Role of Endoglin Insertion and rs1800956 Polymorphisms in Intracranial Aneurysm Susceptibility

A Meta-Analysis

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Abstract: Endoglin is an essential molecule during angiogenesis, vascular development, and integrity. Till now, many studies have investigated the association between endoglin polymorphisms and intracranial aneurysm (IA) risk, with the results remained inconclusive. Therefore, we performed a meta-analysis to summarize the possible association.

We searched PubMed and Embase until June 2015 to identify studies addressing the association between endoglin polymorphisms and IA risk. The summary odds ratios (ORs) and their corresponding 95% confidence interval (CI) were calculated to assess the strength of the association.

Eleven studies with a total of 1501 cases and 2012 controls were finally included in this meta-analysis, with 10 studies investigating endoglin 6-bp insertion (6bINS) polymorphism and 4 studies investigating 1800956 polymorphism. No significant association between endoglin 6bINS polymorphism and IA risk was detected in overall estimation (I vs wt/I + wt/wt; OR = 1.21, 95% CI = 0.87–1.69) or in the subgroup analysis by ethnicity, control source, or ruptured status. However, we observed an association with borderline significance of 6bINS with IA occurrence (I vs wt/I + wt/wt; OR = 1.49, 95% CI = 0.99–2.25, P = 0.058) in studies applying matched controls. Furthermore, we detected a significant association for 6bINS polymorphism of endoglin with increased risk of familial IA (I vs wt, OR = 1.64, 95% CI = 1.10–2.42) but not sporadic IA (I vs wt, OR = 1.09, 95% CI = 0.68–1.45). With regard to rs1800956, our pooled results indicated a significantly decreased IA risk in individuals carrying C allele (C/C vs G/C: OR = 0.45, 95% CI = 0.45–0.94).

This meta-analysis provided no evidence for the association between 6bINS polymorphism with overall IA risk. However, we detected a significant association of 6bINS allele with increased risk of familial IA. Also, we found that rs1800956 was significantly related to IA occurrence. Further, well-designed studies with large sample size are warranted and updated meta-analysis is needed to verify our findings.

INTRODUCTION

Intracranial aneurysm (IAs) is a common disease with an estimated overall prevalence of 3.2% in the general population.1 Rupture of IA is the most common cause of subarachnoid hemorrhages (SAH), accounting for about 85% of all nontraumatic SAH.2 SAH is a catastrophic neurological condition, with a case fatality of ~50% and as many as 46% of survivors may have long-term cognitive impairment.3

Although the pathogenesis of the IA remains unclear,modifiable factors such as hypertension, active smoking, and excessive alcohol consumption have been associated with the risk of IA.4 In addition, accumulating evidence has indicated an important role of genetic component in pathogenesis of IA. First-degree relatives in familial IA families have a relative risk of up to 4.2 when compared with the general population.5 Genome-wide association studies have identified common SNPs on chromosomes 2q, 8q, and 9p, intervals near RBBP8 on 18q11.2 and STARD13-KL on 13q13.1 that significantly associated with IA.6,7

Endoglin is a homodimeric transmembrane glycoprotein (180 kDa) that predominantly expressed in vascular endothelial cells.8 It functions as an accessory coreceptor for TGF-β, and is crucial for maintaining vascular integrity. The endoglin gene is located on chromosome 9q34,9 and some mutations of endoglin gene are responsible for type 1 hereditary hemorrhagic telangiectasia (HHT1), an autosomal dominant vascular disorder characterized by telangiectasia and arteriovenous malformations (AVMs).10,11 Takenaka et al, for the first time in 1999, reported an association between IA development and the 6-bp insertion (6bINS) polymorphism, 5'-TCCCCC-3', in the intron 7 of endoglin gene. Frequency of homozygous 6bINS was found to be significantly higher in IA group than that in the control group.12 However, some following studies failed to replicate these findings.13,14 Another polymorphism, rs1800956 in exon 8 of endoglin, which leads to an aspartic acid to histidine replacement, has been reported to have an effect on IA susceptibility in Korean15 and Chinese Han16 population but not in Japanese population.17

Considering the diverse ethnic background and the relatively small sample size, a single study might have insufficient power to detect the overall effects. Meta-analysis is the method
of choice to overcome this problem by combining data from studies on the same topic. In light of this, we carried out a meta-analysis to evaluate the potential association of *endoglin* polymorphisms on IA risk.

