IL-6 174 G/C Polymorphism in Inflammatory Bowel Disease: A Systematic Review and Meta-Analysis

Danyal Imani[1], Ramazan Rezaei[2], Davood Jafari[1], Bahman Razi[3], Shahab Alizadeh[4], Mohammad Bashashati[5], Nima Rezaei[1,6,7]

[1] Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

[2] Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[3] Department of Hematology, School of Paramedicine, Tehran University of Medical Sciences, Tehran, Iran

[4] Department of Cellular and Molecular Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran

[5] Network of Immunity in Infection, Malignancy, and Autoimmunity (NIIMA), Universal Scientific Education and Research Network (USERN), El Paso, TX, USA

[6] Research Center for Immunodeficiencies, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran

[7] Systematic Review and Meta-Analysis Expert Group (SRMEG), Universal Scientific Education and Research Network (USERN), Tehran, Iran

Received: 26 Jun. 2018; Accepted: 10 Nov. 2018

Abstract- The results of previous studies on the association between IL-6-174G/C (rs1800795) polymorphism and inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC) are not consistent. The present meta-analysis has pooled all eligible studies to understand the relation between this gene polymorphism and IBD risk. A structured search of Medline, EMBASE, and Scopus databases were performed to identify all eligible studies published before June 2017. Odds ratio (OR) and 95% confidence intervals (CIs) were assessed applying fixed- or random-effect models to evaluate the strength of association in recessive model, dominant model, allelic model, heterozygote contrast, and homozygotes contrast. A sum of 9 articles with 1524 IBD cases and 1586 healthy subjects were included in this study. No significant association between the IL-6 -174 G/C polymorphism and overall IBD susceptibility in any tested genetic model was found. Moreover, in the subgroup analysis based on subtypes, the associations between the IL-6 174-G/C polymorphism and CD and UC missed statistical significance. The current meta-analysis suggests that the IL-6-174 G/C polymorphism is not associated with IBD susceptibility. Further and comprehensive studies are necessary to warrant this result.

Acta Med Iran 2018;56(12):740-749.

Keywords: Interleukin-6; Polymorphism; Inflammatory bowel disease; Meta-analysis

Introduction

Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is a usual chronic autoimmune disease of the gastrointestinal tract affecting more than 2.5 million patients in Europe and 1 million in the USA (1). The exact mechanisms which involved in the etiology and pathogenesis of IBD remaining elusive; however, it is believed to occur because of disturbed mucosal immune response in genetically susceptible individuals (2,3). Pro-inflammatory cytokines have been demonstrated to be directly involved in the pathogenesis and linked with the severity of IBD (4,5). IL-6 is an essential mediator of inflammation (6). Accumulating evidence of various studies suggests that this cytokine acts as a key character in the pathogenesis of IBD (7-9).

The IL-6 gene which is located on short arm of chromosome 7, encodes the protein of this pro-inflammatory cytokine. This gene spans 5 kb and includes four introns and five exons (10). Almost 50 SNPs (single nucleotide polymorphisms) in untranslated region of the human IL-6 gene have been described (11,12). The common polymorphisms at position -174 (IL-6 -174G/C) can alter transcriptional process and IL-6 level. While -174 C/C is the low producer genotype, G/G and C/G genotypes are linked with a higher basal level of IL-6 (13). Earlier studies have demonstrated that IL-6 -174

Corresponding Author: N. Rezaei
Research Center for Immunodeficiencies, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran
Tel: +98 21 6692 9234, Fax: +98 21 66929235, E-mail address: rezaei_nima@tums.ac.ir

G/C SNP is associated with the onset and development of several human disorders such as pulmonary tuberculosis, (14) Behcet's disease, (15) juvenile systemic lupus erythematosus, (16) allergic rhinitis, (17,18) atopic dermatitis, (19) neuroblastoma, (20) systemic lupus erythematosus, (21) and nephritis (22).

A relatively large number of studies have evaluated the relation between IL-6-174G/C SNP and IBD susceptibility; nevertheless, the results of these studies were inconsistent, probably due to limited sample sizes and different study populations. Although a group of these studies reported highly significant association between the IL-6 -174G/C SNP and IBD risk, others failed to find statically significant association. Thus, we fulfilled the current meta-analysis on all eligible case-control studies to obtain a better estimate of the effect of IL-6 -174G/C (rs1800795) polymorphisms on the risk of IBD, and more specifically the IBD subtypes CD and UC.

Materials and Methods

The current study was conducted in accordance with MOOSE (Meta-analysis of Observational Studies in Epidemiology) guidelines (23).

Searches and data sources

The Scopus, EMBASE, and Medline databases were systematically examined prior to September 2017 for all studies that had assessed the relation between IL-6 -174G/C (rs1800795) SNP and IBD susceptibility. The following search keywords were exerted: (interleukin6 OR IL-6) AND (inflammatory bowel disease OR IBD OR Crohn's Disease OR ulcerative colitis) AND (polymorphism OR polymorphisms OR SNP OR variation OR mutation). Furthermore, Web of science, google scholar, and cross-references within both review and original articles were evaluated for further literature. The search strategy was limited to articles which were in English language.

