Review

Relationship between Interleukin-6 (–174G/C and –572C/G) Promoter Gene Polymorphisms and Risk of Intracerebral Hemorrhage: A Meta-Analysis

Pradeep Kumar Shubham Misra Arun Kumar Yadav Amit Kumar
Mukesh Sriwastva Kameshwar Prasad

Department of Neurology, All India Institute of Medical Sciences, New Delhi, India

Key Words

Interleukin-6 · Cytokine · Intracerebral hemorrhage · Single nucleotide polymorphisms

Abstract

Background: Polymorphisms of –174G/C and –572C/G in the Interleukin-6 (IL-6) promoter gene can affect both transcription and secretion of IL-6 and may be involved in the inflammatory mechanisms in early and delayed phases after intracerebral hemorrhage (ICH). The role of these polymorphisms remains unclear for the pathogenesis of ICH. **Methods:** PubMed, EMBASE, MEDLINE and Google Scholar searches were conducted from January 1, 1950 to February 29, 2016 and were supplemented with relevant articles identified in the references. The following search terms were used: ('interleukin-6' or 'IL-6') and ('genetic polymorphism' or 'single nucleotide polymorphisms' or 'SNP') and ('intracerebral hemorrhage' or 'ICH') and ('hemorrhagic stroke' or 'HS'). Fixed or random effects models were used to estimate the pooled odds ratios and 95% confidence intervals. Begg's funnel plot was used to assess the potential for publication bias. **Results:** In our meta-analysis, three case-control studies involving 446 ICH cases and 2,322 controls were included. No significant association was observed for the IL-6 (–174G/C and –572C/G) gene polymorphisms with the risk of ICH under dominant, recessive and allelic models. **Conclusion:** Our meta-analysis suggests that IL-6 gene polymorphisms are not associated with the risk of ICH. However, caution must be taken while considering the results of our meta-analysis due to the presence of small sample size. Our results cannot be extrapolated to represent the effect of entire IL-6 genetic polymorphism on stroke patients worldwide. Therefore, further well-designed studies with large sample size are warranted to validate our findings and provide a profound conclusion.

© 2016 S. Karger AG, Basel

Dr. Kameshwar Prasad
Department of Neurology, Neurosciences Centre
All India Institute of Medical Sciences, Room No. 702, 7th Floor
Ansari Nagar, New Delhi 110029 (India)
E-Mail kp0704@gmail.com

Introduction

Stroke is the major leading cause of morbidity and mortality worldwide [1]. Intracerebral hemorrhage (ICH) constitutes about 10–15% of all strokes. The incidence of ICH is higher in Asian countries compared to Western countries [2]. This may be due to the difference in the prevalence of the risk factors as well as lifestyle, environmental and unclear genetic risk factors [3]. There is increasing evidence that the inflammatory mechanisms participate in early and delayed phases after ICH. Inflammation is influenced by various cytokines, such as interleukin-6 (IL-6) which plays an important role in immune regulation and inflammatory inhibition [4–7].

Human IL-6 gene is mapped at chromosome 7p21 which comprises of five exons and four introns and synthesizes a precursor protein of 232 amino acids [8, 9]. IL-6 is a pleiotropic cytokine may be a key mediator in the inflammatory response to ICH [10]. Two functional promoter polymorphisms, –174G/C (rs1800795) and –572C/G (rs1800796) have been identified in the IL-6 promoter region and these two genetic polymorphisms may be associated with the increased level of IL-6 [11]. The mechanisms leading to increased release of anti-inflammatory cytokines in patients with ICH remains unclear. In addition, IL-6 secretion can also be triggered by sympathetic neurons [12]. Few studies have investigated the association of IL-6 (–174G/C and –572C/G) gene polymorphisms in relation to ICH but have shown conflicting findings [13–15]. Meta-analysis of genetic association studies combines the results from independent studies, explores the sources of heterogeneity, and identifies subgroups associated with the factor of interest. Therefore, we conducted this meta-analysis to establish a firm association between the variants (–174G/C and –572C/G) of IL-6 promoter gene polymorphisms and risk of ICH.

