European Review for Medical and Pharmacological Sciences 2019; 23: 5059-5064

IL-10 gene polymorphism in diabetic retinopathy

Y.-L. SHI[1], M.-Y. SHI[2], L.-Z. YIN[3], J.-M. SHANG[4], J.-Y. ZHUANG[1]

[1]Department of Ophthalmology, The First Affiliated Hospital of Xiamen University & Teaching Hospital of Fujian Medical University, Xiamen, China.
[2]Department of Blood Transfusion, Yantaishan Hospital, Yantai, China.
[3]Health Management Center, the People's Hospital of Zhangqiu Area, Jinan, China
[4]Department of Radiology, the People's Hospital of Zhangqiu Area, Jinan, China

Yalin Shi and Mingyan Shi contributed equally to this work

Abstract. – INTRODUCTION: To investigate the relationship between the interleukin-10 (IL-10) gene polymorphism and the pathogenesis of diabetic retinopathy (DR).

PATIENTS AND METHODS: According to the fundus examination report, 420 patients with diabetes were divided into the non-DR group (n=200) and the DR group (n=220). The single nucleotide polymorphisms rs1145612 and rs11567245 in the promoter region of the IL-10 gene were classified by the conformational differential gel electrophoresis.

RESULTS: No correlation was found between the polymorphism rs1145612 and the clinical information of DR patients. The polymorphism rs11567245 had correlations with multiple clinical indicators of DR patients, including body mass index (BMI), systolic blood pressure (SBP), blood urea nitrogen (BUN), fasting blood glucose (FBG), and 2-h postprandial blood glucose (2hBG) (p<0.05).

CONCLUSIONS: The IL-10 gene promoter rs1145612 is not related to the occurrence and development of DR, but a relationship exists between the polymorphism rs11567245 and the occurrence and development of DR.

Key Words
IL-10, Gene polymorphism, Diabetic retinopathy.

Introduction

As one of the crucial complications of diabetes, diabetic retinopathy (DR) is a major cause of blindness around the world[1]. DR is primarily caused by the irreversible damage to the eye micro-vessels triggered by the continuous stimulation of high glucose, including increased vascular permeability, vascular occlusion and sustained accumulation of protein glycosylation products. The joint regulation on DR by genes, various pathogenic factors, and the environment brings certain challenges to the study on its genes[2-4]. Since the 21st century, as the molecular biology and molecular genetics rapidly develops, the susceptible genes and genetic mechanisms of various chronic diseases such as hypertension, hyperlipidemia, and diabetes mellitus have been uncovered. In spite of this, research on the genetics of DR is not comprehensive, so there is a need to search for more susceptible genes to clarify the genetic mechanisms of DR.

An important pathophysiological process in DR is the inflammatory response[5]. Interleukin-10 (IL-10), as an anti-inflammatory cytokine, inhibits the infiltration of various inflammatory cells in the eye micro-vessels and the release of inflammatory mediators by these inflammatory cells, so as to reduce local inflammatory responses[6]. The promoter polymorphism of IL-10 genes can directly influence the expression of IL-10 proteins and eventually affect the occurrence and development of DR[7,8]. Hence, this study aims to investigate the correlations of IL-10 promoters -1002 and -459 with DR by detecting the distribution of them in the DR group and the non-DR group.

Patients and Methods

Materials

According to the diagnostic criteria in the *Guidelines for the Diagnosis and Treatment of Diabetic Retinopathy in China (2014)* issued by the Chinese Medical Association Ophthalmology Branch and the results of the fundus examination, 420 patients with type 2 diabetes mellitus admitted to our hospital from June 2016 to January 2018 were divided into the non-DR group (n=200) and the DR group (n=220). Inclusion criteria:

Corresponding Author: Jingyi Zhuang, BM; e-mail: praq43@163.com

DR: 1) citizen of the Han nationality aged over 30 years old and 2) manifestations in the results of fundus examinations including photographing of the lower microcoria fundus, dilated ophthalmoscopy or fundus fluorescein angiography: a. pre-retinal hemorrhage, b. vitreous hemorrhage in new vessels, c. intraretinal hemorrhage at more than 20 loci in any quadrant, d. microaneurysm, e. intraretinal microvascular abnormalities in two or more quadrants, and f. venous beading changes in three or more quadrants. Patients with any of a-f detected could be diagnosed with DR, or they would be diagnosed without DR. Exclusion criteria: patients with acute complications or other serious systemic diseases. This study was approved by the Ethics Committee of our hospital, and all the subjects included signed the informed consent.

