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A Study of Congenital Protein C Deficiency With Infancy Onset of CADASIL in a Chinese Baby

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Objective: The main objectives of this article were to study a severe congenital protein C deficiency (PCD) in a patient with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and analyze the cause of this case.

Materials and Methods: We had recorded clinical manifestations of the patient, laboratory tests, imaging studies, and gene sequencing of the *PROC* gene and *NOTCH3* gene to study the disease in this family. We checked the change of NOTCH3 protein by immunohistochemistry.

Results: Laboratory studies of the patient had revealed that his PC activity was 3%. Magnetic resonance imaging results showed hyperintense lesions in the cerebral white matter of the patient. *PROC* gene and *NOTCH3* gene sequencing was performed among the family members. The patient was confirmed as homozygous for the (A-G)-12 at the transcription initiation site in the promoter region of the *PROC* gene and heterozygous mutation of the *NOTCH3* gene. Immunohistochemical results showed that NOTCH3 protein was positive in the skin vascular smooth muscle of the patient.

Conclusions: We studied a rare case of an infat boy diagnosed with both congenital PCD and CADASIL; congenital PCD was attributable to a compound that was homozygous for (A-G)-12 at the transcription initiation site in the promoter region of the *PROC* gene, and CADASIL was caused by missense mutation in exon 24 of *NOTCH3*. He was a sporadic patient with congenital PCD and CADASIL; it maybe that the deficiency of protein C led to early onset of CADASIL. The gene sequencing of *PROC* gene and *NOTCH3* gene may have important value for fertility guidance and prenatal diagnosis.

Key Words: PC deficiency, *PROC* gene, CADASIL, *NOTCH3* gene

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Protein C, the key component of the PC anticoagulant system, is an important vitamin K-dependent serine protease zymogen; it is synthesized mostly in the liver and secreted to the plasma as an inactive zymogene and regulates the physiologic coagulation cascade by inactivating factors Va and VIIIa upon activation by thrombin.[1–3] PC is encoded by the protein C gene (*PROC*) on chromosome 2q13-q14, which is composed of 9 exons and spans about 11.2 kb.[4] Heterozygous individuals have an ~7-fold increased risk of venous thrombosis compared with normal individuals. The homozygous (or compound heterozygous) state of protein C deficiency (PCD) is much rarer.[5,6] The prevalence of PCD in the healthy Chinese population is about 0.29%.[7] Although the prevalence of PCD in VTE patients rises to about 14% to 19%, the rate of PCD caused by a missense mutation of *PROC* is about 55% in China.[8,9] Severe congenital PCD is an uncommon yet life-threatening coagulopathy that usually presents with symptoms of purpura fulminans (PF) and severe disseminated intravascular coagulation as early as in the neonatal period.

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is the most frequent hereditary cerebral small vessel disease. CADASIL is caused by mutations in the neurogenic locus notch homolog protein 3 (*NOTCH3*) gene on chromosome 19q12,[10] which is involved in cell signaling and differentiation.[11] There is currently no treatment for this disorder.[12] Magnetic resonance imaging (MRI) studies have established microbleeds and subcortical lacunar lesions as additional radiologic features of CADASIL. At this point, there has been no case of suspected CADASIL with infancy onset that has the characteristics of cerebral white matter lesion and *NOTCH3* mutation.

Here, we studied a rare patient who was diagnosed with congenital PCD and CADASIL. This was a sporadic case diagnosed as both congenital PCD and CADASIL, and the first report of infancy onset of CADASIL.

MATERIALS AND METHODS

A 5-month-old boy was referred to Shenzhen Children's Hospital (China) for evaluation after frequent episodes of refractory PF had developed in his right calf over a month. The patient was a test-tube baby (G4P1), and his mother had a history of abortion and 2 miscarriages. We performed the PC activity testing of this patient and his immediate family, including his parents and all of his grandparents, to investigate the coagulation status; in addition, the testing of *PROC* gene sequencing was performed among this family members.

