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Detection of an Ala601Thr Mutation of Plasminogen Gene in 3 out of 36 Korean Patients with Deep Vein Thrombosis

Plasminogen is a key proenzyme in the fibrinolytic and thrombolytic systems. Congenital deficiency of plasminogen and molecular abnormality of plasminogen (dysplasminogenemia) have been reported in association with the thrombotic tendency in human. In dysplasminogenemia, the level of immunoreactive plasminogen is normal, although the functional activity is reduced. Human plasminogen gene spans about 52.5 kb of DNA and consists of 19 exons. Three types of mutations (Ala601Thr, Val355Phe, and Asp676Asn) have been described in dysplasminogenemia. In this study, we measured the plasminogen activity in patients with deep vein thrombosis and analyzed the DNA sequence to detect three point mutations (Ala601Thr, Val355Phe and Asp676Asn) in patients with hypo/dysplasminogenemia. Dysplasminogenemia was identified in 3 (8.3%) of unrelated 36 patients with deep vein thrombosis and the Ala601Thr mutation was detected in all three patients with dysplasminogenemia. In conclusion, dysplasminogenemia is not rare in deep vein thrombosis, which suggests a risk factor for the thrombosis in Korean population.

Key Words : *Plasminogen; Thrombosis; Mutation; Venous Thrombosis*

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INTRODUCTION

Human plasminogen (PLG), a key proenzyme in the fibrinolytic and thrombolytic systems, is a single chain glycoprotein with a molecular mass of about 93 kDa and consists of 791 amino acid residues (1). Congenital plasminogen deficiency has been classified into two types. In type I, the concentration of immunoreactive PLG is reduced in parallel with functional activity and in type II (dysplasminogenemia, dysPLGemia), the level of immunoreactive protein is normal, while the functional activity is reduced (2). Congenital deficiency of PLG and dysPLGemia have been reported in association with venous thrombosis that develop in patients at relatively young age (3, 4). Human PLG gene spans about 52.5 kb on chromosome 6q26-27 and consists of 19 exons separated by 18 introns (5).

Aoki et al. (6) investigated a patient with recurring thrombosis and the only abnormality was depressed PLG in plasma. Miyata et al. (7) referred to the abnormal PLG as PLG Tochigi and showed that the abnormality is replacement of alanine by threonine (Ala601Thr) due to the nucleotide change of G to A transition in codon 601 of exon XV. Two other types of point mutations (Val355Phe, Asp676Asn) in the PLG gene have been described in patients with dysPLGemia (8).

In this study, we measured the PLG activity in patients with deep vein thrombosis and analyzed the DNA sequence to detect three point mutations (Ala601Thr, Val355Phe, and Asp676Asn) in patients with abnormally low plasma activity of PLG.

MATERIALS AND METHODS

Subjects and samples

Citrated venous blood was drawn from 57 normal individuals (29 males and 28 females, age; mean±SD, 50±18 yr) as well as 36 patients (21 males and 15 females, age; mean ±SD, 51±16 yr) with deep vein thrombosis. The diagnosis of deep vein thrombosis was made by history, physical examination, and diagnostic studies including venogram and ultrasonography. One patient was complicated with pulmonary embolism and three patients suffered from recurrent deep vein thrombosis, renal vein thrombosis, and underlying lymphoma, respectively. Plasma samples were obtained by centrifugation and aliquots were stored at -70°C until analysis. Genomic DNA samples were prepared from leukocytes by the standard technique.

Plasminogen assay

The PLG activity was determined by amidolytic assay using

Table 1. Plasma activity of plasminogen in the patients with deep vein thrombosis (DVT) and in normal subjects

Group	No.	Plasminogen Activity (%)	
		Mean±SD	Range
DVT	36	97±22	38-139
Normal	57	103±11	78-129

automatic coagulyzer (STA) and reagent kits (Stachrom PLG, Diagnostica Stago, France). The plasminogen antigen concentration was measured by immunoturbidimetric assay using Behring Coagulation System (Dade Behring, U.S.A.).

PCR Amplification and Sequence Analysis

Genomic DNA was amplified by employing 3 sets of primers (Exon X; 5′-GTCAGAATTATTCTCAGAGGCTACCGTACT-3′ and 5′-CTACGAATTCTGGGTCTAAGAGAAATTTGG-3′, Exon XV; 5′-TCTGGAATTCTGTACAATGGAGCAGAACAAA-3′ and 5′-CCACGAATTCATCTGTACTGTGTCTTTCTTCT-3′, Exon XVII; 5′-TGAAAGCTTGTGGGTACTGCAGCTGC-3′ and 5′-ACCGAATTCATGGATAGGAATTTGCACAGC-3) in a 50 μL reaction mixture as previously described (4). DNA sequence was obtained using ABI PRISM 3700 DNA analyzer (Applied Biosystems, Foster City, CA, U.S.A.).