**METHODS**

**Search Strategy**

We searched PubMed and Embase until June 2015 to identify studies addressing the association between *endoglin* polymorphisms and IA risk. The following terms were used: “polymorphism,” “genotype,” “mutation,” “variant,” “cerebral aneurysm,” “brain aneurysm,” “intracranial aneurysm,” “subarachnoid hemorrhage,” “SAH,” “endoglin,” and “ENG.” Reference list of eligible studies and relevant reviews were also screened to identify potentially relevant articles. No restriction was imposed.

**Study Selection**

Two reviewers (XH and YF) screened the literatures for relevance independently. Studies were included in meta-analysis if it estimated the association between *endoglin* polymorphism and IA risk. Study employed a case–control design of human subjects; and data was sufficient to calculate the odds ratio (OR) and its corresponding 95% confidence interval (CI). For eligible studies with the same or overlapping subjects, only the one with larger number of patients was included. If the data regarding genotype distribution were insufficient, the effort was made to contact its corresponding author. A third reviewer (YL) was consulted to reach a consensus if any discrepancy occurred.

**Data Extraction**

The following information was extracted from each eligible study using standard forms: first author, publication year, country, ethnicity, mean age, male percentage, source of control, number of cases and controls, match criteria, familial history of IA in IA patients, ruptured status, and genotype distribution in cases and controls.

**Statistical Analysis**

The summary ORs and their corresponding 95% CI were calculated to evaluate the strength of the association between *endoglin* polymorphism and IA risk. Z test was performed to determine the statistical significance of pooled ORs, and \( P < 0.05 \) was considered significant. Cochran’s Q test and the \( I^2 \) statistic were employed to measure the between-study heterogeneity. A \( P \) value of more than 0.05 for the \( Q \) test or \( I^2 \) less than 50% indicated a lack of heterogeneity, and the fixed-effects model (the Mantel–Haenszel method) was subsequently used to calculate the summary ORs.\(^{17} \) Otherwise, the random-effects model (the DerSimonian and Laird method) was applied.\(^{18} \) Hardy–Weinberg equilibrium (HWE) was tested in control subjects using the \( \chi^2 \) goodness-of-fit test. Publication bias was estimated by visually assessing the possible skewness in funnel plots.\(^{19} \) Furthermore, Egger’s test was performed to provide quantitative evidence for the checking of publication bias. Sensitivity analysis was also performed by sequentially omitting individual study to challenge the robustness of the results. \( P < 0.05 \) was considered statistically significant. All the statistical analysis was performed using STATA10.0 (STATA Corporation, College Station, TX). Since this is a meta-analysis of eligible studies, ethical approval was not necessary.

**RESULTS**

**Identification and Characteristics of the Studies**

The process of study selection was summarized in the flow diagram (Fig. 1), which was modified based on the PRISMA Statement.\(^{20} \) Finally, 11 studies with a total of 1501 cases and 2012 controls on IA susceptibility related to 6bINS polymorphism or rs1800956 were included in this study.\(^{12,16–22,26} \) Study characteristics are summarized in Table 1. Of these, 5 studies were conducted in Asian descendants,\(^{12,14–16,21} \) 5 studies in Caucasian descendants,\(^{13,21,22,24,26} \) and 1 study in mixed descendants.\(^{25} \)

Three studies\(^{16,23,24} \) investigated sporadic aneurysm only and 1 investigated familial IA only.\(^{22} \) Two studies did not provide relevant information.\(^{15,21} \) The remaining 5 studies enrolled both sporadic IA and familial IA patients, including 2 studies\(^{12,14} \) providing separate data on genotype distribution for subgroup patients and 1 study\(^{26} \) providing allele data for familial IA only. Peter et al\(^{22} \) provided separate data for ruptured and unruptured IA while Pera et al\(^{21} \) only enrolled patients with ruptured IA. Five studies\(^{1–4,22} \) enrolled population-based controls and 2 studies\(^{14,25} \) enrolled hospital-based controls. In all included studies except 2,\(^{2,25} \) which provided allele data only, genotype distribution of controls was in accordance with HWE.

