Angiopoietin-Like Proteins 4 (ANGPTL4) Gene Polymorphisms and Risk of Brain Arteriovenous Malformation

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Background: Brain arteriovenous malformations (BAVMs) are formed by hypertrophied arterial vessels (afferents, feeders), a large number of arteriovenous shunts which become tangle to form a body (nidus) of malformation, which then expands draining proximal veins. The aim of this study was a replication of single nucleotide polymorphism (SNP) rs11672433 association with BAVM development with the subsequent meta-analysis of published data.

Methods: A total of 252 Russian patients with brain BAVMs and 480 control subjects were included in the present study. Genotyping was performed using real-time polymerase chain reaction with competitive hydrolysis probes.

Results: In our case–control study, we found no significant association with brain arteriovenous malformation for the SNP rs11672433 of ANGPTL4 gene (odds ratio .82, 95% confidence interval = .57-1.17, P value = .27) as well as in meta-analysis (odds ratio 1.18, 95% confidence interval = .81-1.73, P value = .39).

Conclusions: Our data showed that SNP rs11672433 was not associated with the BAVM Russian population and the following meta-analysis did not detect an association in total. Thus, in spite of the fact that ANGPTL4 (protein) participates in the angiogenesis regulation processes, we consider that SNP rs11672433, a high-frequency locus in the ANGPTL4 gene, does not influence the predisposition to BAVM or its effect is too small to be detected in the present size sample set.

Key Words: Arteriovenous malformations—angiopoietin-like proteins 4 (ANGPTL4)—single nucleotide polymorphisms—association—gene.

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Introduction

Brain arteriovenous malformations (BAVMs) are formed by hypertrophied arterial vessels (afferents, feeders), a large number of arteriovenous shunts which become tangled to form a body (nidus) of malformation, which then expands draining proximal veins. The BAVM and cerebral cavernous malformations are the most common vascular malformations with an incidence of approximately 1.1 and .6 per 100,000 adult population per year, respectively. The estimated BAVM detection rate is 1.1-1.34 per 100,000 patient-year. The survival rate of patients with arteriovenous malformation is 85% in the first 10 years and 65%
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in 30 years after diagnosis. In the treatment of BAVM, the main objective of any kind of intervention is the full obliteration of a malformation for prevention of intracranial hemorrhages (ICHs). There are some debates at the ARUBA study (A Randomized Trial of Unruptured Brain Arteriovenous Malformations [AVMs]), which describes treatment methods between vascular lesions. BAVMs are treated by endovascular embolization, stereotactic radiosurgery, and microsurgical resection. Nevertheless, the number of cases and controls was disproportional—167 patients versus 1038 healthy controls. It is possible that the results could have been overestimated and further replications are necessary to prove the role of ANGPTL4 in BAVM predisposition. Thus, the purpose of our research was to carry out a replication of association for SNP rs11672433 in a Russian population with the subsequent meta-analysis of all published research.

Materials and Methods

Patients

The study included 252 patients (mean age: 37.1 ± 15.0, female/male: 111/141) with brain BAVMs. Magnetic resonance imaging and cerebral angiography were performed in the clinical centers in Novosibirsk: Federal Neurosurgical Center, Novosibirsk, Russia, and Novosibirsk Research Institute of Blood Circulation Pathology named after E. N. Meshalkin, Novosibirsk, Russia. Each patient completed a specially developed questionnaire including demographic data (age, gender, nationality) and medical information (age, debut, current type). The average age of patients at the period of manifestation was 33.8 ± 13.5 years. The control group consisted of 480 individuals (mean age: 33.0 ± 11.1, female/male: 199/281) from Novosibirsk without BAVM. The study was approved by the Local Ethics Committee of Center of New Medical Technologies of Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences.