RESULTS

Plasma activities and antigen levels of plasminogen

Table 1 shows the results of plasma PLG activity in normal subjects (n=57) and patients (n=36) with deep vein thrombosis. The mean (±SD) activity of PLG in normal subjects was 103% (±11%) with a range from 78% to 129%. The mean (±SD) activity of PLG in patients with deep vein thrombosis was 97% (±22%) with a range from 38% to 139%. Four (11.1%) of 36 patients had reduced PLG activities. Three (75%) of 4 patients with reduced plasminogen activities had normal antigenic plasma concentrations as shown in Table 2. Therefore, these three (8.3%) cases were consistent with the diagnosis of dysPLGemia.

Genetic analysis

To identify 3 point mutations (Ala601Thr, Val355Phe, and Asp676Asn) in the regions mentioned above, genomic DNAs were sequenced in four patients with hypo/dysPLGemia. In three patients with dysPLGemia, nucleotide sequence analysis revealed a heterozygous G to A transition (Ala601Thr mutation) in exon XV (Fig. 1). The Ala601Thr mutation was not detected in one patient with low PLG activity and reduced antigen, whose diagnosis was consistent with hypo-PLGemia. Two other mutations (Val355Phe and Asp676Asn) were not identified in any of the four patients with hypo/dys-PLGemia.

Table 2. Plasma activities and antigen levels of plasminogen in four patients with hypo/dysplasminogenemia

Case	Age (yr)	Sex	Plasminogen	
			Activity (%)	Antigen (mg/dL)
1	62	M	69	10.0
2	56	F	55	16.8
3	52	M	67	14.4
4	49	F	38	3.8
Normal range (Mean±2SD)			81-125	7.0-13.0

DISCUSSION

Although venous thrombosis often appear to be spontaneous in origin, it is associated with one or more predisposing factors in the great majority of instances. Some common situations are venous stasis, endothelial injury, and circulating activated clotting factors (9). Less often, hereditary or acquired defects of the natural anticoagulants or fibrinolytic defects are involved (10). In this study, fibrinolytic defect due to hypo/dysPLGemia was identified in about 10% of unrelated patients with deep vein thrombosis and heterozygous Ala601Thr mutations were detected in all the patients with dysPLGemia.

Partial human PLG deficiency was first described in a 31 yr-old male patient with a history of repeated episodes of thrombophlebitis, intracranial and mesenteric venous thrombosis, and pulmonary embolism (6). Reduced PLG (50% of normal) in his plasma was traced to a codon 601 missense mutation in exon XV (GCT for Ala→ACT for Thr) (7), and several additional patients with this defect have now been described (3).

Previously, by isoelectric focusing electrophoresis, several workers identified a functionally inactive PLG variant designated PLG M5, and demonstrated that PLG Tochigi and PLG Nagoya II are identical and has a feature common to PLG M5 with an Ala601Thr point mutation (11).

The Ala601Thr mutation is thought to be relatively prevalent and the gene frequency (0.02) is significantly higher among normal Japanese than in other populations (12). Tsutsumi et al. (4) detected an Ala601Thr mutation in 118 (94%) out of 125 unrelated Japanese families with dysPLGemia. Therefore, it was likely that the frequency of the Ala601Thr mutation is high because of the so-called founder's effect (13). It is noteworthy that the Ala601Thr mutation has been found in the Chinese Han population (14). The G to A nucleotide change in the codon 601 of exon XV may be a "hot spot" for mutation, or alternatively, Japanese, Chinese, and Korean families with dysPLGemia may share a single ancestor with

