Cytokine gene polymorphisms in *Blastocystis* carriers

Angelica Olivo-Diaz; Mirza Romero-Valdovinos; Maria Elena Ramirez-Miranda; Williams Arony Martinez-Flores; Fernando Martinez-Hernandez and Pablo Maravilla

The Irritable Bowel Syndrome pathogenesis (IBS) is considered to be multifactorial, some are:

- Psychological distress
- Gastrointestinal dysmotility
- Visceral hypersensitivity
- Microbiota
  - Bacterial overgrowth
  - Altered microbiota
- Infections: Post-infective (PI)-IBS
  - Episode of gastroenteritis can trigger IBS symptoms
- The immune activation in the Intestinal wall: Low-grade inflammation
  - ↑numbers of T cells in lymphoid compartments of the small or large intestine in IBS patients.
  - ↑ proinflammatory cytokines such as interleukin (IL)-1β, IL-6, IL-8 and tumor necrosis factor (TNF)-α in serum of IBS patients.
- Genetics
  - Familiar aggregation (≈33% IBS patients reported a family history of IBS Vs 2% of the control group)
  - **SNPs (Single Nucleotide Polymorphisms)** gene polymorphisms that encoding proteins with immunomodulatory and neuromodulatory features

Cytokine gene polymorphisms are associated with irritable bowel syndrome: a systematic review and meta-analysis

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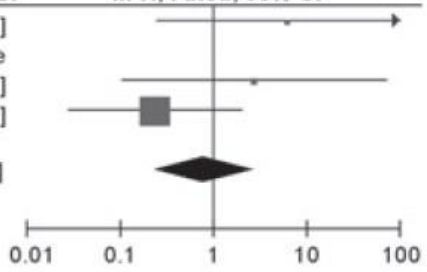
A TNF (-308G/A): AA Genotype

Study or subgroup	IBS Events	IBS Total	Control Events	Control Total	Weight	Odds ratio M-H, Fixed, 95% CI
Barkhordari (15)	1	67	0	137	5.7%	6.20 [0.25, 154.32]
Lee (20)	0	94	0	88		Not estimable
Santhosh (19)	1	23	0	20	8.8%	2.73 [0.11, 70.92]
van der Veek (18)	1	111	6	162	85.5%	0.24 [0.03, 1.99]
Total (95% CI)		**295**		**407**	**100.0%**	**0.80 [0.23, 2.78]**
Total events	3		6			

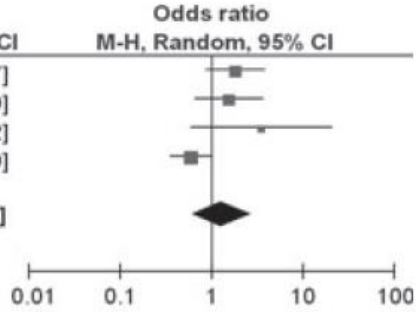
Heterogeneity: $Chi^2 = 3.37$, df = 2 ($P = 0.19$); $I^2 = 41\%$
Test for overall effect: $Z = 0.36$ ($P = 0.72$)



B TNF (-308G/A): GG Genotype

Study or subgroup	IBS Events	IBS Total	Control Events	Control Total	Weight	Odds ratio M-H, Random, 95% CI
Barkhordari (15)	55	67	98	137	28.5%	1.82 [0.88, 3.77]
Lee (20)	83	94	73	88	26.1%	1.55 [0.67, 3.59]
Santhosh (19)	21	23	15	20	12.1%	3.50 [0.60, 20.52]
van der Veek (18)	65	111	114	162	33.2%	0.59 [0.36, 0.99]
Total (95% CI)		**295**		**407**	**100.0%**	**1.30 [0.62, 2.74]**
Total events	224		300			

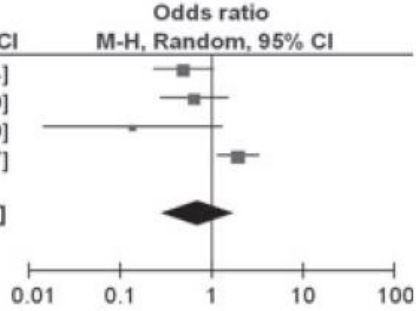
Heterogeneity: $Tau^2 = 0.36$; $Chi^2 = 9.65$, df = 3 ($P = 0.02$); $I^2 = 69\%$
Test for overall effect: $Z = 0.70$ ($P = 0.48$)



