Letter to the Editor

CBS and *SERPINC1* mutation-induced ischemic stroke and multisystem diseases in a young woman: a case description and literature analysis

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Introduction

Globally, there are nearly 2 million new cases of young stroke patients every year (1). An episode of stroke in younger people places a heavy burden on their families, society, and the economy. Epidemiological data shows that the morbidity of younger stroke patients has increased significantly, and it is crucial to identify and address any pathogenic factors for stroke in young people (2). Studies have speculated on the unique risk factors and causes of stroke in young people, and there is a pressing need to improve the relevant examinations to formulate appropriate prevention and treatment programs (2,3). However, some young people experience stroke due to neurological manifestations in the development of systemic diseases. In addition, routine tests and imaging examinations have a limited impact when evaluating a patient's etiology and present diagnostic and treatment challenges for clinicians. With the rapid development of functional genomics and the emergence of new technologies such as gene chips and bioinformatics, the etiology and pathogenesis of stroke in young people can be examined at the gene level. Gene mutation is a very important risk factor for stroke in young adults. This paper reports the case of a young patient with ischemic stroke and multisystem damage caused by cystathionine-β-synthase (*CBS*) and serpin family C member 1 (*SERPINC1*) mutations that were diagnosed following whole exon genome sequencing. To the best of our knowledge, *CBS* and *SERPINC1* mutation-induced ischemic stroke has not been reported previously.

Case presentation

The patient was a 24-year-old female with a history of lower left limb amputation, which was the result of a traffic accident in 2009, and intraocular lens implantation due to lens dislocation in both eyes in 2012. She had no family history of cerebrovascular disease and thrombosis. In 2019, she was admitted to the respiratory department of Huzhou Cent Hospital, the Affiliated Cent Hospital Huzhou University having experienced tightness in her chest over 4 days and 1 day of swelling and pain in the lower right limb. On admission, the patient had edema in the right lower limb, and no abnormalities were observed in the auxiliary examination of the liver, gallbladder, pancreas, and spleen. Her platelet (PLT) content was 164×10^9/L, and the pulmonary computed tomography angiography (CTA) examination suggested pulmonary embolism at the beginning of the left and right pulmonary artery branches (*Figure 1A*,*1B*). The ultrasonography report of the lower extremity suggested deep venous thrombosis on the right side (*Figure 1C*). The levels of D-dimer (17.92 mg/L) and blood homocysteine (>266.5 μmol/L)

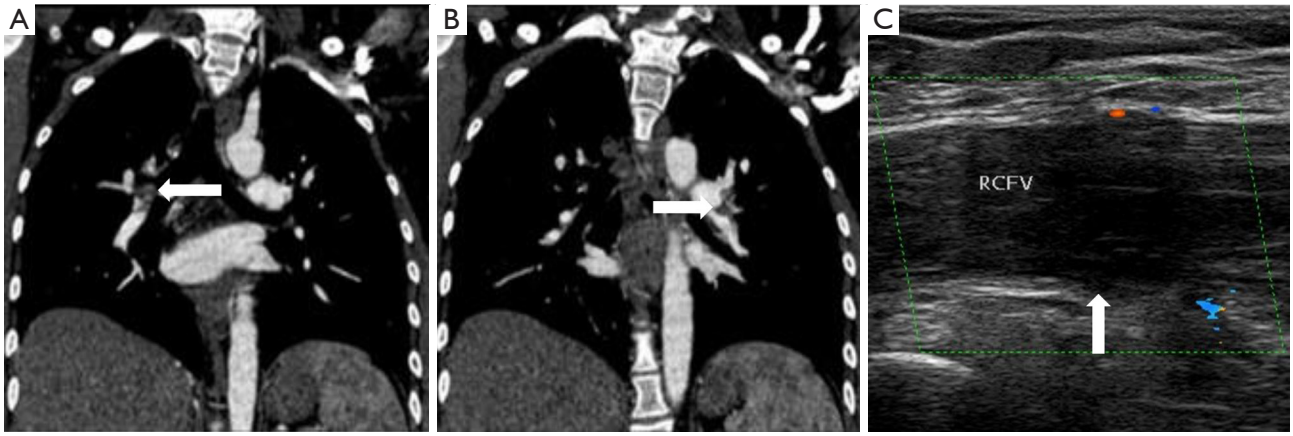

Figure 1 CTA imaging of the patient's pulmonary artery. (A) Thrombosis in the right lung; (B) thrombosis in the left lung; (C) ultrasonography of the right lower limb showed venous thrombosis of the right lower limb. The arrows represent the formation of thrombus. RCFV, right common femoral vein; CTA, computed tomography angiography.

