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Original Article

Hereditary endotheliopathy with retinopathy and encephalopathy: pathological and genetic studies of a family

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Abstract: Background: The objective of this study was to examine the clinical, pathological and genetic features of a family suffering from hereditary endotheliopathy with retinopathy and encephalopathy. Methods: The index case was male, and his symptoms were detected at 18 years of age. The clinical manifestation included recurrent headache, fever, consciousness disturbances and haemiplegia. Bilateral cerebral hemispheric lesions were detected via MRI as low signals on T1 and high signals on T2 and FLAIR, with moderate enhancement. Video EEG revealed an increase in the slow wave frequency. An EMG displayed neurogenic atrophy. Similar clinical and imaging characteristics were detected in his mother and his uncle. Pathological examinations of the brain, muscle and sural nerve were performed on the index case. Sequence analysis of the TREX1 gene was performed on the index case, his sister and his father. Results: A brain biopsy revealed spongiform alterations as well as inflammatory cell infiltration in a few small vessels. Neurogenic muscular atrophy was detected based on a biopsy of the muscle. Demyelination was detected based on a biopsy of the sural nerve. Electron microscopic examination of the sural nerve revealed thickening and delamination of the basement membrane. No reported TREX1 gene mutation was detected for any of the patients. Conclusion: Hereditary endotheliopathy presented with peripheral nerve involvement. Multi-laminar thickening of the basement membrane of the capillaries also appeared in the extracerebral tissue. The involvement of a novel gene should be further examined.

Keywords: Hereditary endotheliopathy, biopsy, vascular disease, TREX-1

Introduction

Hereditary endotheliopathy is characterised by pathological changes in the vascular endothelium. It was first reported in 1988 with features of retinal vasculopathy and frontal-parietal pseudotumour [1]. According to its clinical manifestations, hereditary endotheliopathy can be separated into hereditary endotheliopathy with retinopathy, nephropathy, and stroke (HERNS); cerebroretinal vasculopathy (CRV); and hereditary vascular retinopathy (HVR) [2-4]. The hereditary pattern is consistent with autosomal dominant inheritance. Linkage analysis has identified a locus of the pathogenic gene TREX1 on chromosome 3p21.1-p21.3 [5, 6]. Hereditary endotheliopathy is characterised clinically by the attenuation and exudation of retinal vessels and pathologically by the delamination of the basement membrane. Its diagnosis primarily depends on the clinical manifestations and the pathological finding of microvascular alterations [7]. Genetic analysis can provide further evidence. Hereditary endotheliopathy is a systemic vasculopathy. Based on previous reports, this damage primarily involves the brain, the retina and the kidney. We demonstrated a novel clinical finding of peripheral nerve injury associated with hereditary endotheliopathy.

Patients and methods

Index case (III11)

The proband was a 19-year-old Chinese man who suffered from the onset of symptoms in

February 2006 with no apparent predisposing causes. He presented with fever, pulsatile headache and vomiting and subsequently developed dysphoria, unconsciousness and right limb weakness. Based on a presumptive diagnosis of viral encephalitis, the patient was provided with relevant treatment, which resulted in some clinical improvement in the first week, but his intelligence decline remain unchanged until 2 months later. In May 2006, three months later, the patient suffered from tongue paraesthesia, barylalia, dysphagia and blurred vision. Over the next half an hour, he developed a headache and a fever with a temperature reaching 39-41°C. The symptoms of tongue numbness and visual blurring were relieved 2-3 hours later, with the presence of unilateral extremity weakness. Ten hours later, the extremity weakness abated, followed by relief from the headache and fever. This course continued for 1-2 days. The patient experienced similar attacks more than 10 times over the next year, except that the haemiplegia varied between the right and left limbs. On June 16, 2007, Sixteen months after the onset, the patient experienced an onset of mild headache, extremity weakness and vomiting after morning exercise and subsequently developed dysphoria, unconsciousness and fever. A physical examination indicated a temperature of 39.2°C, occasional eye opening and combined aphasia and dysphoria. Fundoscopy revealed a clear boundary of the optic papilla and constriction of the retinovasculature. A neurological examination revealed a shallow nasolabial fold and limb and facial spasms. Assessments of the muscle were graded as 1/5 in the upper right extremity, 3/5 in the lower right extremity and 4/5 in the left extremities. The bilateral Babinski's sign was positive. After less than 1 month of treatment, the high frequency of seizures was resolved, but the aphasia and the right limb weakness remained. After a follow-up brain MRI demonstrated swelling of the left hemisphere, the patient was treated with corticosteroids and mannitol, which relieved the symptoms. The disease relapsed 3 months later during hospitalisation, with worsening of the limb paralysis. A cranial CT scan revealed remarkably extensive cortex and white matter swelling in the left hemisphere. After treatment with corticosteroids, mannitol and supportive care, the patient regained consciousness and exhibited some improvement in paralysis, with sequelae of paralysis and combined aphasia.

Laboratory testing of the blood, urine, stool and hepatorenal function revealed no abnormalities. Analysis of the CSF, drawn on April 27, 2007, revealed a pressure of 120 mm H_2O, 2×10^6 white blood cells/L, a protein level of 1232 mg/L, a glucose content of 3.30 mmol/L and a chloride content of 117.3 mmol/L. Analysis of the CSF on June 25 revealed a pressure of 75 mm H_2O, 1×10^6 white blood cells/L, a glucose content of 3.30 mmol/L and a chloride content of 115.4 mmol/L. Video EEG revealed an increase in the slow wave frequency and predominant θ activity, with interspersed δ wave and δ activity, which increased during sleep. Electromyography revealed moderate to severe damage to the tibial-peroneal nerves and mild to moderate damage to the bilateral tibial, right ulnar and bilateral median nerves. Evidence of denervation of the anterior tibial and biceps brachii muscles was detected. The bilateral tibial-peroneal nerve exhibited an absence of sensory conduction velocity (SCV) and a decrease in motor conduction velocity (MCV) and action potentials. The right ulnar nerve exhibited decreases in SCV and action potentials. In the bilateral median nerve, the SCV was normal, the MCV was slow, the action potentials were small, and the F-wave was not elicited. The right musculocutaneous nerve exhibited prolonged latency and decreased action potentials. Increases in polyphase waves and duration were noted based on EMG of the anterior tibial and biceps brachii muscles. The N75 P100 and N145 waves were absent from the visual-evoked potentials, which revealed bilateral visual pathway abnormalities.