At the age of 11 months, our patient could not stay sitting up and had not shown any verbal skills. His physical and cognitive development was evaluated with Bayley Scales of Infant Development, with scores of 58 for mental

TABLE 1. The Results of Protein C Activity and Coagulation Function of Family Members

Assay	Age (y)	Protein C Antigen	Prothrombin Time	APTT	Thrombin Time	Fibrinogen (Clauss)	Factor VIII Activity	Factor V Activity
Normal range ± 2SD		70%-130%	10.5-14.5 s	32.2-49.1 s	13.2-20.1 s	2-4 g/L	55%-170%	70%-120%
Propositus	0.4	3	18.6	51.7	36.9	0.6	51	138
Father	36	53	12.6	29.8	17	2.53	125	127
Mother	35	32	14.6	40.5	15.4	3.27	136	119
Grandfather	65	47	13.1	34.1	16.3	2.18	142	130
Grandmother	64	133	12.4	29.2	17.3	2.8	120	91
Maternal grandfather	66	93	14.3	50	17.7	3.2	95	118
Maternal Grandmother	65	87	13.3	48	19.8	2.8	135	89
Aunt	30	79	13.9	36	15.1	2.7	62	73

APTT indicates activated partial thromboplastin time.

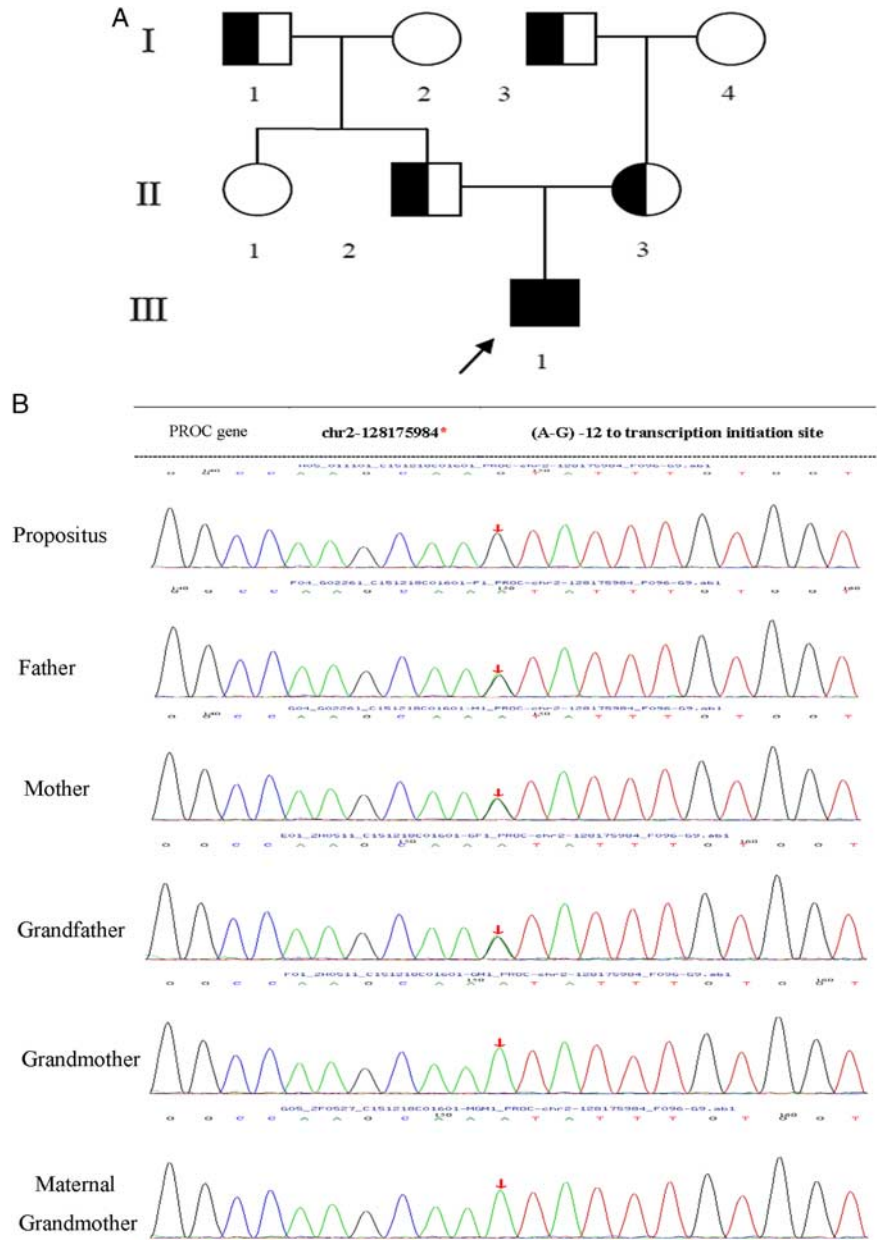

FIGURE 1. A, The pedigree of the family with protein C deficiency. The index patient was indicated with an arrow. B, Results of *PROC* gene sequencing for this family. *indicates the mutation location in this sequencing report.