**Statistical Analysis**

A total of 10 studies involving 1188 cases and 1562 controls examined the association between *endoglin* 6bINS polymorphism and IA risk. Notably, Gregorio et al\(^{12} \) and Santiago-Sim et al\(^{25} \) only provided data regarding allele distribution rather than genotype distribution. Therefore, these studies were only included in the allele model when combining data using meta-analysis. The pooled results are summarized in Table 2. Significant heterogeneity was observed only in the allele model. Overall, no significant association between *endoglin* 6bINS polymorphism and IA risk was detected (I/I + wt/I vs wt/wt: OR = 1.05, 95% CI = 0.89–1.25; I/I vs wt/I + wt/wt: OR = 1.21, 95% CI = 0.87–1.69; I/I vs wt/wt: OR = 1.23, 95% CI = 0.87–1.73; wt/I vs wt/wt: OR = 1.03, 95% CI = 0.86–1.23; I vs wt: OR = 1.20, 95% CI = 0.98–1.47, Fig. 2). When stratified by ethnicity, significant association was detected neither in Asians (I/I vs wt/I + wt/wt: OR = 1.53, 95% CI = 0.64–4.92) nor in Caucasians (I/I vs wt/I + wt/wt: OR = 1.27, 95% CI = 0.63–2.55). When stratified by control source or ruptured status, we failed to detect any significant association (PB: I/I vs wt/I + wt/wt: OR = 1.48, 95% CI = 0.66–3.29; HB: I/I vs wt/I + wt/wt: OR = 0.90, 95% CI = 0.59–1.35; ruptured IA: I/I vs wt/I + wt/wt: OR = 2.39, 95% CI = 0.66–8.72). When stratified based on the match status, we observed an association with borderline significance of 6bINS with IA occurrence (I/I vs wt/I + wt/wt: OR = 1.49, 95% CI = 0.99–2.25, \( P = 0.058 \)) in studies applying matched controls. When focusing on sporadic IA and familial IA separately, we detected a significant association for 6bINS polymorphism of *endoglin* with increased risk of familial IA (I vs wt, OR = 1.64, 95% CI = 1.10–2.42, Fig. 3) but not sporadic IA (I vs wt, OR = 1.09, 95% CI = 0.68–1.45).

With regard to rs1800956, a total of 4 studies including 850 patients and 1028 controls investigated the effect of rs1800956 polymorphism of *endoglin* gene on IA risk. Three studies\(^{14–16} \) were conducted in Asians and one\(^{25} \) in mixed population. It should be mentioned that 2 studies\(^{4,25} \) only provided data on allele distribution. Therefore, they were only included for the
allele model but excluded in other comparison models during analysis. Weak between-study heterogeneity was observed in most comparison models, except the allele model. The pooled results indicated a decreased IA risk in individuals carrying C allele (C/C vs G/C + G/G: OR = 0.65; 95% CI = 0.45–0.94, Table 2).

Sensitivity Analysis
Sensitivity analysis was performed only for the 6bINS polymorphism but not rs1800956 due to the limited number of included studies in the latter analysis. In the sensitivity analysis, the influence of each study on the overall meta-analysis estimate was investigated by repeating the meta-analysis while omitting 1 study in each turn. Statistically similar results were obtained by this procedure, suggesting the stability of the meta-analysis.

Publication Bias
Publication bias was tested only for the 6bINS polymorphism but not rs1800956. The shape of the funnel plot seemed symmetrical, indicating the absence of publication bias (Fig. 4). Furthermore, Egger’s test did not indicate any evidence for the lack of publication bias (I vs wt, t = 1.58, P = 0.154).

DISCUSSION
IA is a common disease that could lead to devastating consequences. Therefore, it is of great importance to identify individuals at high risk of IA, whom should then be screened regularly for IA. The familial aggregation phenomenon suggested the role of genetics in the development and rupture of IA. Moreover, exploring the relevant genetic factors could help to uncover the underlying mechanism of IA development. Candidate gene association studies have been widely applied for detecting genetic factors implicated in disease. Genes were selected for their biological function or the linkage disequilibrium with other associated genes. Till now, various polymorphisms in candidate genes such as SERPINA3, COL1A2, and eNOS have been found to contribute to IA risk. As a component of the TGF-β receptor complex, endoglin is an essential molecule during angiogenesis, vascular development, and integrity. It is highly expressed in vascular endothelial cells during embryogenesis,31 during inflammation and wound healing,32 and upon vascular injury.33 In a knockout mouse model, Li et al reported that endoglin deficient mice died at E10.5-115 from defective vascular development, and loss of endoglin could cause poor vascular smooth muscle development and arrest endothelial remodeling.34 Various mutations in endoglin are found to be causative for HHT1.35 Moreover, the 6bINS polymorphism of endoglin has been previously identified as a risk factor for sporadic intracerebral hemorrhage.36 Therefore, endoglin could be a candidate gene for IA development.

