20A129

Elucidating the Mechanism of rs26232 Association with Rheumatoid Arthritis Susceptibility and Severity

Kevin J. Sheridan[1], Emma R. Dorris[1], Eimear Linnehan[1], Michelle Trenkmann[1], Karen Creevey[1], Doug Veale[1], Ursula Fearon[2] and Anthony G. Wilson[1]

[1]University College Dublin Centre for Arthritis Research, Conway Institute, University College Dublin
[2]Molecular Rheumatology, School of Medicine, Trinity Biomedical Sciences Institute, Trinity College Dublin

Abstract

Introduction

The single nucleotide variant (SNV) rs26232 is located within the first intron of the *Macrophage Immunometabolism regulator* (*MACIR)* gene (formerly *C5orf30*) and is associated with the susceptibility to rheumatoid arthritis (RA), and also with more severe radiological joint damage to the joints of hands and feet. The risk C allele is also associated with greater *ex vivo* invasive activity of RA synovial fibroblasts (RASFs) and higher levels of ICAM-1, IP-10 and MMP14 expression. However, rs26232 genotype is not associated with total *MACIR* mRNA levels or of individual transcript variants. Therefore, the effects of rs26232 on RA pathogenicity do not appear to be mediated via *MACIR*. An investigation of neighbouring genes is therefore warranted to determine which gene elicits the causative effects of the rs26232 SNV.

Aims/Background

This work aims to identify the causative gene related to rs26232 association with RA susceptibility and severity.

Method

RASF were derived from knee biopsies of RA patients taken at arthroscopy. rs26232 genotype was determined by PCR genotyping assay with allelic discrimination analysis. qPCR was used to measure gene expression. Gene knock down was achieved using siRNA technology. Matrigel-coated Boyden transwell chambers were used to measure invasion. Wound healing (scratch assays) were used to measure migration. Proliferation was quantified using BrdU cell proliferation ELISA (Roche). CellEvent Caspase 3/7 green detection reagent was used to measure apoptosis.

Results

qPCR analysis of the expression levels of neighbouring genes showed an association between rs26232 genotype and *PAM* gene expression (p = 0.05) that was not observed with *Gin1* and *PPIP5K2*. This data suggested lower levels of PAM expression is associated with a more severe RA presentation. *PAM* knock-down was achieved in RASFs using siRNA technology and phenotypic analysis of *PAM* knock-down RASFs showed an increase in proliferation (p = 0.043) and invasion (p = 0.039), and decrease in apoptosis (p = 0.024), compared to non-targeting control siRNA treated RASFs.

Conclusions

This data demonstrates that *PAM* levels in RASFs are associated with rs26232 genotype and RA susceptibility and severity. Future work will concentrate on elucidating the exact mechanism of PAM's role in RA.

Background

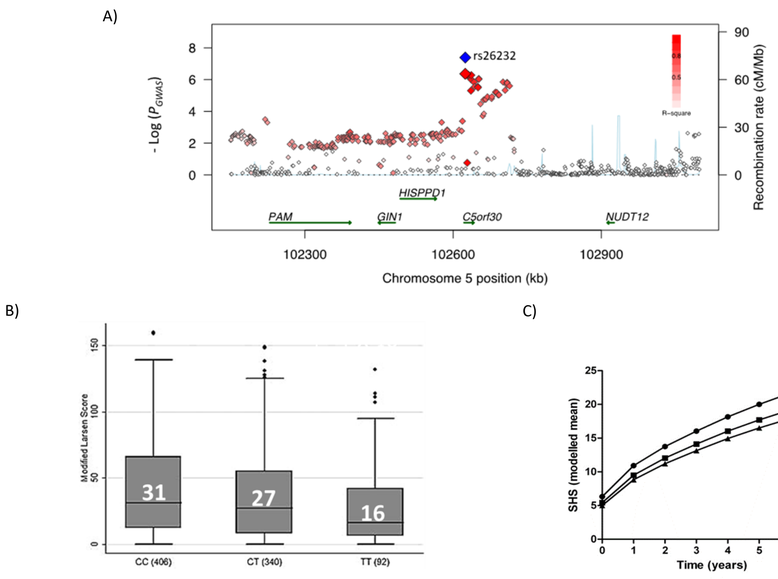

Figure 1: A) Regional association plot showing the strength of association (-Log(P-value)) versus chromosomal position (kilobases, kb) for rs26232[1]. B) Allele-dose association of each genotype of rs26232 with the modified Larsen score of radiologic joint damage in the Genetics of Rheumatoid Arthritis population (Sheffield, UK)[2]. C) Association of each genotype of rs26232 with progression of the modified Sharp/van der Heijde score (SHS) of radiologic joint damage during years 0–7 of follow-up in the Leiden Early Arthritis Clinic cohort[2].