Inclusion criteria

The desired studies were included in the present meta-analysis if they met the subsequent criteria: (a) they were case-control or nested case-control studies that explored the relationship between IL-6 -174G/C polymorphism as exposure and overall IBD, CD or UC as the main outcomes; (b) risk estimates with 95% confidence intervals (CIs) could be extracted or calculated; (c) genotype frequency or allele frequency for the IL-6 -174G/C SNP in both cases and controls, and (d) appropriate IBD identification criteria (according to clinical, endoscopic, radiologic, and pathologic bases) were reported. We excluded reviews, case studies, comments, letters, abstracts, republished studies and studies with overlapped subjects. In addition, studies were excluded if the essential data not available. The selected articles were independently reviewed by two investigators for eligibility based on predefined criteria and discrepancies were determined by discussion among all authors.

Data extraction and quality assessment

In this study, the subsequent data was extracted with the use of a standardized data extraction form: the author's name, country, journal, and year of publication, ethnicity, mean of age, sex, sample size, genotype and allele frequency, and method for genotyping. The NOS (Newcastle–Ottawa Scale) was exerted to evaluate the quality of chosen studies (24). The NOS criteria determines the studies based on a star system, ranging from 0 to 9 stars. Reports, scoring 6 to 9 were classified as "high quality."

Statistical analysis

For each case-control study, deviancy from HWE (Hardy-Weinberg equilibrium) was calculated using a $\chi 2$ test (25). Odds ratio (OR) and 95% CI were applied to measure the extent of association between IL-6 -174G/C SNP and the risk of overall IBD, UC and, CD in allelic model (C vs. G), recessive model (CC vs. GC/GG), dominant model (GC/CC vs. GG), heterozygote contrast (CG vs. GG), and homozygotes contrast (CC vs. GG), respectively. ORs were calculated based on Woolf method (26). Heterogeneity across studies was determined by I^2 test and chi-square-based Q test, and the threshold of significance was set at $P<0.10$. Since heterogeneity was detected in recessive model in IBD and dominant model and heterozygote contrast in UC random effects used. In other genetic models, fixed-effects conducted. Sensitivity analysis was fulfilled to evaluate the exact effect of studies deviated from HWE on the pooled results. Moreover, visual assessment of funnel plot, (27) Egger's and Begg's tests were applied to determine publication bias, in which $P<0.10$ considered bias. The SPSS (version 23.0) and STATA (version 13.0) were used for all statistical analyses.

Results

Characteristics of qualified studies

The procedures for excluding/including possible studies are represented in figure 1. We observed 657 potentially related articles after the initial search of electronic databases. Following screening, 9 eligible studies with 1586 healthy subjects and 1524 IBD cases were included in present meta-analysis (Figure 1) (28-36). The studies were performed in various locations: two studies were carry out in Germany, (32,35) one in Canada, (29) four in other European countries, (28), (33), (31), (36) and two in Middle East (34), (30). According to NOS scale, all of the nine eligible studies had a good procedural quality with a total score ranging from 6 to 8, and none of them obligated all necessities for a high-quality study free of bias. In the overall population, the allele G (62%) and the GC genotype (37%) were the most frequent alleles and genotypes in both patients and healthy subjects groups. The main characteristics and findings of the included studies are presented in table 1 and table 2.

NR, not reported; M, male; F, female; IBD, inflammatory bowel disease; CD, Crohn's disease, UC, ulcerative colitis.

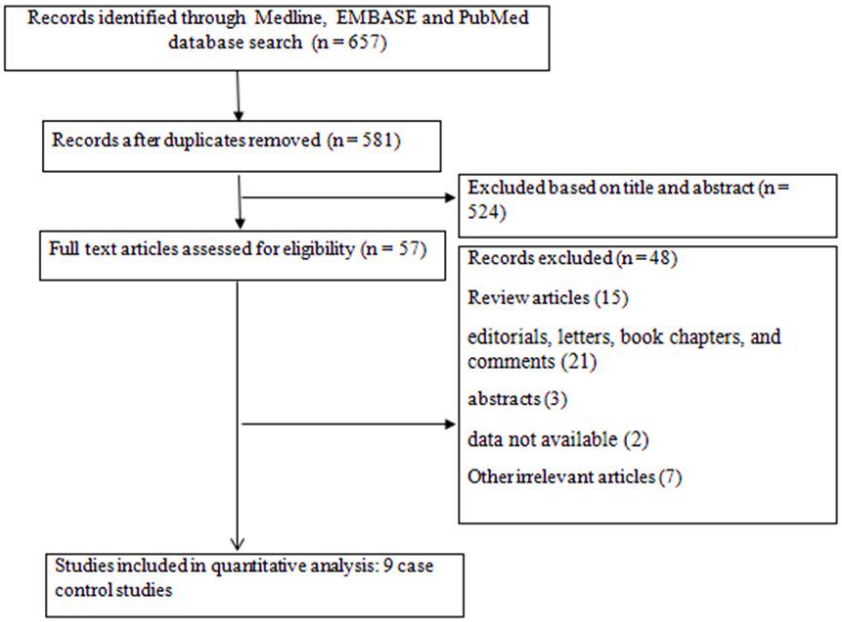

Figure 1. Flow diagram of study selection process.

