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Polymorphisms in *ACVRL1* and *Endoglin* genes are not associated with sporadic and HHT related brain AVMs in Dutch patients

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Abstract

We aimed to replicate the association of the IVS3-35A>G polymorphism in the activin receptor-like kinase (*ACVRL*) *1* gene and the 207G>A polymorphism in the endoglin (*ENG*) gene with sporadic brain arteriovenous malformations (BAVM) in Dutch BAVM patients. In addition, we assessed whether these polymorphisms contribute to the risk of BAVM in patients with hereditary haemorrhagic telangiectasia type 1 (HHT1). We genotyped 143 Dutch sporadic BAVM patients and 360 healthy volunteers for four variants in the *ACVRL1* gene including IVS3-35A>G and two variants in the *ENG* gene including 207G>A. Differences in allele and genotype frequencies between sporadic BAVM patients and controls and their combined effect were analysed with a likelihood ratio test. Furthermore, we compared the allele and genotype frequencies between 24 HHT1 patients with a BAVM with those of a relative with HHT1 without a BAVM in a matched pair analysis using Wilcoxon signed rank test. No significant differences in allele frequency were found between sporadic BAVM cases and controls or between HHT1 patients with and without BAVM for any of the polymorphisms or the combination of *ACVRL1* and *ENG* polymorphisms. Meta-analysis of the current and the two previous studies for the *ACVRL1* IVS3-35A polymorphism showed a persisting association between the *ACVRL1* IVS3-35A polymorphism and risk of sporadic BAVM (OR 1.86; 95% CI 1.32–2.61, p<0.001). We did not replicate the previously found association between a polymorphism in *ACVRL1* IVS3-35A>G and BAVM in Dutch patients. However, meta-analysis did not rule out a possible effect.

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Competing interests None

Keywords

Arteriovenous malformations; Etiology; Genetics; Neurogenetics; Cerebrovascular disease

Introduction

There is increasing evidence for a genetic component in the etiology of brain arteriovenous malformations (BAVM) from candidate gene studies, genome wide expression studies of BAVM tissue and, most recently, gene expression profiling of blood in BAVM patients [1–3]. Familial clustering of supposedly `sporadic' BAVMs has been described [4]. Furthermore, the prevalence of BAVMs is increased in patients with the autosomal dominant disorder hereditary haemorrhagic telangiectasia (HHT) [5–7]. Mutations in the endoglin (*ENG*) gene lead to HHT1 and mutations in the activin receptor-like kinase 1 (*ACVRL1*) gene to HHT2. BAVMs occur in 9–21% of HHT1 patients, yet rarely in patients with HHT2, indicating that mutations in *ENG* may be involved in the occurrence of BAVMs [8]. Both *ENG* and *ACVRL1* play a role in the transforming growth factor-β (TGF-β) signalling pathway involved in angiogenesis, vascular remodelling and regulation of endothelial cell function [9]. Previously, an association between the *ACVRL1* IVS3-35A>G polymorphism and sporadic BAVM has been reported in an American population of 177 Caucasian patients with sporadic BAVM and 129 controls (OR 2.47, 95% CI 1.38–4.44; p-value 0.002) and in a German study on 94 BAVM patients and 202 controls (OR 2.35, 1.16–4.76; p-value 0.018; analysis any A versus GG) as well as a possible modifying role of *ENG* 207G>A [10,11].

We aimed to replicate the association of *ACVRL1* IVS3-35A>G with sporadic BAVMs and the modifying effect of *ENG* 207G>A in a series of Dutch patients with sporadic BAVM and patients with HHT1. In addition, we genotyped additional tagging single nucleotide polymorphisms (SNPs) in both genes to test whether other common polymorphisms might exist in *ACVRL1* or *ENG* that contribute to the risk of a BAVM in sporadic and HHT1 patients.

Patients and Methods

The study was approved by the institutional ethical committee of the University Medical Center Utrecht (UMCU).

Sporadic BAVM Patients and Controls

The study cohort consisted of 143 non-related Dutch patients with sporadic BAVM (55% male, mean age 47±13 years, 61 (42.7%) presenting with haemorrhage), referred to the UMCU between 1991 and 2005. The control group consisted of 360 Dutch healthy blood bank volunteers. Participants were considered to be of Dutch descent if all grandparents were born in the Netherlands.

