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# Association between polymorphisms in the interleukin-10 gene and risk of abdominal aortic aneurysm

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**ABSTRACT.** In this case-control study, we attempted to investigate the role of three common single nucleotide polymorphisms (SNPs; -1082G/A rs1800896, -819T/C rs1800871, and -592A/C rs1800872) in the *IL-10* gene in the development of abdominal aortic aneurysm in a Chinese population. Three hundred and eighty-one patients with abdominal aortic aneurysm and age- and gender-matched healthy controls (N = 381) were collected between March 2012 and March 2014. The *IL-10* -1082G/A, -819T/C, and -592A/C polymorphisms were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Logistic regression analyses revealed that the AA genotype of *IL-10* -1082G/A was associated with an increased risk of abdominal aortic aneurysm compared to the GG genotype in a codominant model [odds ratio (OR) = 1.64, 95% confidence interval (CI) = 1.04-2.60]. Moreover, the GA+AA genotype was correlated with an elevated risk of abdominal aortic aneurysm compared to the GG genotype in a dominant model (OR = 1.34, 95%CI = 1.01-1.79). In conclusion, the results of our study suggested that the A allele of *IL-10*

 

-1082G/A is significantly associated with the development of abdominal aortic aneurysm compared to the wide-type genotype.

**Key words:** Interleukin-10; Polymorphism; Abdominal aortic aneurysm

## INTRODUCTION

Abdominal aortic aneurysm is a chronic inflammatory disease; an estimated 2-5% of males and <1% females aged 65-74 years suffer from this disease (MASS, 2002; Lederle et al., 2008; Svensjo et al., 2011). The development of abdominal aortic aneurysm is a complex process, involving various environmental and genetic factors. Many environmental factors have been reported to play an important role in the development of abdominal aortic aneurysm, such as the gender (male), older age, tobacco smoking, dyslipidemia, and hypertension (Forsdahl et al., 2009). Previous studies have also reported that genetic factors, such as the TGF-β receptor genes, C-reactive protein, and matrix metalloproteinase (MMP)-9 genes, induce susceptibility to abdominal aortic aneurysm (Biros et al., 2011; Duellman et al., 2012; Saratzis et al., 2014).

Inflammatory processes play an important role in the initiation and during the early phase of aneurysm formation (Rasmussen et al., 1997; Rohde et al., 1999). Previous studies have suggested that the expression of pro-inflammatory cytokines, such as IL-6, TNF-α, IL-1β, IFN-γ, and IL-17A, in the plasma could influence the development of abdominal aortic aneurysm (Juvonen et al., 1997; Dawson et al., 2007; Sharma et al., 2012). However, very few studies have reported the association between polymorphisms in the *IL-10* gene and the development of abdominal aortic aneurysm (Bown et al., 2003, 2007, 2014). In this study, we attempted to investigate the role of three common SNPs (IL-10 -1082G/A rs1800896, -819T/C rs1800871, and -592A/C rs1800872) in the *IL-10* gene in the development of abdominal aortic aneurysm in a Chinese population.

## MATERIAL AND METHODS

### Subjects

In this case-control study, we prospectively collected 425 patients with abdominal aortic aneurysm from the Linyi People Hospital (affiliated to Shandong University) between March 2012 and March 2014. All patients were confirmed to have an abdominal aortic diameter >3 cm by ultrasound or computed tomography imaging. Signed informed consent forms were obtained from all patients prior to their participation in the study; finally, 381 patients agreed to participate in our study (participation rate, 89.65%).

In addition, 381 healthy control subjects were randomly selected from the health examination clinic of our hospital between March 2012 and March 2014. Each control subject was age- and gender-matched to the patients.

The demographic and clinical characteristics, including the gender, age, and tobacco smoking status, and presence of hypertension, coronary artery disease, peripheral vascular disease, and cerebrovascular disease, of the patients and controls were determined using a standardized questionnaire. This protocol was approved by the Clinical Research Ethics Committee of the Linyi People Hospital affiliated to Shandong University.

