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長江千愛、瀧 正志	播種性血管内凝固症候群(DIC)の治療	小児内科	53(8)	1067-75	2012

VI.　研究成果の刊行物・別刷

Ⅰ.新生児血栓症

Ⅱ.大賀正一 九州大学大学院医学研究院周産期小児医療学

Ⅲ. 特に伝えたいこと、知って欲しいこと(簡潔に)

1. 小児の血栓症は成人よりまれだが、新生児に最も多く、近年診断される例が増加している。
2. 遺伝性・非遺伝性因子により、頭蓋内病変、電撃性紫斑病などの特徴的な発症様式となる。
3. 凝固線溶能の評価が難しく、血栓の部位を証明する画像検査も施行しにくい。
4. 母体情報と家族歴を把握し、必要に応じて遺伝子解析とカウンセリングを行う。
5. 抗凝固療法に用いる薬剤の使用に関する EBM が少なく、確立された治療法はない。
6. 生命予後のみならず、神経学的予後の不良な例が多い。

Ⅳ. 最新と思われる知見またはトピックス(簡潔に)

1. 日本人小児の分子疫学から、Protein C(PC)欠損症が注目され、先天性のみならず PC 活性の上昇が遅れる後天性 PC 欠損症も確認された。
2. 後天性因子として、早期産児における長期の中心静脈カテーテルの関与は大きい。
3. トロンボモジュリン製剤の有用性に関する情報が蓄積されつつあり、新鮮凍結血漿、活性化 PC 製剤など補充療法の再評価が必要である。

1)定義・病態生理　生後1か月までに、止血機構が過剰に作動して血栓を形成し臓器障害をきたす疾患である。新生児では特に狭い血管腔と高いヘマトクリットによる血流の変化、および凝固制御因子の成熟と内因性線溶活性の低下が、その病態に関与する。誘因・後天性因子として、抗リン脂質抗体症候群(APS)母体、感染症、脱水、多血症、中心静脈ルート、および先天性心疾患術後の血栓性血小板減少性紫斑病などがある。

2)疫学　欧米での頻度はNICU入院の0.15-0.24%と日本より高いが、日本でも近年0.063%と倍増している。遺伝性血栓症は約5%と少ないが、全て PC 欠損症である。小児先天性血栓症の約半数は PC 欠損症で、その半数がホモか複合ヘテロ変異、25 %がヘテロ変異である。

3)診断　部位(脳静脈洞と腎静脈)と病型(胎児水頭症、脳出血性梗塞、電撃性紫斑病)が特徴的である。電撃性紫斑病は末端壊死の出現前に、臀部や足底の紫斑からはじまるものも少なくない。半数以上に先行する頭蓋内病変を合併する。血小板数著減、Fibrinogen 値減少と D-dimer 増加があり、臓器障害を起こす。血栓の画像診断には超音波検査を活用する。PC 欠損症の診断は難しいが、PIVKA Ⅱ陰性でかつ PC と PS 活性の乖離があり、PC 活性が 30%未満の新生児に疑う。家族歴があれば、両親の因子活性を確認し、必要な遺伝子解析を行う。

4)治療　抗凝固療法と血栓溶解療法からなる。APS には、交換輸血と低用量アスピリンを用いる。新生児の深部静脈血栓症の回復は成人より早く再発率も低いが、遺伝性血栓症では進行と再発のリスクが高い。抗凝固療法を継続し、凍結血漿、活性化 PC、AT 製剤などを補充して、トロンボモジュリン製剤も検討する。TPA や新規抗血栓薬の適応が今後の課題である。

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Review Article

Protein C deficiency as the major cause of thrombophilias in childhood

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Abstract Genetic predisposition of thromboembolism depends on the racial background. Factor V Leiden (G1691A) and factor II mutation (G20210A) are the leading causes of inherited thrombophilias in Caucasians, but are not found in Asian ancestries. Protein S (PS), protein C (PC) and antithrombin (AT) activity are reportedly low in 65% of adult Japanese patients with deep vein thrombosis. Approximately half of the patients with each deficiency carry the heterozygous mutation of PS (*PROS1*; 20%), PC (*PROC*; 10%), and AT genes (*SERPINC1*: 5%). Recently, several studies have revealed an outline of inherited thrombophilias in Japanese children. Congenital thrombophilias in 48 patients less than age 20 years consisted of 45% PC deficiency, 15% PS deficiency and 10% AT deficiency, along with other causes. All PS- and AT-deficient patients had a heterozygous mutation of the respective gene. On the other hand, PC-deficient patients were considered to carry the homozygous or compound heterozygous mutation in 50%, the heterozygous mutation in 25%, and unknown causes in the remaining 25% of patients. Half of unrelated patients with homozygous or compound heterozygous *PROC* mutations carried PC-nagoya (1362delG), while their parents with its heterozygous mutation were asymptomatic. Most of the PC-deficient patients developed intracranial lesion and/or purpura fulminans within 2 weeks after birth. Non-inherited PC deficiency also conveyed thromboembolic events in early infancy. The molecular epidemiology of thrombosis in Asian children would provide a clue to establish the early intervention and optimal anticoagulant therapy in pediatric PC deficiency.

Key words intracranial thrombosis, intracranial hemorrhage, purpura fulminans, protein C deficiency.

Introduction

Thromboembolic events less commonly occur in children than in adults. The life-threatening conditions are driven by a wide range of triggers, including infection, trauma and surgery, on a background of individual predispositions. Pediatric thrombosis is being recognized with increasing frequency, although the cause remains elusive. The national registry data from North America, Europe and Australia revealed an incidence of 5–8 venous thromboembolic events (VTE)/10 000 hospital admissions, or 0.05–14/10 000 children.[1] Under 20 years of age, VTE occurs at the highest incidence in neonates and infants, and then at the second peak of incidence during puberty and adolescence. Both peak occurrences are considered to be associated with decreased intrinsic fibrinolytic activity of the blood during these periods. The higher prevalence of adult thromboses in the West than that seen in the East might result from the difference in dietary habits and races. The modern lifestyle is being globally Westernized, but genetic backgrounds are hard-wired to change among races. Factor (F) V Leiden (G1691A) and FII mutation (G20210A) are the major thrombophilic predispositions in Caucasians but not in Asian ancestries.[2] In Japan, two major studies revealed that 65% of adult VTE patients had a low activity of protein S (PS), protein C (PC) or antithrombin (AT), and half of them carried the heterozygous mutation of the respective genes.[3,4] On the other hand, the clinical features and genetic backgrounds of pediatric thrombosis have not been clarified in Japan.

In this review, we first draw an outline of inherited thrombophilias in Japanese children, based on the recent independent studies. Then, we discuss the problems in the diagnosis and treatment of pediatric thromboembolism focusing on PC deficiency.

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Table 1 Definition of the severity of PC deficiency

Subjects	Plasma PC activity	
	IU/mL	%
Healthy adults	0.65–1.35[†]	64–135[‡], 146[§]
PC deficiency		
Mild	>0.2	>20
Moderately severe	0.01–0.2	1–20
Severe	<0.01	<1

Standard values of PC activity were obtained from [†]the reference;[6] [‡]Medical and Biological Laboratories, Tokyo, Japan; and [§]Special Reference Laboratories, Tokyo, Japan. The levels are determined by clot-based assays. PC, protein C.

PC and PC levels in children

PC is a vitamin-K-dependent serine protease, which is synthesized in the liver and circulates at a low concentration in plasma. The anti-coagulant zymogen is activated by the complex formation with thrombin on the endothelial cell receptor thrombomodulin, and more effectively by binding to the endothelial PC receptor. Activated PC cleaves critical sites in the activated procoagulant factor V (FV) and FVIII, and inactivates the two factors.[5] This process is augmented by protein S (PS), FV and lipid cofactors of lipoproteins and phospholipids. PC-deficient individuals have a decreased capacity to control the propagation of thrombin generation by FVa and FVIIIa after the activation of coagulation cascade, according to the circulating amounts of functional PC molecules regardless of the decreased production or increased consumption.

