Congenitally Abnormal Plasminogen in Juvenile Ischemic Cerebrovascular Disease

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**Background and Purpose:** Congenitally abnormal plasminogen is characterized by markedly decreased fibrinolytic activity and has been reported mainly in association with venous occlusive disease.

**Case Description:** We found three young adult patients (34, 45, and 27 years old at onset) with ischemic cerebrovascular disease, all of whom had congenital plasminogen abnormalities but no other known risk factors. Hemostatic tests of all three patients revealed plasma plasminogen activities at almost one half of the normal level despite normal plasma plasminogen antigen levels. They were found to be heterozygotes with abnormal plasminogen (normal Ala-601[GCT] to abnormal Thr-601[ACT]) by DNA sequence analysis after polymerase chain reaction.

**Conclusions:** Congenital plasminogen abnormalities could be one of the risk factors of juvenile ischemic cerebrovascular disease of the arterial as well as venous type. (Stroke. 1993;24:2104-2107.)

**KEY WORDS** • cerebral ischemia • genetics • plasminogen • young adults

Plasminogen is a key proenzyme in the fibrinolytic system. Congenitally abnormal plasminogen exhibits reduced plasma functional activity despite normal antigen level. It is associated with systemic venous thrombosis, but its significance in the pathogenesis of arterial stroke remains unclear. Among 29 patients we have encountered thus far with ischemic cerebrovascular disease of young adult onset (aged younger than 45 years), we have found three with abnormal plasminogen but no other known risk factors. We conducted DNA sequencing analysis using the polymerase chain reaction (PCR) to investigate whether congenitally abnormal plasminogen might be a risk factor for juvenile ischemic cerebrovascular disease.

**Case Reports**

**Case 1**

Patient 1, a 34-year-old man, visited our hospital with mild left hemiparesis and postural tremor of the left hand. His past history revealed sudden onset of left hemiparesis at 8 years of age. His weakness had persisted since the episode, then left-hand tremor developed and gradually worsened. He was referred to us in January 1988. Cerebral magnetic resonance imaging (MRI) showed a small infarct involving the right thalamus, but there was no abnormality in cerebral angiography.

**Case 2**

Patient 2, a 45-year-old man, was admitted in November 1986 because of sudden onset of dysarthria and right hemiparesis including the face. His mother and grandmother had also suffered from cerebrovascular disease. Neurological examinations showed dysarthria, left medial longitudinal fasciculus syndrome, and right pyramidal tract signs. Brain MRI revealed a lacunar infarct at the left ventral pons. Conventional risk factors for stroke (including hypertension, diabetes mellitus, and hyperlipidemia) were all negative.

**Case 3**

Patient 3, a 27-year-old man, was admitted because of recurrent episodes of weakness and paresthesia of the left limbs. These episodes began in November 1990, and each lasted approximately 10 minutes. His father had suffered from vertebrobasilar insufficiency since 40 years of age. On admission, he had mild dysarthria and mild left hemiparesis, which disappeared completely within 24 hours. There was no abnormality in computed tomography, MRI, electroencephalography, or cerebral angiography. A diagnosis of recurrent transient ischemic attack was made. No conventional risk factors for stroke were found.

**Results**

The screening assays of the coagulation and fibrinolysis studies shown in Table 1 were performed by using commercially available assay kits: Testzym PLG kit for plasminogen activity (Daiichi Pure Chemicals Co, Ltd), LP1A Plasminogen kit for plasminogen antigen (Teikoku Hormone Mfg Co, Ltd), Enzygnost TAT kit for thrombin–antithrombin III (Behringwerke AG), Testzym AT III kit for antithrombin III activity (Daiichi), Protein C Clot BW kit for protein C activity (Behringwerke AG), Test of Protein S kit for free protein S (Teijin Ltd), Dimer Test EIA kit for D-dimer (AGEN International Ltd), PIC Test kit for plasmin-α2
plasmin inhibitor complex (Teijin), and PAI-1 ELISA kit for plasminogen activator inhibitor (Monozyme).

The normal control group consisted of 46 subjects (25 men and 21 women, aged 35 to 61 years): 6 healthy male and 2 healthy female volunteers and 38 neurological patients affected by nonvascular diseases unrelated to hemostatic abnormalities (tension-type headache, migraine, epilepsy, essential tremor, Parkinson's disease, myasthenia gravis, dystonia, spastic paraparesis, hemifacial spasm, Adie's syndrome, and cranial neuropathy).

