TLR4 polymorphism is not associated with biopsy proven giant cell arteritis

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ABSTRACT

Objective. *Giant cell arteritis (GCA) is a systemic inflammatory vasculitis affecting the elderly. It primarily affects medium and large arteries of the head and neck and can cause stroke and blindness. The cause of GCA is unknown; however both genetic and environmental factors are likely to be involved. TLR4 is implicated in the pathogenesis of GCA, however previous studies, examining the association between GCA and two* TLR4 *single nucleotide polymorphisms (SNPs), have reported conflicting results. The aim of this study was to determine the association between GCA and range of SNPs spanning the* TLR4 *gene sequence.*

Methods. *A case-control genetic study was performed using DNA from Australian biopsy proven GCA patients (n=139) and population controls (n=130). Samples were genotyped for 8 SNPs tagging common variation across* TLR4. *These SNPs included rs4986790 (+896A/G, Asp299Gly) and rs4986791 (+1196C/T) which have been previously studied in GCA. Allelic and haplotypic variation was analysed by logistic regression assuming an additive genetic model. A random effects meta-analysis of the association between GCA and rs4986790 was performed utilising data from three previous studies.*

Results. *rs4986790 and rs4986791 are in strong linkage disequilbrium and tag one of the five common* TLR4 *haplotypes identified. No associations were observed between* TLR4 *SNPs and/or haplotypes and GCA. A meta-analysis, comprising 577 GCA patients and 1153 controls, did not confirm an association between GCA and rs4986790 (OR 1.29, 95% CI 0.86, 1.92, p=0.22).*

Conclusion. *There is no evidence of an association between* TLR4 *polymorphism and susceptibility to GCA.*

Introduction

Giant cell arteritis (GCA), also known as temporal arteritis, is the most common form of systemic inflammatory vasculitis (1). It primarily affects medium to large extra-cranial arteries of the head and neck, and may result in stroke or blindness. The typical clinical manifestations of GCA are headache, jaw claudication and visual loss (2), and glucocorticoids remain the cornerstone of treatment. GCA primarily affects people aged over 50 years from European and Scandinavian Caucasian backgrounds, and females are 2-3 times more likely to be affected than males (3). Reported incidence rates vary widely, and are highest in people from Scandinavian backgrounds (>17 per 100,000 individuals aged >50 years) (3).

The underlying process of GCA is a granulomatous inflammation accumulating within the vessel wall, typically with granulomas and multinucleated giant cells of a diffuse lympho-monocytic infiltrate (4). However, the pathogenesis of GCA is not understood, although environmental, infectious and genetic risk factors are implicated. Familial ag-

Table I. Demographic and presenting symptoms of patients with giant cell arteritis. Clinical data at presentation was not available for all patients.

Age at diagnosis, median (range)	74 years	(51-94)
Gender, % Female	88/139	(63%)
Temporal artery biopsy positive	139/139	(100%)
Temporal headache	70/77	(65%)
Other headache	44/81	(54%)
Scalp tenderness	37/78	(47%)
Jaw claudication	46/78	(58%)
Visual disturbance	57/78	(73%)
Blindness (monocular and bilateral)	21/71	(30%)
Stroke	2/85	(2%)
PMR	22/43	(51%)

