GENETIC POLYMORPHISMS IN CYTOKINE GENES IN COLOMBIAN PATIENTS WITH OCULAR TOXOPLASMOSIS

Naranjo-Galvis CA [1,2], de-la-Torre A[3,5], Mantilla-Muriel LE[1], Beltrán-Angarita L[4], Elcoroaristizabal-Martín X[6], McLeod R[7], Alliey-Rodriguez N[8], Begeman IJ[7], López de Mesa C[9], Gómez-Marín JE[3], Sepúlveda-Arias JC[1]

[1]Grupo Infección e Inmunidad, Facultad de Ciencias de la Salud, Universidad Tecnológica de Pereira, Pereira, Colombia. [2]Facultad de Ciencias de la Salud, Universidad Autónoma de Manizales, Manizales, Colombia [3]Grupo GEPAMOL, Centro de Investigaciones Biomédicas, Universidad del Quindío, Armenia, Colombia.[4]Grupo de Farmacogenética, Facultad de Ciencias de la Salud, Universidad Tecnológica de Pereira, Pereira, Colombia. [5]Grupo de Investigación en Neurociencias (NeURos), Escuela de Medicina y Ciencias de la Salud, Universidad del Rosario, Bogotá, Colombia. [6]Genetracer Biotech. Santander (Spain) [7]Department of Ophthalmology and Visual sciences and Pediatrics (Infectious Diseases), The University of Chicago, Illinois, USA [8]Department of Psychiatry and Behavioral Neuroscience, The University of Chicago, Illinois, USA.[9]Escuela Superior de Oftalmología, Instituto Barraquer de América, Bogotá, Colombia.

INTRODUCTION

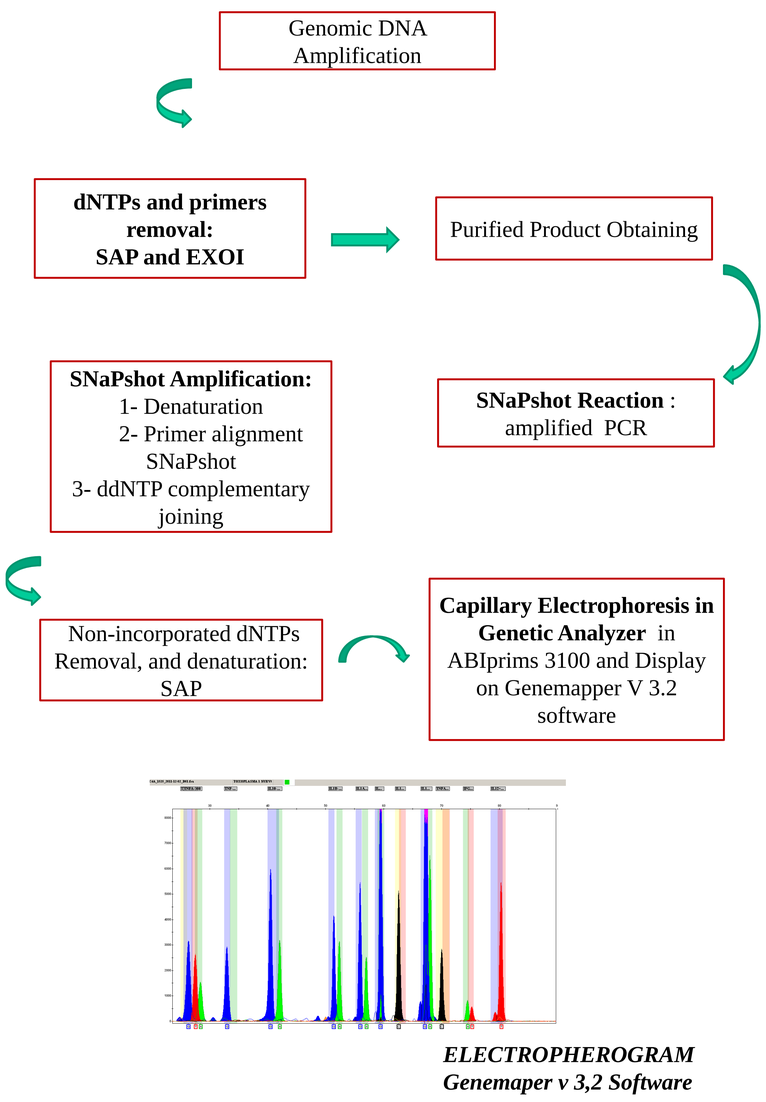
Toxoplasmosis is caused by infection with the protozoan parasite *Toxoplasma gondii* which has the capacity to infect all warm-blooded animals worldwide. It is estimated that 30-70% of the human population is infected with this parasite, and essentially the entire human population is at risk of infection. A limited number of people develop symptoms, suggesting that host susceptibility and strain disparity can play a role in the variability of clinical symptoms. For instance, in Colombia, where seroprevalence studies show that almost half of the population is infected (47% according a national study), the incidence of symptomatic ocular toxoplasmosis (OT) is of three new episodes per 100,000 inhabitants per year[5]. It is estimated that around 10% of newly infected people develop OT.

METHODS

Cases: 61 patients (age 37.37 ± 17.27, mean±SD), with confirmed-diagnosis of OT by aqueous humor PCR, with a male/female ratio = 1.44. Controls: 22 patients with uveitis without OT and 94 healthy individuals with an age of 36.29 ± 13.81 (male/female ratio 0.84) to determine the genetic variation (ancestry) of the Colombian population.

This work evaluates the association between polymorphisms in genes coding for cytokines TNF-α (rs1799964, rs1800629, rs1799724, rs1800630, rs361525); IL-1β (rs16944, rs1143634, rs1143627), IL-1α (rs1800587); IFN-γ (rs2430561); IL-10 (rs1800896, rs1800871), and the presence of ocular toxoplasmosis (OT) in a sample of Colombian population.

