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CCR5 chemokine receptor gene polymorphisms in ocular toxoplasmosis

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ABSTRACT

C–C chemokine receptor type 5 (CCR5) is a chemokine receptor that influences the immune response to infectious and parasitic diseases. This study aimed to determine whether the CCR5Δ32 and CCR5 59029 A/G polymorphisms are associated with the development of ocular toxoplasmosis in humans. Patients with positive serology for *Toxoplasma gondii* were analyzed and grouped as 'with ocular toxoplasmosis' (G1: n = 160) or 'without ocular toxoplasmosis' (G2: n = 160). A control group (G3) consisted of 160 individuals with negative serology. The characterization of the CCR5Δ32 and CCR5 59029 A/G polymorphisms was by PCR and by PCR-RFLP, respectively. The difference between the groups with respect to the mean age (G1: mean age: 47.3, SD ± 19.3, median: 46 [range: 18–95]; G2: mean age: 61.3, SD ± 13.7, median: 61 [range: 21–87]; G3: mean age: 38.8, SD ± 17.9, median: 34 [range: 18–80]) was statistically significant (G1 vs.G2: p-value < 0.0001; t = 7.21; DF = 318; G1 vs.G3: p-value < 0.0001; t = 4.32; DF = 318; G2 vs. G3: p-value < 0.0001; t = 9.62; DF = 318). The Nagelkerke r^2 value was 0.040. There were statistically significant differences for the CCR5/CCR5 (p-value = 0.008; OR = 0.261), AA (p-value = 0.007; OR = 2.974) and AG genotypes (p-value = 0.018; OR = 2.447) between G1 and G2. Individuals with the CCR5/CCR5 genotype and simultaneously the CCR5-59029 AA or AG genotypes have a greater risk of developing ocular toxoplasmosis (4% greater), which may be associated with a strong and persistent inflammatory response in ocular tissue.

1. Introduction

Ocular toxoplasmosis (OT) is the most common cause of posterior uveitis. Its severity may vary according to the immune system of each patient and the reactivation of latent parasites within the retina triggering necrotizing retinopathy and leading to visual impairment (de-la-Torre et al., 2014). The lesions usually heal within two to four months in immunocompetent patients leaving a hyper-pigmented scar. In more than 70% of cases of patients seeking an ophthalmologist, OT lesions that have healed are associated with other injuries (Maenz et al., 2014). Some years ago, we demonstrated that OT represents 27% of ocular diseases among patients from the northwestern region of São Paulo State, Brazil (Ferreira et al., 2014).

CCR5 is a chemokine receptor expressed on several cells with immune function whose role consists in the recruitment and mobilization of cells to sites of inflammation (Silva-Carvalho et al., 2016). The CCR5Δ32 polymorphism, characterized by a deletion of 32 nucleotides, results in a low expression of a non-functional protein on the cell surface (Gupta and Padh, 2015; Silva-Carvalho et al., 2016). Studies have demonstrated that CCR5-deficient murine animals have increased susceptibility to *T. gondii* infection as well as an increase in the number of parasites in the liver and intestine (Bonfá et al., 2014). Individuals with

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Table 1
Characteristics and Frequencies of genotypes and alleles of the polymorphisms of the *CCR5* gene in individuals with ocular toxoplasmosis without ocular toxoplasmosis and controls.

	OT (G1) (n = 160)		Without OT (G2) (n = 160)		Controls (G3) (n = 160)		p
Mean age (± SD)	47.3 ± 19.3[a,b]		61.3 ± 13.7[a,c]		38.8 ± 17.9[b,c]		p-value < 0.0001*
Min/Max	18–95		21–87		18–80		
Median	46		61		34		
	n	%	n	%	n	%	
Genotypes							
CCR5/CCR5	141	88.1	148	92.5	144	90.0	
CCR5/CCR5Δ32	19	11.9	12	7.5	16	10.0	
Alleles							
CCR5	301	94.1	308	96.2	304	95.0	
CCR5Δ32	19	5.9	12	3.8	16	5.0	
Genotypes							
CCR559029 A/A	48	30.0	39	24.4	47	29.4	
CCR559029 A/G	81	50.6	80	50.0	81	50.6	
CCR559029 G/G	31	19.4	41	25.6	32	20.0	
Alleles							
CCR559029 A	177	55.3	158	49.4	175	54.7	
CCR559029 G	143	44.7	162	50.6	145	45.3	

OT = Ocular toxoplasmosis.
a = G1xG2.; b = G1xG3; c = G2xG3.
* G1xG2; G1xG3; G2xG3 p-value < 0.0001.

the AA genotype, which relates to the CCR5 promoter polymorphism 59029, show higher CCR5 expression on the leukocyte surface when compared to the other genotypes (Oliveira et al., 2015).