SNP Selection

Polymorphism in the ANGPTL4 gene region was selected from the analysis based on published data and subsequent sequence database searches (dbSNP). There are 6 SNPs in ANGPTL4 previously studied, with metabolic traits, lipids metabolism and adiposity, coronary disease, BAVM, and severity of post-transplant proteinuria in kidney allograft recipients (Table 1). The set of SNPs was formed by previous investigators based on (1) HapMap genotype and linkage disequilibrium (LD) data (rs4076317, rs2278236, rs1044250, rs11672433, rs11808536) and (2) the previously described effect on the ANGPTL4 function, plasma lipid levels, and cardiovascular risk.

These SNPs covered 100% of the common variants (minor allele frequency [MAF] ≥ 0.05) within the ANGPTL4 gene, with an \( r^2 \geq 0.8 \) according to TAGGER analysis (http://www.broad.mit.edu/mpg/tagger). According to HapMap (data release 21, July 2006 and release 23a/phase II, March 2008) and previously published data, the 4 SNPs (rs4076317, rs2278236, rs1044250, rs11672433) were in weak LD with the others based on \( r^2 \) (\( r^2 < 0.5 \)), but they all lie within high-LD block based on \( D' \) (\( D' > 0.945 \) (Table 2).
From previous association study of BAVM by Mikhak et al, only rs11672433 of ANGPTL4 gene was associated with BAVM. Kremer et al. replicated this association with follow-up meta-analysis. We selected rs11672433 for our replication study at the residents of the West Siberian region.

### Genotyping

DNA was extracted from venous blood using a standard method including isolation and lysis of blood cells, hydrolysis of proteins with proteinase K, and purification of DNA by extraction with a phenol–chloroform...
Genotyping of SNP in the ANGPTL4 gene (rs11672433) was performed using a real-time PCR with competitive hydrolysis probes complementary to the polymorphic DNA sites. Amplification was performed using a CFX-96 cycler (Bio-Rad, Hercules, California, USA). The polymerase chain reaction mixture (25 μL) contained 40–100 ng DNA; 300 nM of each primer (F: 5′-AAGGGAATCTTCTGGAAGACCT-3′, R: 5′-TGCTATGGGCTGGATCAACA-3′); 100–200 nM TaqMan probe (HEX-5′-CTACTACCCGCTGCAGGC-BHQ-3′, FAM-5′-CTACTACCCACTGCAGGCC-BHQ-3′); 200 μM dNTP, buffer for amplification, 1 U of Taq-polymerase. The genotyping success rate was 95%.

Statistical Analysis

The Hardy–Weinberg equilibrium was evaluated using an exact test of Hardy–Weinberg equilibrium for 2-Allele markers in the R package “genetics.” Possible associations between SNPs and disease development were found using logistic regression analysis, as implemented in the “glm” function of the R package for statistical analysis (www.r-project.org). Meta-analysis and estimated heterogeneity were carried out using the “rmeta” package for R (http://cran.r-project.org/web/packages/rmeta/rmeta.pdf). Pooled ORs were computed by the random-effect model for data combined under heterogeneity between studies. Results were considered statistically significant for all statistical calculations if \( P < .05 \).

Results

We performed genotyping SNP rs11672433 in patients with BAVM and controls. The distribution of SNP genotype corresponded to a Hardy–Weinberg distribution in the BAVM patients \( (P = 1.00) \) and controls \( (P = .15) \). Association between ANGPTL4 and the genotype of SNP was estimated using logistic regression. We did not find a significant association between the SNP rs11672433 ANGPTL4 gene and the BAVM \( (OR = .82, 95\% CI = .57 – 1.17, P = .27) \), which was not consistent with previous data on residents of California (Table 3).

We included all previously published data in the meta-analysis (Mikhak et al) and showed that rs11672433 was not significantly associated with BAVM \( (OR = 1.18, 95\% CI = .81 – 1.72, P = .39, P_{het} = .024) \) (Table 3, Fig 1).

Discussion

Vasculogenesis and angiogenesis are important stages in the development of blood vessels during embryogenesis. One of the most important stimulators of angiogenesis is angiopoietin. There are 4 types of angiopoietins—Ang1, Ang2, Ang3 and Ang4. Angiopoietin-like proteins (ANGPTL) are a family of proteins structurally similar to angiopoietins—which are also involved in angiogenesis. The family of angiopoietin-like proteins consists of 8 proteins, which render a pleiotropic effect on angiogenesis and lipid metabolism stimulates development of hematopoietic stem cells in the culture.