C TNF (-308G/A): GA Genotype

Study or subgroup	IBS Events	IBS Total	Control Events	Control Total	Weight	Odds ratio M-H, Random, 95% CI
Barkhordari (15)	11	67	39	137	28.9%	0.49 [0.23, 1.04]
Lee (20)	11	94	15	88	27.5%	0.64 [0.28, 1.49]
Santhosh (19)	1	23	5	20	11.6%	0.14 [0.01, 1.29]
van der Veek (18)	45	111	42	162	32.0%	1.95 [1.16, 3.27]
Total (95% CI)		**295**		**407**	**100.0%**	**0.71 [0.28, 1.80]**
Total events	68		101			

Heterogeneity: $Tau^2 = 0.64$; $Chi^2 = 13.95$, df = 3 ($P = 0.003$); $I^2 = 78\%$
Test for overall effect: $Z = 0.72$ ($P = 0.47$)



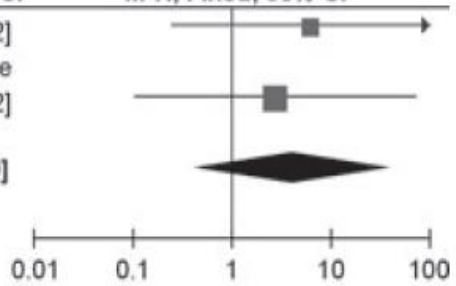
For pooled studies

A TNF (-308G/A): AA Genotype

Study or subgroup	IBS Events	IBS Total	Control Events	Control Total	Weight	Odds ratio M-H, Fixed, 95% CI
Barkhordari (15)	1	67	0	137	39.2%	6.20 [0.25, 154.32]
Lee (20)	0	94	0	88		Not estimable
Santhosh (19)	1	23	0	20	60.8%	2.73 [0.11, 70.92]
Total (95% CI)		**184**		**245**	**100.0%**	**4.09 [0.41, 40.40]**
Total events	2		0			

Heterogeneity: $Chi^2 = 0.12$, df = 1 ($P = 0.73$); $I^2 = 0\%$

Test for overall effect: $Z = 1.21$ ($P = 0.23$)

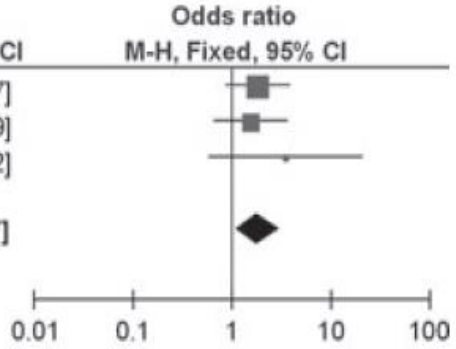


B TNF (-308G/A): GG Genotype

Study or subgroup	IBS Events	IBS Total	Control Events	Control Total	Weight	Odds ratio M-H, Fixed, 95% CI
Barkhordari (15)	55	67	98	137	53.0%	1.82 [0.88, 3.77]
Lee (20)	83	94	73	88	40.6%	1.55 [0.67, 3.59]
Santhosh (19)	21	23	15	20	6.4%	3.50 [0.60, 20.52]
Total (95% CI)		**184**		**245**	**100.0%**	**1.82 [1.08, 3.07]**
Total events	159		186			

Heterogeneity: $Chi^2 = 0.67$, df = 2 ($P = 0.72$); $I^2 = 0\%$

Test for overall effect: $Z = 2.25$ ($P = 0.02$)

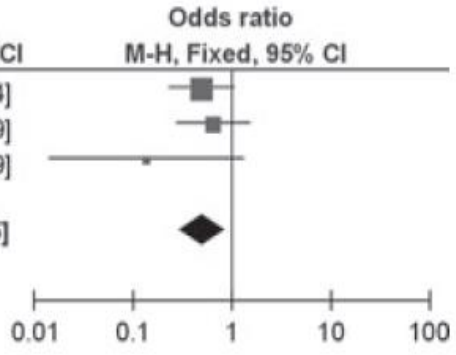


C TNF (-308G/A): GA Genotype

Study or subgroup	IBS Events	IBS Total	Control Events	Control Total	Weight	Odds ratio M-H, Fixed, 95% CI
Barkhordari (15)	11	67	39	137	53.3%	0.49 [0.23, 1.04]
Lee (20)	11	94	15	88	34.0%	0.64 [0.28, 1.49]
Santhosh (19)	1	23	5	20	12.7%	0.14 [0.01, 1.29]
Total (95% CI)		**184**		**245**	**100.0%**	**0.50 [0.29, 0.85]**
Total events	23		59			

Heterogeneity: $Chi^2 = 1.64$, df = 2 ($P = 0.44$); $I^2 = 0\%$

Test for overall effect: $Z = 2.55$ ($P = 0.01$)

