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The MHC2TA –168A/G polymorphism and risk for rheumatoid arthritis: a meta-analysis of 6861 patients and 9270 controls reveals no evidence for association

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Abstract

Background—An association between major histocompatibility complex (MHC) genes, particularly those within the class II HLA region, and rheumatoid arthritis (RA) is well established, and accounts for an estimated 30% of the genetic component in RA. The MHC class II transactivator gene (*MHC2TA*) on chromosome 16p13 has recently emerged as the most important transcription factor regulating genes required for class II MHC-restricted antigen presentation. Previous studies of a promoter region polymorphism (–168A/G, rs3087456) in the *MHC2TA* gene and RA have yielded conflicting results.

Objective—To assess the association of the MHC2TA –168A/G polymorphism (rs3087456) and risk for RA by meta-analysis.

Methods—Meta-analysis was performed for 6861 patients with RA and 9270 controls from 10 case–control studies. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for each study. Summary ORs and 95% CIs were calculated for random effects models.

Results—No effect was observed for the G risk allele (OR 1.02, 95% CI 0.93 to 1.12, p=0.70) or the GG risk genotype (OR 1.14, 95% CI 0.95 to 1.36, p=0.16).

Conclusions—Our results indicate that the MHC2TA –168A/G polymorphism (rs3087456) is not associated with RA yet underscore the importance of including shared epitope allele carrier status, secondary phenotypes and more complete characterisation of *MHC2TA* variation in future studies.

Rheumatoid arthritis (RA) is the most common systemic autoimmune disease with a worldwide prevalence approaching 1%.¹ This chronic inflammatory disease can cause substantial disability from erosive and deforming processes in joints, and is associated with increased mortality.² The prevalence of RA, in conjunction with the impact on the health status of affected individuals, results in tremendous associated costs to patients, their families and society. While the aetiology of RA is unknown, a significant genetic contribution to RA development is established and accounts for an estimated 60% of disease risk.³ A strong association between major histocompatibility complex (MHC) genes, particular those in the class II HLA region,

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and RA has been well established, and accounts for an estimated 30–50% of the genetic component in RA, at least in northern European Caucasians.^{3 4} Thus, non-MHC genes also contribute to disease risk.

The MHC class II transactivator gene (*MHC2TA* or *CIITA*) on chromosome 16p13 is an attractive candidate for genetic studies of RA because it regulates expression of proteins presenting and processing antigens, including MHC class II molecules.⁵ *MHC2TA* encodes the CIITA protein, which chaperones assembly of several transcription factors at MHC promoters.⁶ Swanberg *et al*⁷ initially demonstrated that the G allele of the *MHC2TA* –168A/G promoter polymorphism (rs3087456) increased risk for RA, and that the GG genotype influenced expression of the CIITA protein and MHC class II molecules. Since then studies of *MHC2TA* and RA have focused on this single nucleotide polymorphism (SNP) but have yielded conflicting results; see tables 1 and 2 for a brief summary. Though previous studies describe this SNP as an A→G change, the dbSNP database indicates that the G allele is ancestral; thus this SNP is actually a G→A change.⁸ A meta-analysis was performed to test whether the G risk allele (by comparing AA against GG+GA carriers) or the GG risk genotype (by comparing AA+AG against GG carriers) increased risk for RA.

METHODS

Identification of studies

A literature search of the PubMed database was conducted using the search terms “rheumatoid arthritis” and “*MHC2TA*.” Nine publications were identified before 30 May 2007.^{7 9-15} All languages, geographic areas and publication types were included. Seven publications were original research articles and two were letters to the editor.^{12 14} One letter presented results for three studies.¹⁴ Study selection criteria were: (1) patients with RA fulfilled 1987 American College of Rheumatology classification criteria for RA (excluding juvenile RA);¹⁶ (2) controls were derived from a population in the same geographic area and with the same ethnic background as patients with RA; (3) authors provided original genotype frequencies; and (4) RA patient and control groups did not overlap between studies. Two authors (PB, LB) applied these criteria independently. Ten studies met our selection criteria. The most recent study was not included because it did not provide genotype frequencies.¹⁷