Inherited PC deficiency is an autosomal recessive thrombophilia. Bialellic (homozygous or compound heterozygous) PC gene (*PROC*) mutants incite purpura fulminans (PF) as a consequence of "severe" PC deficiency in the newborn.[6] Heterozygous *PROC* mutants are at risk of venous thromboembolism as "moderately severe" or "mild" PC deficiency in young adults. The severity of PC deficiency is defined according to the levels of activity assessed by chromogenic (amidolytic) or coagulometric (clotting) assay (Table 1).[7] The plasma PC activity in healthy adults ranges from 0.65 to 1.35 IU/mL, corresponding to the % interval of references used in Japan. "Mild," "moderately severe" and "severe" PC deficiencies are defined as the range of >20% (>0.2 IU/mL), 1–20% (0.01–0.2 IU/mL), and <1% (<0.01 IU/mL), respectively. However, the distinction of inherited (heritable) or non-inherited (acquired) PC deficiency is challenging in children who developed acute thrombosis for the following reasons. First, plasma PC activity is physiologically low until adolescence. Second, two major conditions affecting plasma PC levels, vitamin K deficiency and infection, are not rarely found in early infancy. Third, young parents with heterozygous *PROC* mutation are healthy, and the history of the grandparents may be less informative. Fourth, in critically ill children with thrombotic events, the genetic tests are time-consuming in guiding the management, and the constitutional hypercoagulability is hard to assess by functional assays during the treatment course.

Neonatal PC and PS levels are much lower than the adult reference levels. The mean plasma activities of PC and PS in healthy term infants are approximately 35%.[6–8] Preterm infants show much lower levels than term infants because of immaturity of the liver. Both PC and PS levels increase after birth, and reach the lower limit of adult references (~50 IU/dL) during 6 months to 1 year of age (Fig. 1).[8–10] As PC activity often remains below the adult reference ranges until puberty, PC levels in childhood are hard to screen for inherited thrombophilias.[11]

Several factors affect the physiological levels of PC and PS throughout childhood.[12] Both activities are low in the presence of vitamin K antagonist. Vitamin K deficiency precipitates bleeding (i.e. hemorrhagic diseases of the newborn, vitamin K deficiency bleeding in infancy), and also thrombosis (i.e. warfarin-induced skin necrosis/ paradoxical thrombosis). Infection lowers the plasma concentration of PC and PS. The mechanisms of "infectious PF" are involved in antibody-mediated consumption (i.e. post-varicella PF) or toxic effects (i.e. meningococcemia PF).[13] PC/PS deficiency arises from the loss or consumption in patients with nephrotic syndrome, sepsis and/or disseminated intravascular coagulation, and from the impaired synthesis in those with liver dysfunction. The half-life of plasma PC (6–8 h) is shorter than that of PS and other procoagulant vitamin K dependent factors. The true enzymatic activity of PS depends on free PS concentration. However, the interpretation of PS activity in newborns is not complicated because the binding C4b is at very low levels at birth. Acute inflammation reduces PS activity due to binding with C4b. For the diagnosis of PC deficiency in infants,

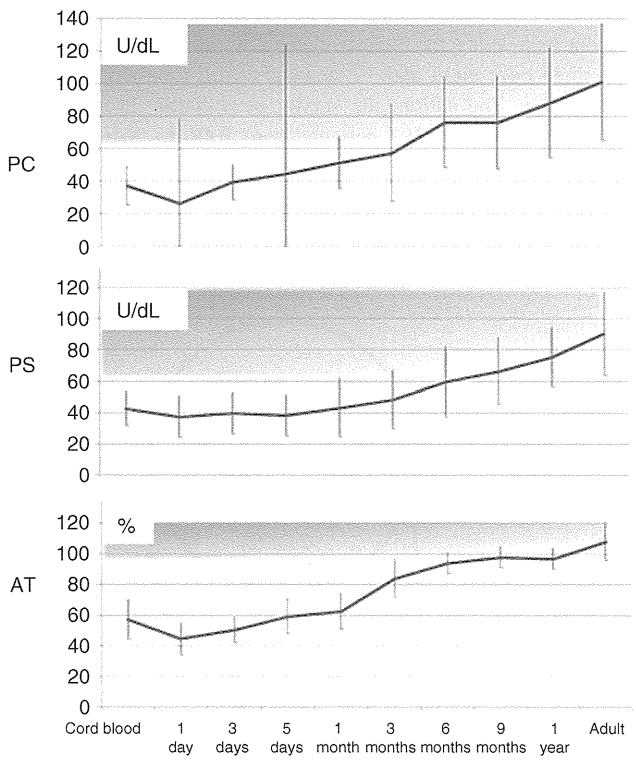

Fig. 1 Changes in the plasma activity of protein C (PC), protein S (PS) and antithrombin (AT) of healthy full-term Japanese infants from birth to 1 year of age. Each bar represents the mean ± SD revised from the data of reference 8.

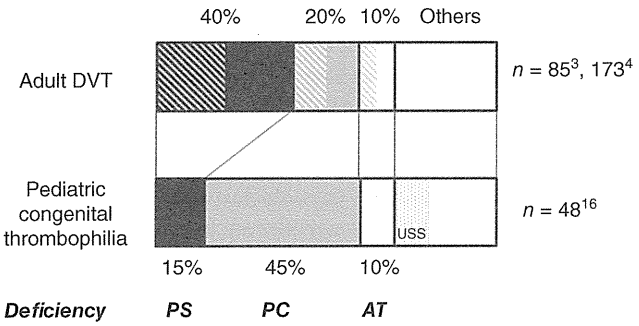

Fig. 2 Proportion of protein S (PS) (black), protein C (PC) (gray) and antithrombin (AT) deficiency (light gray) in adult patients with deep vein thrombosis (DVT) and pediatric patients diagnosed as having congenital thrombophilia in Japan. The hatched column represents patients who carry the heterozygous mutation of the PS, PS or AT gene. Upper columns are based on the data from references 3 and 4, and the lower ones are revised from the data of reference 16. USS (dotted column), Upshaw–Schulman syndrome. Each superscript in the figure refers to the number of reference.

plasma PC activity should be monitored concurrently with PS activity, protein induced by vitamin K absence or antagonists, D-dimer, anti-phospholipid antibodies, and FVII activity. Unexplained dissociation between PC and PS activity may portend a diagnosis of inherited PC deficiency.

Genetic backgrounds of PC deficiency in Japan

Recent reviews by the experts in the UK[14] and North America[15] described only eight and 12 survivors with long-term therapy, respectively. The nationwide survey of pediatric thrombosis in Japan first collected 301 patients from 2006 to 2010.[16] Forty-eight patients (15%) were diagnosed as having congenital thrombophilia due to PC (n = 22; 46%), PS (n = 7, 15%), AT (n = 5; 10%), or ADAMTS13 (n = 5; 10%) deficiency and other causes (n = 9; 19%). This proportion differed from that of adult Japanese deep vein thrombosis cases (Fig. 2). There were no homozygous or compound heterozygous mutations of *PROS1*, *PROC* or *SERPINC1* in adult patients. PS deficiency was the most frequent genetic predisposition of adult thrombosis in Japan. It may reflect the higher frequency of heterozygous *PROS1* mutants in Japanese (1.12–1.8%) than in Caucasians (0.03–0.13%), accounting for the founder effects of PS-tokushima.[17] On the other hand, approximately half of pediatric patients with congenital thrombophilia had PC deficiency. Half of them carried homozygous or compound heterozygous *PROC* mutations, in contrast to no PS or AT-deficient patients with biallelic mutations. Homozygous PS or AT-deficient mice result in fetal loss. Only a few patients with homozygous or compound heterozygous mutations of *PROS1* or *SERPINC1* have ever been reported. The true frequency of Japanese carriers of heterozygous *PROC* mutation may be higher than the prevalence of PC deficiency of 1/700 screened by amidolytic activity.[18] These studies corroborated that *PROC* mutants are the leading cause of inherited thrombophilia in Japanese children.

