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I Marinou, K Walters, M C Dickson, M H Binks, D E Bax and A G Wilson

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# Evidence of epistasis between interleukin 1 and selenoprotein-S with susceptibility to rheumatoid arthritis

I Marinou,<sup>1</sup> K Walters,<sup>1</sup> M C Dickson,<sup>2</sup> M H Binks,<sup>2</sup> D E Bax,<sup>1</sup> A G Wilson<sup>1</sup>

<sup>1</sup> School of Medicine & Biomedical Sciences, The University of Sheffield, Royal Hallamshire Hospital, Sheffield, UK; <sup>2</sup> GlaxoSmithKline R&D, Stevenage, UK

Correspondence to: A G Wilson, Section of Musculoskeletal Sciences, School of Medicine & Biomedical Sciences, The University of Sheffield, Royal Hallamshire Hospital, Sheffield, S10 2JF, UK; [a.g.wilson@shef.ac.uk](mailto:a.g.wilson@shef.ac.uk)

IM and KW contributed equally to this study.

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## ABSTRACT

**Objective:** Selenoprotein-S (SELS) is involved in the stress response within the endoplasmic reticulum (ER) and inflammation. Recently, promoter variants in the SELS gene were shown to be associated with plasma levels of interleukin (IL)6, IL1 $\beta$  and tumour necrosis factor (TNF). It was hypothesised that these variants could influence rheumatoid arthritis (RA) susceptibility and may interact with functional single nucleotide polymorphisms (SNPs) in the genes for IL1, IL6 and TNF.

**Methods:** Genotyping was performed in 988 unrelated healthy controls and 965 patients with RA. Stratified analysis was used to test for interactions. Single gene effects and evidence of epistasis were investigated using the Mantel–Haenszel (M–H) test and the linkage disequilibrium (LD)-based statistic.

**Results:** No association of SELS –105 genotype and RA susceptibility was detected. Stratification of SELS –105 genotypes by IL1 –511 genotypes showed that the disease risk (comparing AA/GA to GG at the SELS –105 locus) in individuals with the GG/AG genotype at the IL1 $\beta$  –511 locus was significantly lower than that in individuals having the AA genotype at the IL1 $\beta$  –511 locus (odds ratio (OR): 0.9 and 2.3, respectively;  $p = 0.004$  by M–H test). Significant epistasis was also detected using the LD-based statistic ( $p < 0.001$ ). No interaction was observed between SELS –105 and IL6 or TNF variants.

**Conclusion:** Our results reveal evidence of strong epistasis in two genes in the IL1 production pathway and highlight the potential importance of gene–gene interactions in the pathogenesis of RA.

Rheumatoid arthritis (RA) is an autoimmune disease with a prevalence of 1%. The contribution of genetic factors on RA susceptibility is shown by twin and population studies. The concordance rates on monozygotic twins ranges from 12% to 15% while in dizygotic twins it is 3% to 4%.<sup>1</sup> The major genetic component influencing RA susceptibility is a group of alleles in the DRB1 gene sharing a sequence motif, namely the “shared epitope” (SE). Recent evidence has also implicated the common allele of the R620W variant in the haematopoietic-specific protein tyrosine kinase protein tyrosine phosphatase non-receptor type 22 (PTPN22) with susceptibility to several autoimmune diseases including RA.<sup>2</sup> The recent genome-wide association study conducted by the Wellcome Trust Case Control Consortium (WTC) screened 1860 RA cases and 2938 healthy controls strongly replicated the association of the PTPN22 with RA susceptibility ( $p < 0.001$ ).<sup>3</sup>

Nine additional new loci of particular interest were also identified with significant ( $< 0.001$ )  $p$  values, such as variants close to the  $\alpha$  and  $\beta$  chains of the IL2R as well as genes involved in the tumour necrosis factor (TNF) pathway and T cell regulation.<sup>3</sup> A replication study of these 9 loci was also performed in an independent cohort of 5063 RA cases and 3849 healthy controls collected from 6 different centres and confirmed the association of rs6920220, located on 6q23 between OLIG3 and TNFAIP3, with increased risk of anti-cyclic citrullinated peptide (CCP)-positive RA as well as the nominal association of the IL2RB gene.<sup>4</sup> TNF receptor-associated factor 1 (TRAF1) and complement component 5 (C5) loci have also emerged as candidate regions by a genome-wide association study and a candidate gene approach study.<sup>5, 6</sup>

The identification of RA susceptibility genes is complicated by a number of factors including genetic and disease heterogeneity, gene–gene and gene–environment interactions. Epistasis is defined as a gene–gene interaction in which the genotype at one locus affects the phenotypic expression of the genotype at another locus. A gene with a weak effect on overall disease risk may be important in combination with other genes. Although the extent of epistasis in autoimmune diseases is still unclear, it is reasonable to postulate that such interactions may be a consequence of functional polymorphisms in genes involved in pathways that are implicated in the disease process.

