be more profoundly depressed during the prenatal course. Additional triggers might precipitate the fetal hypercoagulability. During the maturational but subnormal increase in the plasma PC-activity, no thrombosis recurred without anti-coagulant therapy. *PROC* c.574_576del was determined in our series of adult patients with deep vein thrombosis and other studies in Japan [5]. Recently, it has been recognized as the most common variant of venous thromboembolism in Chinese population [6]. PC amidolytic activities of the variants were similar to those of non-carriers. The PC activity of the patient' father was in normal range. The carrier's and non-carrier's levels might overlap at around the borderline ranges. The unstable activity might easily drop with triggers. No maternal or placental thrombotic factors were found. Perinatal stresses might contribute to the development of stroke. The estimated prevalence of PC-deficiency in general population is 1/200-500. Our observation proposed a risk of post-thrombotic hydrocephalus in the fetuses carrying heterozygous *PROC* mutation.

Contributions to authorship

I.M., O.S., O.M. and F.K. were the principal investigators taking primary responsibility for the paper and wrote the paper. I.M. and T.M. treated the patient. K.D. controlled coagulation and genetic studies. U.M. and H.T. managed and performed the laboratory work for this study. O.S. and H.T. organized the clinical study.

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Clinical Investigation | Articles

Age-specific onset and distribution of the natural anticoagulant deficiency in pediatric thromboembolism

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BACKGROUND: The early diagnosis of inherited thrombophilia in children is challenging because of the rarity and hemostatic maturation.

METHODS: We explored protein C (PC), protein S (PS), and antithrombin (AT) deficiencies in 306 thromboembolic patients aged ≤20 y using the screening of plasma activity and genetic analysis.

RESULTS: Reduced activities were determined in 122 patients (40%). Low PC patients were most frequently found in the lowest age group (0–2 y, 45%), while low PS or low AT patients were found in the highest age group (16–20 y; PS: 30% and AT: 20%). Genetic study was completed in 62 patients having no other causes of thromboembolism. Mutations were determined in 18 patients (8 PC, 8 PS, and 2 AT genes). Six of eight patients with PC gene mutation were found in age 0–2 y (75%), while six of eight patients with PS gene mutation were in 7–20 y. Two AT gene–mutated patients were older than 4 y. Four PC-deficient and two PS-deficient patients carried compound heterozygous mutations. All but one PC gene–mutated patient suffered from intracranial thromboembolism, while PS/AT gene–mutated patients mostly developed extracranial venous thromboembolism.

Q1] **CONCLUSION:** Stroke in low PC infants and deep vein thrombosis in low PS/AT school age children could be targeted for genetic screening of pediatric thrombophilias.

Thromboembolism is a multifactorial disease involving genetic predispositions, underlying disorders, and varied triggers including infection or injury. Vascular, circulatory, and hemostatic conditions are distinctively associated with the development of arterial and venous thromboses. Thromboembolism, formerly recognized as a rare event in children (1), has been increasingly diagnosed as a complication of sepsis, cancer, cardiovascular disease, and therapy-related events. Recent advances in the intensive cares, cardiac surgery, and transplantation medicine along with the imaging diagnosis may contribute to the increased number of pediatric patients with thrombosis (2,3). The established genetic risks of venous thromboembolism (VTE) include protein C (PC), protein S (PS), and antithrombin (AT) deficiency, as well as factor V G1691A (FVL) and prothrombin G20210A (FII) variants (4). The high incidence of VTE in Caucasians (5) is explained by the fact that FVL and FII G20210A carriers were found in 20–60% of adult VTE patients in Caucasian but not Asian ancestries (6–9). PC, PS, and AT deficiencies share the lower prevalence than FVL and/or FII G20210A carriers, but the higher risk of the first and recurrent VTE than the other thrombophilias. On the other hand, the diagnosis is challenging during infancy and early childhood, because the references of increasing anticoagulant activity range widely until adolescence (10,11). The true effect of the natural anticoagulant deficiencies on the development of pediatric thromboembolism remains elusive because of the rare occurrence and the elaborative genetic screenings.

To clarify the clinical impact of the inherited deficiency of natural anticoagulant on children, we conducted the genetic analysis of PC, PS, and AT deficiency after the screening of each activity for pediatric patients with thromboembolism over 20 y in a single institution. The results demonstrated the distinct presentation of PC and PS deficiencies in infants and children, respectively, reflecting the genotype of adult Japanese patients.

RESULTS

Distribution of Thromboembolic Patients

Of 306 patients aged ≤20 y (male: female 1:1.14), 186 suffered from intracranial lesions including ischemic and/or hemorrhagic strokes. The extracranial lesions consisted of renal vein thrombosis (n = 7), purpura fulminans (n = 3), deep vein thrombosis in the leg (n = 15), and pulmonary thromboembolism (n = 9). Patients younger than 3 y were 27% of all patients. The largest age group was <1 y of age (n = 50, 16.3%), and the number of other age groups were similar (median: 12, ranging: 9–17 (3.0–5.5%)).

