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# Association of the KLK1 rs5516 G allele and the ACE D allele with aortic aneurysm and atherosclerotic stenosis

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**Abstract**

**Objective:** Atherosclerosis underlies aortic aneurysm (AA) and atherosclerotic stenosis (AS). Kallikrein-1 (KLK1) and angiotensin-converting enzyme (ACE) are 2 key molecules in kallikrein-kinin systems and renin-angiotensin systems, respectively, which are responsible for maintaining vascular balance and stability, playing important roles in atherosclerosis. We aimed to assess the involvement of single nucleotide polymorphism *rs5516* in *KLK1* as well as the insertion/deletion *rs4646994* polymorphism in *ACE* in the development of AA and AS.

**Methods:** We enrolled Chinese Han patients with AA (N = 408) and AS (N = 432), as well as healthy controls (N = 408). Clinical and demographic characteristics were assessed. Genotypes were analyzed with recessive and dominant models.

**Results:** The *rs5516 G* allele of *KLK1* was significantly associated with AA ($P < 0.001$), and the *D* allele of *ACE* was significantly associated with both AA ($P < 0.001$) and AS ($P < 0.001$). The *GG* and *DD* genotypes were significantly associated with both AA ($P = 0.013$) and AS ($P < 0.001$) in a recessive model, and were synergistic with hypertension in AA patients, but not in AS. Patients with *CC/DD, CG/ID*, or *GG/II* genotypes, which were synergistic with hypertension, had a greater risk of developing AA, while *CC/DD*, *CG/DD, GG/ID*, or *GG/DD* genotypes, which were not synergistic with hypertension, contributed to the development of AS.

**Conclusion:** The *KLK1 rs5516 G* allele is closely associated with AA, and the *ACE D* allele is closely related to AA and AS.

**Abbreviations:** AA = aortic aneurysm, AAA = abdominal aortic aneurysm, ACE = angiotensin-converting enzyme, Ang = angiotensin, AS = atherosclerotic stenosis, CT = computed tomography, KKS = kallikrein-kinin systems, KLK1 = kallikrein-1, OR = odds ratios, RAS = renin-angiotensin systems, SNP = single nucleotide polymorphism, TAA = thoracic aortic aneurysm.

**Keywords:** angiotensin-converting enzyme, aortic aneurysm, atherosclerotic stenosis, kallikrein 1, kallikrein kinin system, polymorphism, renin angiotensin system

## 1. Introduction

Abdominal aortic aneurysm (AAA) and thoracic aortic aneurysm (TAA) are chronic degenerative conditions with serious, but potentially preventable complications,[1,2] including atherosclerotic stenosis (AS), which is the leading cause of death in many countries, causing myocardial infarction and arteriosclerosis obliterans.[3] Aortic aneurysm (AA) and AS share common underlying pathogenic mechanisms, including atherosclerosis, chronic inflammation and proteolytic degradation of the aortic wall.[4]

Angiotensin II (Ang II) has been involved in the pathogenesis of atherosclerosis and arterial response to injury and restenosis, via stimulation of vascular hypertrophy, extracellular matrix production, and induction of cytokines.[5–8] Angiotensin-converting enzyme (ACE), a primary effector of the renin-angiotensin system (RAS), degrades bradykinin (a potent vasodilator) and converts angiotensin I (Ang I) to Ang II. The kallikrein-kinin system (KKS) antagonises the RAS, thus working in concert to stabilize the vascular system.