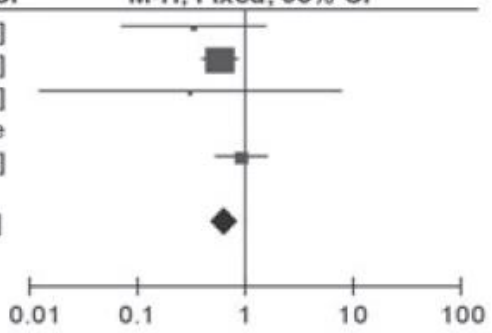


For studies in Asian patients

A IL-10 (-1082G/A): GG Genotype

Study or subgroup	IBS Events	IBS Total	Control Events	Control Total	Weight	Odds ratio M-H, Fixed, 95% CI
Barkhordari (16)	2	66	12	140	6.7%	0.33 [0.07, 1.53]
Gonsalkorale (17)	49	230	135	425	67.4%	0.58 [0.40, 0.85]
Lee (20)	0	94	1	88	1.4%	0.31 [0.01, 7.68]
Santhosh (19)	0	23	0	20		Not estimable
van der Veek (18)	29	111	45	162	24.4%	0.92 [0.53, 1.59]
Total (95% CI)		**524**		**835**	**100.0%**	**0.64 [0.48, 0.87]**
Total events	80		193			

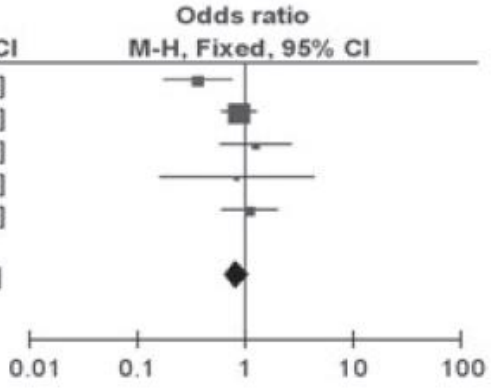
Heterogeneity: Chi² = 2.84, df = 3 (P = 0.42); I^2 = 0%
Test for overall effect: Z = 2.88 (P = 0.004)



B IL-10 (-1082G/A): AA Genotype

Study or subgroup	IBS Events	IBS Total	Control Events	Control Total	Weight	Odds ratio M-H, Fixed, 95% CI
Barkhordari (16)	12	66	53	140	21.7%	0.36 [0.18, 0.74]
Gonsalkorale (17)	60	230	122	425	49.5%	0.88 [0.61, 1.26]
Lee (20)	78	94	70	88	9.6%	1.25 [0.59, 2.65]
Santhosh (19)	19	23	17	20	2.5%	0.84 [0.16, 4.29]
van der Veek (18)	25	111	34	162	16.7%	1.09 [0.61, 1.96]
Total (95% CI)		**524**		**835**	**100.0%**	**0.84 [0.65, 1.08]**
Total events	194		296			

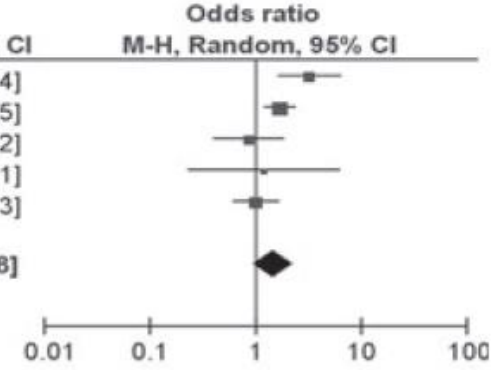
Heterogeneity: Chi² = 7.21, df = 4 (P = 0.13); I^2 = 45%
Test for overall effect: Z = 1.35 (P = 0.18)



C IL-10 (-1082G/A): GA Genotype

Study or subgroup	IBS Events	IBS Total	Control Events	Control Total	Weight	Odds ratio M-H, Random, 95% CI
Barkhordari (16)	52	66	75	140	19.8%	3.22 [1.64, 6.34]
Gonsalkorale (17)	121	230	168	425	30.7%	1.70 [1.23, 2.35]
Lee (20)	16	94	17	88	17.8%	0.86 [0.40, 1.82]
Santhosh (19)	4	23	3	20	6.2%	1.19 [0.23, 6.11]
van der Veek (18)	57	111	83	162	25.6%	1.00 [0.62, 1.63]
Total (95% CI)		**524**		**835**	**100.0%**	**1.46 [0.94, 2.28]**
Total events	250		346			