Another study has recently reported the presentation and genotype of pediatric PC deficiency in Japan, based on the combined data with genetic study, post-marketing survey of plasma-derived activated PC concentrate (AnactC, Kaketsuken & Teijin, Kumamoto, Japan), and literature review.[19] Between 1985 and 2010, this study determined 27 Japanese patients with congenital PC deficiency in Japan. These included one pair of twins, and a pair of cousins. Two patients had died. These patients might appreciably cover the 22 patients reported in the aforementioned nationwide survey.[16] Twenty-four (89%) patients presented within 14 days after birth, including three prenatal hydrocephalies. Of the 27 patients, the first presentation was intracranial lesions (thrombosis, hemorrhage, and/or fetal hydrocephaly) in 19, PF in 16, and both in 10 patients (Fig. 3). Intracranial

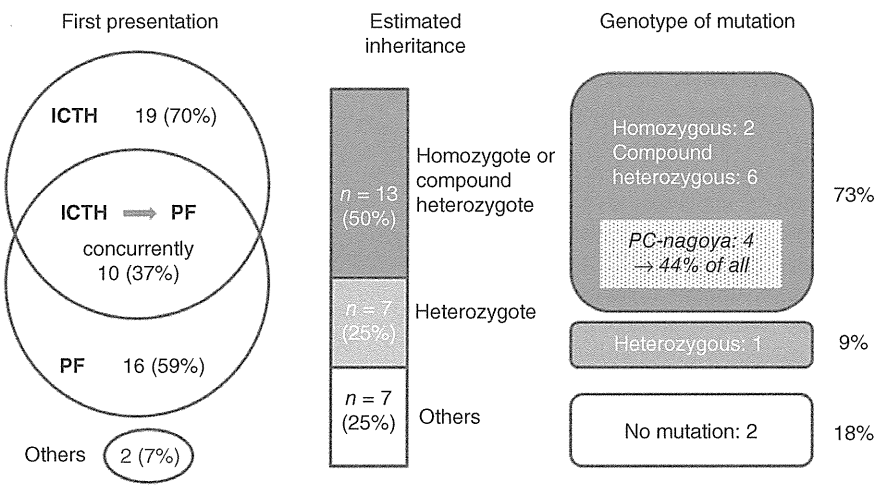

Fig. 3 First presentation at diagnosis, estimated inheritance, and genotype of pediatric patients with protein C (PC) deficiency in Japan. The major form of the disease onset is intracranial thrombosis and hemorrhage (ICTH) and/or purpura fulminans (PF) until 2 weeks of age. Three-quarters of patients were born to healthy parent(s) having low PC activity. Four unrelated patients carried PC-nagoya among 11 patients who received genetic study.

thrombosis/infarction and hemorrhage (ICTH) preceded or concurrently occurred with PF in both affected patients. Low PC activities of 18 mothers and/or 12 fathers indicated 20 heritable PC-deficiencies (two homozygotes, 11 compound heterozygotes, and seven heterozygotes) and seven unidentified causes of PC deficiency. Nine of 11 patients had *PROC* mutations, and four unrelated patients carried PC-nagoya (1362delG). No PC-deficient parents experienced VTE. Of the 18 patients treated with activated PC concentrate, two died and eight evaluable survivors had neurological sequelae. According to a different survey on neonatal thrombosis in Japan between 1999 and 2009,[20] six newborn infants were diagnosed with inherited thrombophilia (all PC deficiency) of 105 patients with neonatal thrombosis. These studies outlined the features of inherited thrombophilia in Japanese children: (i) PC deficiency is the leading cause of inherited thrombophilia; (ii) the first presentation is preceding ICTH and/or PF within the first 2 weeks of life; (iii) PC-nagoya may be prevalent in pediatric but not adult patients with PC deficiency; and (iv) many survivors with PC deficiency have neurological sequelae, visual impairments and amputated extremities.

Neonatal non-inherited PC deficiency

The important observation was that 25% of patients diagnosed with congenital PC deficiency were born to healthy parents with normal PC activity. Their presentations were indistinguishable from those of patients with *PROC* mutations. However, the low PC activity gradually increased and attained the normal range, but did not convey any recurrent VTE by the age of 1 year. In this setting, such patients could be diagnosed as having "neonatal non-inherited PC deficiency."

Manco-Johnson *et al.*[21,22] first reported 11 newborn infants with undetectable PC activity and/or antigen, which proved on subsequent follow up to be acquired. Five of them developed renal, aortic and cerebral thrombosis. Severe to moderately severe PC activity (<0.1 U/mL or <10%) could occur and often led to thrombosis in the stressed newborns as non-inherited PC deficiency. Matsunaga *et al.*[23] recently described a case of neonatal asphyxia and acute renal failure associated with isolated PC deficiency. The term infant had 6% of PC activity, 61% of PS activity, but no mutations in the promoter and coding regions of *PROC*. The hypercoagulability and dissociated PC and PS levels were unexplained by high D-dimer levels, normal FVII activity and absent vitamin K deficiency. During the early neonatal period, plasma PC activity shows prominent wide range compared with PS or AT activity (Fig. 1). The "transient PC deficiency in infancy" should be noticed as a critical thrombophilia showing a mimicking feature of inherited PC deficiency. Further studies are needed to clarify the mechanisms of delayed PC maturation, focusing on the genetic and epigenetic factors regulating PC concentrations.[24,25]

Future directions

Severe or moderately severe PC deficiency occurs in newborn infants, and results in serious conditions. Activated PC products may be limitedly used for the treatment of inherited PC deficiency but not sepsis.[26] However, the clinical dilemma resides in the difficulty in discerning "inherited" from "acquired" PC deficiency at the first presentation. The phenotype and genotype of PC deficiency in Asian children should be clarified more to establish the screening methods, diagnostic guidelines, and optimal managements using PC agents.

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ORIGINAL ARTICLE

Paediatric presentation and outcome of congenital protein C deficiency in Japan

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Summary. Severe heritable protein C (PC) deficiency is quite rare, although heterozygous *PROC* mutation is the second leading cause of genetic predisposition to thrombosis in Japanese adults. The aim of the study was to search the optimal management, the paediatric onset and outcomes of PC deficiency were characterized in Japan. The genetic study, postmarketing survey of activated PC (aPC) concentrate (Anact®C) and intensive review in Japan for 20 years enabled the analysis of the disease onset, genotype, treatment and prognosis. Symptomatic PC deficiency was determined in 27 Japanese children. All but two patients presented within 16 days after birth (three prenatal and six neonatal onsets). Postnatal-onset cases had normal growth at full-term delivery. Of the 27 patients, 19 suffered intracranial thrombosis or haemorrhage (ICTH) (three foetal hydrocephalies), 16 developed purpura fulminans (PF) and 10 had both at the first presentation. ICTH preceded PF in both affected cases. Low PC activities of 18 mothers and/or 12 fathers indicated 20 inherited PC deficiencies (2 homozygotes, 11 compound heterozygotes and 7 heterozygotes) and seven unidentified causes of PC deficiency. Nine of 11 patients studied had *PROC* mutations. Four unrelated patients (50%) carried PC nagoya (1362delG). No PC-deficient parents had experienced thromboembolism. Of the 18 patients with aPC therapy, two died and eight evaluable survivors had neurological sequelae. This first comprehensive study of paediatric PC deficiency suggested that perinatal ICTH was the major presentation, occurring earlier than neonatal PF. PC nagoya was prevalent in paediatric, but not adult, patients in Japan. Early maternal screening and optimal PC therapy are required for newborns at risk of PC deficiency.

Keywords: activated protein C therapy, intracranial thrombosis/haemorrhage, protein C deficiency, purpura fulminans

Introduction

Protein C (PC) is a vitamin K-dependent serine protease zymogen synthesized in hepatocytes. The anticoagulant factor circulates at a low concentration in the plasma, thus being activated by the complex formation with thrombin on the endothelial cell receptor thrombomodulin, and more effectively by binding to the endothelial PC receptor [1]. Activated PC (aPC), augmented by protein S (PS), factor V (FV) and lipid cofactors of lipoproteins and phospholipids, cleaves critical sites in the activated procoagulant FV and

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Abbreviations: aPC, activated protein C; AT, antithrombin; DVT, deep vein thrombosis; ICTH, intracranial thrombosis/haemorrhage; PC, protein C; PF, purpura fulminans; PS, protein S; PT, prothrombin time; VTE, venous thromboembolism.