By combining data from 10 studies including 1188 IA patient and 1562 controls, our meta-analysis did not detect a
| Author          | Year | Country | Ethnicity | Case/Control | Age          | Male Percentage | SNP Investigated | Family History of IA | Control source | Match status | HWE   |
|-----------------|------|---------|-----------|--------------|--------------|-----------------|------------------|---------------------|-----------------|--------------|-------|
| Gregorio26      | 2012 | Brazil  | Caucasian | 40/104       | NA           | NA              | 6bINS, rs1800956 | Familial           | NA              | NA           | NA    |
| Joo15           | 2008 | Korean  | Asian     | 342/253      | 18–82 (case); 19–88 (control) | 34.8 (case); 43.9 (control) | 6bINS, rs1800956 | NA              | HB              | NA           | Yes   |
| Koshy23         | 2006 | India   | Asian     | 102/118      | 50.4 ± 11.4 (case); 49.9 ± 12.8 (control) | 52.9 (case); 50 (control) | 6bINS | Sporadic         | PB              | age, gender, ethnicity, gender | Yes   |
| K rex13         | 2001 | Germany | Caucasian | 121/124      | 23–75 (case); NA (control) | 40.5 (case); 50 (control) | 6bINS | Mixed            | others          | gender | Yes   |
| Lin16           | 2014 | China   | Asian     | 313/450      | 51.5 ± 10.7 (case); 51.9 ± 11.1 (control) | 38.7 (case); 39.8 (control) | rs1800956 | Sporadic         | mixed          | age, gender | Yes   |
| Onda14          | 2003 | Japan   | Asian     | 172/191      | 60.7 ± 9.7 (sporadic IA); 58.9 ± 11.1 (familial IA) | 40.7 (case); 47.4 (control) | 6bINS, rs1800956 | Mixed           | HB              | gender | Yes   |
| Pera21          | 2005 | Poland  | Caucasian | 119/119      | 59.0 ± 16.5 (control) | 50.2 ± 12.1 (case); 59.3 ± 17.5 (control) | 59.7 (case); 59.7 (control) | 6bINS | NA            | PB              | gender, ethnicity | Yes   |
| Peters22        | 2005 | USA     | Caucasian | 98/191       | 50.7 ± 11.7 (case); NA (control) | 20.6 (case); 23 (case) | 6bINS | Mixed            | PB              | ethnicity | Yes   |
| Santiago-Sim25  | 2009 | USA     | Mixed*    | 33/146       | 28–92 (case); NA (control) | 23 (case); 46 (control) | 6bINS, rs1800956 | Familial         | PB              | NA           | NA    |
| Simon24         | 2006 | Germany | Caucasian | 79/202       | 48.8 ± 12.1 (case); 33.5 ± 12.5 (control) | 34.2 (case); 49 (control) | 6bINS | Sporadic         | PB              | ethnicity | Yes   |
| Takenaka12      | 1999 | Japan   | Asian     | 82/114       | 58.8 (case); 60.2 (control) | 40.2 (case); 43.9 (control) | 6bINS | Mixed            | mixed          | gender | Yes   |