Table 2. Linkage disequilibrium statistics (\( D' \), \( r^2 \)) among the 4 previously genotyped SNPs of the 17.75-kb genomic region harboring the ANGPTL4 gene.

<table>
<thead>
<tr>
<th>SNP</th>
<th>rs4076317</th>
<th>rs2278236</th>
<th>rs1044250</th>
<th>rs11672433</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4076317</td>
<td>—</td>
<td>1.0*</td>
<td>—</td>
<td>1.0*</td>
</tr>
<tr>
<td>rs2278236</td>
<td>0.33*</td>
<td>—</td>
<td>1.0*</td>
<td>—</td>
</tr>
<tr>
<td>rs1044250</td>
<td>0.11*</td>
<td>0.33*</td>
<td>1.0*</td>
<td>—</td>
</tr>
<tr>
<td>rs11672433</td>
<td>0.04*</td>
<td>0.11*</td>
<td>0.11*</td>
<td>—</td>
</tr>
</tbody>
</table>

Abbreviation: SNP, single nucleotide polymorphism.

*HapMap (CEU, \( N = 120 \) subjects).
†Staiger et al (\( N = 629 \) overweight nondiabetic subjects from the southwest of Germany).12
‡Legry et al (participants were recruited as part of the World Health Organization [WHO]-MONICA population survey performed from 1995 to 1997 in the Lille Urban Community in Northern France [\( N = 1195 \)].10

mixture, and precipitation of DNA with ethanol. Genotyping of SNP in the ANGPTL4 gene (rs11672433) was performed using a real-time PCR with competitive hydrolysis probes complementary to the polymorphic DNA sites. Amplification was performed using a CFX-96 cycler (Bio-Rad, Hercules, California, USA). The polymerase chain reaction mixture (25 μL) contained 40-100 ng DNA; 300 nM of each primer (F: 5′-AAGGGAATCTTCTGGAAGACCT-3′, R: 5′-TGCTATGGGCTGGATCAACA-3′); 100-200 nM TaqMan probe (HEX-5′-CTACTACCCGCTGCAGGC-BHQ-3′, FAM-5′-CTACTACCCACTGCAGGCC-BHQ-3′); 200 μM dNTP, buffer for amplification, 1 U of Taq-polymerase. The genotyping success rate was 95%.

Statistical Analysis

The Hardy–Weinberg equilibrium was evaluated using an exact test of Hardy–Weinberg equilibrium for 2-Allele markers in the R package “genetics.” Possible associations between SNPs and disease development were found using logistic regression analysis, as implemented in the “glm” function of the R package for statistical analysis (www.r-project.org). Meta-analysis and estimated heterogeneity were carried out using the “rmeta” package for R (http://cran.r-project.org/web/packages/rmeta/rmeta.pdf). Pooled ORs were computed by the random-effect model for data combined under heterogeneity between studies. Results were considered statistically significant for all statistical calculations if \( P < .05 \).

Results

We performed genotyping SNP rs11672433 in patients with BAVM and controls. The distribution of SNP geno-
The human ANGPTL4 gene belongs to the ANGPTL family and is located on chromosome 19p13.3. The gene has 8 exons and 7 introns and encodes the ANGPTL4 protein. ANGPTL4 is a secreted glycoprotein expressed by liver, adipose tissue, pericytes, and smooth myocytes of a vascular wall. ANGPTL4 modulates vascular permeability, inflammatory signaling, angiogenesis, etc. It has been shown that vascular permeability and myocardial infarction severity are increased in ANGPTL4-deficient mice. ANGPTL4-deficient mice also suffer a disorganization of endothelial adherent’s junctions, suggesting that ANGPTL4 could promote the endothelial barrier function at multiple levels.