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FVIII, thus inactivating the enzymes [2]. Therefore, PC-deficient persons have a decreased capacity to control the propagation of thrombin generation by FVa and FVIIIa after the activation of coagulation cascade, according to the circulating functional PC irrespective of the defective production or increased consumption.

Congenital PC deficiency is an autosomal recessive thrombophilia [3]. FVLeiden (G1691A) and FII mutation (G20210A) are the major predisposition to thrombosis in Caucasians, but not in Asian ancestries. Severe PC deficiency is exceptionally rare in all racial backgrounds, despite the frequency of mutation carriers [4–6]. Recent reviews described only eight and 12 survivors with long-term therapy in the United Kingdom [7] and North America [3] respectively. Purpura fulminans (PF) is the major presentation of biallelic *PROC* mutants [7], while it occurs in children with acquired PC deficiency. Infectious PF arises from PC-deficient hypercoagulability incited by the production of lipopolysaccharide or auto-antibodies to PC [8]. However, it remains unclear why PF is an exclusive phenotype in patients with severe PC deficiency. Several reports have uncovered the genetic background of thrombophilic adults [9,10]. Two major studies in Japan demonstrated that 65% of adult patients with deep vein thrombosis (DVT) showed low activity of PS, PC and/or antithrombin (AT), and half of them carried heterozygous mutation of the genes [11,12]. Considering the frequency of each mutant, heterozygous *PROC* mutations may greatly contribute to the development of venous thromboembolisms (VTE) and strokes in Japanese adults. However, there is limited information about PC-deficient children in Japan [13–16].

aPC exerts anticoagulant and cytoprotective effects [17]. The latter actions of anti-inflammation, antiapoptosis and endothelium stabilization are facilitated via the specific receptor expressed on immunocompetent cells [18–20]. aPC may thus be the sole anticoagulant agent that reduced the mortality of sepsis patients [21]. Plasma-derived aPC concentrate (Anact®C, Teijin, Tokyo & Kaketsuken, Kumamoto, Japan) is a PC agent licensed in Japan, and has been used in limited cases with congenital PC deficiency.

In this study, we analysed the clinical features of PC-deficient children based on our experiences with the genetic study, postmarketing study and intensive review of patients in Japan. The diagnosis and use of PC agent at the onset of PC deficiency were discussed.

Materials and methods

Patients and data collection

Clinical data were collected from our genetic study, postmarketing study and literature review. Seven children were diagnosed to have symptomatic PC deficiency as assessed by their plasma PC activity and the genetic study at Kyushu University from 2008 to 2010. This study was certified by the Institutional Review Board of Kyushu University (#232–02). Anact®C is only licensed for the treatment of VTE due to congenital PC deficiency in Japan. Twenty-nine children treated with human plasma-derived aPC concentrate (Anact®C, Teijin, Tokyo & Kaketsuken, Kumamoto, Japan) were collected in the registry between December 2000 and March 2011. The indication for aPC treatment was limited to patients who fulfilled at least one of the five diagnostic criteria for congenital PC deficiency; 1) $\leq$60% PC activity and a ratio of <0.7 for the PC activity to FVII activity, 2) $\leq$60% PC activity and a history of thrombosis, 3) $\leq$60% PC activity and congenital PC-deficient family members, 4) 60~80% PC activity, <0.7 PC to FVII activity or antigen ratio, and a history of thrombosis or congenital PC-deficient family members, or 5) a genetic diagnosis of PC deficiency. We obtained permission to access anonymous data from the postmarketing survey from each attending doctor via the pharmaceutical company. The data covered 66% of all aPC-treated cases. Of the 75 patients who had 99 aPC-treated episodes, 34 patients who did not fulfil the above criteria for congenital PC deficiency were excluded. The data collected included gender, age at each treatment, family history of PC deficiency, previous history of VTE and associated conditions, PC antigen levels and activity, FVII activity, genetic study results, outcomes and complications and the utility, efficacy and adverse events of aPC concentrate treatment. We further reviewed all publications and sentinel sources, including conference presentations in Japan, using Japana Centra Revuo Medicina, PubMed, and Google Scholar for citations published from 1981 to March 2011. The search terms were congenital, inherited, hereditary or heritable PC or PS deficiency, PF and thrombophilia. Finally, we determined that there were 27 PC-deficient patients <20 years of age who could be analysed; seven from our genetic study, and 20 from the aPC agent study and literature review.

Coagulation study

Coagulation tests were performed as described previously [22]. The anticoagulant activities of PC and PS were determined using the Staclot Protein C kit and the Staclot Protein S kit (Diagnostica Stago, Asnieres, France) respectively. A chromogenic substrate was used to assay for AT activity as the heparin-dependent inhibition of bovine thrombin (Chromostrate ATIII kit, Hitachi, Tokyo, Japan). Pooled normal plasma was used as the adult standard. A level within 2SD was regarded to be the reference interval, and a level below 3SD was defined as reduced activity. The PC activity/antigen levels for term and preterm infants

were assessed by previous reports [23,24]. The severity of PC deficiency was defined based on the PC activity [3]; <0.01 U mL^{-1} (<1%) as 'severe', 0.01–0.2 (1–20%) U mL^{-1} as 'moderately severe', and >0.2 (>20%) U mL^{-1} as 'mild'.

Gene analysis of PC, PS and AT

Genomic DNA was extracted from peripheral blood leucocytes after informed consent was obtained. Direct sequencing of the polymerase chain reaction (PCR) products was performed for the coding regions of PC gene (*PROC* exons 1–9) as described previously [11]. When patients had low PS or AT activity, PS (*PROS1* exons 1–15) and AT (*SERPINC1* exons 1–6) genes were also analysed. The exon and exon–intron boundary regions of each gene, including the promoter region, were amplified by PCR, and the products were then subjected to direct sequencing using an ABI 377 (Perkin Elmer Applied Biosystems, CA, USA).

Statistical analysis

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

Results

aPC concentrate-treated children in the postmarketing study

The demographics of 29 patients <20 years of age treated with Anact®C are shown in Table 1. The male/female ratio was 11/18. The median age at presentation was 3 months (range: 3 days–16 years). The median PC activity was 21% (range: 0–57%). There were no adult patients who developed PF included in the registry data. Five of nine PF patients developed ICTH concurrently. Six patients suffered from ICTH but not PF. Fourteen others had pulmonary thromboembolism, DVT and hepatic thrombosis with or without venoocclusive disease during the course of malignancies. The clinical profiles did not differ between the PF and ICTH groups, except for a higher incidence of concurrent infections in PF patients ($P = 0.044$). The ICTH patients had no triggers at the presentation. Although 14 others met the aforementioned criteria of 1) higher PC activity, absent family history and associated conditions suggested a low possibility of inherited PC deficiency. The clinical outcomes after aPC therapy did not differ among the three groups of ICTH, PF and other VTE patients.

Symptomatic congenital PC deficiency in Japanese children

A total of 27 PC-deficient patients <20 years of age were finally collected in Japan for this analysis, including a pair of first cousins (Pt2–1, Pt2–2) and a set of twins (Pt9–t1, Pt9–t2). There was no consanguinity. The patients were divided into two groups; 18 inherited (Table 2) and nine non-inherited deficiencies (Table 3) based on the PC activity of their parents. The patients in each group are listed in order of their age at the first presentation of ICTH (*upper*) or other conditions (*lower*). Of the 19 ICTH patients, 10 had PF that did not precede ICTH. Although three had foetal hydrocephalus (inherited Pt1, Pt2–1; non-inherited Pt1), no antenatal diagnosis of PC deficiency was made. Of the eight others (inherited Pts15–18, non-inherited Pts4–7), PF was the first presentation of six patients. One developed acute renal failure (non-inherited Pt6), and the other suffered from a chance

Table 1. Demographics of activated PC concentrate-treated children according to the major lesions.