Components of the RAS and KKS are expressed within vascular tissues, potentially leading to atherosclerosis and aneurysm.[9] ACE promotes the production of Ang II by inflammatory cells,[10] and the induction of premature senescence of smooth muscle cells.[6] ACE induces endothelial dysfunction, therefore activating monocytes and macrophages, and increasing vascular inflammation, resulting in an enhancement of the atherogenic process.[7] Chronic exposure to high levels of circulating and tissue ACE may accelerate vascular wall remodeling, inducing changes in diameter and thickness.[8]

A genetic predisposition toward aneurysm and stenosis has been suggested by familial and segregation studies.[11,12] Association between the insertion/deletion (*I/D*) polymorphism within *ACE* (rs4646994) and the development of AA or AS has been reported.[13] Besides, the relation between the single nucleotide polymorphism (SNP) rs5516 (g.23591691 C>G) in the gene encoding a KKS serine protease, kallikrein-1 (*KLK1*) and

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the development of AA has also been demonstrated.[9,14,15] In the Chinese Han population, a study revealed that the frequencies of the C and G alleles in *KLK1* were 79% and 21%, respectively,[14] while another study reported frequencies of 89% and 11%, respectively;[15] both studies reported an association between *rs5516* and hypertension.

Intraluminal thrombus and atherosclerotic plaques contain cytokines, neutrophils, proteolytic enzymes, and platelets.[16] Kinins released by kallikrein promote inflammation, which is considered critical in the progression of AA.[17] The transcription and translation of ACE in carotid artery are increased within regions of plaque inflammation.[5] The *I/D* polymorphism is associated with variations in ACE plasma levels. The *DD* genotype and the *D* allele are associated with increased ACE levels in tissue and blood.[18] Both the Ang II type I receptor 1166C polymorphism and *ACE ID* heterozygosity are associated with AA.[19]

Although atherosclerosis might be one of primary pathological mechanisms of both AA and AS, the role of polymorphisms in the RAS and KKS pathways in the development of these diseases has not been precisely defined. Furthermore, previous genetic association studies of *ACE* and *KLK1* polymorphisms with AA and AS yielded conflicting results.[2,9,20] Therefore, we aimed to investigate whether polymorphisms in *ACE* and *KLK1*, 2 major effectors of RAS and KKS pathways, are involved in the development of these 2 diseases, and to investigate the role of these genes in the pathology of AA and AS. The present study might help identify individuals with higher risk of AA and AS.

## 2. Materials and methods

### 2.1. Participants

A total of 408 AA patients, 432 AS patients, and 408 controls (without AA or AS) were enrolled in 2 case–control studies performed at the Drum Tower Hospital, Nanjing, China. Patients were diagnosed with AA by ultrasound, or with AS by computed tomography (CT). AA was defined as diameter more than 3 cm for the infrarenal region of aorta.[21] AS was defined as the presence of stenosis and ischemic symptoms caused by atherosclerosis plaques.[22] From January 2008 to January 2013, all subjects admitted to our hospital were considered eligible if they met the following criteria: AA or AS patients diagnosed by ultrasound or CT, and the control group was selected from patients undergoing routine physical examination; Chinese Han; 18 to 75 years old; and residing for more than 10 years in Nanjing, Jiangsu province. Controls had to be without AA or AS, as confirmed by ultrasound or CT. Exclusion criteria were as follows: history of previous end arterectomy (possible restenosis); arterial tortuosity; cancer; chronic respiratory or liver inflammatory diseases; autoimmune disease; or renal failure. Patients with atrial fibrillation or suspected cardiac emboli were also excluded from the study to avoid confusion between cardiac and arterial sources of ischemic events. The study was approved by the ethics committee of the Nanjing Drum Tower Hospital (Nanjing, China). Written informed consent was obtained from each subject before participation.

The medical history of each patient was recorded, including smoking and drinking habits, history of diabetes, and drug treatment (Table 1). Subjects with a fasting glucose level of 7.0 mmol/L or more or those taking insulin or oral hypoglycemic drugs, were characterized as having diabetes mellitus. Hypertension was defined as a systolic blood pressure more than 140 mmHg, diastolic blood pressure more than 90 mmHg, or currently receiving antihypertensive drugs. The AA patients' major cardiovascular drugs were alprostadil injection, Aescuven Forte Diosmin tablets, both are vasodilators, and antihypertensive drugs. The AS patients' major cardiovascular drugs were warfarin, acetyl salicylic acid, and Aescuven Forte Diosmin.

