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 tangled 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.

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.[1] 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.[2]

The estimated BAVM detection rate is 1.1-1.34 per 100,000 patient-year.[3] 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.[4]

According to published data, various factors could be involved in initiating pathological processes of malformation, including genetic factors, and may predispose individuals to BAVM. A key role in the development of BAVM is played by angiogenesis: (1) BAVMs are formed as a result of angiogenesis disorders in the primary capillary reduction stage, and (2) neovascularization contributes to AVM progression.[5] The angiogenesis genes belong to several families such as the cyclin-dependent-kinase inhibitors (*CDKN2A*, *CDKN2B*), the family of vascular endothelial growth factors (*VEGF*), their receptors (*VEGFR*), fibroblast growth factors (*FGF-2*), angiopoietins (*Angpt-1,2*), TEK receptor tyrosine kinase (*Tie*) 1 and 2, interleukin-8 (*IL-8*), platelet-derived growth factor (*PDGF*), transforming growth factor-beta (*TGF beta*), and angiopoietin-like proteins (*ANGPTL*).[6] The last one represents a good candidate for regulating angiogenesis, so the *ANGPTL4* gene encodes an important angiogenesis factor involved in the postnatal formation of vessels.

For the first time, the association between the polymorphisms of *ANGPTL4* gene and BAVM was established in 2011 by Mikhak et al, in Caucasian individuals in California (216 Caucasian BAVM cases and 246 healthy controls). They studied 4 SNPs: rs2278236, rs1044250, rs11672433, and rs1808536 of *ANGPTL4* gene for association with risk of BAVM or ICH. They showed that the allele A rs11672433 of the *ANGPTL4* gene (odds ratio [OR] = 1.56; 95% confidence interval = 1.01-2.41; $P = .046$) was associated with the risk of BAVM, but not with ICH. Their data confirmed a potential role of the *ANGPTL4* gene in the pathogenesis of BAVM, which could be linked to an angiogenesis dysregulation. The remaining polymorphic variants did not show any association. As the authors specify, the research was limited to a single Caucasian ethnic group. In addition, the sample size was small.[7]

Four years later, Kremer et al conducted a replicative research of SNPs of *ANGPTL4* genes, *IL-1b*, *GPR124*, *VEGFA*, and *MMP-3* with risk of BAVM in Caucasians from the Netherlands (167 Caucasian BAVM cases and 1038 healthy controls) with meta-analysis. Meta-analysis for rs11672433 of *ANGPTL4* gene has shown a significant association with BAVM (OR 1.39; 95% CI 1.10-1.75, P value = .005). The authors concluded that previous studies of the function of the protein ANGPTL4 and association between BAVM and SNP of *ANGPTL4* could demonstrate contribution of protein to the pathogenesis of BAVM.[8] 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[7-12] with metabolic traits,[12] lipid metabolism and adiposity,[10] coronary disease,[11] BAVM,[7,8] and severity of post-transplant proteinuria in kidney allograft recipients[9] (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, rs1808536) and (2) the previously described effect on the ANGPTL4 function,[13] plasma lipid levels, and cardiovascular risk.[11]

These SNPs covered 100% of the common variants (minor allele frequency [MAF] ≥.05) within the *ANGPTL4* gene, with an $r^2 \geq .8$ 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,[10,12] the 4 SNPs (rs4076317, rs2278236, rs1044250, rs11672433) were in weak LD with the others based on r^2 ($r^2 < .50$), but they all lie within high-LD block based on D' ($D' > .945$) (Table 2).