The ICAM-1 E469K gene polymorphism is a risk factor for spontaneous cervical artery dissection

Abstract—In a primary study on proinflammatory genetic profiles in stroke, the authors found the E469K polymorphism of the intercellular adhesion molecule 1 (ICAM-1) highly represented in the subgroup with spontaneous cervical artery dissection (sCAD). They further investigated the same genetic variant in a second group of 65 patients with sCAD. An association between sCAD and EE genotype was confirmed (odds ratio 3.16; $p < 0.01$), indicating that a proinflammatory predisposition is a risk factor for sCAD.

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The mechanisms involved in the pathogenesis of spontaneous cervical arterial dissection (sCAD) are unclear, but recent infection may be a triggering factor in sCAD.[1,2] Increased plasma levels of high-sensitivity C-reactive protein (hsCRP) in the postacute phase after arterial dissection further suggests an involvement of inflammatory mechanisms.[3] In a recent study in young stroke patients regarding polymorphisms of potentially risk-bearing genes, we focused on the E469K polymorphism of the intercellular adhesion molecule 1 (ICAM-1 or CD54) previously described by Pola et al.[4] as a risk factor for stroke in an elderly Italian population affected by cerebral ischemia. Because we found a significant association between the E469K polymorphism and the subgroup of stroke patients with sCAD (unpublished data), we then analyzed the ICAM-1 E469K polymorphism in a second and larger series of sCAD patients in order to confirm this finding.

Methods. The primary, hypothesis-generating study included 157 patients aged 50 years and younger who were consecutively admitted to our university hospital in south Germany with a diagnosis of ischemic stroke. All patients received a full clinical workup including brain imaging (CT or MRI), ultrasound studies, and search for cardiac sources of embolism. Among these 157 stroke patients, 31 had sCAD.

The second group of patients was a consecutive series of 65 sCAD patients from the same hospital. This group did not include sCAD patients of the primary study and might be considered a replicate set. SCAD was confirmed in all patients by means of pathognomonic findings in digital subtraction angiography and/or fat-suppressed MRI..

A total of 204 healthy volunteers without a history of vascular disease served as a control group for both disease groups. Control subjects were randomly selected from the population registries of the same region in southwest Germany.

Non-German descent and the inability to give informed consent or to perform a standardized interview focusing on vascular disease and risk factors were exclusion criteria for patient and control groups. The participation rate of eligible patients was 100% of patients with sCAD; that of control subjects was 71% of all persons contacted by mail. The study was approved by the local ethics board, and all subjects gave written informed consent.

The E469K polymorphism of the ICAM-1 gene was detected as follows: after amplification of the relevant gene sequence using the forward primer 5′GCTTATACACAAGAACCAGAC3′ and the reverse primer 5′GGGGCTGTGGGGAGGATA3′, the PCR products were restricted by incubation with the endonuclease Bsh1236I and fragments were detected by electrophoresis on a 2% agarose gel stained with 0.1% ethidium bromide. The presence of the E allele resulted into two fragments 390 kb and 112 kb in length, whereas that of the K allele yielded an unrestricted fragment of 502 kb.

In 10 randomly selected samples, we consistently confirmed the genotype indicated by the enzymatic restriction by DNA sequencing.

Demographic and clinical variables were compared by χ^2 test, Fisher's exact test, or t test as appropriate. Genotype and allele frequencies were compared by χ^2 test with a 2×3 analysis and χ^2 test for linear trend to obtain an OR. We also performed a pairwise comparison of the single genotypes using the likelihood ratio test (LRT). Finally, regarding a 12% prevalence of the EE genotype in the control group of 204 subjects and a 29% prevalence of the same genotype in the sCAD primary subgroup, for the confirmatory study a patient group size of 65 was calculated using EpiInfo 3.3, with a power of 80% and an α of 0.05.

Results. The demographic data and prevalence of conventional vascular risk factors of the study groups are shown in table 1. Age and gender distribution were not significantly different between patient and control groups; however, male gender was more common in patient groups. Present smoking was significantly less common in patients vs controls, hypertension was more common in the primary, but not in the secondary, larger sCAD group.