Basic information of all the patients included were collected, including gender, age, nation, birthplace, body mass index (BMI), diastolic blood pressure (DBP), systolic pressure (SBP), various blood lipid-related indicators [low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C)] and multiple blood glucose-related indicators [fasting blood glucose (FBG), 2-h postprandial blood glucose (2hBG), hemoglobin A1c (Hb1Ac), triglyceride (TG), total cholesterol (TC), uric acid (UA), serum creatinine (SCr), and blood urea nitrogen (BUN)].

DNA Extraction

Ethylenediaminetetraacetic acid (EDTA) anticoagulant blood (4 mL) was taken from patients, and the genomic deoxyribonucleic acid (DNA) was extracted according to the instructions of the DNA extraction kit (Beijing Solarbio, Beijing, China). 2 μmL blood was taken, whose mass was measured by electrophoresis on a 1.5% agarose gel. Meanwhile, the concentration of the extracted DNA was detected by means of an ultraviolet spectrophotometer.

Polymerase Chain Reaction (PCR) Amplification

The primers of rs1145612, rs11567245, rs1544112, and rs675567 of IL-10 genes were designed to be amplified, and the primer sequences of each locus are shown in Table I. PCR systems (20 μL): 2.0 μL DNA templates, 10.0 μL 2′MIX, 0.4 μL positive and 0.4 μL negative primers and 7.2 μL double distilled water. PCR amplification conditions: at 95°C for 120 s, 94°C for 30 s, 57°C for 90 s, and 72°C for 60 s for a total of 35 cycles, followed by extension at 72°C for 10 min. Subsequently, the amplification of the gene fragment was detected *via* the agarose gel electrophoresis.

Ligase Detection Reaction

The upstream and downstream probes used in this reaction were designed and synthesized by BGI. All the upstream probes were prepared into a probe mixed solution at a concentration of 12.5 pmol/μL modified by 5' terminal phosphorylation. Ligase detection reaction systems (3.05 μL): 0.05 μL ligases, 1 μL buffer, 1 μL PCR product, and 1 μL probe mixture. PCR amplification conditions: at 95°C for 120 s, 94°C for 15 s, and 50°C for 25 s for a total of 30 cycles. After the end of the cycles, the concentration was measured using an ultraviolet spectrophotometer. Subsequently, BGI was entrusted to sequence and analyze the target gene. All data were analyzed using Genemapper (Table II).

Statistical Analysis

All data were analyzed using Statistical Product and Service Solutions (SPSS) 22.0. (IBM, Armonk, NY, USA). Count data were expressed as frequency and percentage, and measurement data were expressed as mean ± standard deviation. The genotype frequencies in the samples were calculated and tested by means of the Hardy-Weinberg genetic equilibrium formula. Count data were detected *via* the chi-square test and

Table I. Primer sequences and the size of products at different loci of IL-10.

Locus	Primer sequence: 5′-3′	Product (bp)
rs1145612	Forward: AGCTTGCAGTCGTAGCGACC Reverse: TGCAGTGCTGGACTTGGAA	201
rs11567245	Forward: AGCTGTGGCATGCTGTAGCC Reverse: TGCGTTAGCTAAGTGCTTGAA	321
rs1544112	Forward: TTGCTAGCTGGATCGATCGATG Reverse: AATGCTAGCTAGTCGATCGTAGCT	302
rs675567	Forward: ATGCTGTAGCTGATCGTAGTTCC Reverse: TGTGTCACCCTGACTAGCTAGTC	244

Table II. Probe sequences of the ligase reaction and the size of products at different loci of IL-10.