## Genotyping

Blood samples were collected from all patients and control subjects in ethylene diamine tetra-acetic acid (EDTA)-coated tubes, and stored at -20°C until use. The *IL-10* -1082G/A rs1800896, -819T/C rs1800871, and -592A/C rs1800872 polymorphisms were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The forward and reverse primers used for this assay were as follows: IL-10 -1082G/A, 5'-CTACTAAGGCTTCTTTGGGAG-3' and 5'-ACTACTAAGGCTTCTTTGGGAA-3'; -819T/C, 5'-TCATTCTATGTGCTGGAGATGG-3' and 5'-TGGGGGAAGTGGGTAAGAGT-3'; and -592A/C, 5'-GGTGAGCACTACCTGACTAGC-3' and 5'-CCTAGGTCACAGTGACGTGG-3'. The PCR program was set as follows: one cycle of DNA denaturation at 94°C for 5 min; 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 2 min; and a final extension 72°C for 5 min. The PCR products were digested with the *Msp*I restriction endonuclease, and analyzed by electrophoresis on a 2% agarose gel stained with ethidium bromide (visualized under UV light).

## Statistical analysis

Differences in the distributions of demographic and clinical characteristics and genotypes of *IL-10* polymorphisms between patients and controls were calculated by the $\chi^2$ test. The Hardy-Weinberg equilibrium (HWE) was calculated using the goodness-of-fit $\chi^2$ test. Multiple-logistic regression models were established to determine the association between *IL-10* -1082G/A rs1800896, -819T/C rs1800871, and -592A/C rs1800872 gene polymorphisms and the risk of abdominal aortic aneurysm; the odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated for this association. All statistical analyses were conducted using the SPSS statistical software package, version 17.0 (SPSS Inc., Chicago, IL, USA). All tests were two-sided, with a significant level considered at P value <0.05.

## RESULTS

The demographic and clinical characteristics of patients with abdominal aortic aneurysm and control subjects are presented in Table 1. As expected, the patients with abdominal aortic aneurysm did not differ significantly from the controls in terms of the age ($\chi^2$ = 0.06, P = 0.81) and gender ($\chi^2$ = 0.00, P = 1.00). Patients with abdominal aortic aneurysm exhibited a greater likelihood of having a habit of tobacco smoking ($\chi^2$ = 23.13, P < 0.001), and suffering from hypertension ($\chi^2$ = 43.02, P < 0.001), coronary artery disease ($\chi^2$ = 25.53, P < 0.001), peripheral vascular disease, and cerebrovascular disease ($\chi^2$ = 38.23, P < 0.001) compared to the control subjects.

The genotype distributions of the *IL-10* -1082G/A, -819T/C, and -592A/C were in conformance with the HWE in the controls (Table 2). Logistic regression analyses revealed that the AA genotype of the *IL-10* -1082G/A polymorphism was associated with an increased risk of abdominal aortic aneurysm compared to the GG genotype in a codominant model (OR = 1.64, 95%CI = 1.04-2.60). The GA+AA genotype of the *IL-10* -1082G/A polymorphism was correlated with an elevated risk of abdominal aortic aneurysm compared to the GG genotype in the dominant model (OR = 1.34, 95%CI = 1.01-1.79). However, we did not find any significant association between the *IL-10* -819T/C and -592A/C polymorphisms and the risk of abdominal aortic aneurysm in the codominant, dominant and recessive models.

**Table 1.** Demographic and clinical characteristics of patients with abdominal aortic aneurysm and control subjects.

| Variables | Patients (%) | Controls (%) | $\chi^2$- test | P value |
|---|---|---|---|---|
| Age (years) | | | | |
| ≥60 | 272 (71.39) | 269 (70.60) | | |
| <60 | 109 (28.61) | 112 (29.40) | 0.06 | 0.81 |
| Gender | | | | |
| Female | 66 (17.32) | 66 (17.32) | | |
| Male | 315 (82.68) | 315 (82.68) | 0.00 | 1.00 |
| Tobacco smoking | | | | |
| Never | 108 (28.35) | 172 (45.14) | | |
| Ever | 273 (71.65) | 209 (54.86) | 23.13 | <0.001 |
| Hypertension | | | | |
| No | 125 (32.81) | 215 (56.43) | | |
| Yes | 256 (67.19) | 166 (43.57) | 43.02 | <0.001 |
| Coronary artery disease | | | | |
| No | 219 (57.48) | 285 (74.80) | | |
| Yes | 162 (42.52) | 96 (25.20) | 25.53 | <0.001 |
| Peripheral vascular disease | | | | |
| No | 266 (69.82) | 314 (82.41) | | |
| Yes | 115 (30.18) | 67 (17.59) | 16.63 | <0.001 |
| Cerebrovascular disease | | | | |
| No | 275 (72.18) | 342 (89.76) | | |
| Yes | 106 (27.82) | 39 (10.24) | 38.23 | <0.001 |