The ICAM-1 E469K genotype distribution and allelic frequencies are shown in table 2. Genotypes were in Hardy-Weinberg equilibrium. Confirming the preliminary results of the primary study group, we found an association between the EE genotype and the sCAD group (odds ratio [OR] 3.16, CI: 1.14 to 6.96 for EE vs KK analysis and OR 2.68, CI: 1.20 to 6.0 for EE vs EK analysis). Regarding the pairwise comparison, the LRT was 8.8241 with a significant $p = 0.0121$, suggesting that EE was the genotype that leads the association.

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Table 1 Demographic data and prevalence of established risk factors in the three study groups

	sCAD group primary study, n = 31	sCAD group secondary study, n = 65	Control group, n = 204
Age, y	38.2 ± 7.4, n.s.	43.4 ± 7.0, n.s.	39.44 ± 9.26
Male	21 (64%), n.s.	37 (56.9%), n.s.	91 (44.6%)
Hypertension	9 (29.0%), $p = 0.0002$	6 (9.2%), n.s.	13 (6.4%)
Diabetes mellitus	2 (6.5%), n.s.	1 (1.5%), n.s.	2 (1.0%)
Cigarette smoking	4 (12.9%), $p = 0.02$	5 (7.7%), $p = 0.0001$	69 (33.8%)
High cholesterol	2 (6.5%), n.s.	5 (7.7%), n.s.	19 (9.3%)
Infection ≥1 wk before sCAD/study inclusion	5 (16.1%), $p = 0.008$	No a priori data available	26 (2.9%)

Significance levels are given as compared to control group.

sCAD = spontaneous cervical artery dissection; n.s. = not significant.

Moreover, the allelic frequency was different from that in the control group ($p = 0.01$). In a post hoc analysis of the combined sCAD groups (n = 96), an almost identical OR of 3.33 ($p < 0.005$) for the EE genotype as compared to the control group was revealed.

Discussion. The EE genotype of the E469K ICAM-1 polymorphism was more prevalent in patients with sCAD, the second most common cause of cerebrovascular disease in subjects younger than age 50 years. Moreover, with the pairwise analysis, we confirmed that the EE genotype is leading the association with sCAD as a recessive model, and, therefore, it might be considered a possible risk factor for the disease. This finding supports the hypothesis that genetic variability regulating the individual inflammatory response to immune challenges is linked to sCAD. This is consistent with the fact that recent infection and persistently elevated plasma levels of hsCRP are associated with sCAD.[1-3] The E469K polymorphism in the human ICAM-1 gene causes a different mRNA splicing pattern, modifying cell-cell interaction and thus producing a different inflammatory immune response.[5] Further, it is known that the E469K polymorphism determines an amino acid substitution (glutamate to lysine) in the immunoglobulin-like domain 5, a site involved in the binding of LFA-1 (the white blood cells ligand of ICAM-1).[6] We might therefore speculate that the E469K polymorphism could lead to a different affinity of ICAM-1 to its ligands. This may cause increased activation of cytokines and proteases, thus inducing extracellular matrix degradation and weakening of the arterial wall. Alternatively, we cannot exclude that the observed association depends on the effect of other unknown genetic variants in the ICAM-1 gene in linkage disequilibrium with the polymorphism under investigation.[7]

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Table 2 Distribution of ICAM-1 E469K genotype and allele frequency in study groups

	sCAD group primary study, n = 31	sCAD group secondary study, n = 65	sCAD groups post hoc combined, n = 96	Control group, n = 204
ICAM-1 E469K genotypes				
EE	9 (29%), $p = 0.03$	18 (27.7%), $p = 0.008$	27 (28.1%), $p = 0.002$	24 (11.8%)
EK	14 (45.2%)	28 (43.1%)	42 (43.8%)	100 (49%)
KK	8 (25.8%)	19 (29.2%)	27 (28.1%)	80 (39.2%)
ICAM-1 E469K allele frequencies				
K	30 (48.4%)	66 (50.2%)	96 (50%)	260 (63.7%)
E	32 (51.6%)	64 (49.8%)	96 (50%)	148 (36.3%)
K/E	0.94, $p = 0.03$	1.03, $p = 0.01$	1.0, $p = 0.005$	1.7

Significance levels are given as compared to control group.

ICAM-1 = intercellular adhesion molecule 1; sCAD = spontaneous cervical artery dissection; n.s. = not significant.

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