G/R 241 Polymorphism of Intercellular Adhesion Molecule 1 (*ICAM-1*) Is Associated with Fuchs Uveitis

Luca Cimino,[1,2] *Luigi Boiardi,*[3] *Raffaella Aldigeri,*[4] *Bruno Casali,*[5] *Davide Nicoli,*[5] *Enrico Farnetti,*[5] *Carlo Salvarani,*[3] *Daniele Cirone,*[2] *Liberatina De Martino,*[2] *Alessandro Pupino,*[2] *and Luca Cappuccini*[2]

Purpose. To investigate potential associations of the *ICAM-1* gene polymorphisms and Fuchs uveitis in a cohort of Italian patients.

Methods. Seventy-one consecutive Italian patients affected by Fuchs uveitis were observed at the Ocular Immunology Unit, Arcispedale S. Maria Nuova (Reggio Emilia, Italy) from 2002 to 2008. Two hundred twenty-six healthy Italian blood donors from the same geographic area were selected as the control group. All Fuchs uveitis patients and control subjects were genotyped by polymerase chain reaction (PCR) and allele-specific oligonucleotide techniques for *ICAM-1* polymorphisms at codon 241 (exon 4).

Results. The frequency of the *ICAM-1* G/R 241 polymorphism was significantly higher in Fuchs uveitis than in the control subjects (16.9% vs. 5.8%; $P = 0.006$, $P_{corr} = 0.012$; odds ratio, 3.3; 95% confidence interval, 1.4–7.7). No significant association between clinical features and *ICAM-1* polymorphisms was found.

Conclusions. This study demonstrates for the first time that the *ICAM-1* G/R 241 polymorphism may represent a candidate gene for Fuchs uveitis susceptibility. (*Invest Ophthalmol Vis Sci.* 2010;51:4447–4450) DOI:10.1167/iovs.09-4669

Intercellular adhesion molecule-1 (ICAM-1; CD54), a 90-kDa member of the immunoglobulin (Ig) superfamily, is expressed on the surface of several cell types including leukocytes, endothelial cells, and proliferating intimal smooth muscle cells. Its ligands are the membrane-bound β2-integrin receptors LFA-1 (CD11a, CD18) and Mac-1 (CD11b, CD18) on leukocytes, although several other ligands have been described.[1] ICAM-1 is critical in the firm arrest and transmigration of leukocytes out of blood vessels and into tissues.[2] ICAM-1 is present on endothelial cells and all leukocytes, where it acts both as an adhesion[2] and a signaling[3–6] molecule, and its expression is enhanced by different cytokines, including tumor necrosis factor (TNF), interferon-γ, and interleukin-1. Endothelial expression of ICAM-1 is increased in atherosclerotic and transplant-associated atherosclerotic tissue and in animal models of atherosclerosis,[7–9] and it has been implicated in the progression of autoimmune diseases, including rheumatoid arthritis, multiple sclerosis, and type I diabetes.[10–13]

Several ICAM-1 coding region biallelic polymorphisms have been described in the human *ICAM-1* gene, including those located in exons 4 and 6, thereby modifying codons 241 and 469, respectively.[14] G241R (glycine/arginine) is located within the third Ig-like domain, shown to be the Mac-1 binding domain,[15] and therefore is of potential importance during leukocyte transmigration. The hypothesis is that, since the *ICAM-1* gene polymorphisms are located in exons and lead to amino acid substitutions, they could alter ligand binding or the stability of the multimeric ICAM-1 on the cell surface and therefore alter signal transduction. Many studies have described associations between *ICAM-1* polymorphisms and chronic inflammatory disorders, including ulcerative colitis and Crohn's disease,[16,17] polymyalgia rheumatica,[18] type I diabetes,[19] multiple sclerosis,[20] chronic allograft vasculopathy,[21,22] peripheral occlusive vascular disease,[23,24] and myocardial infarction.[25] However, there is no consensus as to which alleles are protective and which are detrimental. Interpretation of these clinical associations is hindered by lack of understanding of the functional significance of *ICAM-1* polymorphisms.

Fuchs uveitis is a chronic inflammatory eye disease of unknown etiology that usually presents as a unilateral anterior uveitis in young adults. It is often characterized by an insidious onset and is frequently misdiagnosed; complications include cataract formation and secondary glaucoma. Several theories have been suggested regarding its etiology, including an immunologic disorder characterized by elevated levels of immunoglobulin G in aqueous humor of patients[26,27] and $CD8^+$ cells.[28] Recent findings have suggested that an infectious agent such as *Toxoplasma gondii*[29,30] or Rubella virus[31,32] could act as a trigger of eye inflammation.

ICAM-1 has been shown to play an important role in the induction and development of inflammation in uveitis in both in vitro and in vivo animal studies.[33–36] La Heij et al.[33] found a significantly increased expression of adhesion molecules in the iris of patients affected by uveitis, including Fuchs uveitis, suggesting an immunoregulatory function for adhesion molecules in the pathogenesis of uveitis.