HHT Patients

Between 1982 and 2008, 281 genetically confirmed HHT patients (210 HHT1; 71 HHT2) of 95 families of Dutch descent were referred to the St. Antonius Hospital Nieuwegein. Thirty-three HHT1 patients and none of the HHT2 patients had a BAVM. In four families more than one member had a BAVM.

Of the 33 HHT1 patients with a BAVM, patients with available DNA were included (n=24, 46% male, mean age 45±15 years). Each patient with a BAVM was matched with a relative with HHT1 without a BAVM (54% male, mean age 45±15 years). Furthermore, BAVM-negative patients of BAVM-positive families were compared to BAVM-negative patients of BAVM-negative families (n=41).

Genotyping

For *ACVRL1* we genotyped the variant IVS3-35A>G (rs2071219)[10,11] and three additional SNPs (rs3759178, rs11169953, rs706819), which tagged all known variants with minor allele frequency ≥5% and r^2>0.8 as calculated with Haploview software, using the Hapmap CEU database. For *ENG*, we genotyped 207G>A (rs16930129)(10) and −1742A>G (rs10987759). Genotyping was performed with Taqman assays for ABI 7900 HT Fast Real Time PCR system (Applied Biosystems, Foster City, Ca) according to the specifications of the manufacturer.

Statistical Analysis

All SNPs were tested for deviation from Hardy Weinberg expectations by χ^2 goodness of fit test (threshold α=0.008, equivalent to α=0.05 after Bonferroni correction for six SNPs). We analysed differences in allele and genotype frequencies between sporadic BAVM patients and controls with a likelihood ratio test (UNPHASED v3.0).[12] We studied the combined effect of the genotypes of *ACVRL1* IVS-35G>A (rs2071219) and *ENG* 207G>A (rs16930129) by a two locus allelic combination analysis in UNPHASED. We combined our results with those of the two published studies and assessed possible heterogeneity of the odds ratio (OR) using the Mantel-Haenszel chi-square test.

For the analysis between HHT1 BAVM-positive and BAVM-negative patients within families, we coded genotypes as 0, 1 or 2 minor alleles using a Wilcoxon Signed Rank test.

Results

All polymorphisms were in Hardy-Weinberg equilibrium. We did not find significant differences between patients with sporadic BAVM and controls for any of the SNPs (Table 1).

Furthermore, for the combination of *ENG* 207G>A and *ACVRL1* IVS3-35A alleles, we did not find significant ORs for any allele combination (G-A [55% of BAVM patients, 53% of controls], OR 0.96 95% CI 0.44–2.11; G-G [37% of BAVM patients, 38% of controls], OR 0.91, 95% CI 0.40–2.11; A-A [3% of BAVM patients, 5% of controls], OR 0.59, 95% CI 0.15–2.34 compared to A-G [5% of BAVM patients, 4% of controls] as reference).

However, meta-analysis of the current results and the two previous studies for the *ACVRL1* IVS3-35A polymorphism showed a persisting association between the *ACVRL1* IVS3-35A polymorphism and risk of sporadic BAVM (OR 1.86; 95% CI 1.32–2.61, p<0.001; any A versus GG). We did not find evidence for OR heterogeneity among our study and the two previous studies (p-value 0.203). In HHT1 patients, we did not find significant differences in the number of minor alleles at *ACVRL1* between patients with and without a BAVM or between patients of BAVM-positive and BAVM-negative families (Table 2). We observed a trend for association with the *ENG 207* polymorphism in BAVM-positive families versus BAVM-negative families (p-value 0.056). Genotyping of the rs706819 SNP was technically insufficient to draw any conclusions from.

Discussion

In this study of Dutch sporadic BAVM patients we did not replicate the previously reported association with *ACVRL1* or *ENG* polymorphisms[10,11]. The *ACVRL1* IVS3-35A allele was also not associated with presence of a BAVM in HHT1 patients.

The discrepancy with associations found in the two previous studies could be due to population differences, although all cohorts that were studied consisted of Caucasian patients and demographic characteristics were similar. The proportion of patients who had presented with haemorrhage was slightly lower (42,7%) in our Dutch cohort than that in the American patients 63.8%).[10] The frequency of *ACVRL1* IVS3-35A in our control group was slightly higher than in the control groups of the two previously published studies, but we found no heterogeneity in the observed ORs. Despite the reasonable power (~80%), it is possible that our results are false negative, caused by an overestimate of the effect size in the initial report due to the `winners curse'[13]. We also found no support for the hypothesis that other common polymorphisms in the *ACVRL1* gene contribute to the risk of a BAVM in HHT1 patients, and only weak trends toward association for polymorphisms in the *ENG* gene were found. However, the power of this analysis was limited given the relatively small sample size. Taken together, our results suggest that the pathophysiology of both sporadic and familial BAVMs is complex and other genes or environmental factors might play an important role in the development of BAVMs.