Data extraction

One author (PB) extracted the following data from each study: authors, publication year, journal, publication type, place of study, study design, genotyping method, genotype frequencies, number of cases and controls and sample description (recruitment method, age, race and ethnicity). Cases were described by RA classification criteria, age at onset and RA secondary phenotypes. Controls were described as population-based or matched, and by matching characteristics. All 10 studies had a case–control study design. For the two studies presenting genotype frequencies for a set of matched controls and a larger set of population-based controls, population-based controls were used because genotype frequencies were similar between the two sets of matched and population controls, and a larger sample size provided more power.^{7 15}

Statistical analysis

For each individual study, the odds ratios (OR) and 95% confidence intervals (95% CI) for the G risk allele and the GG risk genotype in patients with RA against controls were calculated. Statistical analyses were performed in R (<http://www.r-project.org/>, accessed October 2006). ORs were calculated using unconditional maximum likelihood estimation. 95% CIs were calculated using normal approximation. Two-tailed p-values were calculated using the χ^2 test of independence. The summary ORs and 95% CIs for the G risk allele and the GG risk genotype

in patients with RA against controls were calculated for random effects models because this model assumes effects are randomly distributed.¹⁸ Between-study heterogeneity was assessed with the Cochran's Q test statistic. Forest plots were generated in R to display the OR and 95% CI for each study (shown by a black square on a horizontal line, respectively) and the summary OR and 95% CI (shown by a dotted vertical line extending from a black diamond, respectively).

RESULTS

Genotype frequencies and study results for the 10 studies used in the meta-analysis are summarised in tables 1 and 2. Studies were weighted by size to contribute to the overall combined result. There was evidence of between-study heterogeneity for the G risk allele ($p=0.04$) but not for the GG risk genotype ($p=0.10$). There was no evidence of association for the *MHC2TA* -168A/G polymorphism (rs3087456) with RA risk when comparing GG+AG against AA carriers (OR=1.02, 95% CI=0.93 to 1.12, $p=0.70$) or when comparing GG against AG+AA carriers (OR=1.14, 95% CI=0.95 to 1.36, $p=0.16$). The forest plots in fig 1 show a schematic representation of the data that are presented in the bottom line of table 2. Results did not differ when the meta-analysis was limited to studies of European populations (data not shown).

DISCUSSION

MHC class II gene expression appears to be regulated almost exclusively by *MHC2TA*, and differential expression of class II genes has shown some evidence for association with both RA susceptibility and progressive disease in a recent study with direct implications for *MHC2TA*.¹⁹ There is substantial evidence for genetic contribution of the MHC to RA. The MHC spans ~4.5 mega base pairs on chromosome 6p21.3 and encodes >180 expressed genes; 40% are related to immune activation and response.^{20 21} The MHC class II gene *HLA-DRB1* demonstrates the strongest association with RA, highlighting antigen presentation and subsequent T cell activation as a potential pathway in RA pathogenesis.⁴ All RA-associated *HLA-DRB1* alleles encode a shared epitope (SE) not present on non-RA associated alleles.²²

Whole-genome microsatellite linkage screens in families with multiple patients with RA have indicated linkage on chromosome 16p, with a stronger effect in families where patients carry two copies of SE alleles.²³ Although this linkage peak may be due to another as of yet unidentified candidate gene on chromosome 16p, thus far only two studies of *MHC2TA* variation and RA have accounted for SE allele carrier status in the data analysis. Because these data were missing from the majority of studies included here, we were not able to incorporate SE carrier status into our analysis. Eyre *et al*¹⁰ did not find any effect of the *MHC2TA* -168A/G polymorphism (rs3087456) on RA susceptibility regardless of SE carrier status. Martinez *et al*¹³ did not find any effect of the *MHC2TA* -168A/G polymorphism (rs3087456) on RA susceptibility but reported two *MHC2TA* haplotypes (comprised of the -168A/G polymorphism (rs3087456) and rs4774) were associated with RA, with a stronger effect revealed in analyses of SE-positive patients.