Table 1. The characteristics of the studies included in the meta-analysis of the overall inflammatory bowel disease, Crohn's disease, and ulcerative colitis

Study author	Year	Ethnicity	Sex	Total cases/controls	Case age/control age (Mean±SD)	Genotype method	Outcome	Quality score
Klein et al.	2001	Caucasian	M/F	302/440	NR/NR	PCR–RELP	IBD, CD, UC	7
Balding et al.	2004	Caucasian	M/F	172/389	30.8±7.1/37.1±11.2	PCR–RELP	IBD, CD, UC	8
Cantor et al.	2005	Caucasian	M/F	193/92	NR/NR	PCR–RELP	IBD, CD, UC	8
Guerreiro et al.	2009	Caucasian	M/F	99/116	40.1±14.6/49.7±11.8	PCR–RELP	IBD, CD	7
Hernandez et al.	2014	Caucasian	M/F	84/135	36±5.22/39±6.2	PCR–SSOP	IBD, CD, UC	8
Stankovic et al.	2015	Caucasian	M/F	167/101	39/38	PCR–RELP	IBD, CD, UC	8
Gok et al.	2014	Caucasian	M/F	69/100	NR/NR	PCR–SSP	IBD, CD	6
Schutle et al.	2001	Caucasian	M/F	105/113	37±13.21/34±11.2	PCR–RELP	IBD	7
Friedgut et al.	2009	Caucasian	M/F	333/100	33/34	PCR–RELP	IBD, CD	8

NR, not reported; M, male; F, female; IBD, inflammatory bowel disease; CD, Crohn's disease, UC, ulcerative colitis

Table 2. Distribution of genotype and allele among IBD, CD and UC patients and controls

Study author	IBD cases					Healthy control					P-HWE	MAF
	GG	GC	CC	G	C	GG	GC	CC	G	C		
Klein et al.	92	141	66	331	273	125	224	91	474	406	0.612	0.46
Balding et al	57	85	30	100	72	123	198	68	222	167	0.444	0.42
Cantor et al	76	89	28	241	145	38	41	13	117	67	0.718	0.36
Guerreiro et al .	44	37	18	125	73	60	52	4	172	60	0.068	0.25
Hernandez et al.	31	44	9	106	62	49	75	11	173	97	0.016*	0.36
Stankovic et al	64	95	18	223	131	61	37	13	159	63	0.057	0.28
Gok et al.	-	-	-	22	11	-	-	-	62	38	-	0.38
Friedgut et al	195	102	13	492	128	63	28	9	154	46	0.036*	0.23
Schutle et al .	32	51	22	115	95	42	52	19	136	90	0.671	0.40
Study author	**CD cases**					**Healthy control**						
	GG	**GC**	**CC**	**G**	**C**	**GG**	**GC**	**CC**	**G**	**C**	**P-HWE**	**MAF**
Klein et al.	48	85	36	181	157	125	224	91	474	406	0.612	0.46
Balding et al.	14	41	9	69	59	123	198	68	222	167	0.444	0.42
Cantor et al	50	70	18	135	106	38	41	13	117	67	0.718	0.36
Guerreiro et al	44	37	18	125	73	60	52	4	172	60	0.068	0.25
Hernandez et al	25	28	4	78	36	49	75	11	173	97	0.016*	0.36
Stankovic et al	24	40	8	88	56	61	37	13	159	63	0.057	0.28
Gok et al.	-	-	-	22	11	-	-	-	62	38	-	0.38
Friedgut et al	195	102	13	492	128	63	28	9	154	46	0.036*	0.23
Study author	**UC cases**					**Healthy control**						
	GG	**GC**	**CC**	**G**	**C**	**GG**	**GC**	**CC**	**G**	**C**	**P-HWE**	**MAF**
Klein et al	47	56	30	103	86	125	224	91	474	406	0.612	0.46
Balding et al.	43	44	21	130	86	123	198	68	222	167	0.444	0.42
Cantor et al	26	19	10	71	39	38	41	13	117	67	0.718	0.36
Hernandez et al	6	16	5	28	26	49	75	11	173	97	0.016*	0.36
Stankovic et al	38	40	45	125	65	61	37	13	159	63	0.057	0.28

P-HWE, *P* for Hardy–Weinberg equilibrium; MAF, minor allele frequency of control group; other abbreviation as table 1.

*studies deviated from HWE, IBD, inflammatory bowel disease; CD, Crohn's disease, UC, ulcerative colitis

Quantitative analysis

Meta-analysis for IL-6 −174G/C polymorphism and the overall IBD

Based on the inclusion criteria, 9 studies with 1524 patients and 1586 healthy subjects' were analyzed. The summarized results and heterogeneity tests for the relation between the IL-6-174 G/C polymorphism and the risk of overall IBD, CD, and UC in five analysis models are illustrated in Table 3. No significant association was found between the IL-6 -174 G/C SNP and overall IBD susceptibility in five genetic models including dominant model (OR=1.10, 95% CI=0.93–1.30, fixed-effects)(Figure 2**)**, recessive model (OR=1.18, 95% CI=0.80–1.75, random effects) (Figure 3**)**, allelic model (OR=1.08, 95% CI=0.96–1.22, fixed effects) (Figure 4**) ,**CC vs. GG model (OR=1.08, 95% CI=0.84–1.40, fixed effects) (Figure 5**)** and GC vs. GG model (OR=1.07, 95% CI=0.90–1.28, fixed-effects) (Figure 6**)**.