6bINS = 6-bp insertion, HB = hospital-based, HWE = Hardy–Weinberg equilibrium, IA = intracranial aneurysm, Mixed* = white, black, Hispanic, Asian, unknown, NA = not available, PB = population-based, SNP = single-nucleotide polymorphism.
| Variables       | N     | OR (95% CI)                  | $P_{het}$ | OR (95% CI)                  | $P_{het}$ | OR (95% CI)                  | $P_{het}$ | OR (95% CI)                  | $P_{het}$ | OR (95% CI)                  | $P_{het}$ |
|-----------------|-------|-----------------------------|----------|-----------------------------|----------|-----------------------------|----------|-----------------------------|----------|-----------------------------|----------|
| 6bINS           |       |                             |          |                             |          |                             |          |                             |          |                             |          |
| I/I + wt/I vs wt/wt | 10    | 1.05 (0.89, 1.25)           | 0.406    | 1.21 (0.87, 1.69)           | 0.189    | 1.23 (0.87, 1.73)           | 0.199    | 1.03 (0.86, 1.23)           | 0.563    | 1.20 (0.98, 1.47)           | 0.019    |
| 6bINS           |       |                             |          |                             |          |                             |          |                             |          |                             |          |
| Ethnicity       |       |                             |          |                             |          |                             |          |                             |          |                             |          |
| Caucasian       | 5     | 1.01 (0.77, 1.32)           | 0.242    | 1.53 (0.64, 4.92)           | 0.802    | 1.62 (0.71, 3.73)           | 0.868    | 0.97 (0.73, 1.28)           | 0.314    | 1.23 (0.85, 1.78)           | 0.023    |
| Asian           | 4     | 1.09 (0.88, 1.35)           | 0.412    | 1.27 (0.63, 2.55)           | 0.035    | 1.16 (0.80, 1.69)           | 0.034    | 1.07 (0.85, 1.34)           | 0.563    | 1.19 (0.86, 1.68)           | 0.074    |
| Source of control |     |                             |          |                             |          |                             |          |                             |          |                             |          |
| PB              | 5     | 1.03 (0.79, 1.36)           | 0.225    | 1.48 (0.66, 3.29)           | 0.714    | 1.51 (0.67, 3.42)           | 0.793    | 1.00 (0.76, 1.32)           | 0.13     | 1.11 (0.89, 1.38)           | 0.429    |
| HB              | 2     | 0.99 (0.77, 1.28)           | 0.486    | 0.90 (0.59, 1.35)           | 0.725    | 0.90 (0.59, 1.38)           | 0.923    | 1.02 (0.77, 1.33)           | 0.396    | 0.97 (0.80, 1.18)           | 0.694    |
| Match           |       |                             |          |                             |          |                             |          |                             |          |                             |          |
| Yes             | 7     | 1.05 (0.86, 1.28)           | 0.301    | 1.49 (0.99, 2.25)           | 0.265    | 1.48 (0.97, 2.27)           | 0.237    | 1.00 (0.81, 1.23)           | 0.376    | 1.10 (0.94, 1.30)           | 0.169    |
| Family history of IA |     |                             |          |                             |          |                             |          |                             |          |                             |          |
| Sporadic        | 4     | 1.10 (0.81, 1.48)           | 0.397    | 1.20 (0.50, 2.88)           | 0.182    | 1.13 (0.49, 2.65)           | 0.228    | 1.12 (0.81, 1.53)           | 0.521    | 1.09 (0.68, 1.65)           | 0.215    |
| Familial        | 4     | 1.23 (0.84, 1.80)           | 0.204    | 2.10 (0.72, 6.15)           | 0.069    | 2.19 (0.64, 7.44)           | 0.049    | 1.05 (0.69, 1.60)           | 0.445    | 1.64 (1.10, 2.42)           | 0.055    |
| Ruptured status |       |                             |          |                             |          |                             |          |                             |          |                             |          |
| Ruptured        | 2     | 0.84 (0.56, 1.28)           | 0.203    | 2.39 (0.66, 8.72)           | 0.485    | 2.28 (0.61, 8.50)           | 0.564    | 0.78 (0.42, 1.47)           | 0.141    | 0.94 (0.65, 1.35)           | 0.337    |
|                 |       |                             |          |                             |          |                             |          |                             |          |                             |          |
|                 |       |                             |          |                             |          |                             |          |                             |          |                             |          |
| rs1800956       | 4     | 0.96 (0.21, 4.45)           | 0.075    | 0.65 (0.45, 0.94)           | 0.45     | 0.53 (0.13, 2.13)           | 0.28     | 1.53 (0.92, 2.52)           | 0.172    | 1.13 (0.60, 2.12)           | 0.011    |