status and 29 for psychomotor status, supporting that the patient had demonstrated developmental delays and cognitive impairment at that point of time. Thereafter, the patient was subjected to cranial MRI and *NOTCH3* gene sequencing. The changes of NOTCH3 protein with skin tissue was performed using an anti-*NOTCH3* antibody (ab23426; Abcam, UK) by immunohistochemistry staining (Supplemental Digital Content 1, http://links.lww.com/JPHO/A291).

RESULTS

Results of Protein C Activity and Coagulation Function of Family Members

The results of protein C activity and coagulation function of these family members had shower in Table 1. All of the tested indicators were abnormal for the patient; protein C activity level of the proband for him, his mother, and his father were reduced to 3%, 32%, and 53%, respectively. His prothrombin time, thrombin time, and activated partial thromboplastin time were increased to 18.6, 36.9, and 51.7 seconds. Factor V activity had increased to 138%, and the remaining indicators were also reduced for the patient.

Analysis of *PROC* Genotype and Plasma Protein C Phenotype

Nucleotide sequence analysis demonstrated that the proband was confirmed as a compound homozygous for (A-G)-12 at the transcription initiation site in the promoter region of the *PROC* gene (Fig. 1). His parents were confirmed as heterozygous. His grandfather, maternal grandfather, mother, and father all had low PC activity but were asymptomatic.

Results of MRI *NOTCH3* Gene Sequencing and Immunohistochemistry

The patient's cranial MRI showed hyperintense lesions in the cerebral white matter on T2-weighted images; cranial MRI of the patient and his mother are shown in Figure 2. On the basis of the above clinical manifestation and imaging findings, hereditary cerebral microangiopathy was suspected for this patient. Molecular analysis of the *NOTCH3* gene was performed, with automatic sequencing of exon 24 showing that the patient had a heterozygous mutations in