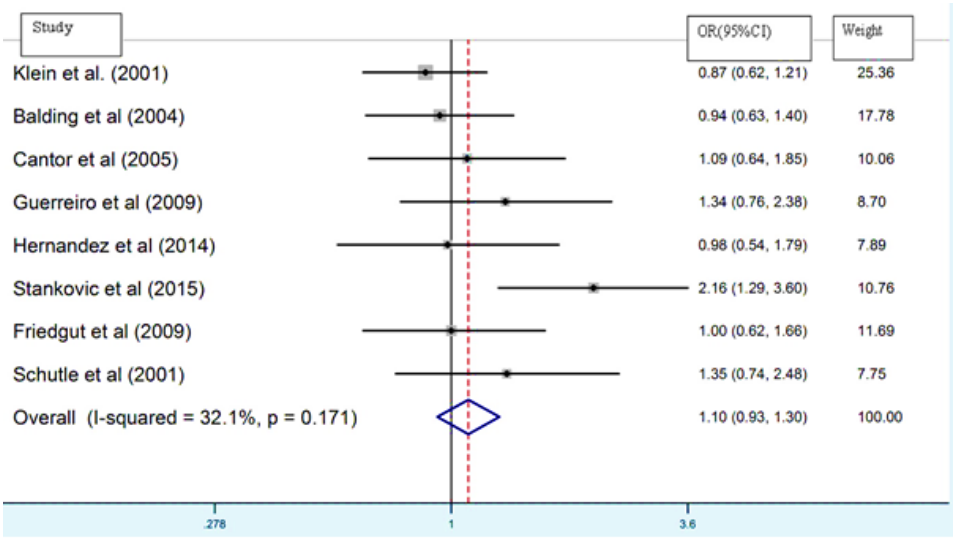

Figure 2. Association between the 174 G/C (rs1800795) SNP in IL-6 and IBD for dominant model

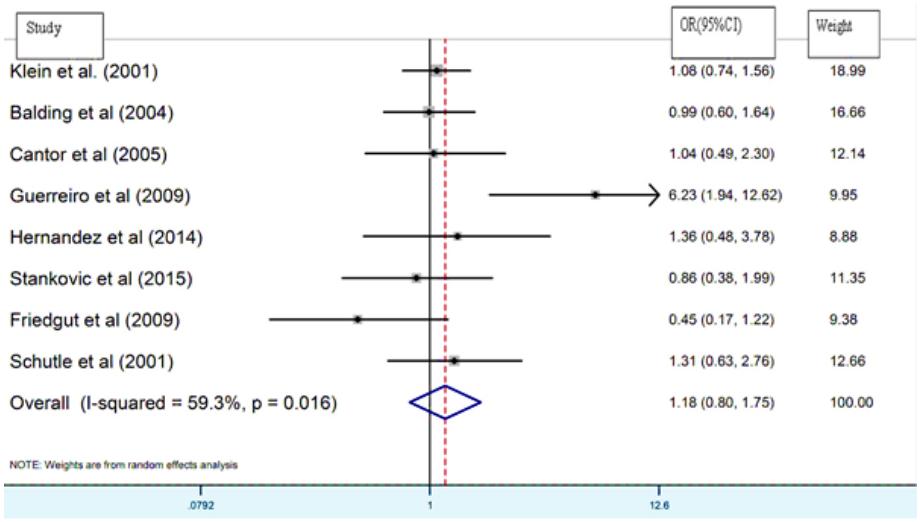

Figure 3. Association between the 174 G/C (rs1800795) SNP in IL-6 and IBD for recessive model

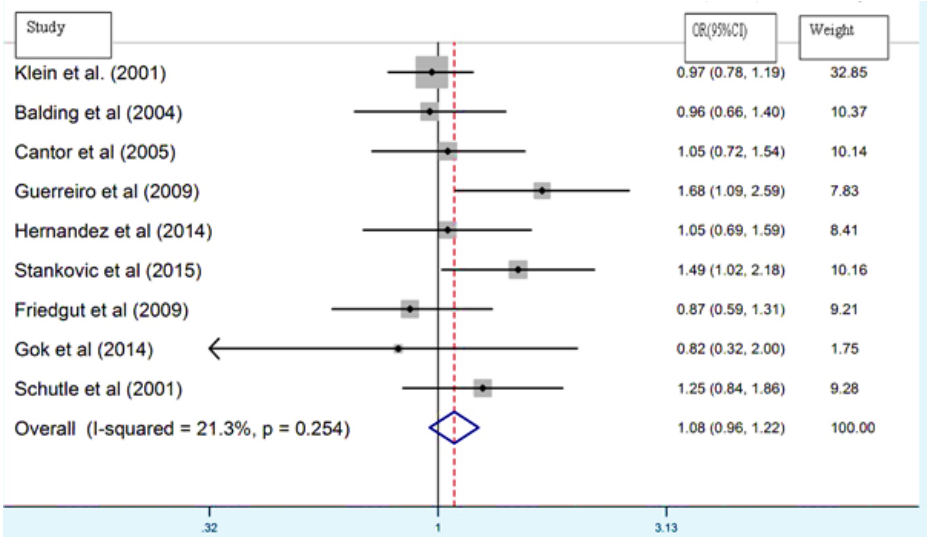

Figure 4. Association between the 174 G/C (rs1800795) SNP in IL-6 and IBD for allelic model.