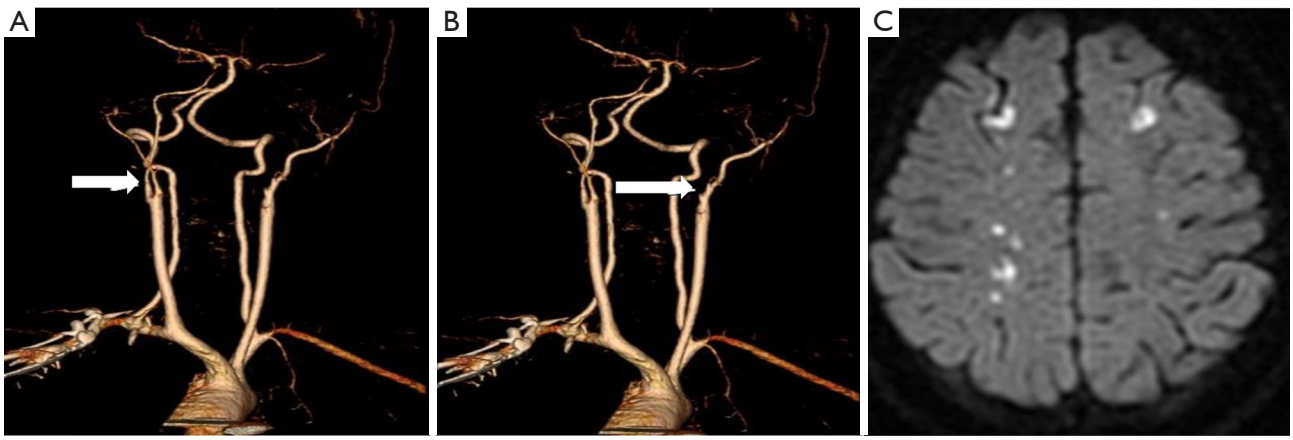

Figure 2 CTA examination of carotid artery. (A) The right ICA was thin, the arrow represents the thin ICA; (B) the left ICA was occluded, the arrow represents ICA occlusion; (C) DWI sequence imaging of the head MRI showed bilateral scattered new cerebral infarction. CTA, computed tomography angiography; ICA, internal carotid artery; DWI, diffusion-weighted imaging; MRI, magnetic resonance imaging.

were abnormal. Erythrocyte sedimentation rate (ESR), both liver and kidney functions, electrolytes, thyroid function tests, autoantibodies, and tumor markers were all normal. However, due to the lack of further analysis and the clinical significance of the serious increase of blood homocysteine, warfarin anticoagulation therapy was initiated after hospitalization on September 1, 2019, but folic acid and vitamin B complex was not given to treat the homocysteinemia.

On August 22, 2020, the patient was admitted to the Department of Neurology after experiencing numbness and weakness in her upper left limb for 5 days. On admission, the muscle strength of the upper left limb was graded 5, the superficial sensitivity in the same area had decreased, and the muscle strength of the right limb was graded 5. A venous ultrasound of the lower extremity showed an old mural thrombus in the right common femoral vein and at the origin of the right superficial femoral vein. The neck CTA showed occlusion of the left internal carotid artery (ICA) and incomplete occlusion of the right ICA (*Figure 2A*,*2B*). Auxiliary examination [head magnetic resonance imaging (MRI)] showed scattered cerebral infarctions in both the bilateral frontal and parietal lobes