**Table 2.** Association between polymorphisms in the interleukin-10 *(IL-10)* gene and risk of abdominal aortic aneurysm.

| Model | *IL-10* gene | Patients | Controls | P for HWE | OR (95%CI) | P value |
|---|---|---|---|---|---|---|
| | -1082G/A rs1800896 | | | | | |
| Codominant | GG | 156 (40.94) | 184 (48.29) | | 1.0 (Ref.) | - |
| | GA | 161 (42.26) | 151 (39.63) | | 1.26 (0.91-1.73) | 0.13 |
| | AA | 64 (16.8) | 46 (12.07) | 0.09 | 1.64 (1.04-2.60) | 0.02 |
| Dominant | GG | 158 (41.47) | 184 (48.29) | | 1.0 (Ref.) | - |
| | GA+AA | 225 (59.06) | 197 (51.71) | | 1.34 (1.01-1.79) | 0.04 |
| Recessive | GG+GA | 317 (83.2) | 335 (87.93) | | 1.0 (Ref.) | - |
| | AA | 59 (15.49) | 46 (12.07) | | 1.36 (0.88-2.10) | 0.15 |
| | -819T/C rs1800871 | | | | | |
| Codominant | TT | 155 (40.68) | 172 (45.14) | | 1.0 (Ref.) | - |
| | TC | 170 (44.62) | 162 (42.52) | | 1.16 (0.85-1.60) | 0.33 |
| | CC | 56 (14.7) | 47 (12.34) | | 1.32 (0.83-2.12) | 0.22 |
| Dominant | TT | 155 (40.68) | 172 (45.14) | 0.36 | 1.0 (Ref.) | - |
| | TC+CC | 226 (59.32) | 209 (54.86) | | 1.20 (0.89-1.62) | 0.21 |
| Recessive | TT+TC | 325 (85.3) | 334 (87.66) | | 1.0 (Ref.) | - |
| | CC | 56 (14.7) | 47 (12.34) | | 1.22 (0.79-1.90) | 0.34 |
| | -592A/C rs1800872 | | | | | |
| Codominant | AA | 135 (35.43) | 146 (38.32) | | 1.0 (Ref.) | - |
| | AC | 186 (48.82) | 168 (44.09) | | 1.20 (0.86-1.66) | 0.26 |
| | CC | 70 (18.37) | 67 (17.59) | 0.13 | 1.13 (0.74-1.74) | 0.56 |
| Dominant | AA | 135 (35.43) | 146 (38.32) | | 1.0 (Ref.) | - |
| | AC+CC | 256 (67.19) | 235 (61.68) | | 1.18 (0.87-1.60) | 0.27 |
| Recessive | AA+AC | 321 (84.25) | 314 (82.41) | | 1.0 (Ref.) | - |
| | CC | 70 (18.37) | 67 (17.59) | | 1.02 (0.69-1.50) | 0.91 |

OR = odds ratio; CI = confidence interval.

The stratification analysis of the demographic and clinical characteristics revealed that the *IL-10* -1082G/A polymorphism was not associated with the habit of tobacco smoking, hypertension, coronary artery disease, peripheral vascular disease, and cerebrovascular disease in the risk of abdominal aortic aneurysm (Table 3).