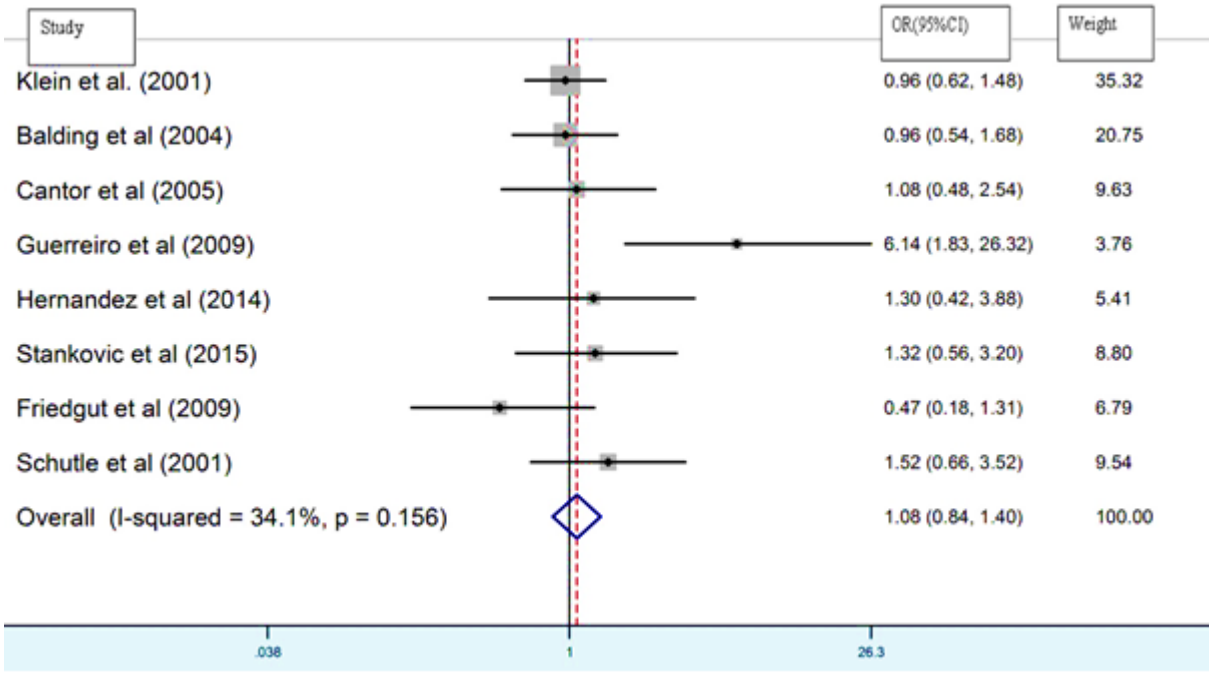

Figure 5. Association between the 174 G/C (rs1800795) SNP in IL-6 and IBD for CC vs. GG model.

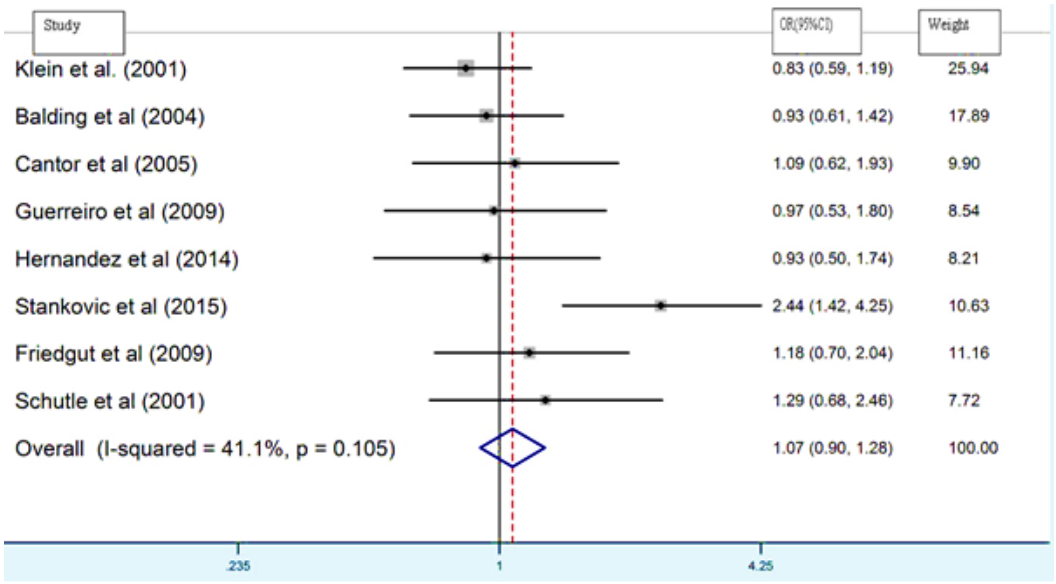

Figure 6. Association between the 174 G/C SNP in IL-6 and IBD for CG vs. GG model.

Meta-analysis for IL-6 −174G/C polymorphism and CD

In subgroup analysis, the association between IL-6 −174 G/C polymorphism and CD was assessed in 8 studies with a total of 1045 cases and 1473 healthy subjects. When all qualified articles were pooled, the quantitative analysis revealed no significant association between IL-6 -174 G/C polymorphism and Crohn's risk under five genetic models containing dominant model (OR=1.19, 95% CI=0.97–1.47, fixed-effects), recessive model (OR=0.98, 95% CI=0.72–1.34, fixed effects), allelic model (OR=1.13, 95% CI=0.98–1.31, fixed effects), CC vs. GG model (OR=1.10, 95% CI=0.78–1.55, fixed effects) and GC vs. GG model (OR=1.20, 95% CI=0.96–1.50, fixed-effects) (Table 3).

Table 3. Main results of the pooled ORs in a meta-analysis of the IL6-174 G/C polymorphism.