Blood was collected from patients undergoing surgery and from healthy control participants during routine medical examination.

### 2.2. Genotyping

Subjects were genotyped by the Genome Research Facility, Drum Tower Hospital of Nanjing (China). Genomic DNA was extracted using the SBS DNA Extraction kit (SBS Genetech Co., Ltd., Shanghai, China), according to the manufacturer's instructions.

The following primers were used to amplify *KLK1* and *ACE* by PCR: *KLK1* forward *5′*-TGCTGTGAAGGTCGTGGAG-*3′*; *KLK1* reverse *5′*- GCACTCATCATTAGGCAGGATT-*3′*; *ACE* forward *5′*-CTGGAGACCACTCCCATCCTTTCT-*3′*; and *ACE* reverse *5′*- GATGTGGCCATCACATTCGTCAGAT -*3′*.

Primers (0.4 μM) were added to12 ng/μL of template DNA, 0.2 mM of dNTP mix, 10× PCR buffer (2 mM $Mg^{2+}$ Plus), and 0.025 units/μL Taq DNA Polymerase (DR001A, Takara Bio, Otsu, Japan).

The PCR protocol was: initial denaturation at 95°C, 40 cycles of 94°C for 30 seconds, 57°C for 30 seconds, and 72°C for 1 minute, and a final elongation at 72°C for 10 minutes, using a 2720 Thermal Cycler (Applied Biosystems, Foster City, CA). PCR products were visualized on agarose gels and using the UVP Bioimaging GDS-8000 System and Labwork Image Acquisition and Analysis Software (version 4.5.00.0 for Windows).

*KLK1* reverse primer was used to sequence *KLK1* genotypes using a DNA Analyzer (3730XL, Applied Biosystems, Foster City, CA). Sequencing results were edited using the BioEdit sequence alignment editor (Ibis Biosciences, Carlsbad, CA).

For *ACE*, an amplicon of approximately 200 bp represented the deletion (*D*) polymorphism, and an amplicon of approximately 500 bp represented the insertion (*I*) polymorphism.

PCR products that appeared to indicate homozygosity for the deletion polymorphism were further processed with insertion-specific primers to identify possible erroneous mistyping of heterozygotes (TTTGAGACGGAGTCTCGCTC): forward *5′*-TGGGACCACAGCGCCCGCCACTAC-*3′*; reverse *5′*-TCGC CAGCCCTCCCATGCCCATAA -*3′*. Amplicons from this reaction were visualized on agarose gels. A 338-bp PCR product indicated presence of the I allele.

### 2.3. Statistical analyses

Univariate analysis of continuous and discrete covariates for AA and AS was performed using *t* tests or *z* tests, chi-square tests, or Fisher exact tests, as appropriate. A dominant model was used to measure the differences between *rs5516 CC* homozygotes and *G* allele carriers, or between *ACE I/D* polymorphism, while a recessive model compared *GG* homozygotes with *C* allele carriers, or *DD* homozygotes and *I* allele carriers. $P < 0.05$ indicated statistical significance.

## 3. Results

### 3.1. Analysis of the KLK1 rs5516 in AA and AS patients

Table 1 presents the characteristics of the participants. There were more hypertensive subjects in AA and AS patients than that in control (54% and 59% vs 21%, respectively), as well as more

**Table 1**

**Characteristics of subjects undergoing KLK1 and ACE genotyping.**

| | Subjects | | | | |
|---|---|---|---|---|---|
| characteristics | AA | *P* | AS | *P* | Control |
| Number | 408 | | 432 | | 408 |
| Women | 49 (12%) | 0.709 | 65 (15%) | 0.302 | 53 (13%) |
| Age, y | 69.5 ± 15.2 | 0.324 | 58.9 ± 14.2 | 0.931 | 58.2 ± 9.2 |
| Hypertension | 220 (54%) | <0.001 | 255 (59%) | <0.001 | 86 (21%) |
| Diabetes mellitus | 24 (6%) | 0.047 | 99 (23%) | <0.001 | 12 (3%) |
| Ever hepatitis | 6 (1%) | | 2 (0.4%) | | 0 |
| Ever tuberculosis | 3 (0.7%) | | 2 (0.4%) | | 0 |
| Ever smoker | 66 (16%) | <0.001 | 72 (17%) | <0.001 | 27 (7%) |
| Ever drinker | 30 (7%) | <0.001 | 4 (1%) | | 6 (1%) |
| Ever drug user | 0 | | 0 | | 0 |