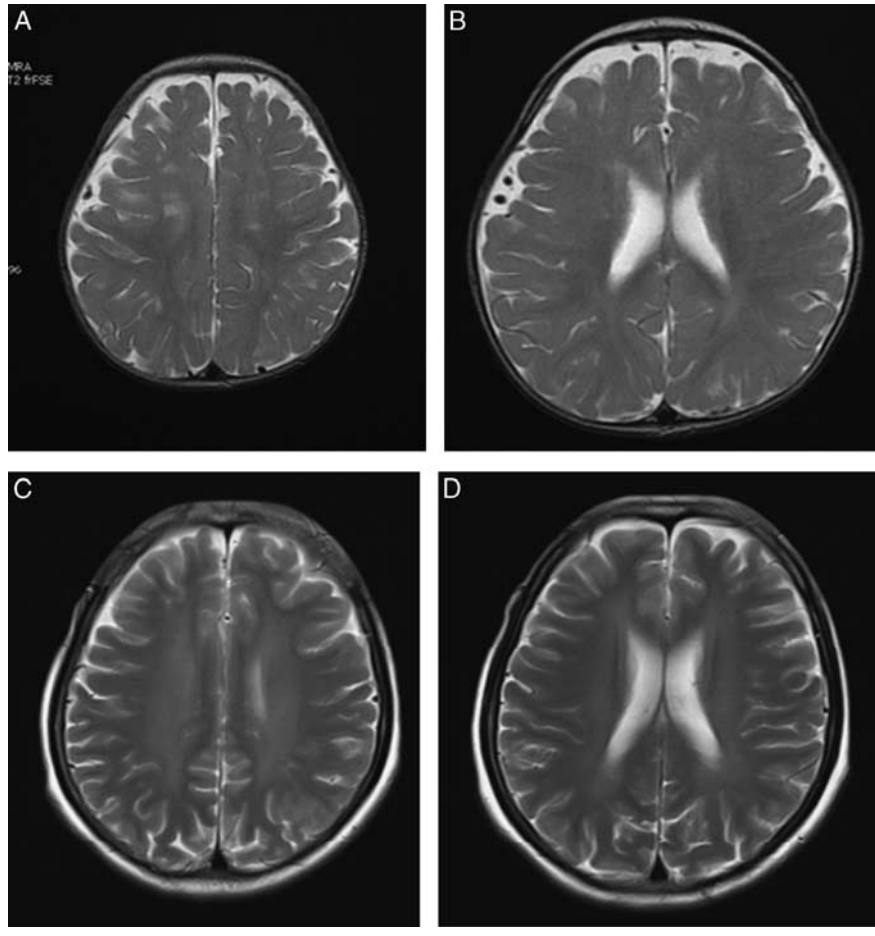

FIGURE 2. A, Magnetic resonance imaging scan of the brain showing multiple hyperintense centrum ovale white matter lesions on T2-weigthed images. B, Magnetic resonance imaging scan of the brain showing multiple hyperintense periventricular white matter lesions on T2-weighted images. C, Mother's magnetic resonance imaging scan of the brain showing multiple hyperintense centrum ovale white matter lesions on T2-weigthed images. D, Mother's magnetic resonance imaging scan of the brain showing multiple hyperintense periventricular white matter lesions on T2-weighted images.

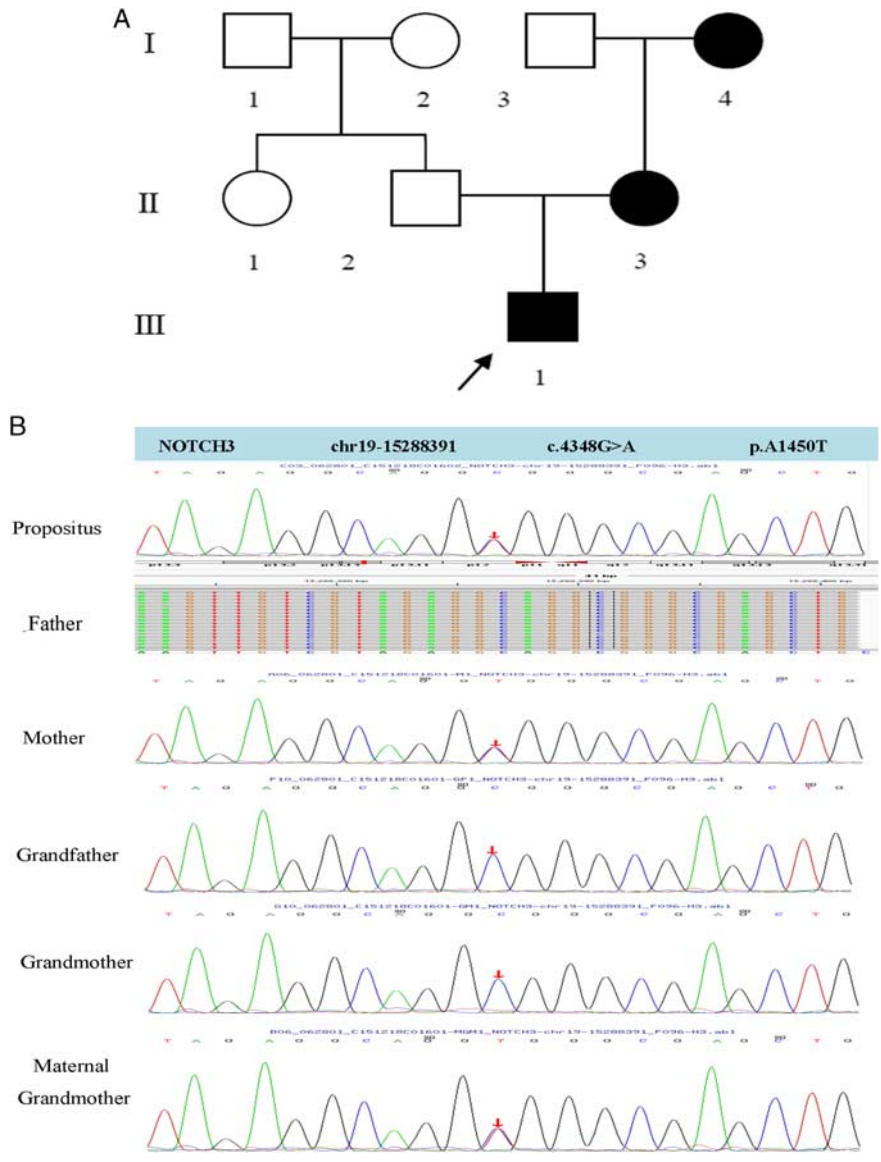

FIGURE 3. A, The pedigree of the family with NOTCH3 protien. The index patient was indicated with an arrow. B, Results of *NOTCH3* gene sequencing for this family.