All three patients showed low functional levels of plasminogen activity and normal levels of plasminogen antigen, as shown in Table 1. Other screening test results were normal except for slightly to moderately increased plasma thrombin–antithrombin III complex levels. From these findings, we suspected that abnormal plasminogen might be present in these patients and might have contributed to the development of ischemic cerebrovascular disease, although plasma levels of plasminogen activity in some family members, who were asymptomatic, were also decreased.

Genomic analysis of abnormal plasminogen was performed by two methods using PCR. Venous blood was drawn from the three patients after informed consent had been obtained, and genomic DNA samples were prepared from leukocytes by standard techniques. First, the samples were screened by means of PCR single-strand conformation polymorphism (PCR-SSCP) analysis. The primers 5'-GGTTGGAATGCACCTCTGTG-3' and 5'-AGACAAGACTTCATGCAAT-3', with the 5' end labeled with [γ-32P]ATP, amplified a 111-base pair fragment including the region coding for Ala-601 of normal plasminogen (94°C for 1 minute, 55°C for 1 minute, 72°C for 2 minutes, 35 cycles; IWAKI thermal sequencer, model TSR-300). Electrophoresis on 6% polyacrylamide gel at room temperature revealed three bands in the DNA of all three patients (Fig 1, line 1). Heterozygote should show four bands, but we consider that the lowest band represents two overlapping bands in these cases.

Next, PCR direct sequence analysis was performed to identify the mutation in the plasminogen gene. In all three patients, guanine in GCT coding for Ala-601 was replaced by adenine, resulting in the substitution of threonine for alanine in plasminogen. All three patients were heterozygotes with abnormal plasminogen (Fig 2, panel 1). Unfortunately, DNA analysis of other members of the patients' families could not be performed because consent was denied.

The DNA sample of homozygote used in Figs 1 and 2 was taken from the patient with multiple cerebral infarction whose plasminogen gene had been analyzed by PCR direct sequencing.

**Discussion**

Plasminogen, a key proenzyme in the fibrinolytic system, consists of 791 amino acid residues and includes

![Fig 1. Polymerase chain reaction–single-strand conformation polymorphism analysis. 1, Heterozygote shows three bands. Heterozygote should show four bands, but the lowest band represents two overlapping bands. All three patients were heterozygotes. 2, Homozygote shows two bands different from normal. 3, Normal gene shows two bands.](image-url)
five homologous kringle structures and a serine protease domain. Miyata et al.7,8 sequenced abnormal plasminogen isolated from three Japanese subjects and found an amino acid substitution, Ala-600 to threonine, in the serine protease portion of the protein. It was first reported erroneously that Ala-600 was replaced by thronine,7,8 but thereafter it has been shown that the exact site of replacement was localized to be Ala-601.9 A genetic analysis of three unrelated patients with plasminogen abnormalities by Ichinose et al.9 revealed two types of amino acid substitutions, Ala-601(GCT) to Thr(ACG) and Val-335(GTC) to Phe(TTC).9

We initially used the rapid and sensitive PCR-SSCP method to detect the presence of base changes in given sequences of genomic DNA. Then PCR direct sequence analysis was performed to identify point mutation in the plasminogen gene. In all three patients, guanine in GTC coding for Ala-601 was replaced by adenine, resulting in the substitution of threonine for alanine. Our result, as well as that obtained by Ichinose et al.,9 suggests that this mutation is the predominant one leading to abnormal plasminogen, at least among Japanese. We concluded that all three patients had congenital heterozygous abnormal plasminogen.

Congenital plasminogen abnormalities have been reported in association with venous thrombosis that develops at a relatively young age.1-4 However, it has not been previously associated with stroke except for two reports.10,11 Shinmyozu et al.12 found no significant difference in the frequencies of plasminogen abnormality between patients with cerebral infarction and normal subjects. We have thus far encountered 29 patients with ischemic cerebrovascular disease of early adult onset (aged younger than 45 years) who have known plasminogen activity and plasminogen antigen levels. Among them, three (10.3%) have abnormal plasminogen but no other known risk factor. This frequency is markedly higher than that in the Japanese population (2% to 3%).12-14 implying that this abnormality may be an important risk factor for ischemic cerebrovascular disease in young adults. Available reports on juvenile ischemic cerebrovascular disease and abnormal plasminogen, including data on our patients, are listed in Table 2.

The frequency of abnormal plasminogen is rather high in the Japanese population (2% to 3%)12-14 compared with other populations (1%).15 We believe that examination for abnormal plasminogen should be made routinely in young adults presenting with ischemic cerebrovascular disease, especially in those without other known risk factors.

References


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