AA = aortic aneurysm, ACE = angiotensin-converting enzyme, AS = atherosclerotic stenosis, KLK1 = kallikrein-1.

diabetes (6% and 23% vs 3%). In addition, more AA and AS patients were past or present smokers (16% and 17% vs 7%). Finally, more patients were past or present drinkers in AA patients than that in control (7% vs 1%).

The genotype distribution of *rs5516* was consistent with Hardy-Weinberg equilibrium in control, but homozygotes for the *G* allele were significantly overrepresented in both AA (odds ratio [OR] = 3.000, 95% confidence interval [CI]: 1.203–7.481, $P = 0.013$, Table 2) and AS patients (OR = 4.722, 95% CI: 1.986–11.227, $P < 0.001$, Table 2), as demonstrated by a recessive model. Furthermore, the frequency of the *G* allele in AA patients was significantly higher than that in control (OR = 1.354, 95% CI: 1.242–1.477, $P < 0.001$, Table 2).

There was a significant difference in the *KLK1* genotype distributions between AA (n = 408) and AS (n = 432) patients (OR = 1.788, 95% CI: 1.396–2.289, $P < 0.001$, Table 2). Furthermore, there was a significant difference in the frequency of the *G* allele between AA and AS patients (OR = 1.283, 95% CI: 1.045–1.575, $P = 0.017$, Table 2). The frequency of *CG* heterozygotes among AA patients (31.86%) was significantly higher than within AS patients (17.82%). However, there was no statistical significance in the frequency of *GG* homozygotes between AA and AS patients.

### 3.2. KLK1 rs5516 in hypertensive patients

AA patients homozygous for the *G* allele were significantly overrepresented among patients with hypertension, as demonstrated by a recessive model (OR = 15.039, 95% CI: 2.036–111.11, $P < 0.001$ Table 3). Furthermore, the frequency of the *G* allele among hypertensive patients was significantly higher than that in nonhypertensive patients (OR = 1.433, 95% CI: 1.080–1.902, $P = 0.011$, Table 3).

Among the AS patients, we observed significant association between the *GG* genotype and hypertension (OR = 1.149, 95% CI: 1.069–1.235, $P < 0.001$, Table 3). The frequency of the *C* allele in hypertensive patients was significantly higher than that in non-hypertensive patients (OR = 1.688, 95% CI: 1.170–2.436, $P = 0.005$, Table 3).

### 3.3. Analysis of the ACE I/D polymorphism in AA and AS patients

The genotype frequencies of *ACE I/D* polymorphism were consistent with Hardy–Weinberg equilibrium in control. There was a significant association between the *DD* genotype and AA or AS, as demonstrated by a recessive model (OR [AA/Control] = 1.517, 95% CI: 1.288–1.788, $P < 0.001$; and OR [AS/Control] = 1.353, 95% CI: 1.144–1.600, $P < 0.001$, respectively, Table 4).

Individuals with at least one copy of the *D* allele were significantly overrepresented in the AA group (OR = 1.517, 95% CI: 1.288–1.788, $P < 0.001$, Table 4), as demonstrated by a dominant model, and the frequency of the *D* allele in AA and AS patients was significantly higher than that in control (OR [AA/Control] = 1.354, 95% CI: 1.242–1.477, $P < 0.001$; and OR [AS/Control] = 1.258, 95% CI: 1.151–1.374, $P < 0.001$, respectively, Table 4).