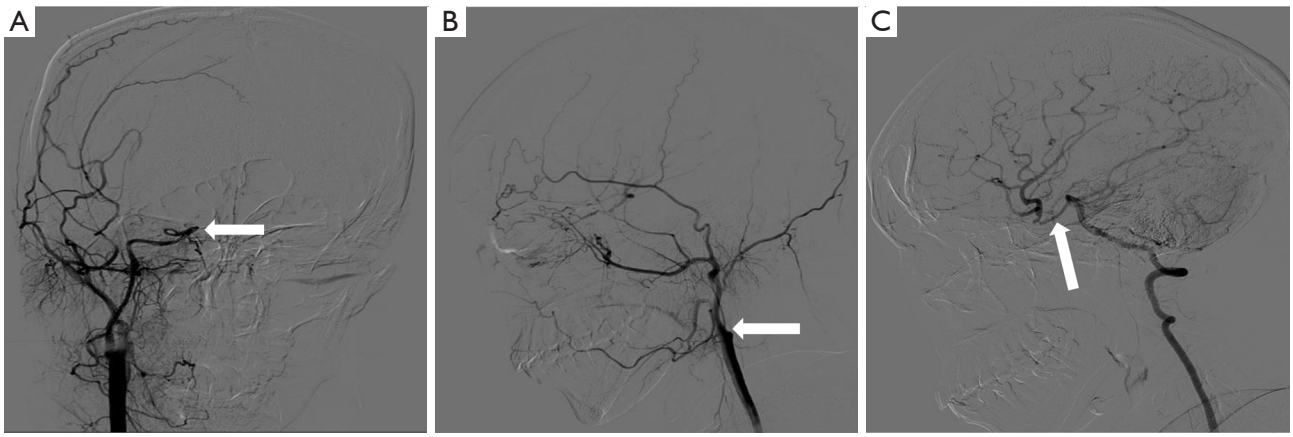

Figure 3 DSA examination. (A) Thinning in the right ICA and occlusion at its end, the arrow represents ICA occlusion; (B) occlusion at the beginning of the left ICA, the arrow represents ICA occlusion; (C) opening of the posterior communicating artery, the arrow represents open arteries. DSA, digital subtraction angiography; ICA, internal carotid artery.

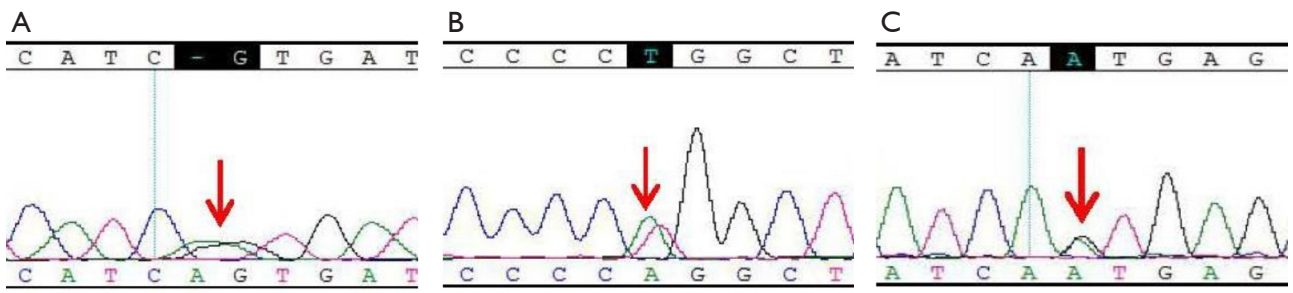

Figure 4 The results of high-throughput sequencing. (A) Change in nucleotide 502 of the coding region codes for adenine than guanine resulting in amino acid 168 to changing from valine to methionine (c.502G>A, V168M); (B) change in coding region 168 from thymine to adenine, resulting in amino acid 230 changing from leucine to glutamine (c.689T>A, p.L230Q); (C) change in nucleotide 719 in the coding region from adenine to guanine, resulting in a change in amino acid 240 from asparagine to serine (c.719A>G, p.N240S). Since forward or reverse sequencing was adopted for sequencing, the base shown in the results may be the reverse complementary sequence of the detected base. The arrows represent gene mutations. V168M, 168 amino acid from valine to methionine.

(*Figure 2C*). Digital subtraction angiography (DSA) showed that the right ICA was thin, and the C7 segment was occluded. The left ICA was also occluded, and the posterior circulation was compensating for the anterior circulation via the posterior communicating artery (*Figure 3A-3C*). The ESR, liver and kidney functions, electrolytes, thyroid function, glycosylated hemoglobin (HbA1c), blood glucose, and tumor markers were all normal. In order to exclude systemic lupus erythematosus, Sjogren's syndrome and vasculitis, autoantibodies [anti mitochondrial antibodies, antinuclear antibodies, anti-kinetochore antibodies, anti-ribosome P protein antibodies, anti-nucleosome antibodies, anti-Sm antibodies, anti-Sjogren's syndrome A (anti-SSA) antibodies, anti-Sjogren's syndrome B (anti-SSB) antibodies, etc.] were tested, the results of which were also normal. Hemoglobin (Hb) was 97 g/L, D-dimer was 0.7 mg/L, and blood homocysteine was more than 250 μmol/L. The blood samples of the patient were tested by the target region high-throughput sequencing method after admission, which showed that her *CBS* gene had 2 variants. The first was a heterozygous variation of c.502G>A in which the 502 nucleotide in the coding region changed from guanine to adenine, resulting in a change in the 168 amino acid from valine to methionine (V168M) (*Figure 4A*). The other variant was a heterozygous missense mutation in nucleotide 689