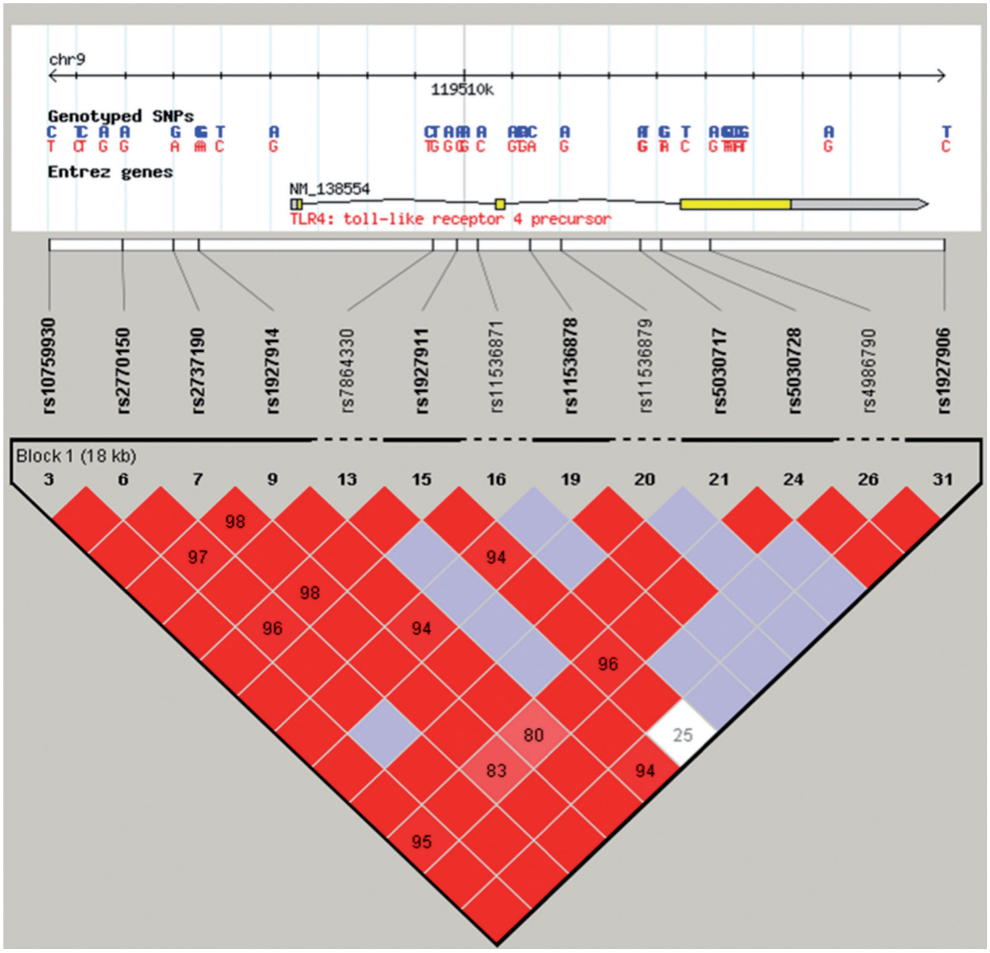

Fig. 1. Pairwise linkage disequilibrium (LD) analysis of Hapmap SNPs (version 3, release 2, CEU population) for the region spanning the TLR4 gene (Chr9, 119498-119520). The intensity of the red shading is proportional to pairwise LD estimates between SNPs. Grey shading denotes rare SNPs. A single linkage disequilibrium block was identified which spanned the entire TLR4 gene.

gregation and established associations with *HLA-DR4* provide evidence for a genetic component to GCA (5-7). Multiple genetic association studies have been performed on a number of immune response genes; however, these studies have utilised, almost exclusively, GCA cohorts of Southern European origin (Spanish and Italian), with little or no replication of positive results in other cohorts (reviewed in (8)). One gene that remains of interest is Toll-like receptor 4 (*TLR4),* with three previous studies reporting on the association between rs4986790 (+896A/G, Asp299Gly) and GCA in Southern European patients, with conflicting results (9-11). We therefore examined the association between GCA and common polymorphism across the entire *TLR4* gene sequence, using a tagging SNP approach, in Australian GCA patients.

Methods

GCA patients

One hundred and thirty-nine Australian biopsy proven GCA patients were recruited through the South Australian Giant Cell Arteritis Registry and The Royal Victorian Eye and Ear Hospital. The characteristics of these patients are reported in Table I. This study has ethics approval from the Queen Elizabeth Hospital, Royal Adelaide Hospital, Repatriation General Hospital and Flinders Medical Centre in South Australia, and the Royal Victorian Eye and Ear Hospital in Victoria, and all participants provided written, informed consent. A total of 130 Australian population controls were used for comparison.

TLR4 genotyping

Eight SNPs spanning the *TLR4* gene sequence were selected for genotyping. The SNPs chosen for analysis were selected on the basis of previous genetic studies in conjunction with a tagging SNP approach utlilising Hapmap version 3 release 2 and CEPH (Utah residents with ancestry from northern and western Europe, CEU) SNP data (12) and Haploview software (13). A single linkage disequilibrium block spanning *TLR4* (Chr9, 119498-119520) was identified (Fig. 1). This region included 13 SNPs with a minor allele frequency >0.05. Tagging analysis selected 7 SNPs, one of which had previously been studied in GCA (rs4986790) (9-11). This tagging approach captured 84% of Hapmap *TLR4* SNP alleles with an r^2 of 0.98. An additional non-Hapmap *TLR4* SNP, previously studied in GCA, was also genotyped (rs4986791, +1196 C/T) (9, 10). Details of these SNPs including rs number, chromosome position and alleles encoded are summarised in Table II. *TLR4* genotyping was performed by the Australian Genome Research Facility (AGRF) (Brisbane, Queensland, Australia) on the SEQUENOM MassARRAY® iPLEX® Gold platform. One DNA sample failed all genotyping tests and was excluded from the analysis; the remaining success rate was 100% across all 8 SNPs.

