

The C242T Polymorphism of the NAD(P)H Oxidase p22^{phox} Subunit Is Associated with an Enhanced Risk for Cerebrovascular Disease at a Young Age

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Key Words

NAD(P)H oxidase · p22^{phox} subunit · Juvenile stroke · Oxidative stress

Abstract

Background and Purpose: Oxidative stress has been proposed as a major contributing factor for vascular disease, that acts independently from its participation in predisposing disorders such as diabetes and arterial hypertension. A functionally relevant C242T polymorphism of the *CYBA* gene encoding the NAD(P)H oxidase p22^{phox} subunit, is supposed to lead to an abnormal reduction in the generation of reactive oxygen species in vascular smooth-muscle and endothelial cells. **Methods:** We investigated the p22^{phox} C242T single-nucleotide polymorphism by polymerase chain reaction in consecutive patients with ischemic stroke or transient ischemic attack under the age of 50 (n = 161) and in population-based control subjects (n = 136). **Results:** Homozygosity for the T variant was associated with an enhanced risk for cerebral ischemia (odds ratio 3.85, confidence interval 1.39–10.64) after adjusting for classical risk factors. Risk for cerebral ischemia was not increased in heterozygous subjects. **Conclusion:** The p22^{phox} C242T single-nucleotide polymorphism is associated with stroke risk. This finding supports the hypothesis that oxidative stress may contribute to stroke pathogenesis.

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Introduction

The vascular NAD(P)H oxidase, a major source of reactive oxygen species, is increasingly being implicated in the pathogenesis of vascular disease [1, 2]. The p22^{phox} subunit, which is essential for the activation of NAD(P)H oxidase, showed enhanced expression in atherosclerotic vessel walls [3]. A functional C-to-T substitution polymorphism of the regulatory p22^{phox} subunit has been reported to go along with a reduction in the generation of superoxide anions (O₂⁻) in the vascular wall [4]. Ultimately, this may lead to secondary dysfunctions, such as enhanced platelet adherence, intima proliferation or inflammation. Previous association studies revealed an increased risk for and enhanced progression of coronary disease in subjects carrying this single-nucleotide polymorphism (SNP) [5, 6]. However, conflicting findings have been published, with some researchers reporting a protective effect of the T allele in coronary artery disease. Moreover, no association of the C242T genotype and other p22^{phox} polymorphisms with cerebral small-vessel disease was detected in a recent study [7]. In a Japanese population, the TC and TT genotypes showed an association with stroke or transient ischemic attack [8]. Nevertheless, we guess that the explanatory power of these previous studies may be limited on account of confounding factors in an aged patient population. Therefore, in this study we

Table 1. Demographic characteristics of the study population and prevalence of conventional vascular risk factors

	Control group (n = 136)	Patients (n = 161)	Odds ratio (95% CI)	p value
Age, years	34.3 ± 9.5	40.4 ± 7.6		<0.001 ¹
Male sex	70 (51.4%)	95 (59%)	1.35 (0.85–2.15)	0.19
Hypertension	8 (5.8%)	54 (33.5%)	8.07 (3.68–17.71)	<0.001
Diabetes mellitus	1 (0.7%)	11 (6.8%)	9.90 (1.26–77.69)	0.008
Cigarette smoking	45 (33.1%)	42 (26%)	0.71 (0.43–1.17)	0.188
Hypercholesterolemia	14 (10.3%)	31 (19.2%)	2.07 (1.05–4.09)	0.032
Family history of stroke	21 (15.4%)	15 (9.3%)	0.56 (0.27–1.14)	0.108

¹ Determined by t test.**Table 2.** Genotype and allele distribution of p22^{phox} in the patient and control groups

	Controls (n = 136)	Patients (n = 161)
p22 ^{phox} genotype		
CC	62 (45.6%)	73 (45.3%)
CT	68 (50%)	66 (40.9%)
TT	6 (4.4%)	22 (13.6%)
p22 ^{phox} allele frequency		
C	192/272 (71%)	212/322 (66%)
T	80/272 (29%)	110/322 (34%)

sought to investigate the association of the p22^{phox} genotype with cerebrovascular disease (CVD) in a well-characterized group of juvenile stroke patients.

Methods

We investigated 161 patients aged less than 50 years (40.4 ± 7.6) who were consecutively admitted to our university hospital with a diagnosis of ischemic stroke (n = 112) or transient ischemic attack (n = 49). All patients received a computed coronal tomography or magnetic resonance imaging scan and a full clinical workup. This included extra- and intracranial ultrasound studies and a search for cardiac sources of embolism, right-to-left shunt and digital arterial angiography, where appropriate. Etiological subgroups were defined as follows: (1) large-artery atherosclerosis (n = 34), defined as stenosis of 50% diameter reduction; (2) cardiac embolism (n = 35), defined following the Trial of Org 10172 in Acute Stroke Treatment criteria of cardiac embolism [9] and additionally including persistent foramen ovale, as diagnosed by transesophageal echocardiogram and ultrasound studies; (3) dissection of brain-supplying arteries (n = 30), as diagnosed by ultrasound and magnetic resonance imaging or digital subtraction angiography; (4) cryptogenic embolism (n = 50), and (5) others