Additionally, common secondary phenotypes (rheumatoid factor (RF) positivity, anti-cyclic citrullinated peptide positivity and erosive joint disease) associated with severe disease outcomes have evidence of specific genetic associations, which may reflect differences in underlying disease mechanisms based partly on the influence of different genetic factors.²⁴ Thus far only two studies of *MHC2TA* variation and RA have examined secondary phenotypes; therefore, we were not able to incorporate secondary phenotypes into our analysis. Harrison *et al*¹¹ reported slight trends when comparing the G risk allele in RF-negative patients with controls (OR 0.8, 95% CI 0.6 to 1.0, $p=0.08$) and 559 RF-positive patients to 159 RF-negative patients (OR 1.3, 95% CI 1.0 to 1.7, $p=0.08$). Yazdani-Biuki *et al*¹⁵ reported no evidence for

association between the MHC2TA –168A/G polymorphism (rs3087456) and RF positivity or anti-cyclic citrullinated peptide positivity.

Although our results indicate that the MHC2TA –168A/G polymorphism (rs3087456) does not increase risk for RA, additional functional variants may exist in *MHC2TA*. Three studies have investigated other *MHC2TA* polymorphisms.^{10–13} Eyre *et al*¹⁰ identified five frequency validated SNPs from public databases mapping to the 5' region of the gene (rs7501204, rs6498114, rs6416647, rs7404672, rs6498116) and performed single-locus and haplotype analyses in 813 patients with RA and 532 controls with negative findings. Swanberg *et al*⁷ evaluated haplotypes comprised of the MHC2TA –168A/G polymorphism (rs3087456) and two additional SNPs (rs4774 and rs34654419 (rs2229320plus27 bp) in exon 11) in 1288 RA cases and 709 controls; single-locus association results were not significant for these SNPs, and association with the MHC2TA –168A/G polymorphism (rs3087456) was not improved by haplotype analysis. These results are in contrast with evidence mentioned above for association with the MHC2TA –168A/G (rs3087456)/rs4774 haplotypes in 350 patients with RA and 509 controls.¹³ Our analysis of haplotype block structure utilising publicly available data suggest that at least 21 haplotype tagging SNPs are needed to fully capture common *MHC2TA* variation.

CONCLUSIONS

In this study, a meta-analysis of 6861 patients and 9270 controls was performed to assess association between the MHC2TA –168A/G promoter region polymorphism (rs3087456) and RA risk. Given that the large sample size of our study provided sufficient power to detect modest effects, our negative results indicate that the MHC2TA –168A/G polymorphism (rs3087456) is not causally associated with RA. Nevertheless, undiscovered functional variants may exist in the *MHC2TA*. In conclusion, a complex role for *MHC2TA* in RA, in conjunction with SE carrier status, remains plausible. Future studies that incorporate SE carrier status, secondary phenotypes and a more comprehensive screening of *MHC2TA* variation with haplotype tagging SNPs will help elucidate what role, if any, *MHC2TA* plays in RA.

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Figure 1.

Summary odds ratio (OR) and 95% confidence interval (CI) for the G risk allele (A) and the GG risk genotype (B) in patients with rheumatoid arthritis and controls in a meta-analysis of the MHC2TA -168A/G polymorphism (rs3087456) and rheumatoid arthritis risk.

Table 1

Genotype and allele frequencies in patients with RA and controls for 10 studies included in a meta-analysis of the MHC2TA -168A/G polymorphism (rs3087456) and RA risk

