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DIFFERENCES IN ALLELIC AND GENOTYPIC FREQUENCIES OF THE RS1333040 POLYMORPHISM OF THE CDKN2B GENE IN ARTERIOVENOUS MALFORMATIONS

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Abstract
Cerebral arteriovenous malformation (AVM) is a congenital anomaly of the brain's vascular system characterized by the formation of abnormal tangles connecting arteries and veins. Cerebral AVMs, being highly complex entities for both neurologists and neurosurgeons, continue to attract considerable attention from researchers and clinicians alike, aiming to identify the most effective treatment strategies and to deepen the understanding of the underlying processes that govern the formation and progression of these vascular malformations.

Keywords: Arteriovenous malformations, brain, vascular pathology, genetics.

Introduction

Arteriovenous malformation (AVM) is a congenital vascular malformation of the brain. The hallmark of cerebral AVMs is arteriovenous shunts forming a “nidus” of malformation. These are characterized by tangles of vessels, including hypertrophied arterial feeders forming the nidus and dilated draining veins. AVMs may manifest with hemorrhage in 50–70% of cases, or present more indolently with seizures, headaches, chronic cerebral hypoperfusion, and focal neurological deficits.

Genetic Research into AVM

Recent genomic studies increasingly explore complex diseases. Genetic predisposition to AVMs is investigated using candidate gene analysis, genome-wide linkage, and genome-wide association studies (GWAS). These studies show that polymorphisms in genes related to inflammation, angiogenesis, endothelial growth factors, and more contribute to AVM pathogenesis. AVMs are thought to arise from sporadic fetal mutations occurring during gestational weeks 4–13.

Role of the **CDKN2B** Gene

Located at chromosome 9p21, **CDKN2B** regulates the cell cycle by inhibiting CDK4 and CDK6, thereby controlling vascular cell proliferation, senescence, and apoptosis. Disruption may contribute to vascular wall abnormalities, including AVM formation.

The **CDKN2B gene** is one of the key regulators of the cell cycle involved in controlling the proliferation, differentiation, and aging of vascular cells. This gene encodes an inhibitor of the

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cyclin-dependent kinases CDK4 and CDK6, which are essential for controlling the transition from the G1 to the S phase of the cell cycle. Dysfunction of this gene may lead to pathological changes in the vascular wall and the development of anomalies such as arteriovenous malformations (AVMs) [3].

The study included **154 participants**, with the main group comprising **94 patients** diagnosed with cerebral arteriovenous malformations (AVMs), and the control group consisting of **60 conditionally healthy individuals** with no signs of cerebrovascular pathology. Thus, the main group made up 61.0% (95% CI: 52.9–68.8), and the control group 39.0% (95% CI: 31.2–47.1).

In terms of gender distribution, **men predominated**—66 individuals (70.2%), while **women accounted for 28 individuals** (29.8%), which generally reflects trends described in the literature regarding the more frequent manifestation of AVMs in males. In the control group, men represented 61.7% (37 individuals), and women 38.3% (23 individuals).

An association analysis was conducted using the **case-control** model. The results of our study on the **allele frequency distribution of the rs1333040 polymorphism** of the **CDKN2B gene** revealed significant differences between the AVM patient group and the control group (Table 1). The **C allele** was significantly more frequent in the AVM group, accounting for 31.98% compared to 17.5% in the control group ($\chi^2 = 6.8$; $p = 0.01$; RR = 1.8; 95% CI: 1.13–2.95; OR = 2.2; 95% CI: 1.22–4.03). The **T allele**, on the other hand, was significantly more common in the control group—82.5% vs. 68.03%. These data suggest an **associative link between the C allele and AVM development**. According to the odds ratio, the presence of this allele variant of the CDKN2B gene significantly increases the risk of AVM by **twofold** ($\chi^2 = 6.8$; $p = 0.01$; RR = 0.5; 95% CI: 0.27–1.13; OR = 0.5; 95% CI: 0.25–0.82).

The distribution frequencies of the **genotypes C/C, C/T, and T/T** of the rs1333040 polymorphism of the CDKN2B gene in the AVM patient group and the control group were:

- **11.5%, 41.0%, and 47.5%** in the patient group
- **3.3%, 28.3%, and 68.3%** in the control group, respectively (Table 2).

The **T/T genotype** was more frequently observed in the control group compared to the AVM group (68.3% vs. 47.5%; $\chi^2 = 5.4$; $p = 0.08$; OR = 0.4; 95% CI: 0.2–0.88). The **heterozygous C/T genotype** was significantly more common in the AVM group than in the control group (41.0% vs. 28.3%; $\chi^2 = 2.1$; $p = 0.30$; OR = 1.8; 95% CI: 0.83–3.74).