(n = 12), including cerebral microangiopathy and vasculitis. A total of 136 control subjects (aged 34.3 ± 9.5 years) without a history of vascular disease were randomly selected from the population registries of the same region in southwest Germany and were contacted by mail. The participation rate of eligible patients was 99.5%, and that of control subjects was 65%. Patients or controls of non-German descent or who were unable to undergo a standardized interview that focused on risk factors of vascular disease were excluded. The study was approved by the local ethics board, and all subjects gave written informed consent. The C242T polymorphism of the p22^{phox} gene was detected by polymerase chain reaction (PCR) amplification from genomic DNA, employing the forward primer 5'-CGCTGGCGTCCGGCCTGATCCTCA-3' and the reverse primer 5'-ACGCACAGCCGCCAGTAGGTAGAT-3'; subsequently the PCR products were digested by incubation with the restriction endonuclease *RsaI*. Fragments were detected by electrophoresis on a 2.5% agarose gel stained with 0.1% ethidium bromide. Amplification products of the wild type of the single-nucleotide polymorphism yielded an unrestricted amplification product of 348 bp. Heterozygous subject DNA digestion led to 3 fragments of 348, 188 and 160 bp in length, while TT genotypes yielded fragments sized 188 and 160 bp. Demographic and clinical variables were compared by Fisher's exact test, or t test as appropriate. Genotype and allele frequencies were compared by a pairwise comparison of the genotypes using a binary logistic regression correcting for age, arterial hypertension, diabetes mellitus and smoking.

Results

The demographic variables and prevalence of major vascular risk factors are presented in table 1. Patients suffered more often from hypertension and diabetes mellitus and were somewhat older than control subjects. The TT genotype of the C242T polymorphism of the p22^{phox} gene was significantly more prevalent in the group of juvenile CVD patients than in the control group (p = 0.009 when compared to the CC/CT genotype; table 2) result-

Table 3. Etiological subgroups of the study population and the frequency of homozygosity for the p22^{phox} C242T genotype

Etiology of cerebral ischemia	Number of patients	TT genotype
Macroangiopathy	34	7 (20.5%)
Microangiopathy	9	0
Cardiogenic embolism ¹	35	5 (14.2 %)
Vasculitis	3	0
Dissection	30	5 (16.6%)
Cryptogenic	50	5 (10%)

The percentage values in parentheses indicate the frequency of the TT genotype in each etiological subgroup.

¹ Includes presumed paradoxical embolism.

ing in an odds ratio of 3.81 (CI 1.38–10.42) for TT carriers (unadjusted odds ratio 3.42, CI 1.34–8.75). The T allele frequency did not significantly differ between the two groups. Heterozygous carriers did not carry an enhanced risk of CVD. In analyses of the etiological stroke subgroups, our data reveal no clear association with a special etiological subgroup (table 3). However, the limited sample size in the subgroups needs to be accounted for.

Discussion

In a well-characterized Caucasian patient and control group we have demonstrated a direct, independent association between the p22^{phox} C242T polymorphism and CVD at a young age. Our findings give further evidence that oxidative stress is implicated in the pathogenesis of CVD. It remains to be clarified why the reduced oxidative stress associated with the T genotype goes along with an

enhanced risk for CVD, especially when considering previous studies demonstrating oxidative stress being increased in vascular pathology [10]. Functionally, an impaired NAD(P)H oxidase activity has been linked to enhanced generation of cytotoxic NO derivatives (e.g. peroxynitrite), abnormal pressor responses [11], enhanced salt sensitivity [12] and a procoagulant state [13] as well as postinfarction inflammation and hypercholesterolemia [14]. From our experience of performing genotype analysis in a typical population of elderly stroke patients, we note that in older subjects weak associations are more likely to be masked by the high prevalence of conventional vascular risk factors than in a population of juvenile patients with a lower burden of risk factors. However, even in a young patient population, the conventional vascular risk factors (arterial hypertension, hypercholesterolemia and diabetes mellitus) are still much more prevalent than in the control group (table 1).

This strategy was also followed by a large Australian study with patients aged less than 45 years, which showed a higher prevalence of the T allele in young patients with coronary artery disease [5]. Moreover, the etiology for ischemic stroke in young people differs from that in the elderly, with nonangiopathic causes being more relevant [15].

The exact mechanism by which an abnormal reduction in NAD(P)H oxidase activity may promote vessel pathology, and ultimately result in stroke, still deserves further investigation. It should be considered that as well as being toxic agents, reactive oxygen species are directly implicated in adaptive responses, including apoptosis or proliferation of smooth-muscle cells, by modulating redox-sensitive gene expression. As the underlying pathomechanisms are far from being understood, we hope that this report will stimulate further investigations.

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