Locus	Probe	Probe sequence: 5′-3′	Product (bp)
rs1145612	rs1145612	P-TGTGACGTAATTTTTTTTTTTTTTTTTT-FAM	78
	rs1145612-A	TTTTTTTTTTTTTTTCATATCCATTTTTTTTTAT	
	rs1145612-C	TTTTTTTTTTTTTTTCATATCCATTTTTTTTTAC	
rs11567245	rs11567245	P-AGGGGCGTGTGACGTAATTTTTTTTTTTTTTTTTT-FAM	101
	rs11567245-G	TTTTTTTTTTTTTTTTTTTTTTTTTTAGCGAAGCTG	
	rs11567245-A	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTAGCGAAGCTGCAT	
rs1544112	rs1544112	P-GCCAGGGGCGTTAATTTTTTTTTTTTTTTTTT-FAM	58
	rs1544112-C	TTTTTTTTTTTTTTTTTTTTTTTTTCCAGTCAATAGCGAAGCT	
	rs1544112-T	TTTTTTTTTTTTTTTTTTTTTTTTTCCAGTCAATAGCGAAGCC	
rs675567	rs675567	P-GCCTCTAAGGCGTTAATTTTTTTTTTTTTTTTTT-FAM	169
	rs675567-T	TTTTTTTTTTTTTTTTTTTTTTTTTCGCGAAAGCGAAGCT	
	rs675567-A	TTTTTTTTTTTTTTTTTTTTTTTTTCCATCGACCGAAGCGAAGCC	

subjected to multiple comparisons. Measurement data were tested *via* the *t*-test and the analysis of variance. $p<0.05$ represented that the difference was statistically significant.

Results

Clinical Data

According to the inclusion and exclusion criteria, a total of 420 patients with type 2 diabetes mellitus met the requirements, including 200 patients in the non-DR group and 220 patients in the DR group. Baseline information of the two groups of patients included gender, age, SBP, DBP, BMI, HbA1c, BUN, FBG, 2hBG, TG, TC, LDL-C, HDL-C, SCr, and UA. There were no statistical differences in all the above indicators ($p>0.05$), so they were comparable (Table III).

Hardy-Weinberg Equilibrium Test

The Hardy-Weinberg equilibrium formula was applied to examine the test results of the linkage disequilibrium at different loci of the IL-10 gene. As shown in Table IV, the r^2 between loci in each group was less than 0.33, so it was considered that these loci accorded with the equilibrium test.

Hardy-Weinberg Equilibrium Test

The genotype frequencies at each gene locus in the two groups of patients are shown in Table V. It could be found that the polymorphism at four loci was not statistically different between the two groups ($p>0.05$) (Table V).

Correlations of Polymorphisms at Each Locus of the IL-10 Gene With Clinical Data of DR

The polymorphisms rs1145612 and rs11567245 of the IL-10 gene were correlated with clinical data of DR patients. No correlation was found between

Table III. Basic clinical information of the two groups of patients.

Group	n	Age	Male-female ratio	BMI (kg/m^2)	DBP (mmHg)	SBP (mmHg)	HbA1c (%)	BUN (mmol)
Non-DR group	200	61.23±12.22	98/102	23.43±2.24	75.13±12.22	130.23±12.43	6.81±1.29	5.13±1.11
DR group	220	59.22±12.89	103/105	23.11±3.19	77.19±8.97	132.55±11.70	6.55±1.62	5.02±1.31
t/Z/C2		0.221	0.126	0.334	-0.562	0.493	0.392	0.446
p		0.656	0.77	0.62	0.51	0.81	0.58	0.71
Group	**FBG (mmol)**	**2hBG (mmol)**	**TG (mmol)**	**TC (mmol)**	**LDL-C (mmol)**	**HDL-C (mmol)**	**SCr (mmol)**	**UA (mmol)**
Non-DR group	6.93±1.22	13.15±2.22	1.41±0.26	4.43±0.24	2.53±0.22	1.23±0.43	60.81±11.29	322.65±23.56
DR group	6.71±2.01	13.26±2.89	1.36±0.22	4.31±0.19	2.49±0.97	1.55±0.70	60.55±11.62	345.14±11.67
t/Z/C2	1.22	0.421	0.626	0.89	0.962	0.393	0.445	1.241
p	0.07	0.756	0.87	0.81	0.71	0.18	0.82	0.09

Table IV. Test results of the linkage disequilibrium at different loci of the IL-10 gene in each group.

Locus	r^2	
	rs1145612	rs11567245
rs1145612	–	0.021
rs11567245	0.021	–

rs1145612 and the clinical data of DR ($p>0.05$), but there was a significant association between rs11567245 and DR. BMI, SBP, BUN, FBG, 2hBG, TG, and clinical indicators in patients with genotype GG were markedly different from those in patients with genotype AA ($p<0.05$), and the clinical manifestations in the former were severer than those in the latter (Tables VI and VII).