PC, PS, and AT Activities in Patients

A total of 122 patients had the reduced activity of either anticoagulant; 95 with low PC (31%), 48 with low PS (16%), and 20 patients with low AT activity (7%; **Figure 1**). Low PC patients

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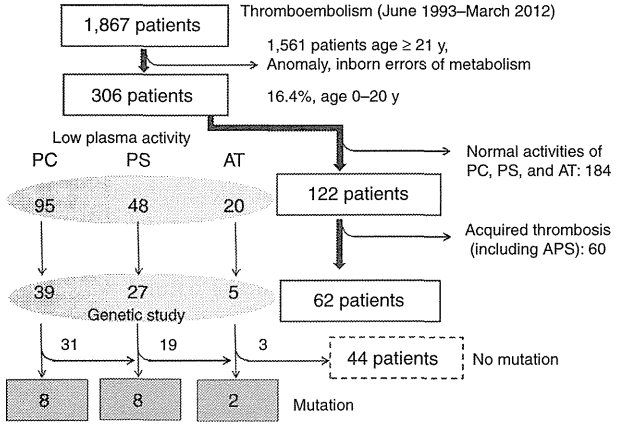
were most frequently determined in the lowest age group (0–2 y, 45%), while low PS or low AT patients were frequently found in the highest age group (16–20 y; PS: 30% and AT: 20%). The number of patients with low activity in age groups are shown in **Figure 2**. The proportions of low PC patients were higher than those of low PS or AT patients in age 0–2 (each <0.00001) and 13–15 y (0.0001, 0.0155), respectively. The proportion of low PC or PS patients (19%) was each higher than that of low AT patients in 7–12 y (0.0005). The number of patients showing both low levels of PC and PS activities were 25 (20%), 5 of whom had the low levels of all three factors (**Supplementary Figure S1** online).



[Q3] **Figure 1.** Flowchart of the genetic study of pediatric thrombophilia in a Japanese institution. During the 20 y, 306 patients (≤20 y of age) were screened by the plasma activities of protein C (PC), protein S (PS), and antithrombin (AT) and underwent the genetic study if they have idiopathic or unusual thrombophilic predispositions.

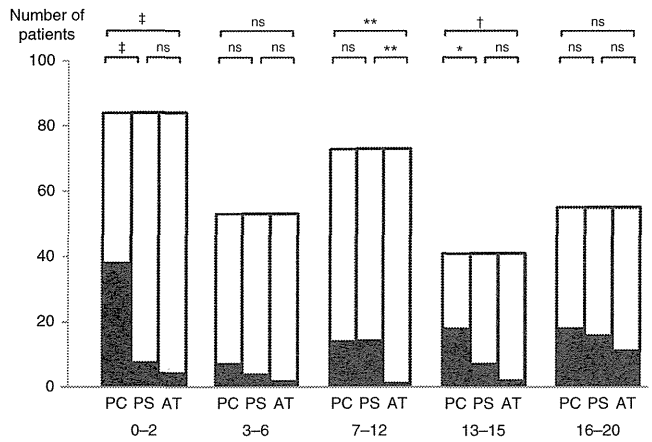


Figure 2. Proportion of patients who showed the low plasma activity of PC, PS, and AT according to the age groups. 0–2 y: infants (n = 84; low PC, 45%; low PS, 8%; low AT, 5%), 3–6 y: preschoolers (n = 53; low PC, 14%; low PS, 8%; low AT, 4%), 7–12 y: elementary school children (n = 73; low PC, 19%; low PS, 19%; low AT, 1%), 13–15 y: junior high school students (n = 41; low PC, 44%; low PS, 17%; low AT, 5%), 16–20 y: high school and college or university students (n = 55; low PC, 33%; low PS, 30%; low AT, 20%). Closed bar represents the patients who showed each low plasma activity. *P = 0.0155; **P = 0.0005, †P = 0.0001; ‡P < 0.00001. ns, no statistical significance.

Mutations of PC, PS, and AT Genes in Patients

Of 62 children who showed the low level of at least one factor, mutation carriers were found in low PC (n = 8/39, 21%), PS (n = 8/27, 30%), or AT (n = 2/5, 40%) patients, respectively. Forty-four patients carried no mutations. Age distribution of 62 patients differed among three deficiencies (0.0042; **Figure 3**). The proportion of age 0–2 y patients with PC deficiency (44%) was the highest among any age group patients with PS or AT deficiency. Six of eight patients with PC gene mutations (75%) were found in the age group 0–2 y, in which only two PS and no AT mutation carriers were determined. Four of eight patients with PS gene mutations (50%) were found in the age group 7–12 y. No ratio of mutated to analyzed patients differed in the age-groups.

Clinical Onset, Genotype, and Activity Levels of PC, PS, and AT in the Mutation Carriers

Eighteen patients with a mutation are listed in **Table 1**. Seven out of eight PC-deficient patients suffered from intracranial thromboembolism and five of them were younger than 2 y. On the other hand, six of eight PS-deficient patients presented in the second decade of life, the onset of which were adult type deep vein thrombosis of pulmonary thromboembolism in five of them. Double allele (homozygous or compound heterozygous) and one allele (heterozygous) mutation(s) were found in half of eight PC-deficient patients, respectively. On the other hand, double allele mutated patients were only two of eight heritable PS-deficient patients, both of whom carried PS-Tokushima. Two other patients had PS-Tokushima. Both patients with heritable AT deficiency carried heterozygote AT mutation. Four of eight patients with PC gene mutation and