Reference	Cases/controls	Genotypes, % (n)			Alleles, %		Effect: OR (95% CI), p value
		AA	AG	GG	A	G	
Akkad <i>et al</i> ⁹	319	55.2 (176)	38.9 (124)	5.9 (19)	74.6	25.4	Neutral
Germany	463	53.8 (249)	39.5 (183)	6.7 (31)	73.5	26.5	
Eyre <i>et al</i> ¹⁰	1401	54.2 (760)	39.8 (557)	6.0 (84)	74.1	25.9	*Neutral
UK	2476	56.2 (1391)	37.2 (922)	6.6 (163)	74.8	25.2	
Harrison <i>et al</i> ¹¹	733	55.0 (404)	37.4 (274)	7.5 (55)	73.8	26.2	Neutral
UK	613	54.8 (336)	39.5 (242)	5.7 (35)	74.6	25.4	
Ikuni <i>et al</i> ¹²	1121	2.8 (31)	22.6 (253)	74.7 (837)	14.0	84.0	Susceptible GG genotype: 1.47 (1.16 to 1.87), p = 0.001
Japan	450	3.3 (15)	30.0 (135)	66.7 (300)	18.3	81.7	
Martinez <i>et al</i> ¹³	350	52.9 (185)	40.3 (141)	6.9 (24)	73.0	27.0	Neutral.
Spain	519	57.0 (296)	37.0 (192)	6.0 (31)	75.5	24.5	[†] Susceptible G allele: 1.81 (1.10 to 2.97), p = 0.01
Orozco <i>et al</i> ¹⁴	287	39.7 (114)	40.8 (117)	19.5 (56)	60.1	39.9	Neutral
Argentina	287	38 (109)	48.0 (139)	13.6 (39)	62.2	37.8	
Orozco <i>et al</i> ¹⁴	748	59.4 (444)	35.0 (262)	5.6 (42)	76.9	23.1	Protective G allele: 0.76 (0.62 to 0.94), p = 0.01
Spain	676	52.7 (356)	40.5 (274)	6.8 (46)	72.9	27.1	
Orozco <i>et al</i> ¹⁴	278	57.6 (160)	33.8 (94)	8.6 (24)	74.5	25.5	Neutral
Sweden	478	55.4 (265)	38.5 (184)	6.1 (29)	74.7	25.3	
Swanberg <i>et al</i> ⁷	1262	57.7 (728)	35.7 (451)	6.6 (83)	75.6	24.4	Susceptible G allele: 1.19 (1.02 to 1.38), p = 0.02
Sweden	1599	61.9 (989)	33.0 (528)	5.1 (82)	78.4	21.6	
Yazdani-Biuki <i>et al</i> ¹⁵	362	50.8 (184)	42.8 (155)	6.4 (23)	72.2	27.8	Protective GG genotype: 0.58 (0.34 to 0.99), p = 0.04
Austria	1709	52.3 (894)	39.1 (669)	8.5 (146)	71.9	28.1	
Meta-analysis	6861	46.4 (3186)	35.4 (2428)	18.2 (1247)	64.1	35.9	
All studies	9270	52.9 (4900)	37.4 (3468)	9.7 (902)	71.6	28.4	

*The minor allele frequency for rs3087456 was reported incorrectly as the major allele frequency (A. Barton, pers. comm.).

[†]In a haplotype with rs4774 in patients with one or more shared epitope alleles.

Table 2

ORs and 95% CIs for the G risk allele and the G risk genotype in patients with RA and controls for 10 studies included in a meta-analysis of the MHC2TA –168A/G polymorphism (rs3087456) and RA risk

Reference	GG+AG vs AA		GG versus AG+AA	
	OR (95% CI)	p Value	OR (95% CI)	p Value
Akkad <i>et al</i> ⁹	0.95 (0.71 to 1.26)	0.70	0.88 (0.49 to 1.59)	0.68
Germany				
Eyre <i>et al</i> ¹⁰	1.08 (0.95 to 1.23)	0.24	0.91 (0.69 to 1.19)	0.47
UK				
Harrison <i>et al</i> ¹¹	0.99 (0.80 to 1.23)	0.91	1.34 (0.86 to 2.08)	0.19
UK				
Iikuni <i>et al</i> ¹²	1.21 (0.65 to 2.27)	0.55	1.47 (1.16 to 1.87)	0.001
Japan				
Martinez <i>et al</i> ¹³	1.18 (0.90 to 1.56)	0.22	1.16 (0.67 to 2.01)	0.60
Spain				
Orozco <i>et al</i> ¹⁴	0.93 (0.66 to 1.30)	0.67	1.54 (0.99 to 2.41)	0.06
Argentina				
Orozco <i>et al</i> ¹⁴	0.76 (0.62 to 0.94)	0.011	0.81 (0.53 to 1.25)	0.35
Spain				
Orozco <i>et al</i> ¹⁴	0.92 (0.68 to 1.24)	0.57	1.46 (0.83 to 2.57)	0.18
Sweden				
Swanberg <i>et al</i> ⁷	1.19 (1.02 to 1.38)	0.024	1.30 (0.95 to 1.78)	0.10
Sweden				
Yazdani-Biuki <i>et al</i> ¹⁵	1.06 (0.85 to 1.33)	0.61	0.73 (0.46 to 1.15)	0.17
Austria				
Meta-analysis	1.02 (0.93 to 1.12)	0.70	1.14 (0.95 to 1.36)	0.16
All studies				