							Test of publication bias			
		Sample size	Test of association		Test of heterogeneity		Begg's		Egger's	
Disease	Genetic model	Case/Control	OR	95%CI	I^2(%)	P	Z	P	t	*P*
IBD	Dominant model	1524/1586	1.10	0.93-1.30	32.1	0.17	1.73	0.08	1.70	1.70
	Recessive model	1524/1586	1.18	0.80-1.75	59.3	0.01	0.25	0.80	0.60	0.32
	Allelic model	1524/1586	1.08	0.96-1.22	21.3	0.25	1.04	0.29	0.61	0.56
	CC vs. GG	1524/1586	1.08	0.84-1.40	34.1	0.15	1.48	0.13	1.29	0.24
	GC vs. GG	1524/1586	1.07	0.90-1.28	41.1	0.10	0.32	0.54	1.34	0.22
CD	Dominant model	1045/1473	1.19	0.97-1.47	30.7	0.19	0.75	0.45	1.12	0.31
	Recessive model	1045/1473	0.98	0.72-1.34	43.2	0.10	0.15	0.88	0.34	0.77
	Allelic model	1045/1473	1.13	0.98-1.31	33.4	0.16	0.49	0.62	0.19	0.85
	CC vs. GG	1045/1473	1.10	0.78-1.55	39.9	0.12	0.75	0.45	0.61	0.56
	GC vs. GG	1045/1473	1.20	0.96-1.50	39.7	0.12	1.05	0.29	1.16	0.29
UC	Dominant model	374/1157	0.96	0.65-1.42	52.7	0.07	0.98	0.32	1.42	0.24
	Recessive model	374/1157	1.17	0.85-1.62	0.0	0.77	1.47	0.14	1.11	0.34
	Allelic model	374/1157	1.05	0.86-1.29	4.8	0.37	1.47	0.15	1.68	0.19
	CC vs. GG	374/1157	1.1	0.71-1.45	0.0	0.53	0.98	0.33	1.09	0.35
	GC vs. GG	374/1157	0.92	0.58-1.45	50.0	0.04	1.96	0.05	3.64	0.03

IBD, inflammatory bowel disease; CD, Crohn's disease, UC, ulcerative colitis.

Meta-analysis for IL-6 −174G/C polymorphism and UC

The association between IL-6−174 G/C SNP and UC was assessed in 5 case-control studies with a totality of 1157 healthy subjects and 374 cases. The association between the IL-6-174 G/C polymorphism and UC susceptibility in all of the genetic model including dominant model (OR=0.96, 95% CI=0.65–1.42, random-effects), recessive model (OR=1.17, 95% CI=0.85–1.62, fixed effects), allelic model (OR=1.05, 95% CI=0.86–1.29, fixed effects) and CC vs. GG model (OR=1.10, 95% CI=0.71–1.45, fixed effects) and GC vs. GG model (OR=1.20, 95% CI=0.96–1.50, random-effects) missed statistical significant threshold (Table 3).

Evolution of publication bias and sensitivity analysis

We evaluated publication bias by using Egger's and Begg's tests and funnel plot. Overall, no result of publication bias was detected in most of the genetics models. In the sensitivity analysis, we removed the study by Hernandez *et al*., (33) and Friedgut *et al*., (34) due to the deviation of the genotypes distribution from HWE in the healthy subjects group, and established that the acquired pooled ORs were not substantially changed, highlighting the reliability of our results.

Discussion

IBD is a heterogeneous autoimmune disease which both environmental and genetic factors are involved in the pathogenesis of this disease (37). IL-6 as a multifunctional cytokine considerably affects both innate and adaptive immune responses and displays a wide spectrum of biological events in inflammation, immune regulation, oncogenesis processes, and tissue hematopoiesis (38-40).

The lamina propria of innate immune cells through the NF-κB pathway secrets IL-6 which induces intestinal injury (38). Furthermore, IL-6 is a key element in effective host defense against extracellular pathogens at mucosal sites and mucosal protection (41).

IL-6 exhibits both activating and inhibiting inflammatory properties in context, and it is currently anticipated as an optimum clinical choice (39). Pro-inflammatory IL-6 is one of the major orchestrators of pathogenicity in IBD and is linked to the differentiation of T helper 1/T helper 2 (37).

IL-6 is also actively involved in controlling the differentiation and sustenance of inflammatory T helper cells (42). Additionally, synapse between IL-6 and its ligand on CD4+ T cells results in STAT3 augmentation and nuclear transformation, inducing the production of anti-apoptotic genes like Bcl-xl. The consequence is a strengthen lamina propria T-cells resistance to apoptosis with subsequent T cell proliferation, that culminates in chronic intestinal inflammation. Thus IL-6 is prominent in the pathogenicity of T cell orchestrated autoimmune diseases, including IBD (40).

Clinical manifestation of IBD has been positively linked to intestinal or circulating IL-6 levels (37,40,43). The disease intensity based on endoscopy and histopathology of active IBD patients has also been associated with the increase in serum IL6 protein and

mRNA in inflamed mucosa (37).

Moreover, the involvement of IL-6 gene SNP in influencing the predisposition and physical manifestation of IBD has also been reported (29,43,44). There are many studies which evaluate the association between IL-6 -174G/ C polymorphism and IBD risk; however, inconsistent and inconclusive results are achieved.

To the best of authors' knowledge, this is the first study evaluating the relation between IL-6 -174G/C polymorphism and IBD risk. Following a comprehensive search and screening of electronic databases, 9 case-control studies fulfilled the inclusion criteria and were evaluated, including a total of 1586 healthy subjects and 1524 IBD patients. The results of current meta-analysis demonstrated that there was no significant association between the IL-6 -174- G/C SNP and IBD susceptibility in all genetic models. AS the results may have been influenced by subgroup analysis, we performed subgroup analysis, including CD and UC, which revealed that the association between IL-6-174- G/C SNP and UC and CD risk missed the statistical significance.