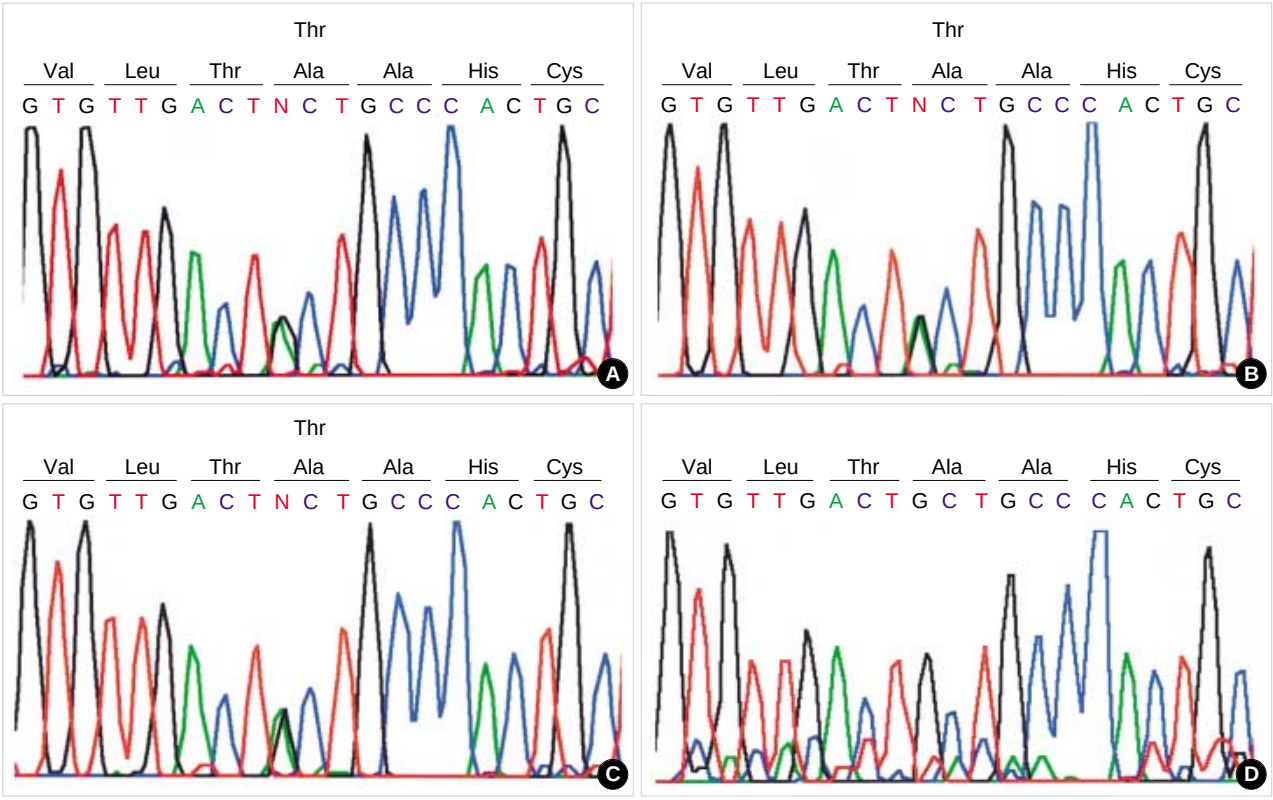

Fig. 1. The results of nucleotide sequencing analysis from three patients with dysplasminogenemia (A, B, C) and a patient with hypoplasminogenemia (D). Nucleotide sequence analysis revealed a heterozygous G to A transition (GCT→ACT: Ala601Thr mutation) in exon XV (A-C).

the Ala601Thr mutation. At any rate, our study suggests that this mutation is not as rare as had been thought in Korean population. Therefore, it may be worth including the determination of plasma PLG activity and this type of mutation in the screening test for genetic defects of inherited thrombophilia.

In a study of a Japanese cohort, approximately 27% of individuals with dysPLGemia had a clinical history of thrombosis (4). Although the role of PLG deficiency in the pathogenesis of venous thromboembolism is debated in the literature (15), several groups clearly favor the association between PLG deficiency and thrombosis (16-19). Moreover, structural studies on a hereditarily abnormal PLG, PLG Tochigi have shown that Ala601 (equivalent to Ala55 in the chymotrypsin numbering system) is very near the active site His57. The Thr at position 55 in PLG may perturb His57 such that the proton transfers associated with the normal catalytic process cannot occur in the abnormal plasmin (7).

The Val355Phe mutation was found in four unrelated Japanese families, indicating that it is a recurring mutation and is not very rare in Japan (4). However, Val355Phe as well as a third mutation Asp676Asn was not found in any of the four cases of hypo/dysPLGemia in this study, indicating these mutations are not as common as Ala601Thr mutation. Ser572Pro and Gly732Arg mutations were also reported in dysPLGemia (4, 20).

One of our cases (malignant lymphoma patient) without Ala601Thr mutation is consistent with hypoPLGemia because both the activity and antigenic concentration of PLG are reduced. Acquired PLG deficiency, as may occur in liver disease, sepsis, and Argentine hemorrhagic fever due to decreased synthesis and/or increased catabolism, has been frequently associated with thrombotic vascular occlusion (21). In this case, other natural anticoagulants such as antithrombin, protein C, and protein S were also found to be decreased, suggesting an acquired condition possibly due to increased catabolism or decreased synthesis. Hereditary hypoPLGemia seems to be rare because only five cases among 164 individuals were classified into hypoPLGemia in Japanese population (4).

The limitation of this study is that the evaluation for the PLG levels and genetic study were not conducted in the family members of our four cases.

Further studies on both symptomatic and asymptomatic family members are required to confirm the relationship of dysPLGemia with Ala601Thr mutation and thrombosis.

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