Abstract. Deficiency of protein C may cause deep venous thrombosis and pulmonary embolism, leading to ischemic stroke. The present study reports on a case of a young adult with recurrent cerebellar infarction due to hereditary heterozygous protein C deficiency and performs a literature review. A 35-year-old Asian woman was admitted to the Department of Neurology of The First Affiliated Hospital of Guangxi Medical University (Nanning, China) due to right limb paralysis and vomiting. The diagnosis of stroke was confirmed by computed tomography and magnetic resonance imaging, which indicated acute cerebral infarction of the right cerebellar hemisphere and cerebellar vermis, as well as a previous cerebral infarction on the left cerebellar hemisphere. This patient had taken aspirin orally for 4 years following surgical therapy for small intestine thrombosis and was regularly taking hydroxychloroquine sulfate to treat systemic lupus erythematosus. The protein C (PROC) levels were 57.6%, while protein S and antithrombin levels were normal. Gene sequencing analysis of the patient and the patient's pedigree revealed a heterozygous mutation, c.565C>T, on the PROC gene in the patient and the patient's father. In conclusion, the clinical manifestations of hereditary PROC deficiency may vary between individuals. The heterozygous mutation locus c.565C>T on the PROC gene is associated with thrombophilia. Awareness of the association between natural anticoagulants and thrombophilia may promote the prevention and therapy of stroke.

Introduction

Stroke is associated with high morbidity and mortality, with ischemic stroke being the most common type (1,2). Cerebellar infarction is a less common subtype of ischemic stroke, which may arise from thrombophilia causing both deep venous thrombosis and pulmonary embolism. Abraham et al (3) indicated that 25% of strokes occur in young patients aged <40 years. Stroke in young individuals combined with disability may cause a heavy burden on their family, as they are frequently the major economic and labor source of their family. Protein C, a glycoprotein encoded by the PROC gene, is synthesized in the liver and activated by thrombin on the surface of endothelial cells. Protein S has an anti-coagulation role in the presence of protein C by inhibiting factors Va and VIIIa (4,5). Studies have indicated that inherited or acquired protein C and protein S deficiency may be a cause of thrombophilia. Patients with protein C/S deficiency usually develop deep venous thrombosis (DVT), including lower limb DVT, while arterial thrombosis is relatively rare. The present study reported on an unusual case of hereditary protein C deficiency due to a c.565C>T heterozygous mutation in the PROC gene in a young woman who experienced recurrent ischemic stroke. Further pedigree validation analysis was performed, which confirmed that the same gene locus mutation was present in the patient's father.

Case report

Methods. A 35-year-old Asian woman was admitted to the First Affiliated Hospital of Guangxi Medical University (Nanning, China) in January 2017, with symptoms of weakness in the right side of her limb and fatigue for 19 days. Initially, she was able to hold objects with her right arm but walked with a dragging step. The weakness of her right limb progressed and she was unable to walk independently and hold chopsticks. At 9 days prior to presentation, she started vomiting and experienced dizziness. It became so severe that she was admitted to the local hospital. She was treated with anti-nausea drugs and stomach protection drugs, but experienced no obvious improvements in the weakness of the
right side of her limb. Her symptoms persisted to the point where ambulation was difficult, thus prompting her to visit the neurological outpatient department.

A review of the patient's medical history revealed the diagnosis of portal vein and superior mesenteric artery occlusion 4 years ago, and a successful surgery had been performed. Furthermore, the patient suffered from systemic lupus erythematosus (SLE) and received regular treatment with aspirin and hydroxychloroquine sulfate pills. She also suffered from hyperthyroidism and was treated with propylthiouracil tablets. No obesity, diabetes or hypertension were present. The patient was allergic to animal hair, but no drug allergies were detectable. Tobacco and alcohol use was denied. Furthermore, no hereditary disease was recorded for the patient's family.

The patient's vital signs were normal when she arrived at the department of neurology with an axillary temperature of 36.3˚C, heart rate of 78 beats per minute, blood pressure of 127/72 mmHg and respiratory rate of 19 breaths per minute. No positive signs were observed on general examination. Nervous system examination indicated vague speech and her tongue lagged to the right side. A muscle strength test indicated that her right-side strength was decreased (level IV, according to the UK Medical Research Council RT01 trial) (6).