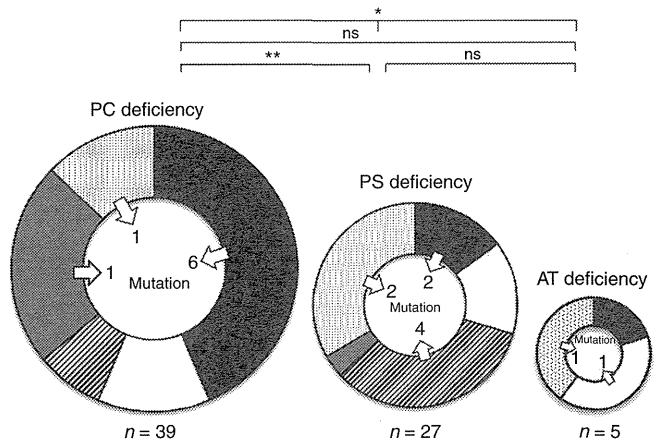


Figure 3. Age distribution of patients with thromboembolism carrying PC, PS, and AT gene mutation. The circle size represents the number of patients. (**a**) PC deficiency (n = 39): 0–2 y, 44%; 3–6 y, 13%; 7–12 y, 8%; 13–15 y, 23%; and 16–20 y, 13%. Six of eight patients with PC gene mutations (75%) were found in the age group 0–2 y. (**b**) PS deficiency (n = 27): 0–2 y, 15%; 3–6 y, 15%; 7–12 y, 33%; 13–15 y, 4%; and 16–20 y, 33%. Four of eight (50%) patients were found in the age group 7–12 y. (**c**) AT deficiency (n = 5): 0–2 y, 20%; 3–6 y, 40%; 16–20 y, 40%. All AT gene mutation carriers were found in the age group more than 3 y. Closed areas indicate 0–2 y of age, open areas indicate 3–6 y of age, hatched areas indicate 7–12 y of age, gray areas indicate 13–15 y of age, and dotted areas indicate 16–20 y of age. *P = 0.0042, **P = 0.0019. ns, no statistical significance. [Q

Table 1. Plasma activity of protein C, protein S, and antithrombin and each gene mutation in pediatric patients

Patient	Sex	Age	Diagnosis	Plasma activity (%) PC	PS	AT	Mutation	Family history	Plasma activity (%) mother/father PC	PS	AT
PC deficiency							*PROC*				
	F	6 d	Cerebral-VTE, twin-1	*<5*	45	111	Ex8; c.887C>T, p.Leu265Phe	Negative	*63/63*	69/99	125/110
							Ex9; c.1360G>C, p.Trp422Cys				
	F	6 d	Cerebral-VTE, twin-2	*<5*	*24*	141	Ex8; c.887C>T, p.Leu265Phe	Negative	*63/63*	69/99	125/110
							Ex9; c.1360G>C, p.Trp422Cys				
	F	9 d	ICH, PF	*<10*	140	60	Ex9; c.1109G>A, p.Val339Met	Negative	*32/72*	68/110	115/120
							Ex9; c.1362delG, p.Gly423ValfxX82[a]				
	F	13 d	Cerebral VTE	<5	*29*	110	Ex4; c.258delT, p.Leu55ArgfsX6	Negative	*46/48*	na/na	na/na
							Ex9; c.905C>T, p.ArG271Trp				
	M	4 mo	Intrahepatic bleeding	*25*	91	104	Ex7; c.671_673delAAG, p.Lys193del	Negative	*64*/93	67/95	97/101
	F	1 y	Meningitis, ICH/CI, PF	*31*	*38*	68	Ex4; c.356G>T, p.Asp88Tyr	Positive	*50*/99	66/83	na/105
	F	14 y	ICH, sinus thrombosis	*32*	62	87	Ex6; c.603A>G, p.Asp170Gly	na	na	na	na
	F	18 y	CI lt. frontal	*39*	101	*79*	Ex3; c.293G>A, p.Glu67Lys	na	na	na	na
PS deficiency							*PROS1*				
	M	3.5 mo	Thalamic bleeding, lt.	*48*	69	85	Ex6; c.927A>G, p.Lys196Glu[c]	na	na	na	na
	F	2 y	Hypoplasia of optic papilla lt.	90	61	116	Ex6; c.927A>G, p.Lys196Glu[c]	na	na	na	na
	F	10 y	DVT, epilepsy	*60*	*10*	na	Ex2; c.418-1G>C, Int1 splice acceptor site	na	na/92	*<10*/84	na
	M	11 y	DVT	*5*[b]	*12*	122	Ex15; c.2361-2delAA, p.Lys674GlufsX24	na	na	na	na
	M	12 y	DVT	*10*[b]	*2*	115	Ex1; c.391T>C , p.Leu17Pro	Positive	102/na	*54*/na	114/na
							Ex6; c.927A>G, p.Lys196Glu[c]				
	F	12 y	IE, multiple CI	*66*	*12*	103	Ex13; c.1884C>T, p.Arg515Cys	Positive	na	69/*low*	na
	F	16 y	DVT	*24*	*5*	90	Ex13; c.1884C>T, p.Arg515Cys	na	103/107	*14*/71	97/97
							Ex6; c.927A>G, p.Lys196Glu[c]				
	M	16 y	Thrombophlebitis, PTE	*21*	*11*	105	Ex12; c.1692C>T, p.Arg451X	na	119/101	*29*/129	109/116
AT deficiency							*SERPINC1*				
	F	4 y	Vasculitis	121	74	*14*	Ex3; c.561T>C, p.Ser148Pro	Positive	104/na	*30*[d]/na	*58*/na
	F	18 y	Pulmonary infarction	76	*47*	*55*	Ex7; c.1438T>C, p.Phe440Ser	na	113/na	67/na	*65*/na