Heterogeneity: Tau² = 0.14; Chi² = 10.29, df = 4 (P = 0.04); I^2 = 61%
Test for overall effect: Z = 1.67 (P = 0.10)



For pooled studies

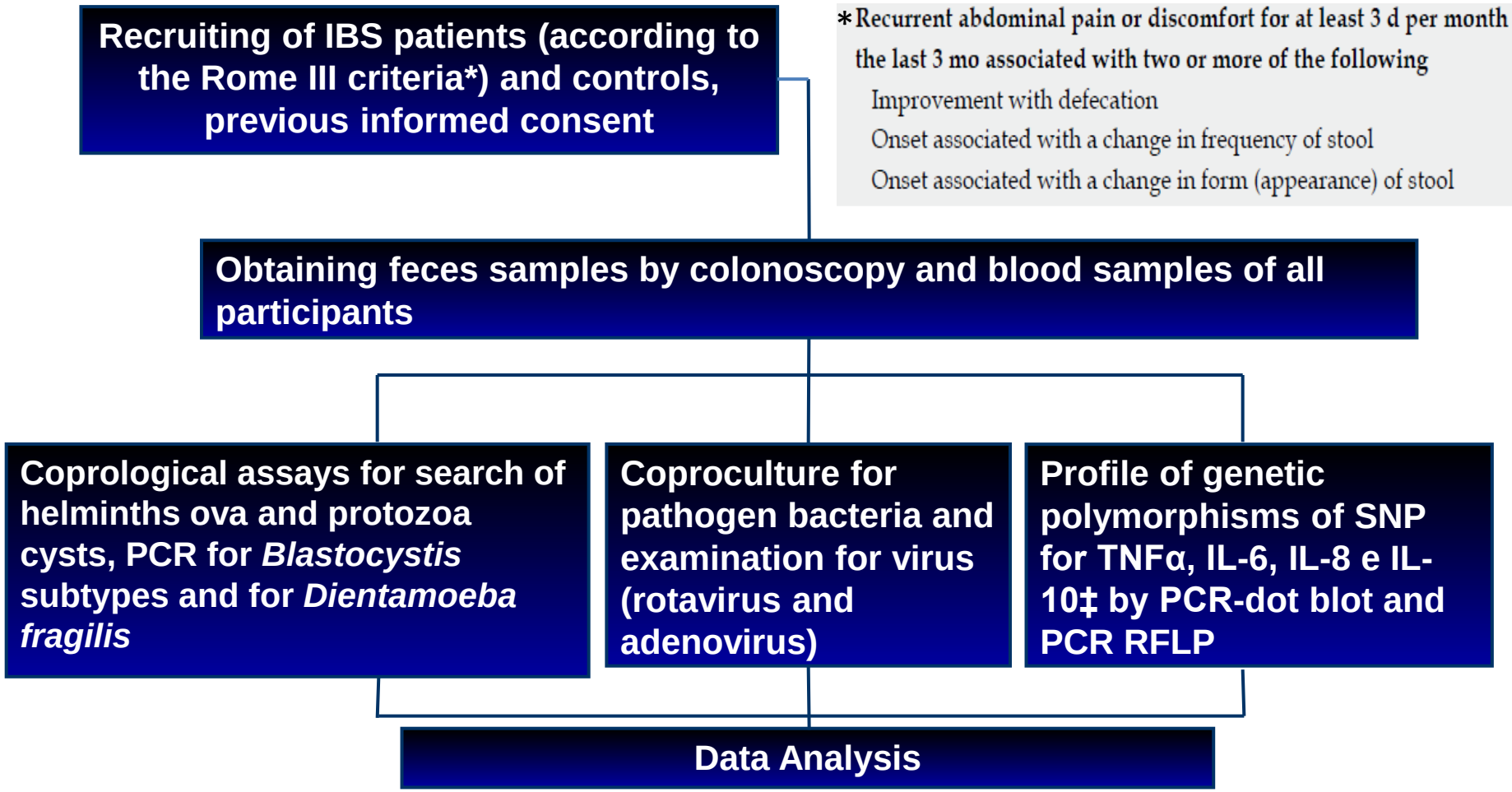
Proinflammatory Cytokine Gene Polymorphisms in Irritable Bowel Syndrome