of the coding region from thymine to adenine, resulting in a change in amino acid 230 from leucine to glutamine (c.689T>A, p.L230Q) (*Figure 4B*). In addition, another variation in *SERPINC1* at nucleotide 719 in the coding region changed from adenine to guanine, inducing amino acid 240 to change from asparagine to serine (c.719A>G, p.N240S) (*Figure 4C*).

After admission, the patient was diagnosed with cerebral infarction and homocystinuria and was deemed to be in a hereditary hypercoagulable state based on her gene sequence analysis. Her treatment regimen comprised aspirin, atorvastatin, rivaroxaban, vitamin B12, and folic acid. After discharge, she continued taking rivaroxaban, folic acid, vitamin B12, and vitamin B6 orally and underwent extensive follow-up (for 2 years) to improve drug compliance. Her blood homocysteine was recorded as having returned to a normal value (12.6 μmol/L) by the third month (normal upper limit is 20 μmol/L), and there were no further events of cerebral infarction or embolism. The patient continued to take folic acid, vitamin B12, vitamin B6, and rivaroxaban. Subsequently, she was re-examined every 3 months, and the results were all normal. All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Declaration of Helsinki (as revised in 2013). Written informed consent was provided by the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

Discussion

The patient's medical history involved multiple systems, such as ectopia lentis, deep vein thrombosis of the lower extremities, pulmonary embolism, and cerebral infarction. The auxiliary examination, which included a carotid DSA examination, showed slenderness in the right ICA and occlusion in the C7 segment, as well as occlusion in the left ICA. Blood homocysteine met the diagnostic criteria for severe hyperhomocysteinemia (>100 μmoL/L); this elevation is often caused by hereditary factors (4). This prompted us to perform gene sequencing, which revealed 2 variants in her *CBS* gene and 1 variant in the *SERPINC1* gene. These 2-locus gene mutations had resulted in hyperhomocysteinemia. This is a heterozygous missense mutation, which was compatible with the clinical phenotype of the patient. Mutations at this site had led to antithrombin III (ATIII) deficiency thrombophilia.

Classical homocystinuria, primarily caused by the presence of missense pathogenic mutations in *CBS*, is a rare autosomal recessive trait and the most common cause of hyperhomocysteinemia (5). Clinical manifestations and the age at onset are related to gene polymorphism and vitamin B6 responsivity (6,7). Usually, the eye, bone and connective tissue, vascular system, and central nervous system (CNS) are commonly involved, manifesting in the patient as myopia, lens ectopia, osteoporosis, lateral spine shift, fragile skin, fine hair, mental retardation, and seizures, among others. Ectopia of the lens is particularly suggestive in pediatric patients and should be further investigated by an ophthalmologist.

In contrast, adults are more likely to present with vascular lesions. High levels of homocysteine can cause diseases by promoting smooth muscle proliferation, resulting in the following: vascular sclerosis, low-density lipoprotein cholesterol oxidation, and vascular atherosclerosis aggravation; the induction of endoplasmic reticulum stress, resulting in loss of protein function; endothelial dysfunction; inhibition of anticoagulant protein activity, which leads to thrombosis and vascular disease (8-11). Decreased cystathionine and cysteine levels are correlated with changes in apoptosis, oxidative stress, and structural proteins. In this case, DSA showed bilateral ICA occlusion, which was presumed to be related to smooth muscle cell proliferation, endothelial dysfunction, as well as abnormal metabolism of low-density lipoprotein caused by high homocysteine levels and carotid artery occlusion that ultimately led to cerebral infarction. Abnormal structural proteins in the zonules of the lens resulted in defective microfibrillar components and ectopia of the lens (12).