Furthermore, the frequency of the *D* allele in AA patients was significantly higher than that in AS patients (OR = 1.077, 95% CI: 1.001–1.159, $P = 0.047$, Table 4), and the frequency of *ID* heterozygotes among AA patients (25.74%) was significantly higher than that in AS patients (18.06%).

### 3.4. I/D polymorphism of the ACE in hypertensive patients

The *ACE DD* genotype was overrepresented in AA patients with hypertension, as demonstrated by a recessive model (OR = 1.241,

**Table 2**

**Multivariable association of rs5516 with AA and AS.**

| | Control (408) | AA (408) | OR (AA/Control, 95% CI) | *P* value | AS (432) | OR (AS/Control, 95% CI) | *P* value | OR (AA/AS, 95% CI) | *P* value |
|---|---|---|---|---|---|---|---|---|---|
| *Genotype* | | | | | | | | | |
| *CC* | 284 | 260 | Reference | | 325 | Reference | | Reference | |
| *CG* | 118 | 130 | 1.102 (0.895–1.356) | 0.361 | 77 | 1.156 (1.072–1.247) | <0.001 | 1.788 (1.396–2.289) | <0.001 |
| *GG* | 6 | 18 | 3.000 (1.203–7.481) | 0.013 | 30 | 4.722 (1.986–11.227) | <0.001 | 0.635 (0.360–1.122) | 0.114 |
| Allele | | | | | | | | | |
| *C* | 686 | 650 | Reference | | 727 | Reference | | Reference | |
| *G* | 130 | 166 | 1.354 (1.242–1.477) | <0.001 | 137 | 0.995 (0.799–1.240) | 0.967 | 1.283 (1.045–1.575) | 0.017 |

AA = aortic aneurysm, AS = atherosclerotic stenosis, CI = confidence interval, N = number of individuals, OR = odds ratio, *P* = 2-sided *P* value.

**Table 3**

**Multivariable association of rs5516 with AA or AS and hypertension.**

| | AA patients | | | | AS patients | | | |
|---|---|---|---|---|---|---|---|---|
| | Hypertension | Nonhypertension | OR (95% CI) | *P* value | Hypertension | Nonhypertension | OR (95% CI) | *P* value |
| *Genotype* | | | | | | | | |
| *CC* | 134 | 126 | Reference | | 195 | 130 | Reference | |
| *CG* | 68 | 62 | 1.021 (0.771–1.352) | 0.886 | 54 | 23 | 0.922 (0.840–1.012) | 0.1 |
| *GG* | 18 | 0 | 15.039 (2.036–111.11) | <0.001 | 6 | 24 | 1.149 (1.069–1.235) | <0.001 |
| Allele | | | | | | | | |
| *C* | 336 | 314 | Reference | | 444 | 283 | Reference | |
| *G* | 104 | 62 | 1.433 (1.080–1.902) | 0.011 | 66 | 71 | 1.688 (1.170–2.436) | 0.005 |

AA = aortic aneurysm, AS = atherosclerotic stenosis, CI = confidence interval, N = number of individuals, OR = odds ratio, *P* = 2-sided *P* value.

95% CI: 1.034–1.606, $P = 0.015$, Table 5). The equivalent association was not observed in AS patients ($P = 0.372$, Table 5). The frequency of the *D* allele in hypertensive AA patients was significantly higher than that in nonhypertensive AA patients (OR = 1.108, 95% CI: 1.001–1.226, $P = 0.046$, Table 5), but there was no significant difference between the frequency of the *D* allele between hypertensive and nonhypertensive AS patients ($P = 0.722$, Table 5).

### 3.5. Combined analysis of KLK1 and ACE genotypes in AA and AS subjects

We observed that the *CC/DD*, *CG/ID*, and *GG/II* genotypes were significantly more frequent among AA patients than that in healthy controls, and that the *CC/DD*, *CG/DD*, *GG/ID*, and *GG/DD* genotypes were significantly more frequent in AS patients than that in healthy controls (Table 6).