	PF[a]	ICTH[b]	Other VTE[c]	*P* value	
				a vs. b	a+b vs. c
Number of Pts, male : female	9, 3 : 6	6, 1 : 5	14, 7 : 7	0.604	0.264
Age*, median (range)	15 days (3–422)	14 days (7–317)	4.4 yrs (58 days–16 yrs)	0.768	<.0001
PC activity, median (range) %	0 (0–31)	18 (0–40)	28 (0–57)	0.195	0.006
Family history positive, %	78	83	0	>.999	<.0001
Associated conditions					
No	4	6	0	0.044	0.0002
Yes infection	5	0	1		
malignancy	0	0	12		
cardiac disease	1	0	1		
Efficacy; yes/no/unknown	5 / 4 / 0	1 / 1 / 3	7 / 4 / 1	>.999	>.999
Clinical utility; yes/no/unknown	3 / 1 / 5	3 / 0 / 3	7 / 0 / 7	>.999	>.999
Survival outcome, (%)	7 (78)	6 (100)	7 (50)	0.486	0.050

*The age at onset of disease was defined as the first time PC activity was determined.

Pts: patients, PF: purpura fulminans, ICTH: intracranial thrombosis and/or haemorrhage, VTE: venous thromboembolism

Other VTE included pulmonary thromboembolism ($n = 1$), DVT with haemothorax ($n = 1$), hepatic thrombosis with or without venoocclusive disease ($n = 12$) during the course of aplastic anaemia, myelodysplastic syndrome, haemophagocytic syndrome, leukaemia, neuroblastoma, or leukodystrophy.

Table 2. Reports of symptomatic children with inherited protein C deficiency in Japan.

		Age at presentation, days			Patient						
Pt	Neonatal asphyxia	ICTH	PF	Ocular bleed	PC-act (%)	*PROC*	type*	Parents low PC-act	FH†	aPC therapy	Outcome
Intracranial lesions											
1	yes	GA33w	3	no	<10	ex9:1235G>A(V339M)	homo	M F	no	yes	died (6 m)
2–1	yes	GA34w	26	46	<10		c.heter	M F	yes	yes	amputation, PMR
2–2	yes	0	no	12	<10		heter	M	yes	yes	PMR
3	no	0	2	yes	3	ex9:1362delG*(stop420→462)	homo	M F	no	yes	NR
4	no	0	0	NR	<10	ex9:1190G>A(V297M)/ex9:1362delG*	c.heter	M	yes	yes	amputation, PMR
5	no	0	7	yes	<10		heter	M	no	no	PMR,VP-shunt
6	no	1	1	NR	5	ex3:296G>A(E26K)/ex9:1362delG*	c.heter	M F	no	no	favourable, PMR
7	no	2	2	yes	<10		c.heter	M F	no	yes	discharge (2 m)
8	NR	>3	3	NR	1.5		c.heter	M F	NR	yes	PMR
9–t1	no	6	no	6	<10	ex8:887C>T(L223F)/ex9:1360G>C(W380C)	c.heter	M F	twin	yes	PMR
9–t2	no	6	no	NR	<10	ex8:887C>T(L223F)/ex9:1360G>C(W380C)	c.heter	(M F)	twin	yes	PMR
10	no	7	no	NR	19		heter	M	no	yes	NR
11	no	13	16	NR	8	ex4:258delT 18Stop/ex9:905C>T(R229W)	c.heter	M F	no	yes	Ep, PMR
12	NR	16	no	no	17		heter	M	yes	yes	NR
13	NR	10 m	no	no	40		heter	F	yes	yes	NR
14	no	13 m	13 m	no	8	ex4:356G>T(D46Y)	heter	M	yes	yes	severe PMR
Extracranial lesions											
15	no	no	0	NR	<5	ex7:725C>T(R169W)/ex9:1362delG*	c.heter	M F	no	no	normal develop
16	no	no	1	yes	20		heter	M	no	no	no PMR, died
17	no	no	2	yes	24		c.heter	M F	NR	yes	NR
18	yes	no	10	yes	<10		c.heter	M F	yes	yes	NR

*The allele type of the *PROC* mutation was estimated by each parent's PC activity unless the genetic study was performed.

†Family history (FH) includes young thrombosis, habitual abortion, foetal/neonatal hydrocephalus and consanguineous marriages.

Pt: patient, PC: protein C, aPC: activated PC, NR: not recorded, ICTH: intracranial thrombosis, haemorrhage and/or hydrocephalus, PF: purpura fulminans, homo: homozygote, c.hetero; compound heterozygote, PMR: psychomotor retardation, Ep: epilepsy, Pt2–1 and Pt2–2 are first cousins. Pt9–t1 and Pt9–t2 are twins.

Four of 11 genetic studies (Pt4, 9–t1, 9–t2, 11 and 14) were completed at Kyushu University. The base number was referred to Accession No. NM 000312 Version 3, 1790np (mRNA) PRI 27-NOV-2011. Four patients among eight unrelated families carried PC nagoya 1362delG* (stop420→462).

Table 3. Reports of symptomatic children with non-inherited protein C deficiency in Japan.

		Age at presentation, days			Patient						
Pt	Neonatal asphyxia	ICTH	PF	Ocular bleed	PC-act (%)	*PROC*	type*	Parents low PC-act	FH†	aPC theropy	Outcome
Intracranial lesions											
1	no	GA36w	no	NR	37		unknown	NR	NR	no	PMR
2	no	3	no	NR	24	no mutation	unknown	normal	no	no	PMR
3	no	5	no	NR	34		unknown	NR	no	no	discharge (18d)
Extracranial lesions											
4	NR	no	0	NR	23		unknown	normal	no	yes	died
5	no	no	1	NR	30		unknown	normal	NR	no	NR
6	yes	no	no	no	6	no mutation	unknown	normal	no	yes	renal failure
7	no	no	no	11	28		unknown	normal	no	no	blindness

*The allele type of the *PROC* mutation was estimated by each parent's PC activity unless the genetic study was performed.
†Family history (FH) includes young thrombosis, habitual abortion, foetal/neonatal hydrocephalus and consanguineous marriages.
Pt: patient, PC: protein C, aPC: activated PC, NR: not recorded, ICTH: intracranial thrombosis, haemorrhage and/or hydrocephalus, PF: purpura fulminans, homo: homozygote, c.hetero; compound heterozygote, PMR: psychomotor retardation.
Two genetic studies (Pt2 and Pt6) were completed in Kyushu University.

vitreous haemorrhage (non-inherited Pt7). Postnatal-onset infants showed normal birthweight (median 2848 g) at full-term delivery. Severe or moderately severe PC deficiency was found in 85% of the patients in the inherited group and 15% in the non-inherited group. The genetic study and parents' PC activity indicated that the 20 inherited patients had two homozygous [14], 11 compound heterozygous [13,16] and seven heterozygous PC deficiency [15]. PC nagoya was found in half of the unrelated patients with genetic diagnosis. There were no genotype-specific features of PC-deficient children. Only five patients declared a family history suggestive of inherited thrombophilias. Mothers ($n = 18$) or fathers ($n = 12$) had mild PC deficiency. None of them had experienced thromboembolism before the diagnosis of their children (Table 2).

The aPC agent was given to 18 patients (67%) for the treatment of PF (100 U kg^{-1} bolus, and 600–800 U kg^{-1} per day continuous infusion for 6 days), or other VTE (200–300 U kg^{-1} per day continuously infusion for 6 days). Of the 18 aPC-treated patients, two died of infection, and eight of 10 survivors with recorded outcomes had neurological sequelae.

Discussion

This study documented that the first presentation of Japanese children with PC deficiency was exclusively ICTH and/or PF within 3 weeks after birth. Although each incidence was similar, PF did not precede ICTH in patients having two lesions. ICTH developed prenatally without apparent triggers, while PF occasionally occurred with infection. PC nagoya was prevalent in Japanese children with PC deficiency. The mothers of >75% of infants showed low PC activity, but only 25% infants had a declared thrombophilic family history at presentation. The mortality rate was 8%, and most of the survivors had neurological deficits irrespective of aPC therapy. Screening for PC-deficient mothers may allow for earlier intervention to improve the outcome of affected newborns.

