Genetics, autoantibodies and clinical features in understanding and predicting rheumatoid arthritis

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Chapter 5

The RAGE G82S polymorphism is not associated with rheumatoid arthritis independent of HLA-DRB1*0401

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The receptor for advanced glycation endproducts (RAGE) has been shown to play a role in several pathologies including Rheumatoid Arthritis (RA) (1). RAGE binding of ligands upregulated in RA synovial tissue, fluid and serum, can lead to increased cell activation, including migration, hyperplasia and increased cytokine production. Several animal studies have described a possible role for RAGE in the onset and severity of arthritis. In these animal studies, blockade of the receptor showed suppression of arthritis, while administration of RAGE ligands induced arthritis in healthy mice (2,3). In addition, increased levels of RAGE ligands have been found in RA patients and correlate with disease severity (4-6).

Previous studies indicated that a gain of function mutation of RAGE correlates with RA. Linkage of RAGE with the HLA-DRB1-DQ region, a region known to associate with RA susceptibility and severity, could account for this correlation. To dissect the possible confounding effects of the HLA-DRB1 region for the possible association of RAGE with RA, we investigated the correlation of a gain of function mutation in RAGE, to HLA-DRB1 alleles and RA.

377 consecutive RA patients of the Leiden Early Arthritis Clinic, an inception cohort for patients with recent onset arthritis (7) (mean age 48 ± 17 years SD, 55% female) and 535 non-RA controls of the same cohort (mean age 57 ± 16 SD, 67% female) were included in the analysis. All RA patients fulfilled the 1987 criteria of the American College of Rheumatology. The study was approved by the local Ethics Committee and written informed consents were obtained from all patients and controls according the declaration of Helsinki. HLA genotyping was available for all patients and controls and in addition for typing of the RAGE G82S polymorphism a PCR was performed, followed by an overnight digestion with *Alu* I. The 82S polymorphism resulted in the formation of an extra *Alu*I restriction site.

A previous report by Hoffman et al (2) described that the RAGE 82S polymorphism correlated with susceptibility for rheumatoid arthritis. However, RAGE is in strong linkage disequilibrium with HLA-DR4 (8), particularly HLA-alleles encoding a common 'shared epitope' within the HLA-DRB1 allele. In the population studied by Hoffman et al, a linkage was found with DR1*0401 and after correction for this allele, the correlation between the RAGE 82S polymorphism and susceptibility for RA was lost. These data were not conclusive, since the number of patients and controls positive for the RAGE 82S polymorphism were very low (5 out of 95 and 2 out of 134 respectively). Here, we identified 46 out of 377 RA patients and 36 out of 535 controls harbouring the RAGE 82S polymorphism. We found an association between the RAGE 82S polymorphism and RA without correction for HLA alleles. However, in patients, RAGE 82S was in linkage (defined as P<0.01) with DRB1*0401 (odds ratio (OR) 6.5 P<0.0001). In controls RAGE 82S was in linkage

Table 1. Frequencies of patients and controls positive or negative for RAGE 82S or HLA-DRB1*0401 and statistic calculation by the method described by Svejgaard et al.

RAGE 82S	DRB1*0401	Patients	Controls
+	+	36	20
+	–	10	16
–	+	78	60
–	–	253	439

Comparison	Patients		Controls		OR (C.I.)	P	Test
RAGE 82S+ vs. RAGE 82S-	82S+ 46	82S- 331	82S+ 36	82S- 499	1.99 (1.19-3.12)	0.0045	RAGE associated?
DRB1*0401+ vs. DRB1*0401-	DRB1*0401+ 114	DRB1*0401- 263	DRB1*0401+ 80	DRB1*0401- 455	2.47 (1.76-3.45)	<0.001	DR0401 associated?
82S+/0401+ vs. 82S-/0401+	82+/0401+ 36	82-/0401+ 78	82+/0401+ 20	82-/0401+ 60	1.38 (0.70-2.77)	0.32	RAGE associated in DR0401+?
82S-/0401+ vs. 82S-/0401-	82+/0401- 10	82+/0401- 253	82+/0401- 16	82+/0401- 439	1.08 (0.45-2.57)	0.84	RAGE associated in DR0401-?
82S-/0401+ vs. 82S+/0401-	82-/0401+ 36	82-/0401+ 10	82-/0401+ 20	82-/0401+ 16	2.88 (1.00-8.45)	0.029	DR0401 associated in RAGE+1?
82S-/0401+ vs. 82S-/0401-	82+/0401+ 78	82+/0401- 253	82+/0401+ 60	82+/0401- 439	2.26 (1.53-3.32)	<0.001	DR0401 associated in RAGE-1?
Association between RAGE and DR0401 in patients	patients 82S+ 36	patients 82S- 10	patients 0401+ 78	patients 0401- 253	11.68 (5.28-26.4)	<0.001	Linkage disequilibrium in patients?
Association between RAGE and DR0401 in controls	controls 82S+ 20	controls 82S- 16	controls 0401+ 60	controls 0401- 439	9.15 (4.26-19.74)	<0.001	Linkage disequilibrium in controls?

with DRB1*0401 (OR 4.43, P<0.00001) and *0901 (OR 5.05, P=0.002). Correction for presence or absence of these HLA alleles was done by the Svejgaard method (9). When the association of RAGE 82S with RA was corrected for the absence or presence of DRB1*0901, the association between RAGE and RA remained present. Conversely, after correction for the presence/absence of HLA DRB1*0401 the association between RAGE 82S and RA was lost (Table 1), indicating that RAGE is not associated with RA independently of HLA DRB1*0401 in this cohort. Also in logistic regression analysis with DRB1*0401 and RAGE as possible explanatory variables and the presence of RA as dependent variable, only DRB1*0401 was independently associated with RA (OR 2.5, P<0.001).

In conclusion, considering the size of our study, it is unlikely that the RAGE 82S polymorphism is associated with RA independently of HLA DRB1*0401.

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