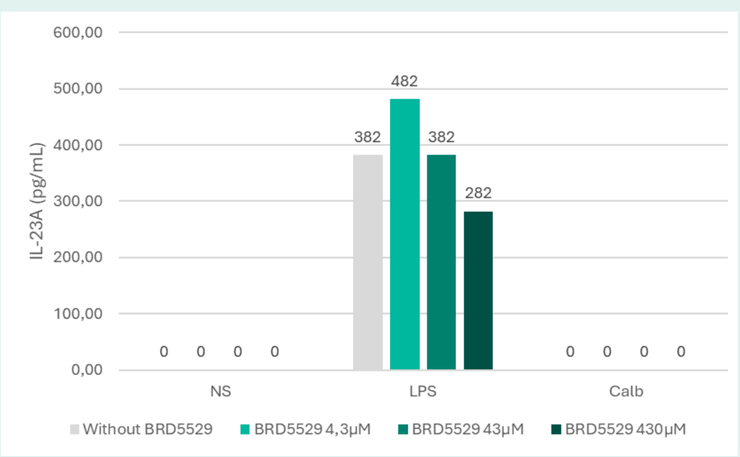


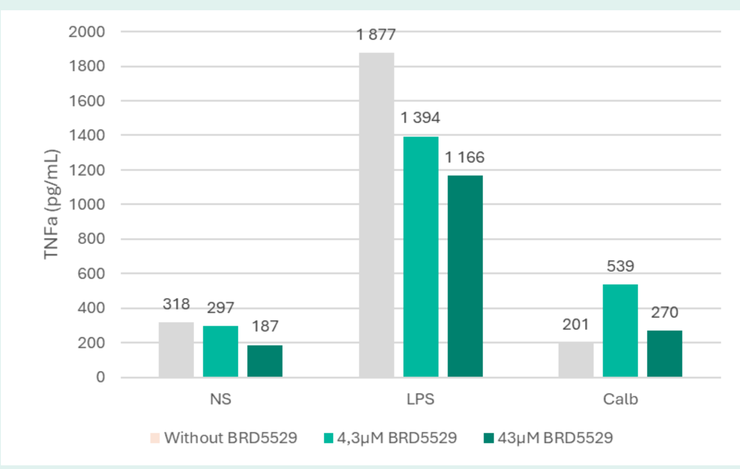

### Ex vivo cytokine production




- Dose-dependent association between susceptibility allele and IL-6 and IL-23 production

### Ex vivo cytokine production




- Trend of a dose-dependent decrease of IL23 and TNFα production after treatment with BRD5529

## CONCLUSION

By improving our understanding of the pro-inflammatory mechanisms linking SNV-CARD9 to SpA, this project opens the way to new targeted therapeutic approaches.

## AUTHOR INFORMATIONS

Eva FRISON: eva.frison@inserm.fr