Neonatal PF is the hallmark of heritable PC deficiency. Of more than 30 reported PF cases, one PS-deficient homozygote developed neonatal PF [25], and five patients had a *PROS* mutation in association with three FVLeiden [25–29]. In our review of Japanese patients, there were no PF cases with inherited PS/AT-deficiency. Four PS-deficient Japanese children developed DVT after the age of 3 years [30]. There is a missing link between the rare *PROC* mutants and the exclusive phenotype of PF neonates. The frequency of *PROS* mutant heterozygotes is higher in the general Japanese population (1.12~1.8%) [31–33] than was seen in Caucasians (0.03~0.13%) [34]. The founder effects of PS-tokushima might account for PS-deficiency as the leading cause of genetic thrombophilia in Japanese adults [12,32]. On the other hand, the frequency of *PROC* mutations in Japanese subjects (0.16%) [35] is similar to that in Caucasians (0.2–0.3%) [4,5]. PC nagoya is one of the five major genotypes of PC deficiency in Japan. However, no PC nagoya but, recurrent mutations related to CG>TG and CG>CA transitions, were found in adult Japanese VTE cases [11,12]. The high frequency of PC nagoya in paediatric, but not adult, patients suggested a distinct contribution of this gene to the development of thrombosis between biallelic mutants and heterozygous ones, as the founder effect in Japan.

Perinatal ICTH and neonatal PF were the exclusive phenotypes of paediatric PC deficiency. ICTH occurred earlier than PF as the first presentation. Hydrocephalus was a unique manifestation in PC-deficient foetuses. Fong *et al.* [36] have recently reported sisters with cerebral palsy who experienced periventricular haemorrhagic infarction caused by heterozygous *PROC* mutations. Postnatal magnetic resonance imaging showed that the brain lesions were consistent with bilateral cerebral intramedullary venous thrombosis occurring at under 28 weeks of gestation for the

older sister and around the time of birth for the younger sister. Mothers with FVLeiden or FII G20210A are at risk of delivering low birthweight infants [37]. On the other hand, in the series of subjects with PC deficiency (Tables 2 and 3), premature birth or intrauterine growth retardation was unremarkable. Postnatal cases had appropriate birthweight at full-term deliveries. This may be corroborated by the fact that homozygous PC-deficient mice are born and die shortly after birth, whereas homozygous PS- or AT-deficient mice result in foetal loss.

PC-deficient heterozygotes were occasionally found in the series of paediatric patients. Of 16 PF children in Turkey (median age: 2 years, range 3.5 months ~12 years) [38], six patients (38%) carried FVLeiden (five heterozygotes and one homozygote), but none had FII G20210A. Of the six PC- and nine PS-deficient patients, only one had inherited thrombophilia of PS-deficiency. All six PC-deficient patients were <2 years of age, and four of them had severe infection. Hypercoagulability of inherited thrombophilia depends on the genetic background of patients [39]. Acute infectious PF occurs across all age groups, and the patients have been rarely assessed by genetic study [15]. Taken together, these data indicate that additional insults, including infection, might contribute to the development of PF in heterozygous PC-deficient infants.

The plasma PC concentration physiologically increases from birth until age 6 months [3]. Vitamin K deficiency predisposes infants to depressed PC activity. Of 11 patients assessed by *PROC* sequencing, two infants (Table 3; Pt2 and Pt6) carried no PC mutation. Selective low PC activity continued in both patients, despite their normal PS/AT/FVII levels and absent protein induced by Vitamin K absence or antagonists-II. Our study demonstrated no polymorphisms in the promoter regions of *PROC* which might have affected the PC levels of patients. The plasma PC and PS levels could be differentially influenced by other factors in early infancy. The presence of borderline PC activity in infancy hardly predicts the PC-deficient heterozygotes. Although the genetic study is essential for the diagnosis of paediatric thrombophilias, severe PC deficiency may be a critical perinatal condition beyond the patient genotype. Although >75% of mothers showed low plasma PC levels, all PC-deficient mothers were healthy up until the delivery. Pt3 in Table 2 underwent successful aPC prophylaxis at surgery, as reported elsewhere [40]. PC replacement may be useful for VTE prophylaxis in pregnancy and Caesarean section. The aPC therapy might be also life saving for Japanese children with PC deficiency; however, most survivors had serious long-lasting sequelae despite treatment. Further studies should be directed towards early maternal screening and optimal PC therapy for newborns at risk of PC deficiency.

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Contributions to authorship

SO was the principal investigator taking primary responsibility for the study. DK organized coagulation and genetic studies. TK, MO, TD, MI, JY and SO treated and enrolled the patients. YK and MU managed and performed the laboratory work for this study. TI, AS, HK and TH helped to collect information of all paediatric/perinatal cases of PC deficiency in Japan. SO wrote the manuscript.

Disclosures

The authors stated that they had no interests which might be perceived as posing a conflict or bias.

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日本小児科学会雑誌　117巻10号　1538～1544（2013年）

日本小児血液・がん学会推薦総説

小児期に発症する遺伝性血栓症～プロテインCの重要性～

九州大学大学院医学研究院周産期・小児医療学

大賀正一

キーワード：血栓性素因，栓友病，電撃性紫斑病，脳静脈洞血栓症，深部静脈血栓症，肺動脈血栓塞栓症

はじめに

止血はすべての動物が備える出血に対する生体反応である．この機序は，感染に対する基本的な生体防御機構である免疫系と深く関わる．凝固系と補体系を司る因子には構造や機能に類似点が多い．好中球の細胞外トラップ（neutrophil extracellular traps：NETs）や感染早期に産生されるフィブリノゲンやサイトカインは，炎症と凝固を結ぶ機構である．血栓には，傷害部位の出血を阻止する生理的血栓と，何らかの病態により血管内で形成され，組織・臓器の循環を障害する病的血栓がある．傷害局所における止血血栓の形成と退縮には凝固線溶系の働きが不可欠だが，それ以外の部位では凝固制御系が全身の不適切な血栓形成を阻止している．

プロテインC（PC）は，プロテインS（PS）およびアンチトロンビン（AT）とともに3大自然抗凝固因子である[1]．PCの先天的欠損あるいは後天的欠乏から，新生児や重症感染症患者は電撃性紫斑病を発症する[2]．PCはトロンボモデュリン（TM）とトロンビン（Factor IIa：FIIa）の複合体によって活性化される．近年，活性化PC（APC）は抗凝固作用のほかに，その直接的な細胞保護作用が，TMの多彩な抗炎症作用とともに注目されるようになった[3]．抗凝固因子関連製剤（AT，APC，TMなど）の敗血症性ショックなどに対する臨床的有用性の評価は難しいが，小児・新生児領域での使用経験も集積されつつある．

小児の血栓症は成人よりまれであるが，新生児と思春期に頻度が高くなる．近年，国内外でその増加が認識されてきた[4]．血栓症は多元病であり，動脈硬化や糖尿病などのいわゆる成人病（non-communicable disease）と深く関連する．栓友病（thrombophilia）という病名は，1965年にEgebergがAT欠損症の家系に，はじめて用いた概念である．現在では，特発性あるいは家族性血栓症と診断されていた患者に遺伝子変異が同定され，遺伝性血栓症の確定診断に至る例が増加している．成人における血栓症の遺伝的背景は，「栓友病」という単一遺伝子病ではなく，寄与の大きさに幅のある「血栓性素因」である．一方，小児では，致死的でかつ重度の障害を残す稀な遺伝病としての「栓友病」を見逃すことはできない．