This study had some limitations. First, the number of eligible studies which fulfilled our inclusion criteria was limited. Therefore, we failed to conduct subgroup analysis based on age, sex, and race and assess possible variations in the population. Second, significant results of publication bias were observed in the heterozygotes contrast for studies exploring the association between IL-6−174G/C SNP and CD. We limited our search to studies in English; thus, those published in non-English languages might be the source of publication bias. Third, because of inaccessibility for original data, we were not able to further assess the potential interactions between gene-gene and gene-environmental factors, which this shortage might affect our results. Fourth, GWA-studies or immunochip-based studies not included in this study. Finally, all of the included studies were conducted in Caucasians, and data regarding other ethnicities were not found. Therefore, further studies are warranted to identify the relationship between this SNP and IBD susceptibility, especially in East-Asian and African populations.

In conclusion, this meta-analysis of currently available evidence suggests that the IL-6-174-G/C SNP is not associated with increased or decreased risk of IBD. Due to the potential paucity of the available data, further large, well-designed epidemiological researches are necessary to allow a more definitive conclusion. Such studies should also take environmental factor as well as mucosal and secretory IL-6 levels into account to gain a more comprehensive understanding of the association between IL-6-174 G/C SNP and susceptibility to CD and UC. Finally, whether this polymorphism of IL-6 could be used as a biomarker of response to therapies in IBD needs further investigation.

Acknowledgments

This study was supported by a grant from Tehran University of Medical Sciences (35541).

References

1. Kaplan GG. The global burden of IBD: from 2015 to 2025. Nat Rev Gastroenterol Hepatol 2015;12:720-7.
2. Liu TC, Stappenbeck TS. Genetics and Pathogenesis of Inflammatory Bowel Disease. Ann Rev Pathol 2016;11:127-48.
3. Kevans D, Silverberg MS, Borowski K, Griffiths A, Xu W, Onay V, et al. IBD Genetic Risk Profile in Healthy First-Degree Relatives of Crohn's Disease Patients. J Crohns Colitis 2016;10:209-15.
4. Powrie F, Leach MW, Mauze S, Menon S, Caddle LB, Coffman RL. Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RBhi CD4+ T cells. Immunity 1994;1:553-62.
5. Neurath MF, Finotto S, Glimcher LH. The role of Th1/Th2 polarization in mucosal immunity. Nat Med 2002;8:567-73.
6. Nguyen DP, Li J, Tewari AK. Inflammation and prostate cancer: the role of interleukin 6 (IL-6). BJU Int 2014;113:986-92.
7. Takac B, Mihaljevic S, Stefanic M, Glavas-Obrovac L, Kibel A, Samardzija M. Importance of interleukin 6 in pathogenesis of inflammatory bowel disease. Coll Antropol 2014;38:659-64.
8. Lochhead P, Khalili H, Ananthakrishnan AN, Richter JM,Chan AT. Association Between Circulating Levels of C-Reactive Protein and Interleukin-6 and Risk of Inflammatory Bowel Disease. Clin Gastroenterol Hepatol 2016;14:818-24.
9. Cekic C, Arabul M, Alper E, Pakoz ZB, Saritas E, Yuksel, et al. Evaluation of the relationship between serum ghrelin, C-reactive protein and interleukin-6 levels, and disease activity in inflammatory bowel diseases. Hepatogastroenterology 2014;61:1196-200.
10. Ataie-Kachoie P, Pourgholami MH, Richardson DR,Morris DL. Gene of the month: Interleukin 6 (IL-6). J Clin Pathol 2014;67:932-7.
11. Pereira DS, Garcia DM, Narciso FM, Santos ML, Dias JM, Queiroz BZ, et al. Effects of 174 G/C polymorphism in the promoter region of the interleukin-6 gene on plasma IL-6 levels and muscle strength in elderly women. Braz J Med