In RA, evidence of interactions comes from linkage and association studies. The first European genome scan provided evidence of RA linkage on chromosome 3 that was stronger in human leukocyte antigen (HLA)-identical sibpairs, suggesting possible interactions between the HLA-DRB1 locus and a region on chromosome 3.<sup>7</sup> The second European genome scan demonstrated RA linkage to the interleukin 1 (IL1) locus that was restricted to HLA-identical sibpairs suggesting that genes of the IL1 cluster may play an additional role only in the presence of RA-related HLA alleles.<sup>8</sup> More recently a gene interaction was detected between PTPN22 R620W and HLA-DRB1 SE alleles, supporting the view that genes with a moderate risk can exert a stronger effect in combination with other risk loci.<sup>9</sup>

Recently, selenoprotein S (SELS) has been identified as an important regulator of IL1, TNF and IL6 production with plasma levels of these proteins correlating with functional promoter single nucleotide polymorphisms (SNPs) in SELS.<sup>10</sup> This gene is involved in the stress response

within the endoplasmic reticulum (ER) and inflammation. We hypothesised that variants in SELS could influence RA susceptibility and may interact with functional SNPs in the genes for IL1, IL6 and TNF that have been previously associated with RA.

## METHODS

### Study populations

A total of 988 Caucasians (healthy unrelated individuals) and 965 individuals with RA participated in this study and have been described previously.<sup>11</sup> All patients fulfilled the American College of Rheumatology (ACR) criteria and had a minimum disease duration of 3 years. The South Sheffield Research Ethics Committee approved this study and informed consent was obtained from all participants.

### SNP genotyping

Blood samples were collected in EDTA anticoagulated tubes and DNA was extracted using standard methods. TaqMan genotyping assays were designed for functional SNPs in IL1 $\beta$ , IL6, TNF and SELS as previously described (table 1).<sup>12</sup> Thermal cycling was performed as follows; after an initial denaturation and enzyme activation of 10 min at 95°C, samples were subjected to 40 cycles of 15s at 95°C for denaturation and 60s at 60°C for annealing/extension. We included multiple positive and negative controls in all genotyping plates and repeated 10% of our samples to minimise genotyping errors. Thermal cycling in 384-well plates was performed on a PTC-225 DNA engine Tetrad (MJ Research, San Francisco, California, USA) and genotypes were determined using an ABI Prism 7900HT (PE Biosystems, Foster City, California, USA).

### Statistical analysis

Deviation from Hardy–Weinberg equilibrium (HWE) was tested for each SNP in cases and controls separately using a  $\chi^2$  test with a threshold of  $p < 0.05$ . The odds ratios (ORs) were calculated with asymptotic 95% confidence intervals (CIs). The mode of inheritance at each locus was determined by the ORs for RA in the three genotype groups.

Gene–gene interactions are often measured as departure from an additive or multiplicative genetic model by, for example, adding a product term between two risk factors in a logistic regression model (statistical interaction), however this method which treats interaction as a residual term in a regression model has no clear biological interpretation. To study gene–gene interactions in addition to the Mantel–Haenszel (M–H) test we have used a recently described test for interactions between two unlinked loci. This model is based on the assumption that two interacting loci will create linkage disequilibrium (LD) even if these loci are unlinked, the level of which depends on the extent of the interaction. This

definition describes the dependence of penetrance for a two-loci haplotype where the penetrance of one locus depends on the genotypes at another locus.<sup>13</sup> The LD-based statistic provides a clearer biological interpretation of epistasis and also an increased power to detect gene–gene interactions under several plausible models of interactions.

Therefore we first stratified study subjects according to IL1 genotypes. Differences in odds ratios of RA (comparing two SELS genotype groups) in the different IL1 genotype groups were determined using the M–H test. In each pairwise test of statistical interaction, the mode of inheritance considered at each locus was that used for the main effects. An alternative method based on measuring the departure from independence of penetrances in two unlinked loci (an LD-based statistic) was also performed. A total of 7 tests for main effects and 21 tests for gene–gene interactions were performed (total of 28 tests). Applying a Bonferroni correction to this number of tests would require a  $p$  value less than 0.002 to ensure a conventional type 1 error rate of 5%. The Bonferroni correction however assumes independent tests and is conservative for correlated tests, as the tests in this study are. All  $p$  values given in the text are unadjusted and should be judged against the adjusted cut-off of 0.002, bearing in mind that it is a conservative threshold. All statistical analyses were carried out using STATA statistical software (V 9.1, STATA, College Station, Texas, USA).