Materials and Methods

Identification of Relevant Studies

PubMed, EMBASE, MEDLINE and Google Scholar searches were conducted from January 1, 1950 to February 29, 2016. The following search terms were used: ('interleukin-6' or 'IL-6') and ('genetic polymorphism' or 'single nucleotide polymorphisms' or 'SNP') and ('intracerebral hemorrhage' or 'ICH') and ('hemorrhagic stroke' or 'HS'). Fixed or random effects models were used to estimate the pooled odds ratios (ORs) and 95% confidence intervals (CIs). Begg's funnel plot was used to assess the potential for publication bias. Meta-analysis was carried out using the Review Manager 5.3 software. The search was done without the limitations of language, but only included those studies that were conducted on human subjects. All references in eligible articles were extensively reviewed to identify additional published articles.

Inclusion and Exclusion Criteria

To be included in the analysis, eligible studies have to meet the following criteria: (1) case-control studies investigating the association between the IL-6 gene polymorphism and ICH; (2) all patients in the candidate gene studies meet the diagnostic criteria for ICH, and (3) studies with sufficient available data to calculate ORs with corresponding 95% CIs. The major reasons for excluding studies were: (1) not a case-control study; (2) duplicate publications with overlapping subjects from the same study, and (3) no available data reported. This meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline [16]. No author was contacted regarding the missing information that was required for the meta-analysis to avoid the risk of retrieval bias.

Data Extraction

According to the PRISMA guideline, two investigators (P.K. and S.M.) independently checked each full-text report for eligibility and extracted the following data from eligible studies: surname of first author, year of publication, country of origin, ethnicity, definition and number of cases and controls, age, sex ratio, geno-

Fig. 1. Flow diagram for the selection of studies and specific reasons for exclusion from the present meta-analysis.

typing method and genotype frequency. Disagreements were solved by discussion between all authors until consensus was reached.

Quality Assessment

We also evaluated the methodological quality of every study which is included in our analysis using the Newcastle-Ottawa scale [17]. Two authors (P.K. and S.M.) independently assessed the quality of included studies. Discrepancies over quality scores were resolved by discussion with all authors and subsequent consensus was reached.

Statistical Analysis

Genotype distributions in the controls were tested for confirmation of Hardy-Weinberg equilibrium using the χ^2 test. The association between the IL-6 genetic polymorphism and ICH was assessed by the pooled ORs with their corresponding 95% CIs. Taking into consideration possible between-study heterogeneity, a statistical test for heterogeneity was first conducted using Cochran's Q statistic and I^2 metric [18]. We considered the presence of significant heterogeneity at the 10% level of significance and values of I^2 exceeding 50% as an indicator of significant heterogeneity. When no heterogeneity was found with $p < 0.10$ or $I^2 < 50\%$, a fixed-effect model was used to estimate the pooled ORs and 95% CIs. Otherwise, a random-effects model was applied. Begg's funnel plot was used to assess the potential for publication bias.

Results

A total of 38 published articles were identified using the pre-specified search strategy. Figure 1 represents a flow chart of retrieved and excluded studies with their reasons for exclusion. Out of 38 retrieved articles, 27 studies were excluded due to their irrelevancy to our interest, one study was excluded as it was a duplicate study, seven studies were excluded as they were not showing any association with ICH and one study was excluded as it was not a case-control design study. Keeping the inclusion criteria in mind, three case-control studies

Fig. 2. Forest plot for the association between IL-6 –174G/C gene polymorphism and risk of ICH. **a** Dominant model (CC + GC vs. GG). **b** Recessive model (CC vs. GG + GC). **c** Allelic model (C allele vs. G allele).