Results

Laboratory test results indicated a low glucose 6 phosphate dehydrogenase activity of 5.02 (normal range, 6.80-20.50). Auto-antibody tests revealed positive results for anti-nuclear antibody. Blood coagulation function and D-dimer level was normal, but clotting factor activity was abnormal. The level of clotting factor XII was decreased (57.5%; normal range, 78-112). However, clotting factor VIII (152%; normal range, 78-128) and factor XI (118.9%; normal range, 82-118) exhibited increased activity. The results of her routine blood test, blood glucose, electrolytes and thyroid function were normal. Electrocardiogram results were normal. The long-range electrocardiogram results revealed sinus arrhythmia. Cerebrovascular Doppler examination was normal.

A computed tomography (CT) scan of the brain was performed immediately and indicated multiple low-density lesions on the bilateral sides of the cerebellar hemisphere and cerebellar vermis (Fig. 1). Magnetic resonance imaging (MRI) was performed to confirm ischemic lesions (Fig. 2), which was indicative of acute cerebral infarction of the right cerebellar hemisphere and cerebellar vermis, and a previous cerebral infarction of the left cerebellar hemisphere. CT angiography (CTA) was also performed to identify whether any of the neck and intracranial arteries were narrow; however, the results indicated that no obvious narrow artery was detectable (Fig. 3).

Considering that the patient had a previous history of portal vein and superior mesenteric artery occlusion, as well as asymptomatic strokes, thrombophilia (acquired or inherited) was considered. Further evidence supported this diagnosis. Protein C and protein S levels were detected, revealing a decreased level of protein C (57.6%; normal range, 70-140%), but normal levels of protein S (71.6%; normal range, 60-130%) and antithrombin III (92.4%; normal range, 75-125%). Gene sequencing analysis associated with thrombosis was performed by Guangzhou Kingmed Diagnostics Group Co., Ltd. (GuangZhou, China), which analyzed the pedigree as well as her mother and father, and revealed a heterozygous mutation c.565C>T on the PROC gene (Fig. 4).

Once a diagnosis is available, a suitable treatment plan is essential. In the present case, the woman was taking the long-term anti-platelet agent aspirin. However, a recurrence of arterial thrombosis still occurred. Heparinization therapy replacing the oral anti-coagulant therapy was considered beneficial for preventing further thrombosis.

Discussion

Ischemic stroke is divided into five types according to the Trial of ORG 10172 in Acute Stroke Treatment classification: Large artery atherosclerosis, cardioembolism, small artery occlusion, and stroke of other determined and undetermined etiology (7). The present case study reported on a young woman who suffered from recurrent cerebellar infarction, which was confirmed by CT and MRI scans. Imaging data suggested that the patient had experienced multiple stroke lesions, including a previous cerebral infarction, which indicated that she had a previous asymptomatic stroke. CTA was performed, and no large artery stenosis was noted. Since the patient had a history of portal vein and superior mesenteric
artery occlusion and recurrent stroke, the possibility of thrombophilia was considered. Protein C levels were decreased, and gene sequencing analysis of thrombosis-associated genes in the patient and her family members indicated one c.565C>T heterozygous mutation in the PROC gene in the patient and the patient’s father. However, the mother had no mutation on the same locus, which indicated that the father was the source of the gene mutation. Nucleotide mutation on Chr2-128183690 of the PROC gene has been reported to be associated with protein C deficiency (8). In the Human Gene Mutation Database, there are >270 types of mutation on different loci of the PROC gene associated with hereditary protein C deficiency were recorded.

A clinical feature of protein C deficiency-associated thrombophilia is recurrent venous thrombosis. However, associated arterial thrombosis, particularly recurrent artery thrombosis, remains relatively rare. Numerous high-risk situations may cause a thrombotic event, including major surgery, oral contraceptives, pregnancy and co-existing rheumatoid immune diseases. The case of the present study had hyperthyroidism and SLE, which may have increased her risk of developing thrombosis. Accordingly, the clinical manifestation of protein C deficiency is variable among individuals and may be complicated by underlying diseases or risk factors. Members of the patient's family, including the patient's father who had the same PROC gene mutation may be asymptomatic. Further studies on pedigrees with PROC gene mutation-associated thrombophilia are required to explore the diversity of clinical episodes of thrombophilia among affected individuals.