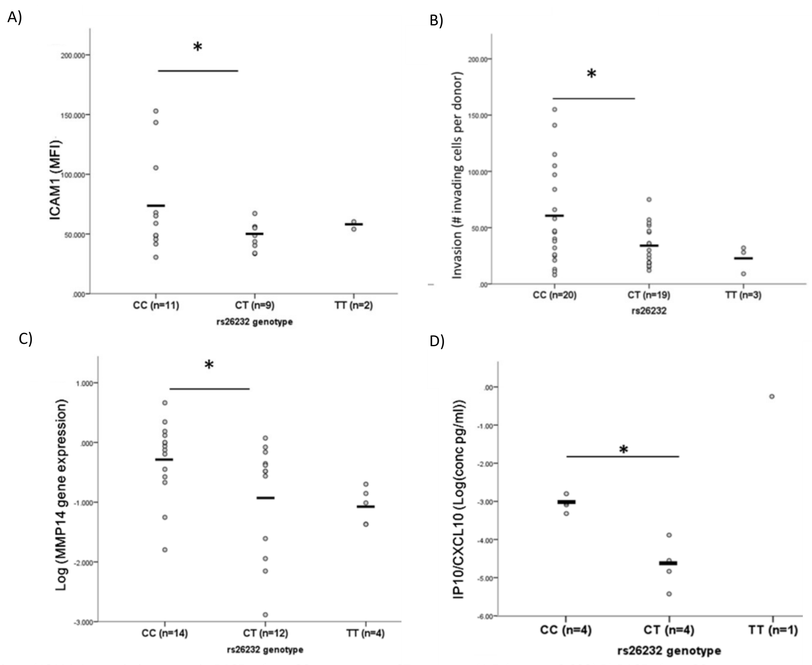

Figure 2: A) Expression of ICAM-1 protein is greater in RASFs of the CC compared to CT genotype (1.5-fold, p = 0.039).B) RASF of the CC genotype are more invasive than CT (p = 0.020). C) MMP14 relative gene expression is higher in CC compared to CT RASFs (1.6-fold, p = 0.021) D) RASFs of CC genotype produce greater IP10 (CXCL10) compared to CT genotype (5-fold, p = 0.011)[3].

Results

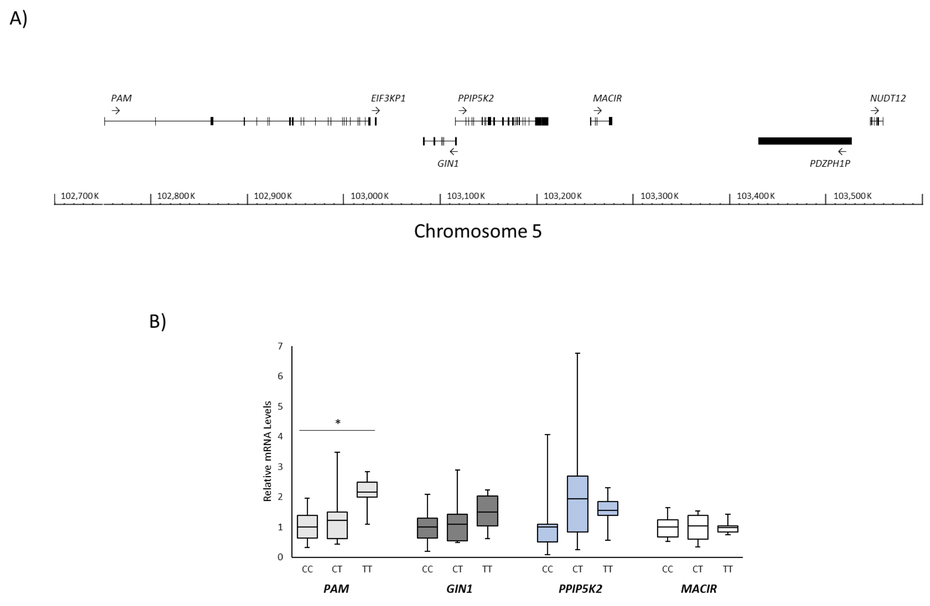

Figure 3: A) Annotation of genes in the locality of rs26232, located within the first intron of *MACIR*. B) Relative expression levels of *PAM, GIN1, PPIP5K2* and *MACIR* by rs26232 genotype. *PAM* shows a significant difference in expression levels between rs26232 genotypes (*P*=0.05), while *MACIR, GIN1* and *PPIP5K2* show no significant variation. Expression levels compared using Kruskal-Wallis test.