本稿では，小児血栓症の遺伝的背景について，感染，炎症と免疫とのかかわりから，とくに新生児・乳児におけるPC欠乏の重要性について解説する．

血栓症と血栓性素因

血栓症とは，「止血機構が過剰に作動して形成された病的血栓による臓器障害」と定義される．まだ血栓を形成していないが，これを生じやすい過凝固の身体的要因が血栓性素因である．血栓傾向の複合的要因が絡み合って，血栓症を発症する．血栓形成には，古典的なVirchowの3徴，①血管壁，②血流の変化，および③凝血能が相互に作用する．動脈血栓の形成には血管壁と血流の変化が，また静脈血栓の形成には凝血能が深く関与する．どこに血栓を起こしやすいか（例えば頭蓋内と外）は，血管壁の構造と走行の特性が関与する．局所ではなく，病的血栓を全身性に形成しやすい要因（誘因，関連する病態および基礎疾患）について遺伝と環境を軸に図1にまとめた．

血友病と対比させ，血栓症のみが唯一の表現型であ

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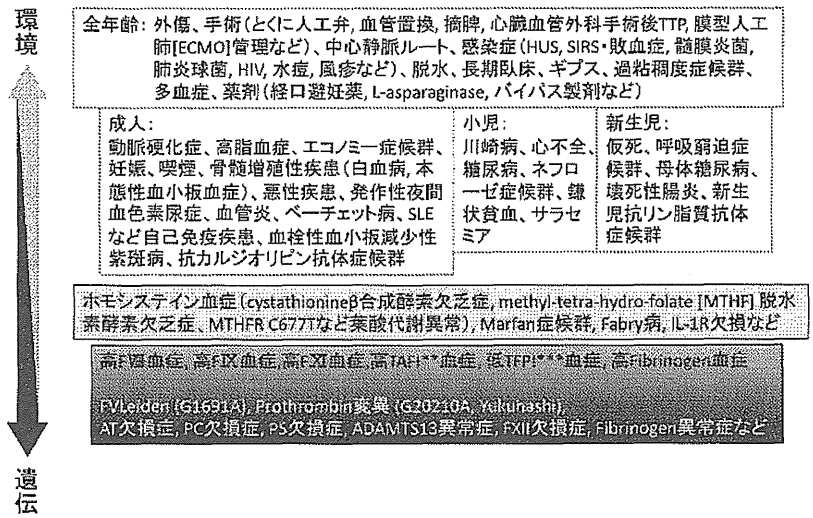

図1 血栓塞栓症をおこす素因（病態）

TTP：thrombotic thrombocytopenic purpura，ECMO：extracorporeal membrane oxygenation，TAFI：thrombin-activatable fibrinolysis inhibitor，TFPI：tissue factor pathway inhibitor

る単一遺伝子病を「栓友病」とすれば，PC，PSおよびAT遺伝子異常による抗凝固因子欠損症（機能喪失）がこれに相当する．両アレル変異や欠失による重症型として電撃性紫斑病が有名である．軽症・中等症の活性低下を示すヘテロ変異保有者は，若年成人になって下肢の深部静脈血栓症（deep vein thrombosis：DVT）や肺血栓塞栓症などの静脈血栓塞栓症（venous thromboembolism：VTE）を発症しやすい「血栓性素因」を有する．両アレル異常によるATとPS欠損は胎生致死とされ，患児の報告は極めて例外的である[5][6]．外因系関連の組織因子（tissue factor：TF）/FVIIa，プロトロンビンとその受容体protease activated receptor (PAR)-1は胎児の成育に必須である．抗凝固因子も妊娠維持に不可欠で，PC，PS及びAT欠損症の母はしばしば不妊症/不育症の病歴を持つ．新生児電撃性紫斑病の報告はほぼすべて両アレル異常の重症PC欠損症だが，その理由は明らかでない．

凝固因子の異常による栓友病（機能獲得）には，APCに抵抗性をもつFactor V Leiden (FVL) がある．FVLは欧米人に最も頻度の高い遺伝性血栓性素因で，静脈血栓塞栓症患者の20～60％をしめる[7]．このヘテロ接合体の頻度はコーカサス人一般集団の5～8％に及び，VTEの発症リスクは4～7倍高い．FVLホモは0.18％とまれだが，VTEリスクは80倍となる．欧米で次に頻度の高い遺伝性血栓性素因はプロトロンビン（PT）非翻訳領域の変異G20210Aで，このヘテロ変異の保有者はVTEリスクが2～3倍高く，ホモではさらに上昇する．一方，日本など東アジア人にはFVLとPTG20210Aはみつからず[8]，中国でもPC異常が主要な血栓性素因である[9]．日本から最近AT抵抗性を示す血栓症家系PT-Yukuhashiが報告された[10]．今後，全ゲノム解析はさらに新規変異/多型を同定するであろう．血栓発症のリスクについては，初発と再発の何れにおいても，PC及びAT欠損者のほうがFVLやPT多型を有する者よりも高い（表1）．このような背景から，血栓症の多い欧米における栓友病thrombophiliaという病名は，日本では一般に遺伝性血栓症と訳されるのであろう．

日本人と欧米人のPS，PCおよびATのヘテロ変異保有者頻度を比較すると，PCとATについては，一般集団と成人DVT患者，いずれにも民族間で差はない．一方，日本人にはPS-Tokushimaが多いため，PS異常の頻度は，一般集団，成人DVTのいずれにおいても欧米人より高い．日本人の成人DVT患者85名について3因子活性をスクリーニングすると65％にいずれかの低下があり，各因子欠乏症例の約半分にヘテロ変異が確認され，全体の33％が遺伝性血栓症の保因者となる[11]．ヘテロ変異保有者の活性値は判別困難なこともあるが，成人DVT173名の3因子を直接遺伝子解析しても変異率は32％と変わらない[12]．

他にも血栓性素因を有する遺伝性疾患がある．高FVIII血症は10％の活性上昇がVTEの発症リスクを10％上昇させるようであるが，遺伝子異常はまだ同定されていない．フィブリノゲン異常症は出血と血栓の

表1　遺伝性血栓性素因を有する小児における血栓塞栓症発症の危険率

遺伝性血栓性素因	危険率（オッズ比）[95% 信頼区間]	
	脳血管閉塞（初回）	深部静脈血栓症（初回，再発）
Protein C 欠損症	9.3 [4.8-18.0]	7.7 [4.4-13.4]，2.4 [1.2-4.4]
Protein S 欠損症	3.2 [1.2-8.4]	5.8 [3.0-11.0]，3.1 [1.5-6.5]
Antithrombin 欠損症	7.1 [2.4-22.4]	9.4 [3.3-26.7]，3.0 [1.4-6.3]
Factor V G1691A	3.3 [2.6-4.1]	3.6 [3.8-4.8]，1.4 [0.4-1.2]
Factor II G20210A	2.4 [1.7-3.5]	2.6 [1.6-4.4]，2.1 [1.0-3.5]
Lipoprotein (a)	6.5 [4.5-8.7]	4.5 [3.3-6.2]，0.8 [0.5-1.4]
LA/aPL	6.6 [3.5-12.4]	4.9 [2.2-10.9]，
3つ以上の遺伝性素因	11.9 [5.9-23.7]	9.5 [4.9-18.4]，4.5 [4.5-6.9]

LA/aPL：lupus anticoagulants/antiphospholipid antibodies（文献4より抜粋）

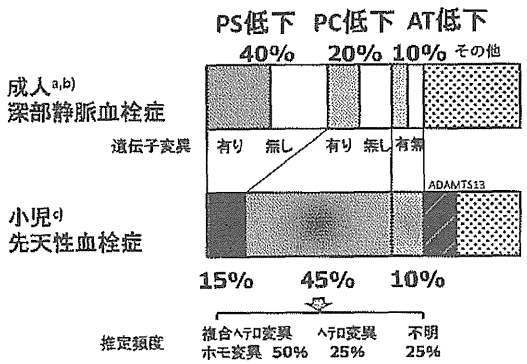

図2　日本人の成人と小児における血栓性素因
（文献11，12，17より改変）

両方をおこす．プラスミノゲン異常症（Tochigi など）は血栓性素因として否定されたが，FXII 欠損症，TM 異常症についてはまだ素因として確立していない．ADAMTS13 欠損症は慢性再発性 TTP として，通常新生児期に重度の溶血と黄疸で発症するが，血栓症の発症は遅れる．凝固・抗凝固・線溶因子の異常以外に，血栓症が特徴的症候となる先天性代謝異常症がある．高ホモシステイン血症は活性酸素による内皮傷害が動脈血栓を誘導する．メチレンテトラヒドロ葉酸還元酵素遺伝子多型（MTHFR C677T）の頻度は日本人に高く，ホモシステイン血症をきたし脳梗塞との関連が示唆されている．先天性ネフローゼも血栓症を合併する．IL-1Ra を含む領域の染色体欠失による新生児自己炎症性疾患にも血栓症が報告された[13]．発作性夜間血色素尿症は小児には極めて稀で血栓症も通常みられない．小児悪性腫瘍治療中の中心静脈ラインの血栓リスクに及ぼす遺伝的素因の寄与は少ないようである[14]．