Further studies to find genetic determinants of development and behaviour of BAVMs are needed and should include large numbers of patients. For a disease as rare as BAVM, studies of large cohorts can only be accomplished through international collaboration.

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Table 1

Genotype and allele frequencies of polymorphisms in *ACVRL1* and *ENG* in patients with sporadic BAVM and controls

Genotype	Sporadic BAVM patients n (%)	Controls n (%)	OR (95% CI)*	allele frequency	Sporadic BAVM patients n (%)	Controls n (%)	OR (95% CI)*
ENG207G>A (rs16930129)				*ENG207G>A*(rs16930129)			
G/G	118(84.9)	298(83.2)	1.06(0.61–1.82)	G	257(92.5)	652(91.1)	1.20(0.72–2.01)
G/A	21(15.1)	56(15.6)	1	A	21(7.6)	64(8.9)	1
A/A	0(0)	4(1.1)	-				
ENG-1742A>G(rs10987759)				*ENG-1742A>G*(rs10987759)			
A/A	0(0)	4(1.1)	-	A	20(7.2)	58(8.2)	0.86(0.51–1.47)
A/G	20(14.3)	50(14.1)	1.00(0.57–1.76)	G	260(92.9)	652(91.8)	1
G/G	120(85.7)	301(84.8)	1				
rs3759178				rs3759178			
G/G	25(17.7)	54(15.3)	1.05(0.60–1.84)	G	105(37.2)	269(38.1)	0.96(0.72–1.28)
G/T	55(39.0)	161(45.6)	0.77(0.50–1.19)	T	437(62.8)	437(61.9)	1
T/T	61(43.3)	138(39.1)	1				
rs11169953				rs11169953			
C/C	65(46.8)	163(46.2)	1.35(0.68–2.67)	C	191(68.7)	472(66.9)	1.09(0.81–1.47)
C/T	61(43.9)	146(41.4)	1.41(0.71–2.81)	T	87(31.3)	234(33.1)	1
T/T	13(9.4)	44(12.5)	1				
ACVRL1 IVS3-35A>G (rs2071219)				*ACVRL1 IVS3-35A>G* (rs2071219)			
A/A	44(31.7)	123(34.8)	1.12(0.62–2.02)	A	161(57.9)	407(57.7)	1.01(0.76–1.34)
A/G	73 (52.5)	161(45.6)	1.42(0.82–2.47)	G	117(42.1)	299(42.4)	1
G/G	22(15.8)	69(19.6)	1				
rs706819				rs706819			
C/C	71(52.2)	194(55.8)	0.70(0.33–1.48)	C	195(71.7)	519(74.6)	0.86(0.63–1.18)
C/T	53(39.0)	131(37.6)	0.77(0.36–1.67)	T	77(28.3)	177(25.4)	1
T/T	12(8.8)	23(6.6)	1				

*Pearson chi-square, expected values were at least >5 in 90% of the cells

Table 2

Allele frequencies in BAVM-positive and BAVM-negative HHT patients

	BAVM (+)		BAVM(−) in BAVM(+) families			BAVM(−) in BAVM(−) families		
	allele frequency	no. patients	allele frequency	no. patients	p-value *	allele frequency	no. patients	p-value †
ENG-1742A>G	0.11	22	0.09	22	0.102	0.02	41	0.095
ENG207 G>A	0.19	24	0.15	24	0.317	0.06	41	0.056
rs3759178	0.42	19	0.35	19	0.660	0.40	41	0.527
rs11169953	0.37	19	0.35	19	0.366	0.30	41	0.683
ACVRL1 IVS3 35A>G	0.57	15	0.60	15	0.763	0.59	41	0.849

*Wilcoxon Signed Rank test: Comparison of BA VM-positive HHT1 patients with their BAVM-negative relatives

†Pearson Chi square test: Comparison of BAVM-negative patients of BAVM-positive HHT1 families versus BAVM-negative patients of HHT1 BAVM-negative families.