**Table 3.** Association between the *IL-10* -1082G/A polymorphism and risk of abdominal aortic aneurysm stratified based on the demographic and clinical characteristics.

| Variables | *IL-10* -1082G/A | | | | OR (95%CI) | P value |
|---|---|---|---|---|---|---|
| | GG | | GA+AA | | | |
| | Patients | Controls | Patients | Controls | | |
| Tobacco smoking | 158 | 184 | 225 | 197 | | |
| Never | 43 | 79 | 65 | 93 | 1.28 (0.77 - 2.16) | 0.32 |
| Ever | 115 | 105 | 158 | 104 | 1.39 (0.95 - 2.03) | 0.08 |
| Hypertension | | | | | | |
| No | 52 | 101 | 73 | 114 | 1.24 (0.78 - 2.00) | 0.34 |
| Yes | 106 | 83 | 150 | 83 | 1.42 (0.94 - 2.14) | 0.08 |
| Coronary artery disease | | | | | | |
| No | 90 | 136 | 129 | 149 | 1.31 (0.90 - 1.90) | 0.14 |
| Yes | 68 | 48 | 94 | 48 | 1.38 (0.81 - 2.37) | 0.21 |
| Peripheral vascular disease | | | | | | |
| No | 111 | 149 | 155 | 165 | 1.26 (0.90 - 1.78) | 0.17 |
| Yes | 47 | 35 | 68 | 32 | 1.58 (0.82 - 3.04) | 0.14 |
| Cerebrovascular disease | | | | | | |
| No | 112 | 166 | 163 | 176 | 1.31 (0.94 - 1.83) | 0.1 |
| Yes | 46 | 18 | 60 | 21 | 1.26 (0.56 - 2.80) | 0.55 |

OR = odds ratio; CI = confidence interval.

## DISCUSSION

The results of this study suggest that the *IL-10* -1082G/A polymorphism could influence the development of abdominal aortic aneurysm, even after accounting for the age, gender, and other confounding factors.

Previous studies have also shown that the *IL-10* -1082G/A gene polymorphism is associated with several types of chronic inflammatory diseases, including systemic lupus erythematosus, rheumatoid arthritis, deep venous thrombosis, and sepsis (Zhang et al., 2011; Ouyang et al., 2013; Schotte et al., 2014; Tang et al., 2014). Zhang et al. (2011) reported that the A allele of *IL-10* -1082A/G polymorphism was associated with an increased risk of rheumatoid arthritis in a Chinese population. Tang et al. (2014), in a case-control study conducted in a Chinese population, reported that the A allele of the *IL-10* -1082G/A polymorphism was associated with an increased risk of deep venous thrombosis, while Ouyang et al. (2013) reported that (in a meta-analysis performed with 11 studies) the A allele of *IL-10* -1082G/A was associated with increased susceptibility to sepsis in a Chinese population.

Bown et al. (2003, 2007) reported that the *IL-10* gene polymorphisms were associated with the development of abdominal aortic aneurysm in two studies: in a case-control study of 100 patients with abdominal aortic aneurysm and 100 control subjects (Bown et al., 2003), they reported that the A allele of *IL-10* -1082G/A was associated with low IL-10 secretion (therefore acting as a risk factor for abdominal aortic aneurysm; in another study (Bown et al., 2007), the A allele of *IL-10* -1082G/A was found to be associated with a lower level of IL-10 in the plasma compared to the G allele, thereby being correlated with an increased risk of abdominal aortic aneurysm. In this study, the A allele of *IL-10* -1082G/A was shown to increase the risk of abdominal aortic aneurysm in a Chinese population, which is in line with these previous results (Bown et al., 2003, 2007). However, larger sample studies must be performed in the future to confirm our results.

Our study has two major limitations: firstly, the patients and control subjects were selected from a single hospital, which may not represent the general population; therefore, selection bias could not be avoided. Secondly, the sample size is relatively small, and the statistical power of

determining the association between polymorphisms in the *IL-10* gene and risk of abdominal aortic aneurysm could be limited. Therefore, further studies with larger sample sizes must be performed to validate our findings.

In conclusion, our study suggests that the A allele of *IL-10* -1082G/A is significantly associated with the development of abdominal aortic aneurysm compared to the wide-type genotype. Further studies with larger sample sizes must be conducted in the future to confirm this association.

## Conflicts of interest

The authors declare no conflict of interest.

## REFERENCES

Biros E, Norman PE, Jones GT, van Rij AM, et al. (2011). Meta-analysis of the association between single nucleotide polymorphisms in TGF-β receptor genes and abdominal aortic aneurysm. *Atherosclerosis* 219: 218-223.

Bown MJ (2014). Genomic insights into abdominal aortic aneurysms. *Ann. R. Coll. Surg. Engl.* 96: 405-414.