These polymorphisms have been correlated with susceptibility to various infectious diseases including HIV and inflammatory diseases such as osteomyelitis, preeclampsia, rheumatoid arthritis and systemic lupus erythematosus (Rao et al., 2014; Silva-Carvalho et al., 2016; Gupta and Padh, 2015; Souza et al., 2015). The aim of this study was to investigate possible associations of the *CCR5*Δ32 (rs333) and *CCR5* 59029 A/G (rs1799987) polymorphisms with the development of OT in humans.

2. Materials and methods

2.1. Ethics information

All individuals, who agreed to participate in this research, were informed about the nature of the study and were required to sign an informed consent form authorizing the use of their samples. The study was approved by the Research Ethics Committee of the Medicine School in São José do Rio Preto (case #1980/2009).

2.2. Sample selection and clinical diagnosis

This study enrolled 320 immunocompetent patients with serologically diagnosed toxoplasmosis (IgG anti-*T. gondii* antibodies) matched by gender, being treated in the Retinopathy Outpatient Service of Hospital de Base of the Medicine School in São José do Rio Preto (FUNFARME) and in the Medical Services Outpatient Clinic (AME) in São José do Rio Preto. Patients were grouped as 'with OT' (G1; n = 160) or 'without OT' (G2; n = 160). Patients 'without OT' had other ocular diseases without any evidence of OT. In order to verify the frequency of the alleles in the study population, a control group (G3) was formed of 160 healthy volunteer blood donors from the blood bank of São José do Rio Preto, whose serology results for antibodies against toxoplasmosis were negative.

The clinical evaluation of subjects was conducted by two experienced physicians using an indirect binocular ophthalmoscope (Binocular Ophthalmoscope ID10, Topcon Corporation, USA), and all were classified according to the ETDRS criteria (ETDRS, 1985).

2.3. Inclusion/exclusion criteria

The inclusion criteria of the patient groups were positive laboratory diagnosis of toxoplasmosis, the presence of ocular scars/lesions (G1) or without ocular scars/lesions due to toxoplasmosis (G2), and being a resident in a municipality in the northwestern region of the state of Sao Paulo. The inclusion criteria for the control group (G3) were negative laboratory diagnosis for toxoplasmosis and living in the same geographical region as the patients.

The exclusion criteria for all groups were other infectious and parasitic diseases, blood dyscrasia, and the use of oral anticoagulants.

2.4. Laboratory analysis

IgG anti-*T. gondii* antibodies were confirmed by enzyme-linked immunosorbent assay (ELISA) according to manufacturer's instructions, performed in duplicate (ETI-TOXO-G PLUS; DiaSorin S.p.A. Italy).

Genomic DNA was attained from peripheral blood using a commercial kit for silica column extraction (QIAamp1DNA Blood Mini Kit, QIAGEN, the Netherlands) following the manufacturer's instructions.

Identification of the deletion of 32 base pairs of the *CCR5* gene (CCR5Δ32) was achieved using the polymerase chain reaction (PCR) technique. The methodology used to identify the CCR5 59029 A/G polymorphism in the promoter region of the *CCR5* gene was polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The A/G alleles were identified by the presence of a restriction site for the Bsp1286I enzyme (FastDigest, Fermentas-Thermo Scientific). The PCR conditions were previously described in detail (de Oliveira et al., 2015).

2.5. Statistical analysis

Genotype and allelic frequencies were obtained by direct counting. Statistical calculations were performed using GraphPad Instat software (version 3.06). The chi-square test was used to compare proportions between groups, adopting a level of significance of 5%. The mean ages were compared using the *t*-test. The Hardy–Weinberg equilibrium was verified using the ARLEQUIN program version 3.11 (http://cmpg.unibe.ch/software/arlequin3/). A binary logistic regression test (stepwise method) was performed using the SPSS program (IBM, version 23)