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REGULAR ARTICLE

Low protein Z levels but not the protein Z gene G79A polymorphism are a risk factor for ischemic stroke ☆

M.P.J. van Goor [a], D.W.J. Dippel [a], K.S.-G. Jie [b,c], M.P.M. de Maat [b], P.J. Koudstaal [a], F.W.G. Leebeek [b,*]

[a] *Department of Neurology of the Erasmus MC, University Medical Center Rotterdam, PO Box 2040 3000 CA, The Netherlands*
[b] *Department of Hematology of the Erasmus MC, University Medical Center Rotterdam, PO Box 2040 3000 CA, The Netherlands*
[c] *Department of Hematology, Atrium Medisch Centrum, PO Box 4446, 6401 CX Heerlen, The Netherlands*

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Abstract

Background: Protein Z (PZ) is a vitamin K-dependent plasma protein that plays a role in both pro-and anticoagulant pathways, but its exact physiological function remains unclear. The aim of this study was to determine the association between the G79A PZ gene polymorphism in intron F, PZ levels and the occurrence of ischemic stroke.
Methods: We performed a case–control study in 118 Caucasian patients with first ever ischemic stroke or TIA confirmed by CT, and 113 age-and sex-matched population controls. Venous blood samples for PZ levels were collected 7 to 14 days and 3 months after stroke onset. Estimates of relative risk (odds ratios) were adjusted for vascular risk factors.
Results: The adjusted relative risk of ischemic stroke associated with PZ levels in the lowest quartile versus the highest quartile was 3.0 (95% CI: 1.1–8.7) at 7–14 days, and 5.1 (95% CI: 1.2–21.9) at 3 months after the stroke. PZ levels in the convalescent sample were significantly lower than in the acute sample. In the convalescent sample, odds ratios increased with lower quartiles of protein Z level (test for trend $p=0.02$). Thirty-nine patients (33%) and 32 (28%) controls were heterozygous for the G79A PZ gene polymorphism and 4 (3%) patients and 4 (4%) controls had the AA-genotype. The PZ levels were significantly lower in subjects with the AA-genotype and intermediate

☆ Column: Protein Z levels and G79A polymorphism in ischemic stroke.

* Corresponding author. Erasmus MC, University Medical Center Rotterdam, Department of Hematology, PO Box 2040, 3000 CA Rotterdam, The Netherlands. Tel.: +31 10 7034935; fax: +31 10 7035814.
E-mail address: f.leebeek@erasmusmc.nl (F.W.G. Leebeek).

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in heterozygote subjects. The odds ratio of ischemic stroke associated with A-allele carriers versus GG-homozygotes was 1.2 (95% CI: 0.7–2.1).
Conclusion: No association between the G79A PZ gene polymorphism and the occurrence of stroke was observed. However, low PZ levels are independently associated with an increased risk of ischemic stroke.

Introduction

Protein Z (PZ) is a vitamin K dependent single-chain glycoprotein of 62 kDa that is synthesized in the liver. Its amino-terminal sequence is highly homologous to the vitamin K-dependent coagulation factors II, VII, IX, X and protein C [1,2]. Although PZ was already purified from bovine plasma in 1977 [3] and later found in human plasma [1], its exact physiological function remains unclear.

Initial data suggested that PZ deficiency was associated with a bleeding tendency [4], but subsequent clinical studies and a PZ deficient mice model failed to confirm these observations [5–7]. More recently, PZ was found to act as a cofactor in the inhibition of factor Xa by a plasma protein called Z-dependent protease inhibitor (ZPI) [2]. Vasse et al. [8] were the first to report a significant association between PZ deficiency and ischemic stroke. Further support for a role of low PZ levels in arterial thrombosis comes from two additional studies in patients with ischemic stroke and acute coronary syndromes [9–11]. Several other clinical case–control studies, mainly among younger patients, have yielded contradictory results both for ischemic stroke [12–15] and coronary heart disease [16].

The gene encoding for PZ has been characterized and several common single nucleotide polymorphisms in the gene have been identified [17,18]. Recently, the minor A allele of the intron F polymorphism G79A (rs3024735) in the PZ gene was described to be protective against ischemic stroke in the young [19]. In that study, PZ level was determined in controls only, and not in patients. In a more recent study the G103A gene polymorphism seemed to be associated with PZ plasma levels, however, the relationship was not as strong as for the G79A polymorphism [20].

To further clarify the role of PZ level in ischemic stroke and to determine to what extent this role is genetically determined, we studied the association of the PZ G79A promoter gene polymorphism and PZ levels with first-ever ischemic stroke. To determine the effect of the acute phase of ischemic stroke on PZ levels, the measurement of the PZ level was repeated in the convalescent phase.

Materials and methods

Study design

We performed a case–control study; cases were consecutively recruited patients aged over 18, with first-ever acute ischemic stroke, who were admitted to the department of neurology of a university hospital. This is an urban area hospital without specific selection criteria for the admission of stroke patients. However, young stroke patients are referred more frequently to this center than to the non-academic centers in the region. Fifty percent of the urban population consists of Caribbean or Mediterranean habitants. Cases and controls in this study were included when they were of the Caucasian race, and this was assumed when they and both parents were born in Northern Europe. We used population controls, i.e. partners, friends or neighbours of the patients. They were age- and sex-matched, of the same race, without a history of stroke and not related to the patient. Our local medical ethics and research board approved the study. Informed consent was obtained from all subjects.

Exclusion criteria

Patients with a definite, sufficient, non-atherosclerotic cause for the stroke, like a mechanical heart valve, endocarditis or carotid dissection were excluded. Other exclusion criteria were use of oral anticoagulants, and age over 75 years, because at high age, atherosclerosis is the predominant cause of stroke.

Definitions and measurements

Ischemic stroke was defined as the acute onset of focal cerebral dysfunction due to cerebral ischemia with symptoms lasting more than 24 h. Patients with TIA (symptoms lasting less than 24 h) were included only if the neurological deficit in the acute phase was observed and described by a neurologist. In all patients a CT scan of the brain was made within 3 days after the onset of symptoms to confirm the diagnosis of ischemic stroke and to rule out hemorrhagic stroke. Clinical stroke subtypes were classified according to the OCSP criteria, adjusted for CT findings [21]. Etiologic stroke type was classified according to the TOAST criteria [22]. We defined large artery atherosclerosis as a stenosis of presumed atherosclerotic origin in the symptomatic carotid or vertebrobasilar arteries of more than 50%. Stroke severity was assessed with the Barthel index at 24 h after inclusion [23].

In both patients and controls, we collected detailed information about cardiovascular risk factors, such as smoking habit, hyperlipidemia, hypertension, diabetes, use of oral contraceptives, and about medical history and family medical history. Patients were screened for cardiac abnormalities by means of standard twelve lead ECG examination. A cardiologist was consulted in female patients aged 55 years or less, in male