exon 24(c.4348G > A), leading to a pathogenic amino acid substitution of p.A1450T (showed in Fig. 3). His maternal grandmother and his mother were asymptomatic but had the same mutation of *NOTCH3* gene. Results of immunohistochemistry showed that NOTCH3 protein was positive in the skin's vascular smooth muscle of the patient (showed in Fig. 4) and indirectly proved that heterozygous mutation of the *NOTCH3* gene caused CADASIL.

DISCUSSION

Hereditary PCD was first reported in 1981. The coagulopathy in PCD is caused by impaired inactivation of factors Va and VIIIa by activated PC after the propagation phase of coagulation activation. There have been > 160 PC gene mutations reported. The incidence of asymptomatic PCD has been reported to be between 1/200 and 1/500 in the population, whereas the incidence of clinically significant PCD has been estimated to be 1 in 20,000 in 1995.[13] The majority of symptomatic heterozygous PC-deficient patients may develop venous thrombosis and/or pulmonary embolism, which usually begins when the patient is between the ages of 15 and 30 years. Homozygous or complex heterozygous *PROC* mutations usually lead to neonatal fulminant purpura, and the patient may soon develop a broad blood clot in the microcirculatory system shortly during the neonatal period.[14] Our patient was confirmed as homozygous for the (A-G)-12 at the transcription initiation site in the promoter region of the *PROC* gene with neonatal fulminant purpura symptom. The implication of this mutation is to be further studied.

Most cases of severe PCD have been managed with combination therapy of PC replacement and warfarin as the preferred anticoagulant.[15,16] As PC concentrate is not available in China, to relieve the patient's thrombosis, we gave the child initially one dose (15 mL/kg) of fresh-frozen plasma (FFP) every day for 40 days until his skin lesions

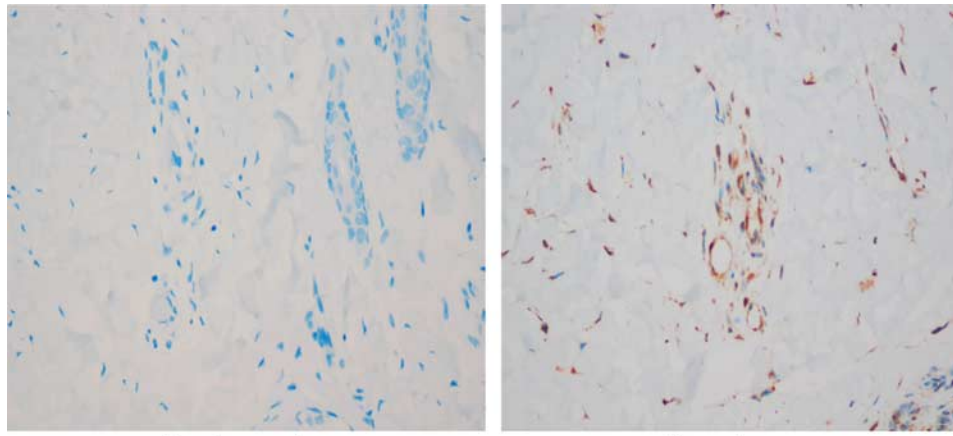

FIGURE 4. Immunohistochemistry of NOTCH3 protein with skin tissue. Immunohistochemistry data showed the NOTCH3 protein was positive in the patient's skin vascular smooth muscle (scar, ×200).

were completely resolved. In response, there was obvious improvement of his coagulation function. However, PC activity of the patient dropped consistently below 13% with a recurrence of PF in his calf, because of which we stopped FFP for 3 days. Consequently, the patient received 1 dose of FFP every day for 5 days and his skin lesions resolved again, but he still presented with thrombosis, because of which combination therapy was stopped; his PC activity was consistently below 10%, which made us speculate that the PC activity did not normalize even with FFP therapy.