The italic formatting indicates low activity according to the conditional limits of age groups.
CI, cerebral infarction; DVT, deep vein thrombosis; ICH, intracranial hemorrhage; lt., ; na, not assessed; PF, purpura fulminans; PTE, pulmonary thromboembolism; VTE, venous thromboembolism.
[a]PC-Nagoya. [b]No mutation of *PROC*. [c]PS-Tokushima. [d]At 19 gestational weeks.

seven of eight patients with PS gene mutation showed reduced activities of PS and PC, respectively.

DISCUSSION

The first survey on PC, PS, and AT deficiency in Japanese thromboembolic children revealed the contribution to 5.9% of pediatric thrombosis, and the age-dependent presentation of each PC, PS, or AT deficiency before adult life. Congenital PC and PS deficiencies were the major thrombophilias in Japanese children with different age distribution. Predominant cerebral thromboembolism in PC-deficiency contrasted sharply with extracranial VTE in the other two deficiencies. The

genotype of PS-deficient children reflected the high prevalence of PS-Tokushima in Japanese general population (1.1–1.8%; **Table 1**) (12,13). The Israeli-German cohort studies (14–16) have recently reported the prevalence of congenital deficiencies PC (7.4%), PS (8.2%), and AT (6.6%) in pediatric patients with thromboembolism aged 0.1–18 y. In the cohorts, the proportion of neonates was also higher in PC deficiency (32%; 8/25 patients) than seen in PS deficiency (10%; 3/30 patients) or AT deficiency (14%; 3/21 patients). Half of PC-deficient newborns presented cerebral sinovenous thrombosis and stroke. In this setting, PC-deficient infants characterized the phenotype of pediatric thrombophilias (purpura fulminans and intracranial lesions) beyond the ethnicity (17). The age-specific prevalence and phenotype of pediatric PC deficiency may be useful in the genetic screening for pediatric thrombophilias.

The first concern is the applicability of the lower limits of PC, PS, or AT activity in age groups for the genetic screening. The ranges of anticoagulant activities often vary until the attainment of adult levels, reflecting the individual maturation and vitamin K status (18,19). Our previous adult study assessed by the same measures reported that half of VTE patients with each low activity carried the heterozygous mutation, which resulted in 33% positive rate of all patients (20). Miyata *et al.* (21) confirmed that 32% of adult VTE patients carried heterozygous mutation of PC, PS, or AT, in the twice sample size, without the screening of plasma activity. In this setting, the concordant mutation rate by the independent studies would warrant the utility of plasma activities for genetic screening in Japanese children. No mutation in >30% of patients raises the possibility of large gene deletions, polymorphisms, and the other genetic variations or certain modifiers affecting the activity and antigen concentration (22). The present measures studied polymorphisms including the promoter regions but not deletions. Large deletions are reported to be 3–6% in these deficiencies (5). Cooper *et al.* (23) revealed that large deletions make up between 7 and 10% of PS and AT mutations and only 1% of PC mutations. The rare occurrence of thrombosis and the wide range of factor activities impeded to define the standard values of "true healthy" children who develop no thromboembolism until the forties. In this study, there was no [Q6] significant difference in the ratios of mutated patients to the low PC patients who underwent the genetic analysis and in the ratios of mutated patients to the low PS patients analyzed in any age groups. Taking into account high safety margins, the limits of factor activity might be practical for the genetic screening of pediatric thrombophilias.

The proportion, genotype, and first presentation of PC, PS, and AT deficiency during childhood were distinct from those of adult patients. Patients with low PC activity were almost twice the number of those showing low PS activity (**Figure 1**). On the other hand, the number of patients who carried either *PROC* or *PROS1* mutation was the same eight. The first nationwide survey for pediatric thrombophilia in Japan suggested a higher prevalence of PC deficiency than expected (24,25). The discrepancy may be explained by the wider range of PC activity than that of PS or AT activity in infant and children (18,26), partly arising from the inherent variation of PC pathway (27,28). Clinical manifestation and thrombin generation differ among the family members having the same *PROC* mutation (29). Otherwise, absolute PC deficiency rather than PS deficiency may contribute to the development of thromboembolism in the newborn and young infants (30). Half of the PS gene–mutated patients carried PS-Tokushima, two of whom had compound heterozygous mutations with one allele PS-Tokushima (**Table 1**). Both patients lacked PS activity and developed deep vein thrombosis at 12 and 16 y of age. These findings suggested that high allele frequency of PS-Tokushima made an impact on the genotype of severe PS-deficient children in Japan. By contrast, six of eight patients with PC deficiency presented in infancy, four of whom carried double allele mutation (one PC-Nagoya) having undetectable plasma activity. The genotypes of pediatric PC and PS deficiency corroborated the previous studies on Japanese adult population.