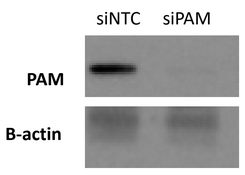

Figure 4: siRNA targeting PAM results in reduced levels of PAM protein in RASFs after 120 h compared to siNTC.

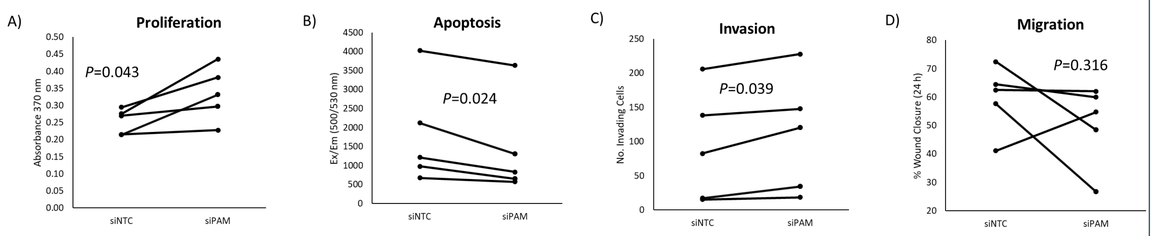

Figure 5: Functional assays for RASFs treated with siRNA targeting PAM (siPAM). A) RASFs treated with siPAM show a significantly higher rate of proliferation (*P*=0.043) compared to RASFs treated with non-targeting control siRNA (siNTC). B) RASFs treated with siPAM show a significantly lower rate of apoptosis (*P*=0.024) compared to RASFs treated with siNTC. C) RASFs treated with siPAM show a significantly higher level of invasion (*P*=0.039) compared to RASFs treated with siNTC. D) RASFs treated with siPAM show no significant difference migration levels (*P*=0.316) compared to RASFs treated with siNTC. *P*-values calculated using paired t-test.

Summary

- rs26232 genotype is associated with severity and susceptibility to RA.
- rs26232 CC genotype is associated with increased invasion of RASF and expression of ICAM-1, MMP14 and IP10/CXCL10. These factors are associated with RA severity.
- rs26232 is located within the first intron of *MACIR*, however *MACIR* gene expression levels are not associated with the rs26232 genotype. This indicates that *MACIR* does not mediate the phenotypes associated with rs26232.
- *PAM* gene expression levels are associated with rs26322 genotype in RASFs, with *PAM* expression highest in TT genotype and lowest in CC. This indicates that lower PAM expression may be associated with susceptibility and severity of RA.
- siRNA mediated knockdown of PAM in RASFs results in an increase in proliferation and invasion, while also decreasing apoptosis.
- This data indicates that lower levels of PAM result in a more aggressive phenotype in RASFs and would explain the link between rs26232 genotype and RA.

References

1. Stahl, Eli A., et al. "Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci." Nature genetics 42.6 (2010): 508-514.
2. Teare, M. Dawn, et al. "Allele-dose association of the C5orf30 rs26232 variant with joint damage in rheumatoid arthritis." Arthritis & Rheumatology 65.10 (2013): 2555-2561.
3. Dorris, Emma R., et al. "Association of the Rheumatoid Arthritis Severity Variant rs26232 with the Invasive Activity of Synovial Fibroblasts." Cells 8.10 (2019): 1300.
4. https://www.ensembl.org/index.html

Acknowledgements

Thanks to UCD Genomics for qRT-PCR assistance. Dr. Carl Orr for access to samples. Karen Creevey for sample preparation.

Arthritis Ireland
Medical Research Charities Group
HRB Health Research Board

Contact

Kevin.Sheridan@ucd.ie

rheumatology@ucd.ie

@UCD_CAR

http://www.ucd.ie/car/