The mini–sequencing technique or “ddNTP primer extension” was used, in which 12 fragments, that contain the evaluated single nucleotide polymorphisms (SNPs), were expanded . The results of this reaction were evaluated by means of capillary electrophoresis in the ABI Prism 3100-Avant sequencer.**(Fig 1).** Functional effect predictions of SNPs were done using FuncPred.



ELECTROPHEROGRAM Genemaper v 3,2 Software

Figure 1

FINANCING

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RESULTS AND DISCUSSION

We investigated the distribution of the twelve SNPs in 61 Colombian OT-infected patients (cases) and 116 (controls). Genotype distribution of all polymorphisms did not deviate significantly from the Hardy-Weinberg equilibrium. The Association analysis of MAF alleles in cytokines polymorphism in patients with OT and Healthy controls are presented in **Table 1.**

TABLE 1. ASSOCIATION ANALYSIS OF MAF ALLELES IN CYTOKINES POLYMORPHISM IN PATIENTS WITH OT AND HEALTHY CONTROLS

CHR	GENE	BP	CHISQ	P	OR	L95	U95	BONF
1	IL-10-819G/A	206773288	0.1851	0.6671	0.8962	0.5439	1.477	1
1	IL-10-1082A/G	206773551	45.04	1,93E-08	5.27	3.18	8.739	3,48E-07
2	IL-1α-889G/A	112785382	0.473	0.4916	0.8401	0.5111	1.381	1
2	IL-1β+3954G/A	112832812	0.1384	0.7099	1.118	0.6212	2.012	1
2	IL-1β-31G/A	112836809	3.546	0.05969	1.526	0.9819	2.371	1
2	IL-1β-511G/A	112837289	1.892	0.169	1.361	0.8768	2.112	1
6	TNF-α-1031T/C	31574530	0.03925	0.843	0.9425	0.5244	1.694	1
6	TNF-α-863C/A	31574698	0.3567	0.5503	1.236	0.6161	2.48	1
6	TNF-α-857G/A	31574704	0.2151	0.6428	1.143	0.6489	2.015	1
6	TNF-α-308G/A	31575253	0.04365	0.8345	1.095	0.4687	2.556	1
6	TNF-α-238C/T	31575323	0.001451	0.9696	0.982	0.3853	2.503	1
9	IFN-γ +874T/A	68158741	30.48	3.37E-05	4.2	2.478	7.12	6,07E-04

Most of the SNPs analyses had no significant effect, while the differences proved to be significant for the SNPs rs1800896 and rs2430561 after application of the Bonferroni correction. In our study, the -1082 G allele (P= 1.93E-08; OR=5.27; 95%CI=3.18-8.73; pBONF =3.48E-07) and the +874 A allele (P= 3.37E-05; OR=4.2; 95%CI=2.478-7.12; pBONF =6.07E-04) were higher and significantly represented in OT patients, compared with controls. In the case of the rs1143627 polymorphism, it initially showed a significant effect (OR=1.53 (95% CI 0.98 to 2.37) p= 0.05969), that disappeared when the Bonferroni correction was applied (pBONF =1,00).

Haplotype distribution

We observed five haplotype combinations (TGGCC, TGACC, CGGAC, TAGCC, CGGCT). However, a significant association in the distribution of the haplotype frequencies between cases and healthy controls was not found. The haplotype “GAA” of the IL-1β gene promoter polymorphisms (rs16944, rs1143634, rs1143627) appeared to be significantly associated with susceptibility to ocular toxoplasmosis (p=0.038). The haplotypes “GG and GA” of the IL-10 gene promoter polymorphism (rs1800896, rs1800871), appeared to be significantly associated with OT (p=4.0132E-07 and p=1.1072E-06, respectively. The frequencies are showed in **Table 2**.

Our study provides evidence that common genetic variants in Th1 (IL-1, IFN-γ, TNF-α) and Th2 (IL10) genes are associated with risk to develop OT in patients from Colombia

TABLE 2. HAPLOTYPE FREQUENCIES OF CYTOKINES GENES IN OT

Haplotype	Frequency	p Value
rs1800896 rs1800871 (IL-10 gene region)		
GG	0.405	4.0132E-07
GA	0.319	1.1072E-06
AA	0.231	0.0321
AG	0.045	0.001
rs1799964-rs11800629-rs1799724-rs1800630-rs361525 (TNF-α gene region)		
TGGCC	0.569	NA
TGACC	0.162	0.6
CGGAC	0.111	0.72
TAGCC	0.07	0.6
CGGCT	0.04	0.73
rs16944-rs1143634-rs1143627 (IL-1β gene region)		
AGG	0.458	NA
GGA	0.357	O.61
GAA	0.159	0.038
GGG	0.017	0.71

CONCLUSIONS

Polymorphism in IL-10 gene-promoter (-1082G/A) was significantly more prevalent in OT patients than in controls (P = 1.93E-08; OR=5.27E+03; 95%CI=3.18-8.739; pBONF =3.48E-07). In contrast, the haplotype “AG” of IL-10 gene promoter polymorphism (rs1800896, rs1800871), was present with lower frequency in OT patients [P= 7e-04, OR (95%CI) 0.10 (0.03-0.35)]. The polymorphism (+ 874 A/T) of IFN-γ was associated with OT (P= 3.37E-05; OR=4.2; 95%CI=2.478-7.12; pBONF =6.07E-04). The haplotype “GAG” of IL-1β gene promoter polymorphism (rs1143634, rs1143627, rs16944), appeared to be significantly associated with OT (p=0.0494). The IL-10, IFN-γ and IL-1β polymorphisms influence the development of OT in the Colombian population.

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