6bINS = 6-bp insertion; HB = hospital-based, I = insertion, IA = intracranial aneurysm, N = number of studies in the meta-analysis, $P_{het}$ = $P$ value of Q-test for heterogeneity test, PB = population-based, wt = wild type.
significant association between the 6bINS polymorphism of endoglin gene and IA risk. Krex et al reported a racial difference between white population and Japanese population. However, when stratified by ethnicity, we failed to detect the significant role of insertion polymorphism in IA susceptibility in Caucasians or Asians. Interestingly, we found a significant association of 6bINS polymorphism with familial but not sporadic IA. This might suggest a different effect of 6bINS allele on individuals with and without family history of IA. Another evidence suggesting different roles of endoglin variant in subsets of IA was reported by Santiago-Sim et al, which found that p.A60E, a rare variant in endoglin, is significantly associated of 6bINS polymorphism with familial but not sporadic IA. This might suggest a different effect of 6bINS allele on individuals with and without family history of IA. Another evidence suggesting different roles of endoglin variant in subsets of IA was reported by Santiago-Sim et al, which found that p.A60E, a rare variant in endoglin, is significantly associated

| Study ID             | OR (95% CI)     | Weight |
|----------------------|-----------------|--------|
| Gregorio (2012)     | 2.66 (1.51, 4.68) | 7.76   |
| Joo (2008)           | 1.00 (0.78, 1.29) | 15.00  |
| Koshy (2006)         | 1.09 (0.67, 1.77) | 9.29   |
| Krex (2001)          | 1.03 (0.62, 1.71) | 6.83   |
| Onda (2003)          | 0.92 (0.67, 1.27) | 13.25  |
| Pera (2005)          | 0.79 (0.47, 1.32) | 8.71   |
| Peters (2005)        | 1.02 (0.65, 1.59) | 10.06  |
| Takenaka (1999)      | 1.81 (1.18, 2.77) | 10.50  |
| Simon (2006)         | 1.36 (0.87, 2.14) | 9.95   |
| Santiago–Sim (2009)  | 1.59 (0.84, 3.01) | 6.65   |
| Overall (I–squared = 54.7%, p = 0.019) | 1.20 (0.98, 1.47) | 100.00 |

NOTE: Weights are from random effects analysis

FIGURE 2. Forest plot for intracranial aneurysm risk associated with endoglin 6bINS polymorphism (I/I + wt/I vs wt/wt). The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares indicates the study-specific weight (inverse of the variance). The diamond represents the pooled OR and 95% CI.

| Study ID             | OR (95% CI)     | Weight |
|----------------------|-----------------|--------|
| Onda (b) (2003)      | 1.07 (0.73, 1.56) | 30.08  |
| Takenaka (b) (1999)  | 1.80 (1.16, 2.78) | 27.67  |
| Gregorio (2012)      | 2.66 (1.51, 4.68) | 22.42  |
| Santiago–Sim (2009)  | 1.59 (0.84, 3.01) | 19.83  |
| Overall (I–squared = 60.6%, p = 0.055) | 1.64 (1.10, 2.42) | 100.00 |

NOTE: Weights are from random effects analysis

FIGURE 3. Forest plot for familial intracranial aneurysm risk associated with endoglin 6bINS polymorphism (I vs wt). The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares indicates the study-specific weight (inverse of the variance). The diamond represents the pooled OR and 95% CI.
with familial IA but not with sporadic IA. Future work should perform further analysis focusing on familial and sporadic IA separately to elucidate the possible discrepancy. It was suggested that ruptured and unruptured IAs were genetically different. Therefore, we performed subgroup analysis focusing on the ruptured aneurysm, but did not find any significant association. This might be due to the small sample size since only 2 studies were included.

As for rs1800956, we detected a significant association between this polymorphism and decreased IA risk in the recessive model. Although it is known that rs1800956 could result in an amino acid change, its functional importance has not been systematically assessed. It was also possible that rs1800956 was in linkage disequilibrium with other associated variants. Notably, the allele distribution was quite different and even opposite among the included studies. The C allele frequency of rs1800956 in IA patients was reported to be 4.4% and 4.8% in Korean and Japanese, respectively. In contrast, it was reported as 92.1% in Chinese. In a study on other topic, frequency of C allele was 97.8% in Africans and 100% in Caucasians. Therefore, there might be significant difference in allele frequency of rs1800956 among different populations. More studies are needed before any conclusion could be made on this issue. Besides, the significant association did not exist when the study by Onda et al and Santiago-Sim et al was added (the allele model). Therefore, findings about the effect of rs1800956 on IA risk should be taken with cautions.