- The rs1333040 polymorphism of **CDKN2B** is a promising **genetic marker** for predicting AVM risk and severity.
- Clinically, **C allele carriers**, especially with C/C genotypes, may have higher risk of severe symptoms (seizures, neurological impairment).
- These results justify large-scale multicenter studies across various ethnicities to validate findings and incorporate them into personalized prognosis and therapy.

A homozygous C/C genotype was more common among patients in the main group than in the control group (11.5% vs. 3.3%; $\chi^2 = 2.9$; $p = 0.18$; OR = 3.8; 95% CI: 0.82–17.2). According to the odds ratio, the risk of AVM in carriers of the T/T genotype is not significantly increased—about a threefold change.

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Table 2 – Allele and genotype frequencies of the rs1333040 polymorphism of the CDKN2B gene in the AVM patient group and the control group:

Allele/Genotype	AVM group (n, %)	Control (n, %)	χ^2	p	RR	95% CI	OR	95% CI
C allele	39 (32.0%)	21 (17.5%)	6.8	0.01	1.8	1.13–2.95	2.2	1.22–4.03
T allele	83 (68.0%)	99 (82.5%)	6.8	0.01	0.5	0.27–1.13	0.5	0.25–0.82
C/C genotype	7 (11.5%)	2 (3.3%)	2.9	0.18	3.4	1.58–7.52	3.8	0.82–17.2
C/T genotype	25 (41.0%)	17 (28.3%)	2.1	0.30	1.4	0.73–2.85	1.8	0.83–3.74
T/T genotype	29 (47.5%)	41 (68.3%)	5.4	0.08	0.7	0.35–1.38	0.4	0.2–0.88

In a comparative subgroup analysis, a pronounced correlation was found between the frequency of the unfavorable T allele and disease severity.

Allele and genotype frequencies in hemorrhagic vs. seizure subgroups

In the hemorrhagic and seizure subgroups (Table 3), the unfavorable C allele was significantly more common in the seizure subgroup (40.0%) compared to the hemorrhagic subgroup (21.4%; $\chi^2 = 3.3$; $p = 0.20$; RR = 0.5; 95% CI: 0.18–1.63; OR = 0.4; 95% CI: 0.16–1.07).

Table 3 – rs1333040 allele and genotype frequencies in hemorrhagic vs. seizure AVM patients:

Allele/Genotype	Hemorrhagic (n, %)	Seizure (n, %)	χ^2	p	RR	95% CI	OR	95% CI
C allele	9 (21.4%)	16 (40.0%)	3.3	0.20	0.5	0.18–1.63	0.4	0.16–1.07
T allele	33 (78.6%)	24 (60.0%)	3.3	0.20	1.9	0.81–4.28	2.4	0.94–6.38
C/C genotype	2 (9.5%)	4 (20.0%)	0.9	0.55	0.5	0.05–4.73	0.4	0.07–2.51
C/T genotype	5 (23.8%)	8 (40.0%)	1.2	0.42	0.6	0.13–2.63	0.5	0.12–1.78
T/T genotype	14 (66.7%)	8 (40.0%)	2.9	0.18	1.7	0.45–6.17	3.0	0.85–10.56

The wild-type T allele was significantly more common in the hemorrhagic subgroup compared to the seizure subgroup (78.6% vs. 60.0%; $\chi^2 = 3.3$; $p = 0.20$; OR = 2.4; 95% CI: 0.94–6.38).

Hemorrhagic vs. other neurological deficit subgroups

Allele and genotype analyses in hemorrhagic vs. other neurological deficit subgroups (Tables 1 and 4) show:

- C allele: 21.4% hemorrhagic vs. 35.0% neurological deficit ($\chi^2 = 1.9$; $p = 0.28$; RR = 0.6; 95% CI: 0.21–1.83; OR = 0.5; 95% CI: 0.19–1.34).
- T allele: 78.6% hemorrhagic vs. 65.0% neurological deficit ($\chi^2 = 1.9$; $p = 0.28$; RR = 1.6; 95% CI: 0.7–3.84; OR = 2.0; 95% CI: 0.74–5.23).