The other concern is the distinct affected site between PC-deficient (intracranial lesion) and PS-deficient children (extracranial VTE). Premature infants with perinatal complications are liable to bleed in the brain. However, intracranial lesions including sinovenous thrombosis were also observed in adolescence (**Table 1**). The expression levels of endothelial PC receptor and thrombomodulin are constitutively low in the brain compared with other organs. In contrast to the basilar arteries or choroid plexus, intracerebral vascular endothelium does not express thrombomodulin (31). Free PS works as a cofactor of activated PC in the PC pathway. Circulating free PS molecules are relatively high in infancy because of physiologically low C4-binding protein levels (26). It may explain the preferential cerebral lesions in PC-deficient children. Activated PC and thrombomodulin have homeostatic signals critical in regulating coagulation, inflammation, endothelial barrier function, and neuroprotection (32). PC-deficient children might be vulnerable to the brain damage in association with prothrombotic triggers.

One of the limitations in this report was that the results were obtained from a single center study. The number of mutation carriers was small even in the prolonged study. The other limitation was that the consumption effects and/or the plasma replacement therapy might not be completely excluded in assessing the activity levels of the natural anticoagulants. The shorter biological half-life of PC than that of PS or AT might influence the dissociated low activity of PC in infants. However, these findings do not explain the distinct presentation between PC deficiency in infancy and PS deficiency in adolescents or young adults. Multiplex ligation-dependent probe amplification and/or single-nucleotide polymorphism array are needed to search the deletion in the future study. Further comparative study on PC- and PS-deficient infants may shed some light on the pathophysiology and optimal management of pediatric thrombophilias.

In conclusion, PC gene–mutated patients mostly presented [C with intracranial thromboembolism than 2 y of age. On the other hand, PS/AT gene–mutated patients were preferentially found in school age children with extracranial VTE.

[Q8] The genetic screening of pediatric thrombophilias could be effectively targeted to stroke in low PC infants and deep vein thrombosis in low PS /AT school age children.

METHODS

Patients and Screening Protocol

From 1993 to 2012, 1,867 patients with thrombosis in Kyushu University and affiliated institutions were consecutively assessed for thrombophilic predispositions (**Figure 1**). After the exclusion of patients with anomaly and inborn errors of metabolism, 306 patients aged less than 21 y of age (16.4%) were enrolled for the study. Clinical information of thromboembolic events and/or coagulopathy was collected from the medical records and the interviews of attending doctors. Of 122 patients showing the low plasma activity, 60 were excluded because of acquired thrombotic disorders including vasculopathy, antiphospholipid syndrome, and autoimmune diseases. Finally, 62 patients underwent the genetic analysis because they had no explainable causes of thromboembolism other than PC, PS, or AT deficiency. Informed consent was obtained from parents of children aged <21 y of age. This study was certified by the Institutional Review [Q9] Board of Kyushu University (#232-02, #448-00).

Coagulation Study

To exclude the consumption effects at the diagnosis of thromboembolism, the antigen/activity levels of PC, PS, and AT were repeatedly assessed when the clinical conditions were stabilized a couple of weeks to months after the onset of thromboembolism. The measurement of anticoagulation factors were performed as described previously (20,33). Anticoagulant activities of PC and PS were determined using the Staclot PC kit and the Staclot PS kit (Diagnostica Stago, Asnieres, France), respectively. A chromogenic substrate was used to assay for AT activity as heparin-dependent inhibition of bovine thrombin (Chromostrate ATIII kit; Hitachi, Tokyo, Japan). The adult references were first standardized by using pooled normal plasma, in which a level below 3 SD was defined as reduced activity. According to the data in Japanese children (18,26), the lower limits of each activity were defined as follows: in age <90 d: PC 60%, PS 60%, and AT 65% of the adult reference levels (PC 75%, PS 60%, and AT 80%); 90 d–2 y: PC 85%, PS 85%, AT 65% of the adult levels; 3–6 y: each 85% of adults; and 7–20 y: same as the adults (**Supplementary Table S1** online).

Gene Analysis of PC, PS, and AT

Genomic DNA was extracted from peripheral blood leukocytes. Direct sequencing of PC (*PROC* exon 1–9), PS (*PROS1* exon 1–15), [Q10] and AT (*SERPINC1* exon 1–6) genes was performed as previously described (20). The exon and exon–intron boundary regions of each gene including promoter region were amplified by PCR, and the products were then subjected to direct sequencing using ABI 377 (Perkin Elmer Applied Biosystems, Foster City, CA).

Statistical Analysis

The median of continuous variables was assessed by the Mann–Whitney *U*-test. The distribution difference of countable variables was analyzed by chi-square test or Fisher's exact test. *P* values less than 0.05 were considered significant.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/pr

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Successful living domino liver transplantation in a child with protein C deficiency

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Abstract: PC is produced in the liver and inhibits blood coagulation by catalyzing active factors V and VIII. PC deficiency causes abnormal blood clotting that is difficult to regulate by anticoagulative treatments. Four reports of PC deficiency treated with LTx have been published; however, no report of DLT as a therapy for PC deficiency is available. We describe a case of a 23-month-old girl who received DLT for compound heterozygous PC deficiency. Her PC activity was below 5%. She developed intracranial lesion and frequent refractory purpura fulminans. Both her parents had heterozygous mutations of PC genes and were excluded as living donors. Furthermore, she was a low priority on the waiting list of deceased-donor transplantation. We performed living DLT using the liver from a patient with MSUD. Activated PC concentrate safely supported the perioperative period. After DLT, she maintained normal PC activities and BCAA levels. This is the first case of PC deficiency successfully treated by living DLT with MSUD. We propose that DLT using liver from patients with MSUD is a treatment option for PC deficiency.