We postulated that the polymorphism of the *ICAM-1* gene (−241) represents a candidate gene for disease susceptibility in Fuchs uveitis.

Materials and Methods

Subjects

The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the local institutional review board. Informed consent was obtained from patients and control subjects before they were included in the study.

From the [1]Ocular Immunology Unit, [2]Ophthalmology Unit, [3]Rheumatology Unit, and [5]Molecular Biology Laboratory, Arcispedale S Maria Nuova Reggio Emilia, Italy; and the [4]Institute of Ophthalmology, University of Parma, Parma, Italy.

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Corresponding author: Luca Cimino, Ocular Immunology Unit, Ophthalmology, Unit, Arcispedale S Maria Nuova Reggio, Viale Risorgimento, 80 Reggio, Emilia, 42123 Italy; l.cimino@libero.it.

Table 1. Demographic and Clinical Features of Patients with Fuchs Uveitis

	Patients (n = 71)	
	n	%
Male	39	54.9
Female	32	45.1
Mean age in years ± SD	42 ± 15	—
Keratic precipitates	53	74.6
Iris atrophy	65	91
Heterochromia	35	49.3
Cataract	26	36.6
Glaucoma	9	12.7
Vitreitis	63	88.7
Unilateral	65	91.5
Vitrectomy	4	5.6

From May 2002 to June 2008, 71 consecutive patients with Fuchs uveitis were examined at the Immunology Ocular Unit, Arcispedale S. Maria Nuova Hospital (Reggio Emilia, northern Italy). Demographic data and ocular findings (Table 1) and laboratory tests were recorded in a computer database.

Details on laterality, chronicity, and ocular signs were included. Patients with uveitis and associated underlying systemic diseases were excluded. The study included 226 healthy subjects who were unrelated blood donor volunteers.

The diagnosis of Fuchs uveitis was based on the presence of the following clinical features: unilateral uveitis involving the anterior segment and the vitreous body, absence of acute symptoms (pain, photophobia), characteristic Fuchs stellate keratic, sparsely distributed precipitates, diffuse iris stromal atrophy with or without heterochromia, vitritis, absence of synechiae, and the absence of cystoid macular edema.[37]

All the study subjects were Caucasians residing in Italy for at least one generation. No ethnic differences were present between patients and control subjects.

Molecular Analysis of the *ICAM-1* Polymorphism

Genomic DNA was extracted by phenol-chloroform standard method from 500 μL whole blood collected in edetic acid. To detect the substitution of arginine for glycine responsible for the *ICAM-1* polymorphism G/R 241 in exon 4 of the *ICAM-1* gene, we developed an allele-specific polymerase chain reaction (ASPCR) method. We designated allele-specific primers 5′-CGTGGTCTGTTCCCTGGACG-3′, 5′-CGTGGTCTGTTCCCTGGACA-3′ (nucleotide number 638-657), using published sequence information on the point mutation of the *ICAM-1* gene,[38] and common primer 5′-GTCGTTGCCATAGGTGACTG-3′8. As an internal positive control, an additional primer pair for the glycoprotein IIIa gene[39] was used in all ASPCRs, with a pair of primers consisting of an allele-specific primer and a common primer, according to the method of Bein et al.[40] The positive control primer pair amplified the 247-bp fragment of glycoprotein IIIa gene. The ASPCR was performed in a total volume of 50 μL that contained 0.2 μg genomic DNA, each primer pair consisting of 20 picomoles of allele-specific primer and 20 picomoles of common primer, 15 picomoles of each positive control primer, 200 μM each dNTP, 10 mM Tris-HCl (pH 8.3), 50 nM KCl, 1.5 mM $MgCl_2$, and 1.5 units of DNA polymerase (AmpliTaq; Perkin-Elmer, Waltham, MA). The PCR reaction was performed for 35 cycles, each consisting of a denaturation step at 95°C for 30 seconds, 68°C for 30 seconds, and 72°C for 30 seconds in a PCR system (GeneAmp PCR System 9600; Perkin Elmer). The amplified PCR products were analyzed by 2% agarose gel electrophoresis followed by ethidium bromide staining and ultraviolet visualization.

Statistical Analysis

The frequencies of the alleles and genotypes between patient and control groups were compared by means of χ^2 test. Odds ratios (ORs) were calculated together with 95% confidence intervals (CIs). Corrected P values were calculated by multiplying P by the number of alleles compared (SPSS statistical package; SPSS Inc., Chicago, IL).

Statistical analysis to define population sample size and statistical power was performed with PAWE software (PAWE version 1.2, February 2003 written by Derek Gordon assisted by Michael Nothnagel, Rockefeller University, New York, NY).[41–45]

Results

The demographic and clinical features of patients are reported in Table 1. Thirty-nine (55%) patients were male and 32 (45%) were female. The mean age was 42 years (range, 8–78 years). Eye findings included stellate keratic precipitates in 53 (75%) cases, vitritis in 63 (89%), iris atrophy in 65 (91%), and heterochromia in 35 (49%). Fuchs uveitis was mostly unilateral (91%). Twenty-six (14%) patients presented with cataract and nine (13%) with glaucoma.