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Table 4 – rs1333040 allele and genotype frequencies in hemorrhagic vs. neurological deficit subgroups

Allele/Genotype	Hemorrhagic (n, %)	Deficit (n, %)	χ^2	p	RR	95% CI	OR	95% CI
C allele	9 (21.4%)	14 (35.0%)	1.9	0.28	0.6	0.21–1.83	0.5	0.19–1.34
T allele	33 (78.6%)	26 (65.0%)	1.9	0.28	1.6	0.7–3.84	2.0	0.74–5.23
C/C genotype	2 (9.5%)	1 (5.0%)	0.3	0.67	1.9	0.35–10.3	2.0	0.17–23.03
C/T genotype	5 (23.8%)	12 (60.0%)	5.5	0.08	0.4	0.08–1.86	0.2	0.06–0.77
T/T genotype	14 (66.7%)	7 (35.0%)	4.1	0.10	1.9	0.51–7.07	3.7	1.04–13.2

There was a trend toward higher C/C genotype frequency in the hemorrhagic group compared to the neurological deficit group (9.5% vs. 5.0%). The odds ratio suggests a twofold increased risk of neurological deficit with this genotype ($\chi^2 = 0.3$; $p = 0.67$).

Hemorrhagic subgroup vs. Control group

Statistical analysis showed differences in allele/genotype frequencies between hemorrhagic AVM patients (C: 21.4%, T: 78.6%) and controls (C: 17.5%, T: 82.5%); $\chi^2 = 0.3$; $p = 0.68$; OR = 0.8; 95% CI: 0.32–1.86). The C/C genotype was significantly less frequent in the hemorrhagic subgroup vs. controls (9.5% vs. 3.3%; $\chi^2 = 1.3$; $p = 0.42$; OR = 3.1; 95% CI: 0.44–21.27), suggesting a possible protective effect against hemorrhage. The frequencies of the C/T genotype were identical (28.3%) in both groups, and the T/T genotype showed no difference (68.3% vs. 66.7%).

Seizure vs. Neurological Deficit subgroups

Results (Tables 1 & 6) show:

- C allele: 40.0% (seizure) vs. 35.0% (deficit), $\chi^2 = 0.2$; $p = 0.77$; RR = 1.1; OR = 1.2
- T allele: 60.0% vs. 65.0%, $\chi^2 = 0.2$; $p = 0.77$; RR = 0.9; OR = 0.8

Genotype frequencies:

- C/C: 20.0% vs. 5.0% (seizure vs. deficit); $\chi^2 = 2.1$; $p = 0.29$; RR = 4.0; OR = 4.8
- C/T: 40.0% vs. 60.0%, $\chi^2 = 1.6$; $p = 0.48$; RR = 0.7; OR = 0.4

Seizure subgroup vs. Control group

(Table 1 & 7):

- C allele: 40.0% vs. 17.5%; $\chi^2 = 8.5$; $p = 0.01$; RR = 2.3; OR = 3.1
- T allele: 60.0% vs. 82.5%; $\chi^2 = 8.5$; $p = 0.01$; RR = 0.4; OR = 0.3

Genotypes:

- C/C: 20.0% vs. 3.3%; $\chi^2 = 6.0$; $p = 0.08$; RR = 6.0; OR = 7.3
- C/T: non-significant difference; $\chi^2 = 1.0$; $p = 0.56$; RR = 1.4; OR = 1.7
- T/T: more common in controls ($\chi^2 = 5.1$; $p = 0.07$; RR = 0.6; OR = 0.3)

Neurological Deficit vs. Control group

(Table 1 & 8):

- C allele: 35.0% vs. 17.5%; $\chi^2 = 5.4$; $p = 0.08$; RR = 0.5; OR = 0.4

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- T allele: 65.0% vs. 82.5%; $\chi^2 = 5.4$; $p = 0.08$; RR = 0.5; OR = 0.4

Genotype:

- T/T: Significantly more common in controls ($\chi^2 = 6.9$; $p = 0.01$; RR = 0.5; OR = 0.2)
- C/T: 60.0% vs. 28.3%; $\chi^2 = 6.5$; $p = 0.09$; RR = 2.1; OR = 3.8
- C/C: 35.0% (deficit) vs. 3.3% (control); $\chi^2 = 6.9$; $p = 0.01$; RR = 0.5; OR = 0.2

Overall Summary

- The C allele and C/C genotype are associated with increased risk and severity of AVM, especially in seizure and neurological deficit presentations.
- The T allele and T/T genotype appear protective or neutral, especially in hemorrhagic AVMs and overall control comparisons.
- These findings reinforce the importance of rs1333040 in AVM pathogenesis and suggest its value as a potential prognostic genetic marker.