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Key words: protein C deficiency – protein C concentrate – domino liver transplantation – neonatal purpura fulminans – maple syrup urine disease

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Abbreviations: 3D-CT, three-dimensional computerized tomography; aPTT, activated partial thromboplastin time; BCAA, branched-chain amino acid; DDLT, deceased-donor liver transplantation; DLT, domino liver transplantation; FAP, familial amyloidotic polyneuropathy; FFP, fresh frozen plasma; HA, hepatic artery; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HV, hepatic vein; LDLT, living-donor liver transplantation; LHV, left hepatic veins; LTx, liver transplantation; M + LHV, middle and left hepatic veins; MHV, middle hepatic vein; MSUD, maple syrup urine disease; PBC, primary biliary cirrhosis; PC, protein C; PFIC, progressive familial intrahepatic cholestasis; PSC, primary sclerosing cholangitis; PT, prothrombin time; PV, portal vein; RHV, right hepatic vein.

PC is a vitamin K-dependent serine protease synthesized and secreted by liver cells as a zymogen. Activated PC downregulates the blood coagulation cascade through its proteolytic effect on factors Va and VIIIa, in conjunction with its cofactor protein S (1, 2). PC deficiency is inherited as an autosomal dominant disorder, and in most cases, derived from heterozygous mutations. Homozygous or compound heterozygous patients often suffer severe and life-threatening complications from birth. The most common presentation is purpura fulminans, a rapidly progressing hemorrhagic necrosis of the skin, which

can occur within the first few hours of birth (1, 2). Cerebral and ophthalmic damage can occur in utero, and children are often born with visual impairment. In addition, patients may suffer from venous thromboembolism (3).

Historically, the standard treatment for PC deficiency was the replacement of FFP and administration of warfarin (1, 2). In 1983, the first case of severe PC deficiency associated with neonatal purpura fulminans successfully managed by warfarin was reported (4). Since 1988, the use of PC concentrate has been widely adopted and reports of successful long-term management using PC concentrate have been published (5). Warfarin, FFP, and PC concentrate, however, are associated with thrombosis and can sometimes be difficult to administer. Hence, there is rising interest in LTx as a therapy for PC deficiency, as the liver produces PC (6–9).

To date, there are 10 reports of DLT of a recipient's metabolically defective, but structurally and functionally normal, explanted liver into a second recipient. The most common indication for DLT is FAP. With an increasing need for organs, livers explanted from patients with rare metabolic diseases, such as primary hyperoxaluria, acute intermittent porphyria, MSUD, and homozygous familial hypercholesterolemia, are being used for DLT (10–12). DLT has since become an option for increasing organ availability.

Here, we report the case of a child with severe compound heterozygous PC deficiency who was successfully treated by DLT using liver from an MSUD patient.

Case presentation

The patient was a 23-month-old girl (body weight: 9.5 kg) who was delivered by C-section because of fetal heart rate deceleration (37w 6d, 2048 g). She was the only child in the family. Multiple cerebral bleeding, subcutaneous bleeding, purpuric skin lesions on lower limbs, and vitreous hemorrhages (ultimately resulting in complete blindness) were observed shortly after birth (Fig. 1). PC activity of the patient was <5%. Genetic test performed on a blood sample confirmed that she inherited an inability to produce PC. Two heterozygous missense mutations of PC in the proband were identified c.296G>A, p.E68K and c.1109G>A, p.V339M. A low PC activity and low antigen level led to the diagnosis of compound heterozygous PC deficiency. The PC activity of the parents was also low (66% and 77%, respectively). The patient's grandmother died of cerebral bleeding, but the details were unclear. At one month of life, an ophthalmectomy was performed due to bilateral vitreous hemorrhages. Initially, the patient was managed with warfarin (0.07 mg/kg/day) and heparin (225 U/kg/day). At 14 months of age, she