The allele and genotype frequencies of G/R 241 in patients and controls are reported in Table 2.

Allele R was significantly more frequent in the uveitis patients than in the control subjects (P = 0.001, P_{corr} = 0.002; OR, 3.7; 95% CI, 1.7–7.9), and the carriage rate of R241 was significantly higher in the patients than in the control subjects (16.9% vs. 5.8%; P = 0.006; P_{corr} = 0.012; OR, 3.3; 95% CI, 1.4–7.7). Furthermore, the distribution of the G/R 241 geno-

Table 2. Frequencies of Alleles, Genotypes, and Carriage Rates of *ICAM-1* G/R 241 Polymorphism in Fuchs Uveitis Cases and Controls

	Fuchs Uveitis (n = 71)		Controls (n = 226)				
	n	%	n	%	P	P_{corr}	OR (95% CI)
Allele							
R	15/142	10.6	14/452	3.1	0.001	0.002	3.7 (1.7–7.9)
G	127/142	89.4	438/452	96.9			
Genotype							
R/R	03/71	4.2	01/226	0.4	0.005	0.015	
R/G	09/71	12.7	12/226	5.3			
G/G	59/71	83.1	213/226	94.2			
Carriage rate							
R/R+R/G	12/71	16.9	11/226	5.8	0.006	0.012	3.3 (1.4–7.7)
G/G	59/71	83.1	213/226	94.2			
R/R	3/71	4.2	1/226	0.4	0.044	0.088	9.9 (1.0–97.0)
R/G+G/G	68/76	95.8	225/226	99.6			

type differed significantly between the patients and control subjects ($P = 0.005$; $P_{corr} = 0.015$).

Sample size and the allele frequencies of the G/R 241 polymorphism provided a statistical power of more than 90% for genotypic test and more than 95% for allelic test. No association was found between clinical features and the frequencies of genotypes.

Discussion

Intercellular adhesion molecules play an important role in the recruitment of specific T lymphocytes from the blood stream into inflamed tissue. ICAM-1 has been proven to be important in the induction and development of inflammation in uveitis, whether or not it is associated with systemic disease, both in vitro and in vivo animal studies. Furthermore, Whitcup et al.[46] reported that serum ICAM-1 levels were higher in patients with uveitis than in normal control subjects and were significantly higher in patients with uveitis associated with an underlying systemic disease than in those without systemic disease. In contrast, other studies reported that serum ICAM-1 levels in accompanying systemic disease were similar to those in isolated uveitis.[47–49] To date, ICAM polymorphisms, particularly G/R 241 polymorphism, have been studied in Behçet disease, polymyalgia rheumatica/giant cell arthritis, and rheumatoid arthritis.[50–52] We hypothesized that G/R 241 polymorphism of ICAM-1 could be associated with Fuchs uveitis. The molecular analysis of ICAM-1 gene confirmed that G/R 241 polymorphism frequency was higher in patients affected by Fuchs uveitis than in control subjects, confirming that it could represent a risk factor for this ocular entity.

A recent experimental study on the functional significance of *ICAM-1* polymorphisms, in terms of cell surface expression of ICAM-1 and leukocyte adhesion to human endothelial cells, confirmed that the genotype G241/E469 was associated with greater cell surface expression, leading to greater adhesion of leukocytes.[53] This finding could explain the described associations of ICAM polymorphisms with chronic inflammatory disease.

In their study on the correlation between genetic polymorphism and circulating soluble ICAM-1 in a healthy population, Ponthieux et al.[54] confirmed a significant association between R241 allele and lower serum ICAM-1 levels, probably due to the binding impairment of ICAM-1 to leukocyte integrin Mac-1 protein. We found a significant increase in the R241 allele in Fuchs uveitis in our population compared with that in the control subjects, and this increase may explain the chronic low-grade inflammation response in this disease.

Most Fuchs patients present with minimal anterior chamber inflammation, and therapy is not required. Symptomatic flare-ups may require short-term topical corticosteroids. However, long-term therapy is not indicated. Theoretically, variations of serum protein levels may be influenced by disease activity, systemic manifestations or systemic therapy. These fluctuating influences may explain why our results are different from those in earlier studies of serum ICAM-1 levels. On the other hand, disease activity and systemic therapy should not affect gene polymorphisms, as reported in our study.

To our knowledge, an association between *ICAM-1* polymorphisms and Fuchs uveitis has never been reported. Since Fuchs uveitis represents a frequent cause of uveitis in our region, and the etiology is still unknown, this study could be a first step in understanding whether this polymorphism represents a risk factor for this ocular disease.

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