Table 1. Characteristic of studies included in the meta-analysis of the association of IL-6 (–174G/C and –572C/G) promoter gene polymorphism with the risk of ICH

Study No.	First author, year	Origin	Ethnicity	Variant assay	Sample size (case/control), n	PCR method	Matching criteria	Male, female (case/control), n	Age (case/control), years	HWE p value	Source of control	NOS score
1.	Kumar, 2016 [13]	India	Asian	–174G/C –572C/G	100/100	PCR-SNaPShot	Age, sex	74, 26 74, 26	50.56 ± 11.58 50.50 ± 11.46	yes	HB	9
2.	Banerjee, 2008 [14]	India	Asian	–174G/C	64/212	PCR-RFLP	Age, sex, geography	41, 23/ 143, 69	58.6 ± 14.2/ 57.4 ± 8.8	yes	HB	8
3.	Yamada, 2006 [15]	Japan	Asian	–572C/G	282/2,010	PCR-Array	NA	179, 103/ 844, 1,166	60.6 ± 11.3/ 63.0 ± 11.4	yes	HB	8

HWE = Hardy-Weinberg equilibrium; HB = hospital based; PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism; NA = not available; NOS = Newcastle-Ottawa scale.

Fig. 3. Forest plot for the association between IL-6 –572C/G gene polymorphism and risk of ICH. **a** Dominant model (GG + CG vs. CC). **b** Recessive model (GG vs. CC + CG). **c** Allelic model (G allele vs. C allele).

including one common study for –174G/C and –572C/G [13], one for –174G/C [14] and one for –572C/G gene polymorphisms [15], were included in our meta-analysis. All the three studies were in the Asian population. The publication years of the included studies ranged from 2006 to 2016. All the studies in this meta-analysis had controls in Hardy-Weinberg equilibrium. The quality scores of all included studies were moderately high. All the three studies had a hospital-based source of control. Table 1 gives a summary of the characteristics and methodological quality of all the included studies.

Three case-control studies involving 446 cases and 2,322 controls were included in our meta-analysis. The study did not find any statistical significance of IL-6 –174G/C gene polymorphism with the risk of ICH under dominant (CC + CG vs. GG; OR, 1.14; 95% CI, 0.72–1.79), recessive (CC vs. CG + GG; OR, 1.17; 95% CI, 0.31–4.38) and allelic (C vs. G; OR, 1.11; 95% CI, 0.74–1.67) models (fig. 2). Similarly, we did not find any statistical significance of IL-6 –572C/G gene polymorphism with the risk of ICH under dominant (GG + CG vs. CC; OR, 1.28; 95% CI, 0.29–5.62), recessive (GG vs. CG + CC; OR, 0.77; 95% CI, 0.44–1.33) and allelic (G vs. C; OR, 1.3; 95% CI, 0.36–4.65) models (fig. 3). The shape of the Begg's funnel plots suggested no significant publication bias (fig. 4).

Fig. 4. Begg's funnel plot for the assessment of publication bias for IL-6 –174G/C (**a**) and –572C/G (**b**) gene polymorphisms.

Discussion

IL-6 is a pleiotropic inflammatory cytokine of low molecular weight, approximately 26 kDa, with a biological half-time of less than 1 h [19]. Generally, IL-6 exerts pro-inflammatory properties including stimulation of the growth of mature B cells and promotion of the synthesis of C-reactive protein by the liver during tissue injury or infection [19, 20]. Two functional promoter polymorphisms, –174G/C (rs1800795) and –572C/G (rs1800796) have been identified in the IL-6 promoter region and these two genetic polymorphisms may be associated with the increased level of IL-6 [11]. High circulating IL-6 levels are associated with various diseases, including cardiovascular disease, type 2 diabetes mellitus, cancer growth, acute cerebral ischemia, and acute brain injury [21–24].

ICH has been shown to be influenced by environmental, life style and genetic risk factors, including hypertension, and a polymorphism of IL-6 was also significantly associated with the prevalence of ICH, with the –572C allele being a risk factor for this condition [15]. The –174G/C polymorphism of IL-6 was previously found to be associated with ICH at brain arteriovenous malformations [25]. Previous findings have suggested a high plasma level of IL-6 was shown to be an independent predictor of early hematoma growth of ICH [26].

Individual study might be too underpowered to show any association between IL-6 (–174G/C and –572C/G) gene polymorphisms and risk of ICH; hence, we decided to pool the results from multiple studies. Meta-analysis of genetic association studies is an effective tool for garnering a greater understanding of complex diseases and potentially provides new insights into gene-disease associations. To the best of our knowledge, this is the first meta-analysis on this subject. Our meta-analysis did not find any significant association with either –174G/C or –572C/G gene polymorphism of IL-6 with the risk of ICH. A recent meta-analysis by Kumar et al. [27] consisting of 16 case-control studies involving a total of 3,317 ischemic stroke (IS) patients and 3,432 healthy controls for –174G/C polymorphism and three case-control studies with a total of 2,001 IS patients and 2,027 healthy controls for –572C/G IL-6 promoter gene polymorphism concluded that there was no significant association with the risk of IS [27].