Table II. Details of the eight SNPs selected for genotyping showing rs number, chromosome, base pair position and alleles encoded.

rs Number	Gene	Chromosome	Base pair position	Minor Allele	Major Allele	Alleles captured
rs2770150	TLR4	9	120463139	C	T	rs2770150, rs5030728
rs1927914	TLR4	9	120464725	C	T	rs1927914, rs2737190
rs1927911	TLR4	9	120470054	T	C	rs1927911
rs11536878	TLR4	9	120471553	A	C	rs11536878
rs5030717	TLR4	9	120473834	G	A	rs5030717
rs4986790	TLR4	9	120475302	G	A	rs4986790, rs7864330
rs4986791*	TLR4	9	120475602	T	C	NA
rs1927906	TLR4	9	120480115	G	A	rs1927906

*rs4986791 is a non-Hapmap SNP.

Table III. *TLR4* SNP allele frequencies in GCA patients and controls.
Odds ratios (OR) and *p*-values were derived assuming an additive genetic model.

		Minor Allele Frequencies (n=269)			
SNP	Minor Allele	Controls (n=130)	GCA (n=139)	OR (95% CI)	*p*-value
rs2770150	C	0.32	0.26	0.72 (0.49, 1.06)	0.09
rs1927914	C	0.28	0.35	1.36 (0.94, 1.97)	0.11
rs1927911	T	0.20	0.26	1.44 (0.94, 2.19)	0.09
rs11536878	A	0.09	0.12	1.36 (0.79, 2.34)	0.27
rs5030717	G	0.09	0.10	1.16 (0.64, 2.09)	0.63
rs4986790	G	0.08	0.08	0.93 (0.49, 1.74)	0.81
rs4986791	T	0.09	0.09	0.97 (0.53, 1.80)	0.93
rs1927906	G	0.10	0.12	1.16 (0.68, 1.97)	0.59

Statistical analysis

TLR4 SNPs were analysed by logistic regression assuming an additive genetic model using Plink (version 1.07) software (14). *TLR4* haplotypes were defined and analysed using the R library haplo.stats (15), again assuming an additive genetic model. Random effects meta-analysis of the association between GCA and rs4986790 was performed using the R library metafor (16), under an additive genetic model. Effect sizes were reported as odds ratios (OR) with 95% confidence intervals (95% CI).

Results

TLR4 SNP and haplotype frequencies are comparable between GCA patients and controls

All *TLR4* SNPs were in Hardy-Weinberg equilibrium in both GCA patients and controls. Allele frequencies for all 8 *TLR4* SNPs are comparable between GCA patients and controls (Table III). The 8 *TLR4* SNPs formed five common population haplotypes (with overall frequency greater than 2%, Table IV). The two SNPs, previously studied in GCA, rs4986790 and rs4986791, were in strong linkage disequilibrium (D'= 1.0, $r^2 = 0.93$), and the minor alleles of these SNPs tagged haplotype 5. There were however no differences in haplotype frequencies between either GCA patients or controls (Table IV).

Meta-analysis of the association between TLR4 rs4986790 and GCA

The results of this study for the rs4986790 association with GCA were combined with three previous studies ((9-11)] for a meta-analysis (Fig. 2), utilising a total of 577 GCA patients and 1153 controls. While the two earlier studies, Boiardi 2009 (10) and Palomino Morales 2009 (11), in Italian and Spanish patients, respectively, were suggestive of an association between GCA and rs4986790 G (10, 11), our study in Australian Caucasian patients was comparable to the more recent Alvarez 2011 study (9), also in Spanish patients, which reported no association between TLR4 rs4986790G and GCA. In the combined random-effects meta-

Table IV. TLR4 haplotype frequencies in GCA patients and controls. Haplotype frequencies were estimated using the expectation-maximisation (EM) algorithm. Odds ratios (OR) and *p*-values were derived assuming an additive genetic model. Minor alleles for each SNP are depicted by dark shading. Five common haplotypes (with a frequency >2%) were identified. Haplotype 1, the most common haplotype, carried the major alleles for each SNP, Haplotype 2 was tagged by the minor allele of rs2770150, Haplotype 3 was tagged by the minor allele of rs11536878, Haplotype 4 was tagged by the minor allele of rs5030717, and Haplotype 5 was tagged by the minor alleles of rs4987690, rs4986791 and rs1927906. There were no significant differences between haplotype frequencies between GCA patients and controls.