J Clin Immunol (2010) 30:74–79

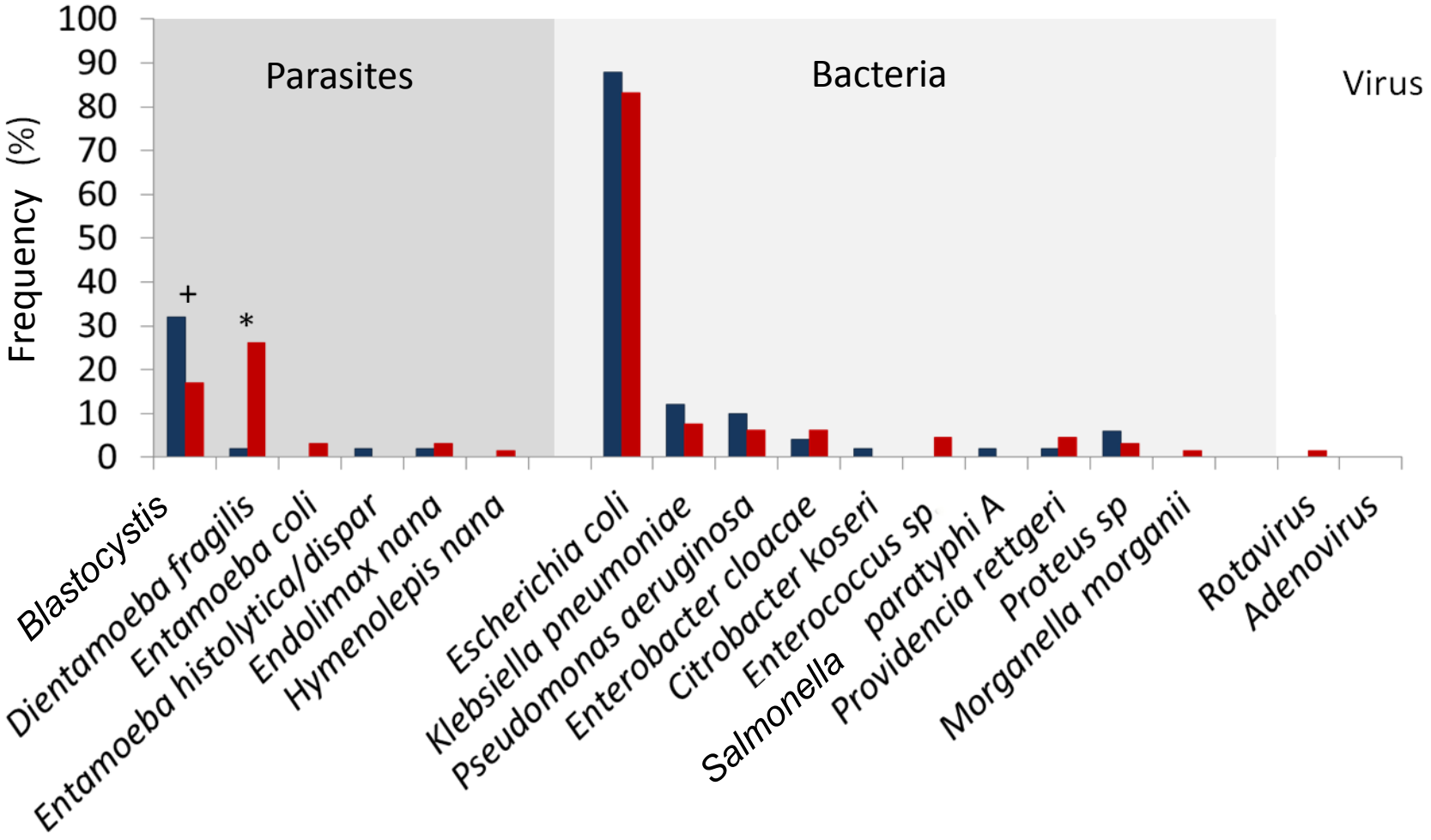
Elham Barkhordari · Nima Rezaei · Bita Ansaripour · Pegah Larki · Maryam Alighardashi · Hamid Reza Ahmadi-Ashtiani · Mahdi Mahmoudi · Mohammad-Reza Keramati · Peiman Habibollahi · Mohammad Bashashati · Naser Ebrahimi-Daryani · Ali Akbar Amirzargar