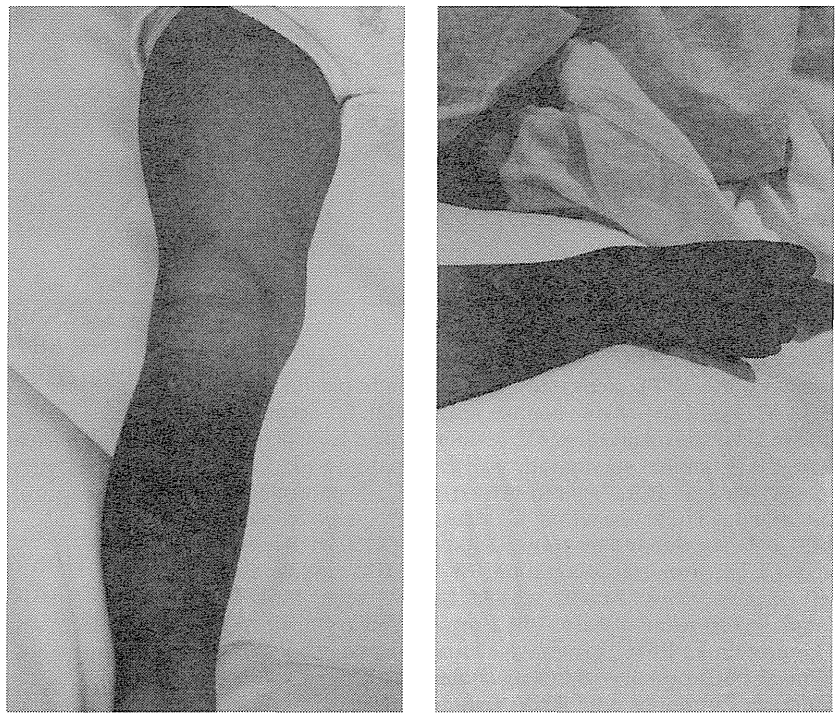

Fig. 1. Skin lesions were observed on the right foot and ankle arising from peripheral thrombotic events.

suffered from symptomatic West syndrome because of past cerebral bleeding; warfarin was changed to dabigatran to control phenobarbital. However, frequent episodes of bleeding and thrombosis such as subcutaneous hematoma persisted, and FFP and activated PC concentrate were required. In addition, there was an increasing difficulty of venous line access. She was evaluated as a candidate for LTx. Both her parents had heterozygous mutations of PC genes and were excluded as living donors. Furthermore, she was a low priority on the waiting list of deceased-donor transplantation. A decision for DLT was made.

At 22 months of age, the patient was transferred to our hospital for LTx. Pre-operatively, therapy with FFP (120 mL) was instituted on alternate days until operation. There were no thrombosis and bleeding episodes before operation. In addition, she was also given dabigatran (36 mg/kg/day) and was monitored to maintain an aPTT of 60–80 s. The final dose (360 mg) was administered 48 h before induction of anesthesia and the initiation of intravenous heparin. Soon after, thrombocytopenia was recognized; heparin was discontinued, and continuous intravenous infusion of activated PC concentrate (300 U/h) commenced.

At 23 months of age, the patient underwent DLT. The first recipient was a 12-month-old-girl with MSUD, who required LDLT of the left lateral segment from a living donor because of high leucine acid, frequent metabolic acidosis, and growth impairment. As size matching between the MSUD patient and our patient was suitable, the whole liver was used as the domino graft. The whole MSUD liver was placed in the abdominal cavity and vascular reconstruction initiated. We decided the transection site of the vessels based on 3D-CT of the first donor and recipient pre-operatively. Because the left HA of the first donor was diverged from the right gastric artery, along with sufficient anastomosis length, the gastroduodenal artery was ligated and the common HA was transected in the first recipient. As well, the RHV, the common channel of the M + LHV, was transected and the PV was transected proximal to the bifurcation. Vascular plasty of the HV was conducted on the back table. RHV, MHV, LHV, and the left superficial vein were sutured together to create one orifice. The unified graft HV was anastomosed with that of the recipient to create one orifice using all of the HV. The PV was anastomosed with the branch patch in an end-to-end fashion. The graft liver was reperfused before microsurgical reconstruction of the HA. Roux-en-Y anastomosis was employed for biliary reconstruction.

Activated PC concentrate was administered during surgery and continued until post-operative day 7. The level of PC activity increased gradually and reached 64% on post-operative day 4. In spite of the discontinuation of activated PC concentrate, PC activity levels were adequately maintained at 80–90% with PT/aPTT constantly within the normal range (Fig. 2). The recipient received a standard immunosuppressive regimen consisting of low-dose steroids and tacrolimus. On post-operative day 20, she had an episode of severe acute cellular rejection and was treated with intravenous bolus steroids therapy. On post-operative day 26, she acquired an infection of cytomegalovirus and was treated with intravenous foscarnet sodium hydrate. On post-operative day 68, she was discharged. She did not develop any BCAA imbalances or symptoms of MSUD on a normal diet with full protein

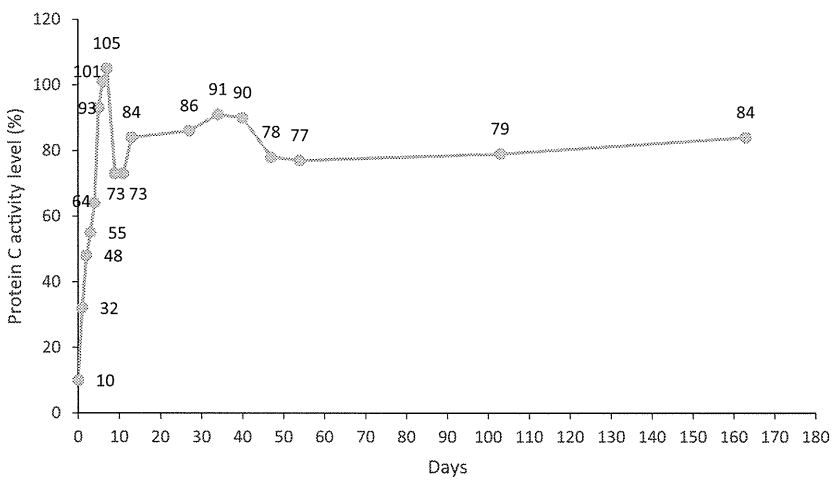

Fig. 2. Plasma PC activity levels with time after DLT.