However, there are some limitations which need to be taken into consideration before accepting the results of our meta-analysis. First, a lower number of studies were included. Second, it summarizes the findings of only two SNPs of a single gene. Third, inadequate power of the included studies to draw solid conclusions and finally the heterogeneity in some of the models was a major concern when interpreting the results of meta-analysis in overall ICH studies.

Conclusion

Our meta-analysis suggests that IL-6 gene polymorphisms are not associated with the risk of ICH. However, caution must be taken while considering the results of our meta-analysis due to the presence of small sample size. Our results cannot be extrapolated to represent the effect of entire IL-6 genetic polymorphism on stroke patients worldwide. Therefore, further well-designed studies with large sample size are warranted to validate our findings and provide a profound conclusion.

Disclosure Statement

There is no potential conflict of interest.

References

- 1 Feigin VL, Forouzanfar MH, Krishnamurthi R, Mensah GA, Connor M, Bennett DA, Moran AE, Sacco RL, Anderson L, Truelsen T, O'Donnell M, Venketasubramanian N, Barker-Collo S, Lawes CMM, Wang W, Shinohara Y, Witt E, Ezzati M, Naghavi M, Murray C; and Global Burden of Diseases, Injuries, and Risk Factors Study 2010 (GBD 2010) and the GBD Stroke Experts Group: Global and regional burden of stroke during 1990–2010: findings from the Global Burden of Disease Study 2010. *Lancet* 2014;383:245–254.
- 2 Das SK, Banerjee TK: Stroke: Indian scenario. *Circulation* 2008;118:2719–2724.
- 3 Bevan S, Markus HS: Genetics of common polygenic ischaemic stroke: current understanding and future challenges. *Stroke Res Treat* 2011;2011:179061.
- 4 Seino Y, Ikeda U, Ikeda M, Yamamoto K, Misawa Y, Hasegawa T, Kano S, Shimada K: Interleukin 6 gene transcripts are expressed in human atherosclerotic lesions. *Cytokine* 1994;6:87–91.
- 5 Castillo J, Rodríguez I: Biochemical changes and inflammatory response as markers for brain ischaemia: molecular markers of diagnostic utility and prognosis in human clinical practice. *Cerebrovasc Dis* 2004;17(suppl 1):7–18.
- 6 Tso AR, Merino JG, Warach S: Interleukin-6 174G/C polymorphism and ischemic stroke: a systematic review. *Stroke J Cereb Circ* 2007;38:3070–3075.
- 7 Ridker PM, Rifai N, Stampfer MJ, Hennekens CH: Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation* 2000;101:1767–1772.
- 8 Sehgal PB, Zilberstein A, Ruggieri RM, May LT, Ferguson-Smith A, Slate DL, Revel M, Ruddle FH: Human chromosome 7 carries the beta 2 interferon gene. *Proc Natl Acad Sci USA*, 1986;83:5219–5222.
- 9 Zilberstein A, Ruggieri R, Korn JH, Revel M: Structure and expression of cDNA and genes for human interferon-beta-2, a distinct species inducible by growth-stimulatory cytokines. *EMBO J* 1986;5:2529–2537.
- 10 Suzuki S, Tanaka K, Suzuki N: Ambivalent aspects of interleukin-6 in cerebral ischemia: inflammatory versus neurotrophic aspects. *J Cereb Blood Flow Metab* 2009;29:464–479.
- 11 Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, Woo P: 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;102:1369–1376.
- 12 März P, Cheng JG, Gadiant RA, Patterson PH, Stoyan T, Otten U, Rose-John S: Sympathetic neurons can produce and respond to interleukin 6. *Proc Natl Acad Sci USA* 1998;95:3251–3256.
- 13 Kumar P, Kumar A, Sagar R, Misra S, Farooq M, Suroliya V, Vivekanandhan S, Srivastava AK, Prasad K: Association between interleukin-6 (–174 G/C and –572 C/G) promoter gene polymorphisms and risk of intracerebral hemorrhage in North Indian population: a case control study. *Acta Medica Int* 2016;3:132–136.
- 14 Banerjee I, Gupta V, Ahmed T, Faizaan M, Agarwal P, Ganesh S: Inflammatory system gene polymorphism and the risk of stroke: a case-control study in an Indian population. *Brain Res Bull* 2008;75:158–165.
- 15 Yamada Y, Metoki N, Yoshida H, Satoh K, Ichihara S, Kato K, Kameyama T, Yokoi K, Matsuo H, Segawa T, Watanabe S, Nozawa Y: Genetic risk for ischemic and hemorrhagic stroke. *Arterioscler Thromb Vasc Biol* 2006;26:1920–1925.
- 16 Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group: Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009;6:e1000097.
- 17 Stang A: Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol* 2010;25:603–605.
- 18 Higgins JPT, Thompson SG, Deeks JJ, Altman DG: Measuring inconsistency in meta-analyses. *BMJ* 2003;327:557–560.
- 19 Brown JM, Grosso MA, Harken AH: Cytokines, sepsis and the surgeon. *Surg Gynecol Obstet* 1989;169:568–575.