Discussion

DR is one of the common and serious complications of diabetes mellitus[9]. At present, it is considered that the main mechanisms of DR mainly involve: 1) abnormal secretion of matrix metalloproteinases, 2) vascular endothelial cell damage triggered by the inflammation and oxidative stress, 3) mitochondrial damage, 4) endoplasmic reticulum stress, 5) epigenetic modification and 6) disorders in vascular endothelial progenitor cells[10-13]. The incidence and development processes of DR are often accompanied by aggregation and infiltration of leukocytes in the lesion area, and at the same time, high glucose stimulation can trigger the secretion of a large amount of pro-inflammatory factors such as tumor necrosis factor-alpha, various interleukins, vascular endothelial growth factors, pigment epithelium-derived factors, cyclooxygenases, nitric oxide, and a variety of adhesion molecules by these inflammatory cells. On the one hand, these inflammatory cytokines can catalyze the clogging of capillaries by white blood cells, and on the other hand, they can promote the microvascular contraction and accelerate the thrombosis. Furthermore, they can directly stimulate vascular endothelial cells, thereby causing endothelial cell damage and aggravating the retinal microvascular occlusion[14-16].

Table V. Distribution of different genotypes of IL-10 gene and DR.

Group	rs1145612			rs11567245		
	CC	CA	AA	AA	AG	GG
Non-DR group	25.1%	60.1%	14.8%	20.1%	50.9%	29.0%
DR Group	24.6%	58.2%	17.2%	19.3%	51.2%	29.5%
C^2	0.688			0.823		
p	0.721			0.682		

Table VI. Correlations of different genotypes at rs1145612 of the IL-10 gene with clinical data of DR.

Group	Age	Male-female ratio	BMI (kg/m²)	DBP (mmHg)	SBP (mmHg)	HbA1c (%)	BUN (mmol)
CC	59.24±10.82	25/28	21.43±3.44	74.36±10.14	134.67±11.69	6.33±1.08	5.31±0.88
CA	60.58±11.96	82/75	21.51±2.68	73.59±9.67	133.67±10.88	6.18±2.64	5.21±1.45
AA	60.13±12.33	105/105	20.68±1.88	74.28±8.73	133.78±10.35	6.37±2.09	4.95±1.18
t/Z/C2	0.621	0.351	0.624	0.557	0.493	0.784	0.73
p	0.86	0.88	0.52	0.91	0.81	0.46	0.60

Group	FBG (mmol)	2hBG (mmol)	TG (mmol)	TC (mmol)	LDL-C (mmol)	HDL-C (mmol)	SCr (mmol)	UA (mmol)
CC	6.93±1.22	12.15±2.22	1.41±0.26	4.04±0.34	2.88±1.22	1.41±0.67	61.14±10.41	342.65±15.67
CA	6.88±0.79	13.11±1.42	1.36±0.22	4.45±0.29	2.58±1.97	1.58±0.73	62.01±12.64	345.14±11.67
AA	6.71±2.01	13.46±1.54	1.11±0.42	4.14±0.54	2.58±1.68	1.45±1.21	61.29±10.66	325.99±14.70
t/Z/C2	0.822	0.723	0.672	0.89	0.563	0.453	0.546	1.893
p	0.14	0.82	0.58	0.53	0.81	0.73	0.11	0.09

Table VII. Correlations of different genotypes at rs1145612 of the IL-10 gene with clinical data of DR.

Group	Age	Male-female ratio	BMI (kg/m^2)	DBP (mmHg)	SBP (mmHg)	HbA1c (%)	BUN (mmol)
AA	58.16±8.34	23/30	20.12±1.22	76.13±9.25	121.35±15.29	6.19±1.49	5.31±0.88
AG	59.25±11.45	80/77	23.51±1.11	75.56±9.44	134.67±15.88	6.08±1.55	6.11±0.34
GG	60.03±10.74	103/107	25.18±2.23	73.23±6.99	140.78±16.21	6.19±1.24	8.21±0.53
t/Z/C2	0.71	0.351	1.44	0.452	1.32	0.616	-1.92
p	0.44	0.83	0.03	0.49	0.02	0.25	0.03