Cytokine	Position	Allele	Patients (n=71) n (%)	Controls (n=140) n (%)	P value	Odds ratio (95% confidence interval)
IL-6	−174	C	27 (20.76%)	101 (36.3%)	0.002	0.46 (0.27–0.77)
		G	103 (79.23%)	177 (63.7%)		2.18 (1.30–3.66)
IL-6	nt565	A	25 (19.23%)	50 (18.0%)	0.868	1.09 (0.62–1.91)
		G	105 (80.76%)	228 (82.0%)		0.92 (0.52–1.63)
TNF-α	−308	A	13 (9.7%)	39 (14.2%)	0.257	0.65 (0.31–1.31)
		G	121 (90.29%)	235 (85.8%)		1.54 (0.76–3.18)
TNF-α	−238	A	8 (5.97%)	59 (21.5%)	<0.001	0.23 (0.10–0.52)
		G	126 (94.02%)	215 (78.5%)		4.32 (1.92–10.13)
IL-1α	−889	C	97 (68.3%)	186 (68.4%)	0.923	1.00 (0.63–1.58)
		T	45 (31.7%)	86 (31.6%)		1.00 (0.63–1.59)
IL-1β	−511	C	71 (50%)	154 (55.4%)	0.344	0.81 (0.53–1.23)
		T	71 (50%)	124 (44.6%)		1.24 (0.81–1.90)
IL-1β	+3962	C	96 (67%)	198 (70.7%)	0.586	0.86 (0.55–1.37)
		T	46 (32%)	82 (29.3%)		1.16 (0.81–1.90)
IL-1R	Pst-I	C	104 (74%)	174 (62.1%)	0.017	1.76 (1.10–2.83)
	1970	T	36 (26%)	106 (44.2%)		0.57 (0.35–0.91)
IL-1RA	Mspa-I	C	27 (19%)	64 (22.9%)	0.521	0.82 (0.48–1.40)
	11100	T	111 (80%)	216 (77.1%)		1.22 (0.71–2.08)

Objective: To evaluate the role of SNP for IL -6, 8, 10 and tumor necrosis factor-alpha (TNF-α) in IBS patients and controls, with or without *Blastocystis* infection.



‡-174 for IL-6; -251, +396, +781, +1633 for IL-8; -1082, -819, -592 for IL-10, as well as -238 and -308 for TNF-α



Frequency of microorganisms identified by CPS, coproculture, virus screening and PCR for *Blastocystis sp.* (p=0.048) and *D. fragilis* (p=0.0004)

Blastocystis subtypes

Group/Subtype	ST 1	ST 2	ST 3	ST 1/3	ST 2/3	Total
IBS	2	1	2	10	1	16
Control	0	0	3	7	1	11

Some associations between variables

Association	χ2	*p*	OR	95% CI
Blastocystis/IBS	3.57	0.048	2.31	1.113-5.567
ST2/IBS	6.072	0.065	12	1.027-140.192
Blastocystis/P. aeruginosa	2.996	0.083	0	0-1.87
Blastocystis/K. pneumonae	3.071	0.079	0.187	0.024-1.488
Dientamoeba/IBS	13.53	0.0004	0.05	0-0.52

Statistical differences within SNPs

Relationship	Alleles o genotypes	*p*	OR (95%CI)[b]	EF[c]
IBS vs. control	**Allele**			
	IL-8+396 (G)	0.0437	1.78 (1.01-3.12)	**0.291**
	IL-10-1082 (A)*	0.0267	1.99 (1.08-3.69)	**0.358**
	Genotypes			
	IL-8+396(GG)	<0.0001	10.13 (2.73-37.55)	**0.313**
	IL-8+781(CT)	0.0248	2.45 (1.11-5.39)	**0.348**
	IL-10-1082(AA)*	0.0039	3.64 (1.49-8.89)	**0.353**
Presence or absence of *Blastocystis*	**Allele**			
	IL-8+781 (T)	0.0448	2.00 (1.01-3.97)	**0.228**
	Genotype			
	IL-8+396(GG)	0.0025	4.64 (1.63-13.19)	**0.290**
IBS and *Blastocystis* carrier vs. IBS and absence of *Blastocystis*	**Allele**			
	IL-10-592 (C)†	0.0166	3.28 (1.22-8.79)	**0.454**
	Genotype			
	IL-8+396(GG)	0.0272	4.22 (1.15-15.5)	**0.429**
Control and *Blastocystis* carrier vs. control and absence of *Blastocystis*	**Genotype**			
	IL-10-819 (CC)†	0.0426[d]	0.12 (0.01-1.05)	**-**

[a] Mantel–Haenszel test; [b] Odds ratio (95% confidence interval); [c] Etiologic fraction; [d] Fisher's exact test

*Allele A has been associated as low producer of IL-10; † Allele A has been associated as higher producer of IL-10.

Discussion

✓An association between *Blastocystis* infection and development of IBS was identified, supports its pathogenic role.

✓*Blastocystis* subtypes 1 and 3 were the most frequent, according with other studies; interestingly, many cases with co-infection were observed.

✓No associations among *Blastocystis* and symptoms, presence with other microorganisms, age and gender were found.