A literature search of the PubMed database for studies on cases of stroke in young individuals (<45 years old) associated with protein C/S deficiency was then performed without any publishing time limits but restriction to English language. The Chinese National Knowledge Internet and Wangfang databases were also searched for relevant studies in Chinese. A total of 9 English case reports were acquired (9-17), but no relevant Chinese case report was retrieved. A total of 4 studies reported on patients without any history of disease or risk factors who suffered a stroke (9,12,15,17). Matsushita et al (13) reported on a young woman taking long-term oral anti-cancer drugs prior to experiencing a stroke. Risk factors were noted in several patients: A history of miscarriage or DVT (14), and accompanying transient ischemic attack (10) or myocardial infarction (16) were present in certain cases. Most patients had familiar protein C deficiency, but no further gene sequencing test was performed. To investigate the novelty of the mutation locus on the PROC gene identified in the present study, the single nucleotide

---

Figure 2. Magnetic resonance imaging of the brain indicated acute ischemic stroke in the right cerebellar hemisphere and cerebellar vermis, and a previous cerebral infarction in the left cerebellar hemisphere. (A) T1 weighted image revealed a low signal in the right cerebellar hemisphere. (B and C) T1 Flair weighted image showed multiple high signals in the right cerebellar hemisphere. (D) T2 weighted image revealed a high signal in the left cerebellar hemisphere. (E and F) T2 weighted image revealed several different high signals in the right cerebellar hemisphere.
polymorphism database (https://www.ncbi.nlm.nih.gov/snp/) was searched. A total of 4 public variants were retrieved using the NM_000312.3 transcript reference sequence: PROC:c.‑50A>T, PROC:NM_000312.3:c.‑50A>T, PROC:c.423G>T and PROC:NM_000312.3:NM_000312.3:c.423G>T, not including the mutation locus identified in the present case. Therefore, in the present study, the c.565C>T heterozygous mutation on the PROC gene (chromosomal location, chr2-128183690; transcript reference no. NM_000312) was discovered in the patient and the patient’s father for the first time, and pathogenicity analysis indicated a pathogenic nature, which further confirmed the diagnosis of hereditary protein C deficiency. However, additional study is required to discover the mechanisms of gene mutation.

In summary, the etiology and pathogenesis of stroke are complex, and hematologic abnormalities may contribute to stroke in young individuals. For young stroke patients, detection of protein C and protein S levels, as well as sequencing analysis of pathogenic genes and validation in their family, is recommended, even in the absence of a recorded family history of thrombotic events. Further prospective studies should be

Figure 3. Computed tomography angiography indicated a complete Willis ring.

<table>
<thead>
<tr>
<th>Result</th>
<th>PROC</th>
<th>chr2-128183690</th>
<th>c.565C&gt;T</th>
<th>p.R189W</th>
</tr>
</thead>
</table>

A: patient
heterozygous mutations

B: patient’s father
heterozygous mutations

C: patient’s mother
no mutations

Figure 4. Family genealogical analysis on PROC gene of the patient and her parents. (A) Heterozygous nucleotide mutation on coding region no. 565 from cytosine to thymine on PROC gene was detected in the patient (c.565C>T). (B) Same heterozygous nucleotide mutation on coding region no. 565 from cytosine to thymine on PROC gene was detected in the patient’s father (c.565C>T). (C) No nucleotide mutation on coding region no. 565 on PROC gene was detected in the patient’s mother. PROC, protein C.
performed to explore the link between heterozygous protein C deficiency and ischemic stroke, as well as the underlying mechanisms.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

CQ designed the case report and revised the manuscript critically for important content. PL acquired and analyzed the data and drafted the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Patients provided consent for publication.

Competing interests

The authors declare that they have no competing interests.

References