CADASIL was initially thought to be a rare disorder, but increasing numbers of families have been identified worldwide.[17–21] The disease is clinically characterized by transient ischemic attacks, strokes, progressive subcortical dementia, migraine with aura, and mood disturbances. For a typical CADASIL patient, widespread areas of increased signal in the white matter are associated with focal hyperintensities in the basal ganglia, thalamus, and brain stem in MRI; the extent of white matter signal abnormalities is highly variable.[22] MRI signal abnormalities can also be detected during a presymptomatic period of variable duration.[23] CADASIL was an autosomal dominant inherited disease, characterized by midadult onset of cerebrovascular disease and dementia, but the majority of mutations are found in exon 4. Most studies indicate that pathogenic mutations have been found throughout exons 2 to 24, which are the exons that encode the EGF reporter. However, the mutation hotspots of CADASIL are located in exon 4 of the *NOTCH3* gene;[24] the prevalence of mutations in other exons varies between countries; in the French, German, and English CADASIL population, exon 3 is the second most frequently mutated exon, whereas, in Dutch CADASIL patients, exon 11 is the second most frequently mutated exon.[25] These identified mutations were clustered in exons 3, 4, 5, and 11 in mainland Chinese patients,[26,27] but the exon 11 was a mutational hotspot in Taiwanese patients.[28] In this study, we had reported a heterozygous mutation in exon 24 (c.4348G > A), which leads to a pathogenic amino acid substitution of p.A1450T. Immunohistochemistry results revealed that the NOTCH3 protein was positive in the skin tissue of our patient, which showed that these mutations had caused CADASIL. Nevertheless, his maternal grandmother and his mother also had the same mutation of the *NOTCH3* gene, but no clinical features of CADASIL such as cognitive impairment or migraine with aura were shown, which suggests that heterozygous mutations of c.4348G > A(p.A1450T) in exon 24 were either sex-dependent pathogenic mutations or not pathogenic mutations. Chen et al[27] found that most patients of Chinese origin were carrying p.Arg607Cys and p.Arg544Cys mutations, not p. Ala 1450Thr mutations. This was consistent with Rutten et al's[25] study, which demonstrated that missense mutations in *NOTCH3* that are not altering a cysteine residue are unlikely to be pathogenic. However, according to the research of Li et al,[29] a novel pathogenic variant of the *NOTCH3* gene, which was a heterozygous mutation of c.128G > C in exon 2, caused a cysteine to serine substitution at codon 43 in 2 Chinese CADASIL patients.[29] Moreover, it explains the phenomenon why his maternal grandmother and mother were not CADASIL. We suspected PCD to cause or aggravate CADASIL. Further research and analysis may help unravel the reasons of early onset in this patient.

The mean age of CADASIL patients who have the clinical symptoms is 45 years, and the duration of the disease varies between 10 and 40 years, and can also be observed as early as 20 years of age.[23] Mosca et al[30] had reported 140 CADASIL patients from Italy and China who were in the age group of 21 to 73 years. Abramycheva et al[31] had reported 30 white CADASIL patients who were in the age group of 22 to 73 years. Cognitive decline initially manifests with a decrease in executive function, followed by a slowly progressive or stepwise deterioration in cognitive function, which becomes apparent while performing daily activities around the age of 50.[32] In this study, we reported the case of an 11-month-old boy with CADASIL who had also been diagnosed with PCD earlier. The infant maybe the youngest among CADASIL patients reported in the world. The case was noted if cognitive decline occurred in an infant or baby; the diagnosis about CADASIL maybe necessary.

CONCLUSIONS

In conclusion, we studied a rare case in which both congenital PCD and CADASIL were diagnosed in an infant boy; congenital PCD was attributable to a compound that

was homozygous for (A-G)-12 at the transcription initiation site in the promoter region of the *PROC* gene, and CADASIL was caused by missense mutation in exon 24 of *NOTCH3* and PCD. He was a sporadic patient with congenital PCD and CADASIL in the world; it maybe that the deficiency of protein C led to early onset of CADASIL. Moreover, it supports that CADASIL patients can be encountered even at the infant stage. In contrast, it shows that the gene sequencing of *PROC* gene and *NOTCH3* gene may have important value for genetic guidance and prenatal diagnosis.

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