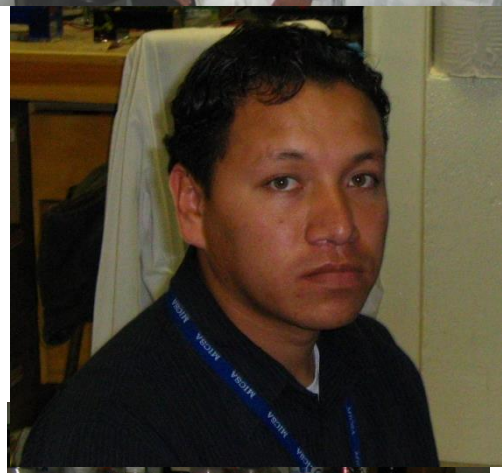
✓Polymorphisms in SNP of IL-6 and TNF-α promoters might not contribute for the susceptibility to IBS in our population.

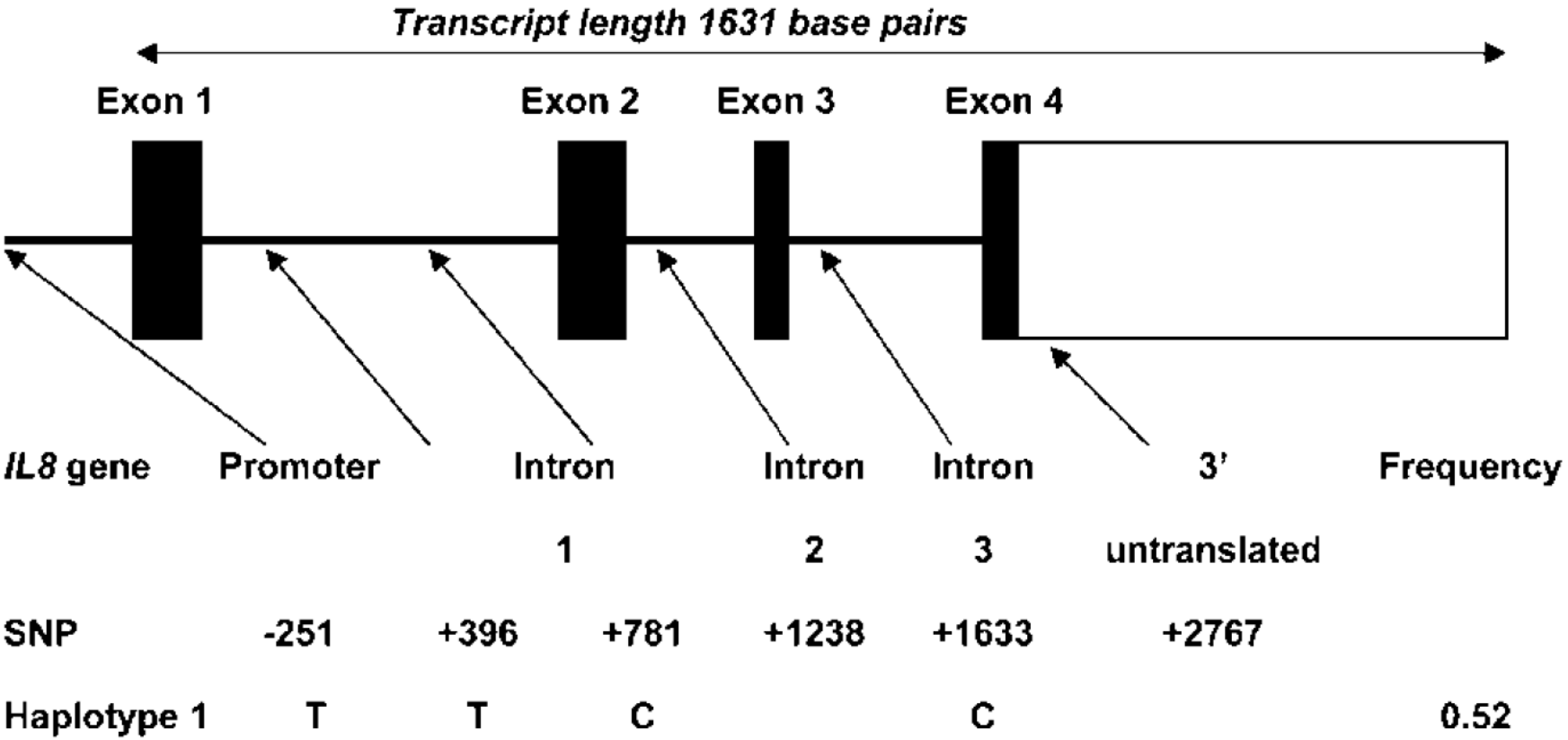
- ✓ In contrast, we found that alleles G and A, as well as the homozygous variant for IL-8 at position +396 and IL-10 at position −1082, respectively, were relevant to develop IBS.