Table 1. Amino acid levels before and after DLT

Amino acids	Normal range nmol/mL	DLT donor*		DLT recipient†	
		Before	After	Before	After
Leucine	80.9–154.3	154.6	137.9	114.4	125.3
Isoleucine	41.3–84.9	149.3	100.5	63.6	60.2
Valine	158–287.7	282.3	155.2	250.3	156.7
Alloisoleucine	ND	ND	ND	ND	ND

*Living-donor liver recipient with MSUD.
†Domino liver recipient with PC deficiency.

intake (Table 1). Six months after the DLT, PC activity was maintained at more than 80%. She remained symptom free.

All the patients and their parents gave informed consent. The study was approved by the Japanese Liver Transplantation Society and the ethical committee of each institute.

Discussion

This case highlighted two important clinical issues: PC deficiency can be successfully treated by living DLT with MSUD, and activated PC concentrate is useful and supported a safe perioperative period.

A total of 10 DLTs using livers from patients with MSUD have been reported (Table 2) (13–19). Domino livers were first considered as marginal grafts because the disease could manifest in the domino recipient (13). However, recipients of liver grafts from MSUD donors are not likely to develop protein intolerance because 60% of branched-chain ketoacid dehydrogenase activity occurs in the muscle (14). The patient and graft survival rates were 100%, and all the livers functioned normally. BCAA homeostasis was maintained with an unrestricted protein diet. MSUD livers maintained nearly normal levels of plasma amino acids and a favorable evolution with no disease development, demonstrating structurally normal liver parenchyma with hepatic function preserved. The literature reveals four previous reports of successful LTx for PC deficiency, but there have been no reports of DLT for PC deficiency (6–9). To the best our knowledge, this is the first case of LDLT for a pediatric recipient with MSUD, who in turn became a donor for a pediatric recipient with PC deficiency.

The use of whole liver for DLT can pose increased technical difficulty during operation (20). Livers obtained from patients with MSUD who had undergone LDLT inherently lack the retro-hepatic inferior vena cava and have multiple vessel and bile duct orifices. In this case, the MSUD patient and her living donor underwent 3D-CT before the operation to evaluate the anatomy of their HV, PV, and HA. We determined the cutting sites of the vessels based on the 3D-CT findings. The HV pedicle in the graft from the MSUD patient was short, and reconstruction was required in the second recipient. There were no surgical complications, and both recipients had good post-operative functional recovery.

Activated PC concentrate proved to be useful for perioperative management of PC deficiency. Prior to the transfer to our hospital, the patient had experienced thrombotic events and bleeding on many occasions while on FFP and dabigatran. While FFP and activated PC concentrate replacement may prevent thrombosis, they can cause fluid overload and pose a high risk of infection. Oral anticoagulant therapy, meanwhile, may risk fatal hemorrhage and often restricts normal childhood activities. Therefore, we decided to perform LTx for our patient.

Initiation of high-dose activated PC concentrate restored levels of PC in the perioperative period, allowing LTx to be performed safely in

Table 2. Outcome of DLT using MSUD

No.	LT for MSUD				DLT recipient				Study
	LTx	Age (yr)	Observation (m)	Outcome	Indication	Age (yr)	Observation (m)	Outcome	
1	DDLT	25	7	Alive	HCC and HCV	51	7	Alive	Khanna et al. (14)
2	DDLT	33	–	Alive	PSC	67	38	Alive	Gopasetty et al. (15)
3	DDLT	11	–	Alive	PFIC	24	25	Alive	Gopasetty et al. (15)
4	DDLT	18	–	Alive	Cystic fibrosis	20	18	Alive	Gopasetty et al. (15)
5	DDLT	23	–	Alive	Congenital hepatic fibrosis	22	20	Alive	Gopasetty et al. (15)
6	DDLT	22	–	Alive	PBC	52	5	Alive	Gopasetty et al. (15)
7	DDLT	5	–	Alive	Embryonal carcinoma	7	2	Alive	Gopasetty et al. (15)
8	LDLT	1	12	Alive	Biliary cirrhosis	2	12	Alive	Mohan et al. (16)
9	DDLT	24	30	Alive	Hemophilia A	52	30	Alive	Badell et al. (18)
10	LDLT	2	13	Alive	Biliary atresia	2	13	Alive	Feier et al. (19)
11	LDLT	1	6	Alive	PC deficiency	1	6	Alive	Present case

our patient. Notably, PC activity level reached 64% by post-operative day 4. Thereafter, PC activity steadily rose to adequate levels and the patient is doing well without any thrombotic events for over three months following the transplantation.

Based on the excellent results observed in this report, domino grafts from patients with MSUD could potentially be used in recipients who are low priority on the transplant waiting list and are likely to die without a transplant. Although elective DLT for PC deficiency remains controversial, reports of good outcome have established it as an acceptable therapeutic option. Furthermore, the continued success of DLT may help to mitigate existing ethical concerns. Nevertheless, prospective studies with long-term outcomes are needed to accurately determine the validity of DLT for PC deficiency.

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Conflict of interest

The authors of this manuscript have no conflict of interest.

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