Biol Res 2011;44:123-9.
12. Nie G, Xie CL, Cao YJ, Xu MM, Shi X, Zou AL, et al. Meta-analysis of IL-6 -174G/C polymorphism and psoriasis risk. Genet Mol Res 2016;15.
13. Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest 1998;102:1369-76.
14. Amirzargar AA, Rezaei N, Jabbari H, Danesh AA, Khosravi F, Hajabdolbaghi M, et al. Cytokine single nucleotide polymorphisms in Iranian patients with pulmonary tuberculosis. Eur Cytokine Netw 2006;17:84-9.
15. Amirzargar A, Shahram F, Nikoopour E, Rezaei N, Saeedfar K, Ziaei N, et al. Proinflammatory cytokine gene polymorphisms in Behcet's disease. Eur Cytokine Netw 2010;21:292-6.
16. Ziaee V, Tahghighi F, Moradinejad MH, Harsini S, Mahmoudi M, Rezaei A, et al. Interleukin-6, interleukin-1 gene cluster and interleukin-1 receptor polymorphisms in Iranian patients with juvenile systemic lupus erythematosus. Eur Cytokine Netw 2014;25:35-40.
17. Nasiri R, Movahedi M, Amirzargar AA, Hirbod-Mobarakeh A, Farhadi E, Ansaripour B, et al. Association of interleukin 6 single nucleotide polymorphisms with allergic rhinitis. Int J Pediatr Otorhinolaryngol 2014;78:1426-9.
18. Nieters A, Linseisen J,Becker N. Association of polymorphisms in Th1, Th2 cytokine genes with hayfever and atopy in a subsample of EPIC-Heidelberg. Clin Exp Allergy 2004;34:346-53.
19. Gharagozlou M, Farhadi E, Khaledi M, Behniafard N, Sotoudeh S, Salari R, et al. Association between the interleukin 6 genotype at position-174 and atopic dermatitis. J Investig Allergol Clin Immunol 2013;23:89-93.
20. Totaro F, Cimmino F, Pignataro P, Acierno G, De Mariano M, Longo L, et al. Impact of interleukin-6 -174 G>C gene promoter polymorphism on neuroblastoma. PloS One 2013;8:e76810.
21. Santos MJ, Fernandes D, Capela S, da Silva JC,Fonseca JE. Interleukin-6 promoter polymorphism -174 G/C is associated with nephritis in Portuguese Caucasian systemic lupus erythematosus patients. Clin Rheumatol 2011;30:409-13.
22. Spasojevic-Dimitrijeva B, Zivkovic M, Stankovic A, Stojkovic L,Kostic M. The IL-6 -174G/C polymorphism and renal scarring in children with first acute pyelonephritis. Pediatr Nephrol 2010;25:2099-106.
23. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. JAMA 2000;283:2008-12.
24. Wells G, Shea B, O'connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. (Accessed January 2018, 12, at http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp).
25. Wigginton JE, Cutler DJ, Abecasis GR. A note on exact tests of Hardy-Weinberg equilibrium. Am J Hum Genet 2005;76:887-93.
26. Woolf B. On estimating the relation between blood group and disease. Ann Hum Genet 1955;19:251-3.
27. Lau J, Ioannidis JP, Terrin N, Schmid CH, Olkin I. The case of the misleading funnel plot. BMJ 2006;333:597-600.
28. Balding J, Livingstone WJ, Conroy J, Mynett-Johnson L, Weir DG, Mahmud N, et al. Inflammatory bowel disease: the role of inflammatory cytokine gene polymorphisms. Mediator Inflamm 2004;13:181-7.
29. Cantor MJ, Nickerson P, Bernstein CN. The role of cytokine gene polymorphisms in determining disease susceptibility and phenotype in inflammatory bowel disease. Am J Gastroenterol 2005;100:1134-42.
30. Gok I, Uçar F, Ozgur O, Efe H. Analysis of Cytokine Gene Polymorphism Allelic Variation in the Turkish Population with Inflammatory Bowel Disease. Biomed Res 2014;25:551-9.
31. Guerreiro CS, Ferreira P, Tavares L, Santos PM, Neves M, Brito M, et al. Fatty acids, IL6, and TNFα polymorphisms: an example of nutrigenetics in Crohn's disease. Am J Gastroenterol 2009;104:2241-9.
32. Klein W, Tromm A, Griga T, Fricke H, Folwaczny C, Hocke M, et al. The polymorphism at position− 174 of the IL-6 gene is not associated with inflammatory bowel disease. Eur J Gastroenterol Hepatol 2001;13:45-7.
33. López-Hernández R, Valdés M, Campillo J, Martínez-García P, Salama H, Bolarin J, et al. Pro-and anti-inflammatory cytokine gene single-nucleotide polymorphisms in inflammatory bowel disease. Int J Immunogenet 2015;42:38-45.
34. Sagiv-Friedgut K, Karban A, Weiss B, Shaoul R, Shamir R, Bujanover Y, et al. Early-onset Crohn disease is associated with male sex and a polymorphism in the IL-6 promoter. J Pediatr Gastroenterol Nutr 2010;50:22-6.
35. Schulte CM, Goebell H, Rõher HD, Schulte KM. Genetic determinants of IL-6 expression levels do not influence bone loss in inflammatory bowel disease. Dig Dis Sci 2001;46:2521-8.
36. Stankovic B, Dragasevic S, Popovic D, Zukic B, Kotur N, Sokic-Milutinovic A, et al. Variations in inflammatory

genes as molecular markers for prediction of inflammatory bowel disease occurrence. J Digest Dis 2015;16:723-33.
37. Atreya R, Neurath MF. Involvement of IL-6 in the pathogenesis of inflammatory bowel disease and colon cancer. Clin Rev Allergy Immunol 2005;28:187-95.
38. Kishimoto T. IL-6: from its discovery to clinical applications. Int Immunol 2010;22:347-52.
39. Hunter CA, Jones SA. IL-6 as a keystone cytokine in health and disease. Nat Immunol 2015;16:448-57.
40. Grivennikov S, Karin E, Terzic J, Mucida D, Yu GY, Vallabhapurapu S, et al. IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. Cancer Cell 2009;15:103-13.
41. Dann SM, Spehlmann ME, Hammond DC, Iimura M, Hase K, Choi LJ, Hanson E, Eckmann L. IL-6-dependent mucosal protection prevents establishment of a microbial niche for attaching/effacing lesion-forming enteric bacterial pathogens. J Immunol 2008;180:6816-26.
42. Kimura A, Kishimoto T. IL-6: Regulator of Treg/Th17 balance. Eur J Immunol 2010;40:1830-5.
43. Todhunter CE, Sutherland-Craggs A, Bartram SA, Donaldson P, Daly A, Francis R, et al. Influence of IL-6, COL1A1, and VDR gene polymorphisms on bone mineral density in Crohn's disease. Gut 2005;54:1579-84.
44. Senhaji N, Serrano A, Badre W, Serbati N, Karkouri M, Zaid Y, et al. Association of inflammatory cytokine gene polymorphisms with inflammatory bowel disease in a Moroccan cohort. Genes Immun 2016;17:60-5.