小児血栓症の分子疫学と臨床像

日本人小児に発症する遺伝性血栓症の全体像はこれまで明らかではなかった．石黒らは本邦で初めて小児血栓症に関する全国調査を行い，2006～10年の5年間に20歳未満に発症した先天性血栓症48例を集積した．その約70%は成人 DVT と同様に3大抗凝固因子異常であった（図2）．しかし，その内訳は異なり，半数以上は PC 異常症で，PS 異常 15%，AT 異常 10% と続いた．これは瀧らの凝固異常症全国調査および高橋らの新生児血栓症の全国調査[15]の結果を反映している．先天性 PC 欠損症と診断された小児に関して，活性化 PC 製剤（アナクト®C，化血研/帝人）の市販後調査，九州大学病院で約20年継続してきた血栓性素因解析の小児例，さらに会議録などの文献を網羅的に解析し，国内に23家系25例の PC 異常症を確認することができた[16]．11例に遺伝子解析が行われ7例が PC 遺伝子（*PROC*）のホモ（1例）または複合ヘテロ変異（6例）であった．両親の PC 活性から，患児の20%は *PROC* ヘテロ変異による PC 欠乏と推定されたが，変異のない例も確認された．出生時に血栓症の家族歴が明らかであったものは25%にすぎず，75%の患児の母は分娩までに血栓症を起こしていない PC 活性低下例であった．以上より，先天性 PC 欠損症と診断された小児の半数が *PROC* ホモか複合ヘテロ変異，25% がヘテロ変異，残りが原因不明と推定される（図2）．両アレル変異を有する患者の半数は PC-Nagoya（1362delG）を保有していたが，これは興味深いことに成人 DVT 例にはほとんど見つからない[11][12]．このことは成人血栓症では PC-Nagoya の寄与は大きくないが，小児血栓症においてはこれが重症 PC 欠損症をおこす重要な変異であることが示唆される．

小児 PC 欠損症の臨床像として，ほとんどが生後1か月以内に頭蓋内出血/梗塞（在胎33週～生後15日）か電撃性紫斑病で発症すること，60%は両者を合併し，頭蓋内病変が先行すること，眼病変でたまたま見つかる例があること，妊娠後期に先天性水頭症を指摘されている例があること，子宮内発育遅延はまれでほ

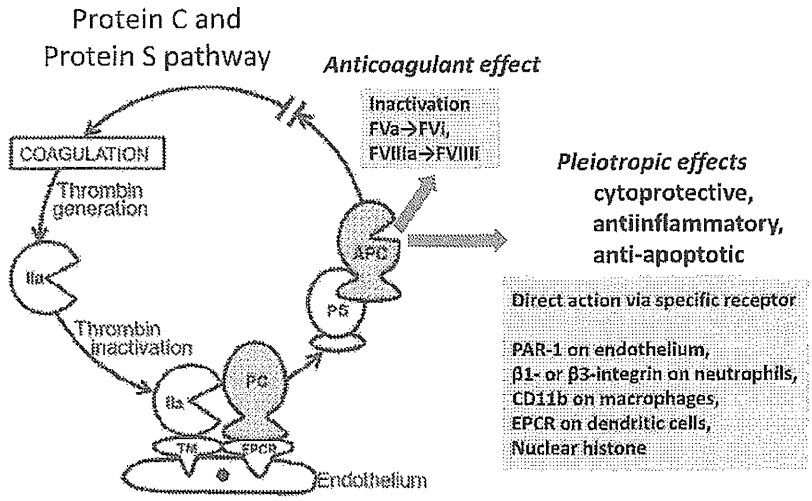

図3　プロテインCの活性化経路とその機能
(文献3及びAnderson JA et al. Can J Cardiol. 2013；29：75-88より改変)

とんどが満期産成熟児であること，などが明らかとなった[17]．さらに，PC活性の単独低値にもかかわらず，変異のない新生児腎不全の症例も確認された[18]．PC欠損症にはDirect sequencingのみでは同定できない大欠失も報告されている．しかし，私たちはこの変異が確認されなかった複数例に，PC活性のゆるやかな上昇と正常化を確認している．FXIとPAI-1の加齢による血中濃度の増加は，age-related stability element (ASE) とage-related increase element (AIE) の発現から説明され，PC濃度の安定化にもASEが関与する[19]．最近，ゲノムワイド解析から血漿PC濃度に影響する内皮PC受容体 (EPCR) 多型などが明らかになった[20]．新生児早期にPC活性が異常低値を示す「新生児一過性PC欠乏症」がどの程度血栓発症に関与するのか，遺伝要因か環境要因か，そしてどのような治療管理が適切かを今後明らかにする必要がある．

胎児水頭症，新生児頭蓋内出血・梗塞，脳静脈洞血栓症，硝子体出血，腎不全は，電撃性紫斑病以外に遺伝性PC欠損症を疑う症候である．乳児では感染に伴う電撃性紫斑病に遺伝性PC欠損症が隠れている可能性がある[21]．新生児から乳児期早期にはPC欠損症を，それ以降はPS欠損症とAT欠損症を考えて血栓性素因をスクリーニングするのが妥当であろう．

プロテインC (PC) の機能と活性化機構

PCは肝臓で合成されるセリンプロテアーゼ (serpin) の酵素前駆体で，ビタミンK依存性抗凝固因子である (図3左)．凝固の活性化がトロンビン (IIa) 産生を誘導すると，過剰なトロンビンが内皮細胞上に発現したTMに結合する．TMに結合したトロンビンは基質特異性が変化して，凝固促進作用を失いPCを強力に活性化する．EPCRはPCと結合して，TM結合IIaとともに活性化PC (APC) へと変化させる．APCは活性化した血小板に結合し，PSを補酵素として，FVaとFVIIIaを選択的に不活化することによりトロンビン産生を抑制して抗凝固作用を発揮する．また，血中APCはPAI-1を不活化して抗凝固作用をもたらす．このようにAPCはトロンビン生成に応じて産生されるので，凝固活性に反応してPC/PS制御系が活性化される．従って，PCとPS濃度の選択的低下はいずれも過凝固となる．可溶性EPCRは逆にAPC産生と機能を障害して過凝固を誘導する．この遺伝子*PROCR* variant (Ser219Gly) がPC経路の活性化を抑制し，血栓性素因であることが最近報告された[22]．

PCをコードする遺伝子*PROC*は染色体2q13-q14に局在する9つのexonからなる．遺伝性PC欠損症はPC抗原量・活性ともに低下するTypeIと抗原量は正常で活性のみ低下するTypeIIに分類される．250以上の血栓発症に関与する変異が同定されその部位は分散している[23]．FVLは，APCの限定分解部位の置換Arg506Glnにより不活化されなくなる．APCとPCはEPCRに同等の親和性を有するので，APCはEPCRから一部解離して作用する．EPCRと結合したAPCはPAR-1を介して，内皮に直接細胞保護作用 (抗炎症と抗アポトーシス) をもたらす．APCは好中球，マクロファージ，樹状細胞などの免疫担当細胞に，また神経細胞にも特異的受容体 (LRP8, S1P1など) を介して