- ✓ Association between IL-8 and IL-10 gene polymorphisms to the pair IBS–*Blastocystis* carrier suggests their individual or additive role as significant factors in the etiology of this disease.

- ✓ Finally, *Dientamoeba fragilis* showed a prevalence of 2% in IBS patients and 28% in controls, it is probably that this phenomenon is due to a selection bias as IBS patients are multi-treated

Thank you!!!!



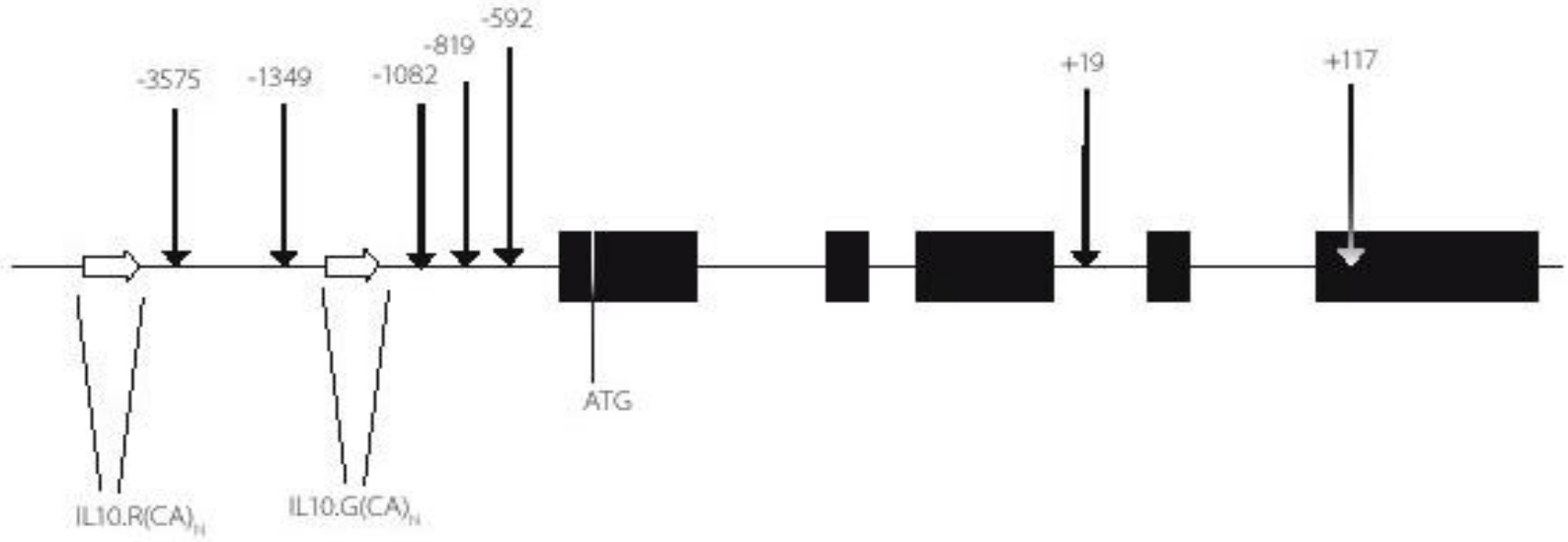


Genes Immun. 2004 Jun;5(4):274-82.

Haplotypes	IBS group ($n = 45$; %)	Control group ($n = 137$; %)	*P* value	*Pc* value[c]	OR (95 % CI)[d]
IL-8[a]					
AGCT	21.34	22.16	0.896	6.272	0.95 (0.47–1.94)
TTCC	3.48	19.09	0.003	0.025	0.15 (0.03–0.63)
AGTT	8.24	9.02	0.898	6.289	0.93 (0.32–2.68)

[c] Bonferroni correction

IL-10 SNPs



SNP -1082 -819 -592

Haplotypes	IBS group ($n = 45$; %)	Control group ($n = 137$; %)	*P* value	*Pc* value[c]	OR (95 % CI)[d]
IL-10[b]					
ATA	32.7	28.3	0.498	3.486	1.19 (0.71–1.99)
GCC	17.71	31.84	0.011	0.075	0.46 (0.26–0.84)
ACC	26.43	7.94	<0.001	<0.001	4.17 (2.19–7.89)

[c] Bonferroni correction