Several limitations should be addressed when interpreting the findings of our meta-analysis. First, only 10 studies for the insertion polymorphism and 4 studies for rs1800956 were included in this study. The total sample size was relatively small, which might result in insufficient power to detect a slight but real effect of endoglin polymorphisms on IA risk. Second, some studies did not present complete data (only allele distribution available). Therefore, they were not included in the comparison models other than the allele model, which might potentially influence the overall pooled results. Third, Peter et al found a lack of association of insertion polymorphism with IA existed even after adjusting for hypertension and smoking. However, we could not reach an adjusted result since data on environmental risk factors were not provided in details in most included studies. Also, analysis on the gene-environment interaction was not feasible.

In conclusion, this meta-analysis provided no evidence for the association between 6bINS polymorphism with overall IA risk. However, we detected a significant association of 6bINS allele with increased risk of familial IA. Also, we found that rs1800956 was significantly related to IA occurrence. Further well-designed studies with large sample size are warranted and updated meta-analysis is needed to verify our findings. Also, future research targeting the role of protein endoglin in IA formation and rupture might bring important information.

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REFERENCES
1. Vlak MH, Algra A, Brandenburg R, et al. Prevalence of unruptured intracranial aneurysms, with emphasis on sex, age, comorbidity, country, and time period: a systematic review and meta-analysis. Lancet Neurol. 2011;10:626–636.
2. van Gijn J, Kerr RS, Rinkel GJ. Subarachnoid haemorrhage. Lancet. 2007;369:306–318.
3. Suarez JI, Tarr RW, Selman WR. Aneurysmal subarachnoid hemorrhage. N Engl J Med. 2006;354:387–396.
4. Rinkel GJ. Intracranial aneurysm screening: indications and advice for practice. Lancet Neurol. 2005;4:122–128.
5. Ronkainen A, Miettinen H, Karkola K, et al. Risk of harboring an unruptured intracranial aneurysm. Stroke. 1998;29:359–362.
6. Bilguvar K, Yasuno K, Niemela M, et al. Susceptibility loci for intracranial aneurysm in European and Japanese populations. Nat Genet. 2008;40:1472–1477.
7. Yasuno K, Bilguvar K, Bijlenga P, et al. Genome-wide association study of intracranial aneurysm identifies three new risk loci. Nat Genet. 2010;42:420–425.
8. Letamendia A, Lastres P, Botella LM, et al. Role of endoglin in cellular responses to transforming growth factor-beta. A comparative study with betaglycan. J Biol Chem. 1998;273:33011–33019.
9. Fernandez-Ruiz E, St-Jacques B, Bellon T, et al. Assignment of the human endoglin gene (END) to 9q34->qter. Cytogenet Cell Genet. 1993;64:204–207.
10. Lu Y, Zhu Y, Shi L, et al. A novel endoglin mutation in hereditary hemorrhagic telangiectasia type 1: a case report. Mol Med Rep. 2015;12:510–512.
11. Damjanovich K, Langa C, Blanco FJ, et al. 5’UTR mutations of ENG cause hereditary hemorrhagic telangiectasia. Orphanet J Rare Dis. 2011;6:85.
12. Takenaka K, Sakai H, Yamakawa H, et al. Polymorphism of the endoglin gene in patients with intracranial saccular aneurysms. J Neurosurg. 1999;90:935–938.
13. Krex D, Ziegler A, Schackert HK, et al. Lack of association between endoglin intron 7 insertion polymorphism and intracranial aneurysms in a white population: evidence of racial/ethnic differences. Stroke. 2001;32:2689–2694.
14. Onda H, Kasuya H, Yoneyama T, et al. Endoglin is not a major susceptibility gene for intracranial aneurysm among Japanese. Stroke. 2003;34:1640–1644.
15. Joo SP, Lee JK, Kim TS, et al. A polymorphic variant of the endoglin gene is associated with increased risk for intracranial aneurysms in a Korean population. *Surg Neurol.* 2008;70:39–44.