## 4. Discussion

In this study, we found that both *rs5516* and the *I/D* polymorphism of the *ACE* ere associated with AA and AS. Furthermore, the *GG* genotype as well as the *DD* was associated with AS and AA. Comparing the genotyping of the patients with AA versus AS, there was a significant association between *CG/GG* genotypes and the patients with AA in a dominant model. This analysis also showed that the *rs5516* minor (*G*) allele was associated with AA in a statistically significant manner. However, the difference between the frequency of GG genotype of the AA patients and that of the AS patients was not significant. This follows for the *ID/DD* genotypes using a dominant model, and the *D* allele as well.

We found that the *ACE D* and the *KLK1 G* alleles were associated with AA and AS, likely via the proinflammatory and vascular remodeling properties of Ang II and kinins. Furthermore, the patients with *CC/DD*, or *CG/ID* or *GG/II* genotypes, which were synergistic with hypertension, had a greater risk of developing AA, while *CC/DD* or *CG/DD*, or *GG/ID* or *GG/DD* genotypes, which were not synergistic with hypertension, contributed to the risk of developing AS.

We believe that these *ACE* and *KLK1* genotypes affect the extent of the inflammatory reaction, influencing AA and AS development in different manners. The KKS and RAS pathways are mutually antagonistic,[9] and thus the physiological roles of the *D* and *G* alleles would be expected to oppose one another. We hypothesize that these 2 factors may be synergistic in certain pathological conditions, such as in artery stenosis caused by atherosclerosis.

High blood pressure in the aorta contributes to vascular remodeling and inflammation of the arterial wall.[4] In this way, hypertension is likely to contribute to the occurrence of AA.[4] We found that individuals with either the *GG* or *DD* genotypes in combination with hypertension were overrepresented in AA patients. However, this was not the case in patients diagnosed with AS, suggesting that the modulated inflammatory process caused by alteration of KLK1 and ACE activity is likely to contribute to the development of AS independently of hypertension.

Previous studies have examined the hypothesis that the *KLK1-GG* genotype is related to aneurysmal disease of the abdominal aorta and denotes a predisposition for AAA, but studies reached conflicting conclusions with inconsistent results. Indeed, Baas et al[2] reported that no *KLK1* SNPs were association with AAA in 1024 Dutch subjects, while Biros

**Table 4**

**Multivariable association of ACE I/D polymorphism with AA and AS.**

| | Control (408) | AA (408) | OR (AA/Control, 95%CI) | *P* value | AS (432) | OR (AS/Control, 95% CI) | *P* value | OR (AA/AS, 95% CI) | *P* value |
|---|---|---|---|---|---|---|---|---|---|
| *Genotype* | | | | | | | | | |
| *II* | 107 | 88 | Reference | | 130 | Reference | | Reference | |
| *ID* | 207 | 105 | 1.621 (1.123–2.34) | 0.01 | 78 | 1.834 (1.522–2.210) | <0.001 | 1.451 (1.167–1.804) | 0.001 |
| *DD* | 94 | 215 | 1.517 (1.288–1.788) | <0.001 | 224 | 1.353 (1.144–1.600) | <0.001 | 1.121 (1.007–1.248) | 0.037 |
| Allele | | | | | | | | | |
| *I* | 421 | 281 | Reference | | 338 | Reference | | Reference | |
| *D* | 395 | 535 | 1.354 (1.242–1.477) | <0.001 | 526 | 1.258 (1.151–1.374) | <0.001 | 1.077 (1.001–1.159) | 0.047 |

AA = aortic aneurysm, ACE = angiotensin-converting enzyme, AS = atherosclerotic stenosis, CI = confidence interval, N = number of individuals, OR = odds ratio, *P* = 2-sided *P* value.