Group	FBG (mmol)	2hBG (mmol)	TG (mmol)	TC (mmol)	LDL-C (mmol)	HDL-C (mmol)	SCr (mmol)	UA (mmol)
AA	4.16±1.43	12.15±2.22	1.43±0.26	4.04±0.34	2.48±1.16	1.11±0.27	60.248±9.21	334.65±25.67
AG	5.44±0.92	13.14±2.32	1.86±0.31	4.45±0.29	2.14±0.91	1.49±0.83	62.57±10.34	335.45±21.67
GG	7.42±1.32	15.36±2.54	2.11±0.34	4.14±0.54	2.38±1.34	2.11±1.13	61.83±10.67	324.26±24.52
t/Z/C2	1.921	-1.451	1.642	0.89	0.398	1.343	0.467	0.472
p	0.02	0.04	0.01	0.53	0.79	0.03	0.34	0.59

IL-10 is a vital member of the interleukin family, which inhibits the adhesion and infiltration of various inflammatory cells as well as the release of various pro-inflammatory cytokines by monocytes and macrophages, so as to ultimately exert an anti-inflammatory effect. The polymorphism of IL-10 has a correlation with the incidence, development, and prognosis of various diseases. For example, Hepatitis B virus patients with C allele genotype at 592 in the IL-10 gene promoter region may possess stronger ability to clear HBV[17]. Additionally, the occurrence and development of cirrhosis after HBV infection are also directly affected by polymorphisms at -592, -819, and -1082 loci in the promoter region[18]. Besides, the IL-10 gene promoter region-592A/A and -819T/T genotypes may have correlations with the development and exacerbation of diarrhea-induced irritable bowel syndromes[19]. According to researches, the lower the level of IL-10 in human is, the higher the probability of DR in patients will be, and the severer the disease will be, indicating that the IL-10 gene polymorphism may be related to DR to a certain degree. Therefore, in this study, the association between genotypes and clinical data of DR patients was analyzed by detecting the polymorphisms rs1145612 and rs11567245 in the promoter region of the IL-10 gene, which revealed that no statistical correlation was found between the polymorphism rs1145612 and various clinical indicators. However, the polymorphism rs11567245 was correlated with multiple clinical indicators such as BMI, SBP, BUN, FBG, 2hBG, and TG, and patients with genotype GG had severer clinical manifestations than those with genotype AA. However, some limitations exist in this study: 1) The clinical sample size is relatively small. 2) Since the follow-up data are lost, the course of the disease is included at the end of this study, which may be an important indicator of the correlation between the gene polymorphism and the disease. 3) The subjects of this study are the Han population, so the results may be different in the European and American populations.

To sum up, this study confirmed that rs1145612 in the promoter region of the IL-10 gene has no relationship to DR, and patients with GG at rs11567245 may have severer clinical symptoms and worse prognosis than patients with AA.

Conclusions

Some relationship exists between the polymorphism rs11567245 and the incidence and development of diabetic retinopathy, and it can be a potential target molecule in the treatment of diabetic retinopathy.

Conflict of Interests

The authors declare that they have no conflict of interest.

References

1) Dow C, Mancini F, Rajaobelina K, Boutron-Ruault MC, Balkau B, Bonnet F, Fagherazzi G. Diet and risk of diabetic retinopathy: a systematic review. Eur J Epidemiol 2018; 33: 141-156.