- 20 Fillman SG, Weickert TW, Lenroot RK, Catts SV, Bruggemann JM, Catts VS, Weickert CS: Elevated peripheral cytokines characterize a subgroup of people with schizophrenia displaying poor verbal fluency and reduced Broca's area volume. *Mol Psychiatry* 2015, Epub ahead of print.
- 21 Cesari, M Penninx BWJH, Newman AB, Kritchevsky SB, Nicklas BJ, Sutton-Tyrrell K, Rubin SM, Ding J, Simonsick EM, Harris TB, Pahor M: Inflammatory markers and onset of cardiovascular events: results from the Health ABC study. *Circulation* 2003;108:2317–2322.
- 22 Fassbender K, Rossol S, Kammer T, Daffertshofer M, Wirth S, Dollman M, Hennerici M: Proinflammatory cytokines in serum of patients with acute cerebral ischemia: kinetics of secretion and relation to the extent of brain damage and outcome of disease. *J Neurol Sci* 1994;122:135–139.
- 23 Folsom AR, Chambless LE, Ballantyne CM, Coresh J, Heiss G, Wu KK, Boerwinkle E, Mosley TH, Sorlie P, Diao G, Sharrett AR: An assessment of incremental coronary risk prediction using C-reactive protein and other novel risk markers: the atherosclerosis risk in communities study. *Arch Intern Med* 2006;166:1368–1373.
- 24 Miñambres E, Cemborain A, Sánchez-Velasco P, Gandarillas M, Díaz-Regañón G, Sánchez-González U, Leyva-Cobián F: Correlation between transcranial interleukin-6 gradient and outcome in patients with acute brain injury. *Crit Care Med* 2003;31:933–938.
- 25 Pawlikowska L, Tran MN, Achrol AS, McCulloch CE, Ha C, Lind DL, Hashimoto T, Zaroff J, Lawton MT, Marchuk DA, Kwok P-Y, Young WL; UCSF BAVM Study Project: Polymorphisms in genes involved in inflammatory and angiogenic pathways and the risk of hemorrhagic presentation of brain arteriovenous malformations. *Stroke J Cereb Circ* 2004;35:2294–2300.
- 26 Silva Y, Leira R, Tejada J, Lainez JM, Castillo J, Dávalos A; Stroke Project, Cerebrovascular Diseases Group of the Spanish Neurological Society: Molecular signatures of vascular injury are associated with early growth of intracerebral hemorrhage. *Stroke J Cereb Circ* 2005;36:86–91.
- 27 Kumar P, Yadav AK, Kumar A, Sagar R, Pandit AK, Prasad K: Association between interleukin-6 (G174C and C572G) promoter gene polymorphisms and risk of ischaemic stroke: a meta-analysis. *Ann Neurosci* 2015;22: 61–69.