The conducted study makes an important contribution to understanding the genetic mechanisms underlying the development of cerebral arteriovenous malformations (AVMs). The significant predominance of the C allele and the homozygous C/C genotype among AVM patients supports the potential of this polymorphism as a promising genetic marker for predicting both the risk and severity of the disease.

Thus, the results of this study demonstrate a significant association between the rs1333040 polymorphism of the CDKN2B gene and the risk of AVM development, particularly with severe clinical manifestations such as epileptic seizures and persistent neurological deficits. These findings justify the need for further research involving larger cohorts and diverse ethnic groups to gain a deeper understanding of the genetic basis of AVMs and to integrate identified markers into clinical practice for personalized prognosis and therapy.

A comparison of our findings with those of other researchers shows that existing data in the scientific literature also support an association between the rs1333040 polymorphism of the CDKN2B gene and vascular pathologies. For example, a study by Sturiale and colleagues established a statistically significant link between the rs1333040 polymorphism and the risk of sporadic brain AVMs. The authors demonstrated that the T allele variant is associated with an increased risk of vascular malformation formation and influences the angioarchitectural characteristics of AVMs, including size, type of venous drainage, and likelihood of hemorrhage [3].

Similar results were obtained in a large-scale study by other researchers, who confirmed the association of the CDKN2B-AS1 gene rs1333040 polymorphism with the risk of developing brain AVMs [4]. In their study, this genetic variant was linked to increased susceptibility to cerebral vascular anomalies, with an odds ratio (OR) of 1.194.

Furthermore, substantial data exist regarding the significance of the 9p21 locus, where the CDKN2B gene is located, in the pathogenesis of various cerebrovascular and cardiovascular diseases. Several genome-wide association studies (GWAS) have confirmed the role of this locus in the development of ischemic stroke, coronary and carotid artery atherosclerosis, peripheral vascular disease, and intracranial aneurysms [5,6]. These findings highlight the importance of this

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gene not only in the development of vascular pathologies in different locations but also in the general regulation of vascular remodeling processes.

Therefore, our findings regarding the rs1333040 polymorphism of the CDKN2B gene are consistent with and expand upon previously published data, emphasizing the significant role of this genetic variant in the risk and clinical presentation of cerebral AVMs [7]. Our study underscores the necessity of further multicenter studies with larger sample sizes to more precisely determine the role of CDKN2B in AVM pathogenesis and to develop individualized strategies for prognosis and complication prevention.

References

1. Mouchtouris N. et al. Biology of cerebral arteriovenous malformations with a focus on in fl ammation. Nature Publishing Group, 2014. № June. P. 1–9. Sturiale C.L. et al.
2. Rangel-Castilla L. et al. Molecular and cellular biology of cerebral arteriovenous malformations: a review of current concepts and future trends in treatment. // Neurosurg. Focus. 2014. Vol. 37, № 3. P. E1.
3. Kim H. et al. Genetic considerations relevant to intracranial hemorrhage and brain arteriovenous malformations. // Acta Neurochir. Suppl. NIH Public Access, 2008. Vol. 105. P. 199–206.
4. Wooderchak-Donahue, W.L.; Akay, G.; Whitehead, K.; Briggs, E.; Stevenson, D.A.; O'Fallon, B.; Velinder, M.; Farrell, A.; Shen, W.; Bedoukian, E.; et al. Phenotype of CM-AVM2 caused by variants in EPHB4: How much overlap with hereditary hemorrhagic telangiectasia (HHT)? Genet. Med. 2019, 21, 2007–2014, doi:10.1038/s41436-019-0443-z.
5. Gilbert, P.; Dubois, J.; Giroux, M.F.; Soulez, G. New Treatment Approaches to Arteriovenous Malformations. Semin. Interv. Radiol. 2017, 34, 258–271, doi:10.1055/s-0037-1604299.
6. Felipe G. Rendón-Elías,a,* Marely Hernández-Sánchez,b Rosemberg Albores-Figueroa,a Fernando Félix Montes-Tapia,c and Luis Humberto Gómez-Danésa. Congenital vascular malformations update. Medicina Universitaria 2014;16(65):184-198.
7. Lawton MT, Rutledge WC, Kim H, Stapf C, Whitehead KJ, Li DY, Krings T, terBrugge K, Kondziolka D, Morgan MK, et al. Brain arteriovenous malformations. Nat Rev Dis Primers. 2015;1:15008. doi: 10.1038/ nrdp.2015.8.

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