Hereditary ATIII deficiency is not inherited, with a morbidity of about 2/10,000 in the general population (13). The main clinical manifestations are venous thromboembolism, which includes deep venous embolism and pulmonary embolism. This is a functional defect caused by the change in protein conformation due to a mutation in the ATIII gene *SERPINC1*. *SERPINC1* is located at 1q23-25.1, spans 13,477 bp, and contains 7 exons and 6 introns (14). SERPINC1-encoded ATIII belongs to the serine protease inhibitor family, which is the most important inhibitor of coagulation factors and can inactivate thrombin, coagulation factors, trypsin, and so on. Clinically, it can be divided into 2 types: type I, when the activity of the ATIII antigen and antithrombin simultaneously reduces by about 50% and there are no protein variants; and type II, which is caused by the abnormal structure of ATIII (15). The

low polymorphism of ATIII suggests that this molecule is sensitive to minor genetic defects, and any missense mutation may lead to changes in antithrombin function and induce venous thrombosis and pulmonary embolism. The young patient in this case developed venous thrombosis due to a mutation in *SERPINC1*.

The diagnosis and treatment in this case can positively guide clinicians. Although there are various reports on hyperhomocysteinemia caused by *CBS* mutation, there are only a few reports about the concurrent mutations of *CBS* and *SERPINC1*. A study has reported that patients with *CBS* deficiency have lower antithrombin levels and severe hyperhomocysteinemia (16). Similarly, mild hyperhomocysteinemia has been reported in pedigrees with antithrombin type I deficiency (17). It has been reported that the incidence of deep vein thrombosis in patients with both hyperhomocysteinemia and prothrombin *G2010A* mutation is 10–20 times higher than that in patients with a prothrombin *G2010A* mutation alone (18). This suggests a synergistic effect between hyperhomocysteinemia and hypercoagulability. Both genetic testing and the clinical manifestations in this case support this hypothesis; however, the exact mechanism remains unclear, and thus, future case reports are needed to further investigate the relationship between these factors.

Secondly, 191 pathogenic *CBS* mutation sites have been reported in cases of patients with ectopia lentis, connective tissue lesions, and CNS involvement who are unresponsive to vitamin B6 treatment (19,20). However, 2 heterozygous mutation sites reported in this paper have clinical lens ectopia and CNS involvement but are responsive to vitamin B6 treatment, which suggests that we are yet to comprehensively understand homocystinuria.

Thirdly, as the pathological mechanism of homocystinuria, which leads to ischemic stroke, includes atherosclerotic thrombosis and hypercoagulability, the patient was given antiplatelet therapy including aspirin and atorvastatin to inhibit vascular inflammation, and rivaroxaban anticoagulation treatment during hospitalization. The long-term combined use of antiplatelet drugs and anticoagulants after discharge is not supported in evidence-based studies and might increase the risk of bleeding. Consistent with this, in cases of *CBS* mutation combined with *SERPINC1* mutation, and those in which the main pathogenic factors are blood homocysteine and hypercoagulability, long-term secondary prevention after discharge is still mainly an etiological treatment without antiplatelet therapy.

Our patient's follow-up demonstrated overall recovery, so we support the identification of the underlying causes and risk factors along with secondary prevention of the complications in young stroke patients. Finally, consideration of factors such as amputation, young age at onset, and hereditary diseases highlights that drug compliance is crucial. Since long-term follow-up requiring numerous visits to the hospital can cause difficulties in completing proper follow-up, engaging nurses in the community where the patient resides who could carry out a long-term follow-up substantially improves the patient's medication and follow-up compliance. It is also suggested that this could be useful in exploring patients with chronic diseases who have limited or no mobility. However, a limitation of this case report is that the gene detection method was performed by target region high-throughput sequencing technology, which presently cannot completely cover all exon regions and the overall coverage rate can reach >95%, so there may have been some errors.

Conclusions

The etiology of ischemic stroke in young people is complex, especially in those with multisystem damage and recurrent attacks. Clinicians need to consider the possibility of gene mutation, improve gene detection techniques, guide the formulation of reasonable secondary prevention strategies, and apply individualized treatment to avoid disease recurrence and reduce the burden on patients, their families, and society.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://qims.amegroups.com/article/view/10.21037/qims-23-255/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures

performed in this study were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Declaration of Helsinki (as revised in 2013). Written informed consent was provided by the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

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