## RESULTS

### Genotype and allele distributions

Baseline characteristics of the study populations are summarised elsewhere.<sup>12</sup> Allele and genotype frequencies for all SNPs were in HWE for RA cases and controls. Table 2 shows the genotype frequencies of each SNP in healthy controls and RA cases. A marginal association was detected between IL1 $\beta$  –511 and RA susceptibility. The –511AA genotype was under-represented in RA cases compared to healthy controls (OR 0.7, 95% CI 0.5 to 1.0,  $p = 0.04$ ). Genotypes frequencies were not significantly different for any other variant.

### Interaction between IL1 $\beta$ –511 and SELS –105

The genotype frequencies for different combinations of IL1 $\beta$  –511 and SELS –105 for RA cases and controls are shown in table 3. The presence of a significant interaction was detected using a stratified analysis (M–H test). The OR comparing individuals with the AA/GA genotype to the GG genotype at the SELS –105 locus in participants who were GG/AG at the IL1 $\beta$  –511 locus was 0.9 (95% CI 0.7 to 1.1,  $p = 0.3$ ). In contrast, the presence of at least one copy of the A allele at the SELS –105 locus (compared to no copies) was associated with an increased risk of RA in subjects who were AA at the IL1 $\beta$  –511 locus (OR 2.3, 95% CI 1.2 to 4.5,  $p = 0.007$ ) (table 2). Applying a M–H test of homogeneity of odds ratios suggests that the risk of RA to carriers of the AA/GA genotype compared to the GG genotype at the SELS –105 locus depends upon their genotype at the IL1 $\beta$  –511 locus ( $p = 0.004$ ). While this falls just short of the Bonferroni threshold it is clearly suggestive of a statistical interaction. The LD test statistic yielded stronger, highly significant evidence of a gene–gene interaction between the SELS –105 and IL1 $\beta$  –511 loci ( $p = < 0.001$ ). This considerably exceeds the Bonferroni threshold and together with the M–H analysis provides compelling evidence for a statistical interaction between the SELS –105 and IL1 $\beta$  –511 loci in RA. No interaction was detected between SELS –105 and

**Table 1** Details of individual single nucleotide polymorphisms (SNPs)

dbSNPID	Gene	Description
rs17561	IL1A	Ser114Ala
rs16944	IL1 $\beta$	Promoter (–511)
rs1143634	IL1 $\beta$	Synonymous coding, Phe105Phe
rs1800795	IL6	Promoter (–174)
rs28665122	SELS	5' UTR (–105)
rs4965814	SELS	Intron 5 (+3705)
rs4965373	SELS	3' UTR (+5227)

The dbSNPID accession number (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp>) and details of location and functional effects of variant are given. UTR, untranslated region.

**Table 2** Genotype frequencies and disease status

Gene	Genotype	Cases n (%)	Controls n (%)	OR (95% CI)	p Value
IL1 -511	AA	90 (9.6%)	117 (12.5%)	0.7 (0.5 to 1.0)	0.04
	GA/GG	852 (90.4%)	820 (87.5%)	1.0	
IL1 +3954	TT	40 (4.5%)	49 (5.5%)	0.8 (0.5 to 1.3)	0.3
	TC/CC	850 (95.5%)	842 (94.5%)	1.0	
IL6 -174	CC/CG	656 (69.9%)	621	1.2 (1.0 to 1.4)	0.1
	GG	282 (30.1%)	314	1.0	
TNF -308	AA	19 (4.1%)	24 (5.4%)	0.7 (0.4 to 1.4)	0.4
	GG/AG	442 (95.6%)	418 (94.6%)	1.0	
SELS -105	AA/GA	235 (26.7%)	250 (26.6%)	1.0 (0.8 to 1.2)	0.9
	GG	644 (73.3%)	690 (73.4%)	1.0	
SELS +3705	TT	24 (2.5%)	28 (2.9%)	0.9 (0.5 to 1.6)	0.6
	TC/CC	920 (97.5%)	936 (97.1%)	1.0	
SELS +5227	GG/GA	504 (56.2%)	490 (55.4%)	1.1 (0.9 to 1.3)	0.3
	AA	393 (43.8%)	395 (44.6%)	1.0	

OR, odds ratio.

the other cytokine SNPs ( $p = 0.6$  for IL6-174;  $p = 0.2$  for IL1+3954;  $p = 0.2$ ; for IL1+4845;  $p = 0.9$ ;  $p = 0.6$  for TNF308 by M-H test).