We had no luck to replicate the association of ANGPTL4 gene polymorphism with BAVM development. This can have a number of explanations. The ANGPTL4 protein is cleaved at the N-terminal-coiled-coil domain (CCD) and the C-terminal fragment—the fibrinogen-like domain (FLD) by several types of pro-protein convertases in the cellular matrix or in blood plasma. The N-terminal domain (CCD) is functionally active, binds heparan and dermatan sulfates, and inhibits endothelial cell adhesion, motility, and tubule-like formation. The SNP rs11672433 (p.Pro389Pro) is localized in the not functionally active C-terminal domain. Moreover, SNP rs11672433 does not change the amino acid sequence and therefore does not

### Table 3. Association of SNP rs11672433 with BAVM.

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Race/Ethnicity</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
<th>Case/Control*</th>
<th>MAF control</th>
<th>MAF case</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mikhak et al</td>
<td>Caucasian</td>
<td>1.65</td>
<td>1.15-2.36</td>
<td>.006</td>
<td>216(143 + 63 + 10)/246(190 + 50 + 6)</td>
<td>.13</td>
<td>.19</td>
</tr>
<tr>
<td>Kremer et al</td>
<td>Caucasian</td>
<td>1.21</td>
<td>.89-1.65</td>
<td>.23</td>
<td>167/1038</td>
<td>.15</td>
<td>.173</td>
</tr>
<tr>
<td>Present study</td>
<td>Mixed</td>
<td>.82</td>
<td>.57-1.17</td>
<td>.27</td>
<td>247 (203 + 42 + 2)/448 (356 + 83 + 9)</td>
<td>.11</td>
<td>.09</td>
</tr>
<tr>
<td>Meta-analysis</td>
<td></td>
<td>1.18</td>
<td>.81-1.72</td>
<td>.39</td>
<td>630/1732</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BAVM, brain arteriovenous malformation; CI, confidence interval; MAF, minor allele frequency; OR, odds ratio; SD, standard deviation; SNP, single nucleotide polymorphism.

*In the column’s cells, the distribution by genotypes is given: total in the AVM group (GG carriers + GA carriers + AA carriers)/total in the control group (GG carriers + GA carriers + AA carriers).

Figure 1. Meta-analysis of the association between rs11672433 and brain arteriovenous malformations. CI, confidence interval; OR, odds ratio.
change the protein function. Also, according to the database Blood eQTL browser (http://genenetwork.nl/bloodeqtlbrowser/), Single nucleotide polymorphism rs11672433 is annotated like expression quantitative trait locus (eQTL) for the MARCH2, ELAVL1 genes. So it would be unlikely that SNP rs11672433 has a direct functional effect on ANGPTL4.

There are few high-frequency SNPs in ANGPTL4 gene (MAF > .05). Practically all of them have been previously investigated and an association with BAVM was not detected, except for SNP rs11672433 in Mikhak et al. study. The results of our study support no significant association of SNP rs11672433 of the ANGPTL4 gene with BAVM in the residents of the West Siberian region of Russia. The meta-analysis has also not revealed an association with BAVMs for rs11672433 in ANGPTL4 (OR 1.18, 95% CI = 0.81-1.73, P = .3). Taking into account that we and Kremer et al have not found a significant association, and as it has been found only in a pilot research, perhaps we had deal with the phenomenon “winner’s curse.”

The limitation of all ANGPTL4 association studies is the small sample size. The statistical power to detect association in the provided meta-analysis with OR = 1.18 and P = .05 is only 29%. For 80% statistical power, it would be necessary to examine about 2200 person cases and 2200 controls.

Finally, our data showed that SNP rs11672433 was not associated with BAVM in the residents of the West Siberian region of Russia, and the following meta-analysis also did not detect an association. Thus, in spite of the fact that the ANGPTL4 (protein) participates in the angiogenesis regulation processes, we conclude that the high-frequency SNP rs11672433 in ANGPTL4 gene does not influence the predisposition to BAVM and processes of angiogenesis.

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References