Haplotype	Sequence	rs2770150	rs1927914	rs1927911	rs11536878	rs5030717	rs4986790	rs4986791	rs1927906	Haplotype Frequencies (N = 269)		OR (95% CI)	p-val
										Controls (N=130)	GCA (N=139)		
Haplotype 1	TTCCAACA									0.40	0.39	1	0.87
Haplotype 2	CTCCAACA	■								0.31	0.26	0.81 (0.53, 1.24)	0.14
Haplotype 3	TCTAAACA		■	■	■					0.09	0.12	1.37 (0.76, 2.46)	0.23
Haplotype 4	TCTCGACA		■	■		■				0.09	0.10	1.12 (0.59, 2.13)	0.64
Haplotype 5	TCCCAGTG		■				■	■	■	0.08	0.08	0.93 (0.48, 1.80)	0.76

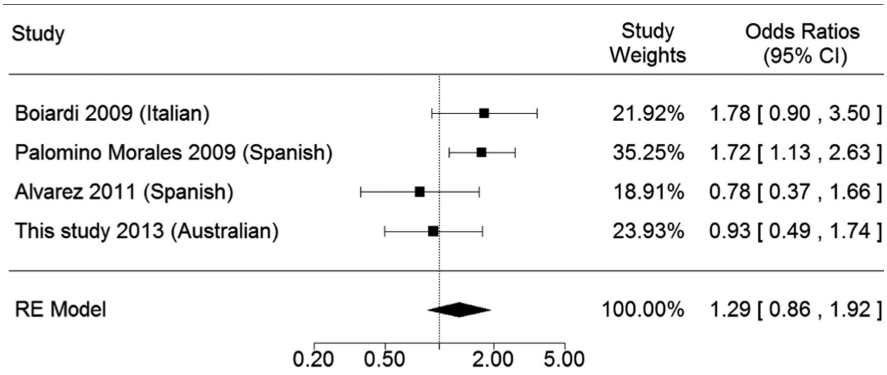

Fig. 2. Random Effects meta-analysis of the association between the rs4986790 G allele and Giant Cell Arteritis. Meta-analysis was performed utilising three previous studies with results from the present study, resulting in a total of 577 GCA patients and 1153 controls. GCA patients from the three previous studies (Boiardi 2009 (10), Palomino Morales 2009 (11) and Alvarez 2011 (9)) were of southern European origin. Odds ratios were derived for each study assuming an additive genetic model. The overall association was not significant, with a combined odds ratio of 1.29 (95% CI 0.86, 1.92, p=0.22). The residual heterogeneity (T2) between studies was 0.07.

analysis, there was no evidence of an association between rs4986790G and GCA (odds ratio 1.29, 95% CI 0.86, 1.92, p=0.22).

Discussion

TLR4 activation has been strongly implicated in the pathogenesis of GCA. Recent data suggests that innate immune reactions, driven by vascular dendritic cells (DC), induce arteritis and shape the subsequent T-cell mediated inflammatory response. Medium and large human arteries populated by DCs exhibit vessel specific profiles of pathogen-sensing Toll-like receptors (TLR). This repertoire is highly predisposed towards the sensing of bacterial pathogens, with both TLR2 and TLR4 abundantly expressed (17). TLR4 is further implicated by the observation that lipopolysaccharide (LPS, a TLR4 ligand) treatment induces vasculitis in human temporal arteries subcutaneously implanted into NOD-SCID mice (18). Genetic studies, focussing on the rs4986790 SNP, have also implicated *TLR4* in the pathogenesis of GCA in Southern European patients, although with conflicting results (9-11).

In this study, we investigated the relationship between *TLR4* polymorphism and GCA, in Australian patients. In addition to rs4986790 and rs4986791, previously studied in GCA, we evaluated six additional tagging SNPs to comprehensively evaluate common polymorphism across the entire *TLR4* gene sequence. We observed that rs49867790 and rs4986701 are in strong linkage disequilibrium and tag one of the 5 common haplotypes identified. However, no associations between *TLR4* SNPs or haplotypes and GCA were identified. Further, in the combined random-effects meta-analysis of this, and three previous studies, there was no evidence of an association between rs4986790G and GCA (odds ratio 1.29, 95% CI 0.86, 1.92, p=0.22). However, an association of small effect size cannot definitively be excluded. Overall, whilst TLR4 activation has been clearly implicated in GCA pathogenesis, the weight of current evidence suggests that common genetic polymorphism in *TLR4* does not confer risk for susceptibility to GCA.

A limitation of our study is the relatively small sample size. In general, genetic association studies with GCA have been hindered by the difficulties in collection of DNA samples from elderly patients in an essentially rare, late onset, disease. Previously published genetic studies for GCA all have similarly small patient samples sizes, and indeed, there is a paucity of different GCA patient cohorts for this type of research. International collaboration will be essential to collect large patient datasets and samples, with prospective recruitment at the time of diagnosis optimal for capturing appropriate samples and accompanying clinical and laboratory data.

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