2) Vilsboll T, Bain SC, Leiter LA, Lingvay I, Matthews D, Simo R, Helmark IC, Wijayasinghe N, Larsen M. Semaglutide, reduction in glycated haemoglobin and the risk of diabetic retinopathy. Diabetes Obes Metab 2018; 20: 889-897.
3) Vingolo EM, Fragiotta S, Mafrici M, Cutini A, Marinelli C, Concistre A, Iannucci G, Petramala L, Letizia C. Vitreous and plasma changes of endothelin-1, adrenomedullin and vascular endothelium growth factor in patients with proliferative diabetic retinopathy. Eur Rev Med Pharmacol Sci 2017; 21: 662-668.
4) Sachdeva R, Schlotterer A, Schumacher D, Matka C, Mathar I, Dietrich N, Medert R, Kriebs U, Lin J, Nawroth P, Birnbaumer L, Fleming T, Hammes HP, Freichel M. TRPC proteins contribute to development of diabetic retinopathy and regulate glyoxalase 1 activity and methylglyoxal accumulation. Mol Metab 2018; 9: 156-167.
5) Dehdashtian E, Mehrzadi S, Yousefi B, Hosseinzadeh A, Reiter RJ, Safa M, Ghaznavi H, Naseripour M. Diabetic retinopathy pathogenesis and the ameliorating effects of melatonin; involvement of autophagy, inflammation and oxidative stress. Life Sci 2018; 193: 20-33.
6) Ip W, Hoshi N, Shouval DS, Snapper S, Medzhitov R. Anti-inflammatory effect of IL-10 mediated by metabolic reprogramming of macrophages. Science 2017; 356: 513-519.
7) Da SPB, Polina ER, Crispim D, Sbruzzi RC, Canani LH, Dos SK. Interleukin-10 -1082A>G (rs1800896) polymorphism is associated with diabetic retinopathy in type 2 diabetes. Diabetes Res Clin Pract 2018; 138: 187-192.
8) Wang C, You Q, Cao X, Guo H, Gao X, Peng X. Micro RNA-19a suppresses interleukin-10 in peripheral B cells of patients with diabetic retinopathy. Am J Transl Res 2017; 9: 1410-1417.
9) Zhang SJ, Chen X, Li CP, Li XM, Liu C, Liu BH, Shan K, Jiang Q, Zhao C, Yan B. Identification and characterization of circular RNAs as a new class of putative biomarkers in diabetes retinopathy. Invest Ophthalmol Vis Sci 2017; 58: 6500-6509.
10) Duh EJ, Sun JK, Stitt AW. Diabetic retinopathy: current understanding, mechanisms, and treatment strategies. JCI Insight 2017 Jul 20;2(14). pii: 93751. doi: 10.1172/jci.insight.93751. [Epub ahead of print].
11) Li C, Miao X, Li F, Wang S, Liu Q, Wang Y, Sun J. Oxidative stress-related mechanisms and antioxidant therapy in diabetic retinopathy. Oxid Med Cell Longev 2017; 2017: 9702820.
12) Obara EA, Hannibal J, Heegaard S, Fahrenkrug J. Loss of melanopsin-expressing retinal ganglion cells in patients with diabetic retinopathy. Invest Ophthalmol Vis Sci 2017; 58: 2187-2192.
13) Durbin MK, An L, Shemonski ND, Soares M, Santos T, Lopes M, Neves C, Cunha-Vaz J. Quantification of retinal microvascular density in optical coherence tomographic angiography images in diabetic retinopathy. JAMA Ophthalmol 2017; 135: 370-376.
14) Capitao M, Soares R. Angiogenesis and inflammation crosstalk in diabetic retinopathy. J Cell Biochem 2016; 117: 2443-2453.
15) Chen W, Zhao M, Zhao S, Lu Q, Ni L, Zou C, Lu L, Xu X, Guan H, Zheng Z, Qiu Q. Activation of the TXNIP/NLRP3 inflammasome pathway contributes to inflammation in diabetic retinopathy: a novel inhibitory effect of minocycline. Inflamm Res 2017; 66: 157-166.
16) Jiang T, Chang Q, Cai J, Fan J, Zhang X, Xu G. Protective effects of melatonin on retinal inflammation and oxidative stress in experimental diabetic retinopathy. Oxid Med Cell Longev 2016; 2016: 3528274.
17) Ahmadabadi BN, Hassanshahi G, Arababadi MK, Leanza C, Kennedy D. The IL-10 promoter polymorphism at position -592 is correlated with susceptibility to occult HBV infection. Inflammation 2012; 35: 818-821.
18) Yan Z, Tan W, Zhao W, Dan Y, Wang X, Mao Q, Wang Y, Deng G. Regulatory polymorphisms in the IL-10 gene promoter and HBV-related acute liver failure in the Chinese population. J Viral Hepat 2009; 16: 775-783.
19) Olivo-Diaz A, Romero-Valdovinos M, Gudino-Ramirez A, Reyes-Gordillo J, Jimenez-Gonzalez DE, Ramirez-Miranda ME, Martinez-Flores WA, Martinez-Hernandez F, Flisser A, Maravilla P. Findings related to IL-8 and IL-10 gene polymorphisms in a Mexican patient population with irritable bowel syndrome infected with Blastocystis. Parasitol Res 2012; 111: 487-491.