Bown MJ, Burton PR, Horsburgh T, Nicholson ML, et al. (2003). The role of cytokine gene polymorphisms in the pathogenesis of abdominal aortic aneurysms: a case-control study. *J. Vasc. Surg.* 37: 999-1005.

Bown MJ, Lloyd GM, Sandford RM, Thompson JR, et al. (2007). The interleukin-10-1082 'A' allele and abdominal aortic aneurysms. *J. Vasc. Surg.* 46: 687-693.

Dawson J, Cockerill GW, Choke E, Belli AM, et al. (2007). Aortic aneurysms secrete interleukin-6 into the circulation. *J. Vasc. Surg.* 45: 350-356.

Duellman T, Warren CL, Peissig P, Wynn M, et al. (2012). Matrix metalloproteinase-9 genotype as a potential genetic marker for abdominal aortic aneurysm. *Circ. Cardiovasc. Genet.* 5: 529-537.

Forsdahl SH, Singh K, Solberg S and Jacobsen BK (2009). Risk factors for abdominal aortic aneurysms: a 7-year prospective study: the Tromso Study, 1994-2001. *Circulation* 119: 2202-2208.

Juvonen J, Surcel HM, Satta J, Teppo AM, et al. (1997). Elevated circulating levels of infl ammatory cytokines in patients with abdominal aortic aneurysm. *Arterioscler. Thromb. Vasc. Biol.* 17: 2843-2847.

Lederle FA, Larson JC, Margolis KL, Allison MA, et al. (2008). Abdominal aortic aneurysm events in the women's health initiative: cohort study. *BMJ* 337: a1724.

Multicentre Aneurysm Screening Study (MASS) (2002). Cost-effectiveness analysis of screening for abdominal aortic aneurysms based on four year results from randomized controlled trial. *BMJ* 325: 1135.

Ouyang L, Lv YD, Hou C, Wu GB, et al. (2013). Quantitative analysis of the association between interleukin-10 1082A/G polymorphism and susceptibility to sepsis. *Mol. Biol. Rep.* 40: 4327-4332.

Rasmussen TE and Hallett JW (1997). New insights into inflammatory aortic aneurysms. *Eur. J. Vasc. Endovasc. Surg.* 14: 329-332.

Rohde LE, Arroyo LH, Rifai N, Creager MA, et al. (1999). Plasma concentrations of interleukin-6 and abdominal aortic diameter among subjects without aortic dilatation. *Arterioscler. Thromb. Vasc. Biol.* 19: 1695-1699.

Saratzis A, Bown M, Wild B, Sayers RD, et al (2014). C-reactive protein polymorphism rs3091244 is associated with abdominal aortic aneurysm. *J. Vasc. Surg.* 60: 1332-1339.

Schotte H, Willeke P, Becker H, Poggemeyer J, et al. (2014). Association of extended interleukin-10 promoter haplotypes with disease susceptibility and manifestations in German patients with systemic lupus erythematosus. *Lupus* 23: 378-385.

Sharma AK, Lu G, Jester A, Johnston WF, et al. (2012). Experimental abdominal aortic aneurysm formation is mediated by IL-17 and attenuated by mesenchymal stem cell treatment. *Circulation* 126: S38-S45.

Svensjo S, Bjorck M, Gurtelschmid M, Djavani Gidlund K, et al. (2011). Low prevalence of abdominal aortic aneurysm among 65-year-old Swedish men indicates a change in the epidemiology of the disease. *Circulation* 124: 1118-1123.

Tang B, Chen YK, Luo WJ, Fu J, et al. (2014). Association between interleukin-10 -1082A/G, -819C/T and -592C/A polymorphisms with deep venous thrombosis. *Hum. Immunol.* 75: 203-207.

Turner DM, Williams DM, Sankaran D, Lazarus M, et al. (1997). An investigation of polymorphism in the interleukin-10 gene promoter. *Eur. J. Immunogenetics* 24: 1-8.

Zhang J, Zhang Y, Jin J, Li M, et al. (2011). The -1082A/G polymorphism in the Interleukin-10 gene and the risk of rheumatoid arthritis: a meta-analysis. *Cytokine* 56: 351-355.