Subjects were stratified according to the IL1 $\beta$  -511 genotype and odds ratios for the SELS -105 gene were calculated in both strata separately. The genetic models used were those previously assigned to each polymorphism and the M-H test was used.

## DISCUSSION

In this study we report strong statistical evidence of an interaction between two functional polymorphisms in the IL1 $\beta$  and SELS genes with increased risk of developing RA. There is a large body of evidence implicating IL1 in RA; it is found at high levels in serum and synovial fluid of patients with RA and evidence has shown that, together with TNF $\alpha$ , it upregulates the expression of matrix metalloproteinases (MMPs) and cell adhesion molecules (CAMs) that are important in bone cartilage and bone resorption.<sup>14</sup> Deletion of the IL1 receptor antagonist gene in Balb/C mice results in an inflammatory polyarthritis.<sup>15</sup> A number of polymorphisms in the IL1 cluster have been extensively associated with inflammatory conditions. The IL1 $\beta$  -511 polymorphism has been shown to result in loss of a putative activating protein 2 (AP-2) binding site, suggesting its functional importance.<sup>16</sup> Furthermore a haplotype consisting of -511T and the -31C has been associated with a twofold to threefold increase in LPS-induced IL1 $\beta$  protein production.<sup>16</sup> The SELS -105 variant is located within a putative endoplasmic reticulum (ER) stress response element (ERSE) and the A allele is associated with impaired SELS expression, leading to increased production of proinflammatory mediators.<sup>10</sup>

SELS is a gene involved in ER stress response. It encodes a membrane protein that removes misfolded proteins from the ER to the cytosol and prevents stress responses that lead to

activation of the inflammatory cascade.<sup>10</sup> Genetic variation of SELS has been associated with circulating levels of the proinflammatory cytokines IL1 $\beta$ , IL6 and TNF. The promoter variant (-105G>A) significantly impairs SELS expression resulting in increased cytokine production and differential ER stress response, suggesting it to be a strong candidate in the pathogenesis of common inflammatory diseases.<sup>10</sup> However we did not detect a significant association of SELS promoter variants with susceptibility to RA.

As genetic variation of SELS influences circulating levels of proinflammatory cytokines, the presence of epistasis with functional cytokine variants was investigated. Using stratification and LD-based methods significant interaction was observed between the IL1 $\beta$  -511 and SELS -105 loci, with the latter method detecting much stronger evidence of epistasis. This may be due to the greater statistical power of the LD-based test statistic compared to the M-H test statistic. The LD-based statistic has been shown to have greater power than logistic regression under several plausible models of epistasis and so it is reasonable to expect that it would also have greater power than the M-H test statistic. Of course other functional genetic variants in high LD with either SNP may be the causative polymorphisms that contribute to increased disease susceptibility. Our data clearly demonstrate that these two genes interactively increase the risk of developing RA. Given the observation of Curran *et al* and the role of IL1 in RA the increased frequency of the -105A and -511A alleles is expected in RA cases and is in line with previous findings.

Our data suggest that the A allele at the SELS -105 locus is a new candidate for RA pathogenesis in individuals who are homozygous for the rare allele at the IL1 $\beta$  -511 locus. Although our results are based on small numbers, they confirm the importance of epistatic interactions in the pathogenesis of complex diseases and support the hypothesis that polymorphisms in genes with either moderate or no main effects can have

**Table 3** Stratification analysis according to IL1 and SELS genotypes

SELS -105	IL1 $\beta$ -511							
	GA/GG				AA			
	RA	Controls	OR (95% CI)	p Value	RA	Controls	OR (95% CI)	p Value
AA/GA	191	214	0.9 (0.7 to 1.1)	0.3	35	26	2.3 (1.2 to 4.5)	0.007
GG	579	571	1.0	-	47	81	1.0	-

Mantel-Haenszel test of homogeneity, p value 0.004.

IL, interleukin; OR, odds ratio; RA, rheumatoid arthritis; SELS, selenoprotein S.

significant effects when combined with polymorphisms at other susceptibility loci.

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**Competing interests:** None.

**Ethics approval:** The South Sheffield Research Ethics Committee approved this study and informed consent was obtained from all participants.

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