**Table 5**

**Multivariable association of ACE I/D polymorphism with AA or AS and hypertension.**

| | AA patients | | | | AS patients | | | |
|---|---|---|---|---|---|---|---|---|
| | Hypertension | Nonhypertension | OR (95% CI) | *P* value | Hypertension | Nonhypertension | OR (95% CI) | *P* value |
| *Genotype* | | | | | | | | |
| *II* | 52 | 36 | Reference | | 65 | 65 | Reference | |
| *ID* | 34 | 71 | 1.797 (1.310–2.466) | <0.001 | 67 | 11 | 3.507 (1.980–6.212) | <0.001 |
| *DD* | 134 | 81 | 1.241 (1.035–1.606) | 0.015 | 123 | 101 | 1.075 (0.916–1.262) | 0.372 |
| Allele | | | | | | | | |
| *I* | 138 | 143 | Reference | | 197 | 141 | Reference | |
| *D* | 302 | 233 | 1.108 (1.001–1.226) | 0.046 | 313 | 213 | 1.020 (0.914–1.138) | 0.722 |

AA = aortic aneurysm, ACE = angiotensin-converting enzyme, AS = atherosclerotic stenosis, CI = confidence interval, N = number of individuals, OR = odds ratio, *P* = 2-sided *P* value.

**Table 6**

**The genotype distributions of KLK1 and ACE.**

| | *II* | | | | | *ID* | | | | | *DD* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Genotype** | **AA** | ***P*** | **AS** | ***P*** | **Control** | **AA** | **P** | **AS** | ***P*** | **Control** | **AA** | ***P*** | **AS** | ***P*** | **Control** |
| *CC, n* | 10 | 0.537 | 127 | | 13 | 44 | | 50 | | 189 | 206* | <0.001 | 148* | <0.001 | 82 |
| *(%)* | 2.5 | | 31.1 | | 3.2 | 10.8 | | 11.6 | | 46.3% | 63.7 | | 34.3 | | 20.1 |
| *CG, n* | 60 | 0.034 | 3 | | 88 | 61* | <0.001 | 20 | | 18 | 9 | | 54* | <0.001 | 12 |
| *(%)* | 14.7 | | 0.7 | | 21.6 | 15.0 | | 4.6 | | 4.4 | 2.2 | | 12.5 | | 2.9 |
| *GG, n* | 18* | 0.016 | 0 | | 6 | 0 | | 8* | <0.001 | 0 | 0 | | 22* | <0.001 | 0 |
| *(%)* | 4.4 | | 0 | | 1.5 | 0% | | 1.9 | | 0% | 0 | | 5.1 | | 0 |

* $P < 0.05$.

AA = aortic aneurysm, ACE = angiotensin-converting enzyme, AS = atherosclerotic stenosis, KLK1 = kallikrein-1.

et al[9] found that *rs5516* was associated with large AAA, but not with small ones. Similarly, reports on the influence of the *ACE DD* genotype on AAA development have been inconsistent. Hamano et al[20] reported no difference in the genotype distributions in well-matched cohorts for age, sex, and atherosclerotic risk factors, but Fatini et al[23] found an independent relationship between the *ACE DD* genotype and AAA (17). The *ACE D* allele was found to be associated with carotid intima-media thickness, and DD homozygotes had significantly greater carotid intima-media thickness than subjects carrying the *II* or *ID* genotype.[24] However, whether the D allele is associated with the presence of carotid plaques[25] or not[23] remains unclear.

Antoniou et al[1] found an increased prevalence of *I/D* heterozygotes in patients with aneurysm and/or hernia; however, they found no correlation between the *ACE DD* genotype, the *D* allele and the presence of AAA. Unlike the study by Antoniou et al,[1] our study analyzed the combined effect of the *ACE* and *KLK1* polymorphisms. Our data failed to demonstrate an association between AA and the *ID* or *CG* genotype, or synergistic effects of the *ID* or *CG* genotype with hypertension in AA patients, probably due to the small sample size. Additionally, we were unable to recruit patients diagnosed with AA and AS simultaneously. The observed ORs were not so large, suggesting that these polymorphisms contribute to AA and AS development, but that they are not the major causes.

## 5. Conclusion

The *KLK1 rs5516 G* allele is closely associated with AA, and the *ACE D* allele is closely related to AA and AS.

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