16. Lin Y, Yu H, Song W, et al. A variant in the endoglin gene is associated with the development of sporadic intracranial aneurysms. *Curr Neuropathol Res.* 2014;11:294–301.

17. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst.* 1959;22:719–748.

18. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials.* 1986;7:177–188.

19. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics.* 1994;50:1088–1101.

20. Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med.* 2009;6:e1000097.

21. Pera J, Slowik A, Dziedzic T, et al. Endoglin gene insertion polymorphism not associated with aneurysmal subarachnoid hemorrhage. *J Neurosurg.* 2005;102:879–881.

22. Peters DG, Kassam AB, Chang YF. A DNA sequence polymorphism in the endoglin gene is not associated with intracranial aneurysm or aneurysmal subarachnoid hemorrhage. *Cerebrovasc Dis.* 2005;20:96–100.

23. Koshy LV, Easwer HV, Bhattacharya RN, et al. Lack of association of Endoglin insertion polymorphism in intracranial aneurysm in South Indian population. *Indian J Hum Genet.* 2006;12:111–115.

24. Simon M, Franke D, Ludwig M, et al. Association of a polymorphism of the ACVRL1 gene with sporadic arteriovenous malformations of the central nervous system. *J Neurosurg.* 2006;104:945–949.

25. Santiago-Sim T, Mathew-Joseph S, Pannu H, et al. Sequencing of TGF-(beta) pathway genes in familial cases of intracranial aneurysms. *Stroke.* 2009;40:1604–1611.

26. Caranci F, Briganti F, Cirillo L, et al. Epidemiology and genetics of intracranial aneurysms. *Eur J Radiol.* 2013;82:1598–1605.

27. Li DY, Easwer HV, Bhattacharya RN, et al. Lack of association of Endoglin insertion polymorphism in intracranial aneurysm in South Indian population. *Indian J Hum Genet.* 2006;12:111–115.

28. Simon M, Franke D, Ludwig M, et al. Association of a polymorphism of the ACVRL1 gene with sporadic arteriovenous malformations of the central nervous system. *J Neurosurg.* 2006;104:945–949.

29. Yang C, Qi ZY, Shao C, et al. Association between three eNOS polymorphisms and intracranial aneurysms risk: a meta-analysis. *Medicine.* 2015;94:e452.

30. Zhu Y, Li W, Ge M, et al. Polymorphism rs42524 of COL1A2 and sporadic intracranial aneurysms in the Chinese population. *J Neurosurg.* 2008;109:1060–1064.

31. Jonker L, Arthur HM. Endoglin expression in early development is associated with vasculogenesis and angiogenesis. *Mech Dev.* 2002;110:193–196.

32. Torsney E, Charlton R, Parums D, et al. Inducible expression of human endoglin during inflammation and wound healing in vivo. *Inflamm Res.* 2002;51:464–470.

33. Botella LM, Sanchez-Elsner T, Sanz-Rodriguez F, et al. Transcriptional activation of endoglin and transforming growth factor-beta signaling components by cooperative interaction between Sp1 and KLF6: their potential role in the response to vascular injury. *Blood.* 2002;100:4001–4010.

34. Li DY, Sorensen IK, Brooke BS, et al. Defective angiogenesis in mice lacking endoglin. *Science.* 1999;284:1534–1537.

35. Alberts MJ, Davis JP, Graffagnino C, et al. Endoglin gene polymorphism as a risk factor for sporadic intracerebral hemorrhage. *Ann Neurol.* 1997;41:683–686.

36. Alberts MJ, Davis JP, Graffagnino C, et al. Endoglin gene polymorphism as a risk factor for sporadic intracerebral hemorrhage. *Ann Neurol.* 1997;41:683–686.

37. Khurana VG, Sohni YR, Mangrum WI, et al. Endothelial nitric oxide synthase gene polymorphisms predict susceptibility to aneurysmal subarachnoid hemorrhage and cerebral vasospasm. *J Cereb Blood Flow Metab.* 2004;24:291–297.

38. Savas S, Schmidt S, Jarjanazi H, et al. Functional nsSNPs from carcinogenesis-related genes expressed in breast tissue: potential breast cancer risk alleles and their distribution across human populations. *Hum Genomics.* 2006;2:287–296.