On April 24, 2007, Brain MRI of the patient in the remission stage displayed patchy T1, T2 and FLAIR signal abnormalities without enhancement in the bilateral frontal, right parietal, temporal-insular or occipital lobes, the right basal ganglia of the Pons. On June 19, 2007, A MRI scan 3 days after onset, demonstrated a patchy high FLAIR signal without apparent enhancement in the bilateral white matter surrounding the cortex-medulla junction, a moderate T1 signal and enhancement in the frontal lobe. The imaging lesions did not resolve after the onset on June 16. A brain MRI performed on July 28 demonstrated left cerebral hemispheric swelling based on high T1, T2 and FLAIR signals without enhancement as well as a reduction in the lesions in the right hemisphere based on a patchy high T2 signal with-

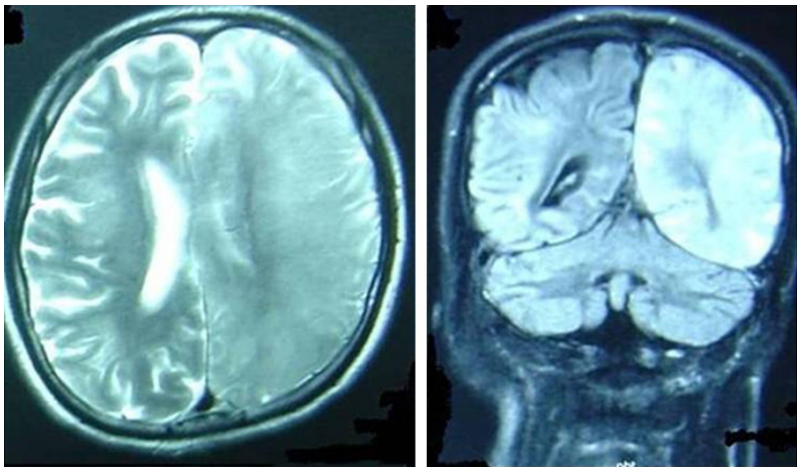

Figure 1. Brain MRI of the proband showing diffuse swelling in the right hemisphere.

out enhancement. On August 10, the brain MRI preceding a stereotactic brain biopsy revealed swelling in the left cerebral hemisphere and a few patchy enhancements, while the lesions in the right hemisphere were further reduced. On September 6, 20 days after the biopsy, the patient developed vomiting and dysphoria; MRI revealed diffuse oedema in the left hemisphere, a large area of finger-like oedema in the white matter, a diffuse high T2 signal in the cortex and one site with T2 signal abnormalities in the right hemisphere (**Figure 1**).

A review of the medical records of the family (**Figure 2**) revealed a similar clinical profile and imaging results. The proband's mother suffered from headaches at 30 years old and presented with neurologic complaints at 43 years of age. She exhibited left extremity weakness and psychiatric symptoms. After providing treatment based on a presumptive diagnosis of stroke, the patient exhibited improvement in the extremity weakness, but the psychiatric disturbance persisted. She subsequently developed loss of vision. A brain MRI displayed patchy lesions in the right temporal lobe, based on a low signal on T1 and a high signal on T2 and FLAIR. The proband's deceased uncle had a history of headaches and was diagnosed with encephalitis based on MRI features of bilateral cerebral hemispheric cortical and subcortical lesions, including low signal on T1 and high signal on T2 and FLAIR. The living environment in the proband's village is found without any infectious disease or aggregation of disease, and no similar cases have been identified.

Nerve biopsies

The research protocol was approved by the Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University. The family provided their informed consent to participate in this study.

The sural nerve biopsy specimens were collected following the patients' informed consent. Semi-thin sections were fixed using 10% formalin and were embedded in paraffin, followed by haematoxylin-eosin (HE), Luxol fast blue (LFB) or Congo red staining for light microscopy. A subset of the specimens were processed as semi-thin sections via fixation using 2.5% pentodialdehyde and post-fixation using osmic acid, followed by Toluidine blue staining for light microscopy. Other specimens were utilised to generate ultra-thin sections, followed by lead and uranium staining for electron microscopy. A muscle biopsy specimen of the proband was processed in the standard manner, and enzyme histochemical staining was performed for light microscopy.

Brain biopsies

The proband underwent stereotactic brain biopsy on August 10, 2007. One portion of the obtained specimen was fixed using 10% formalin and was embedded using paraffin, followed by HE or LFB staining for light microscopy. Another portion of the specimen was fixed using 2.5% pentodialdehyde and was embedded for electron microscopy. Alternatively, post-fixation using osmic acid was performed on a portion of the specimen, ultra-thin sections were generated, and lead and uranium staining was performed for electron microscopy.

Genetic studies

The TREX1 gene was amplified via PCR using the primers 5'-ATGTGCTGGTCCCACTAAG-3' and 5'-GGACAGCCAGCAGGCACAG-3'. The PCR assay contained 25 μL of buffer, 8 μL of dNTPs, 50-500 ng of DNA, 200 ng of each primer and

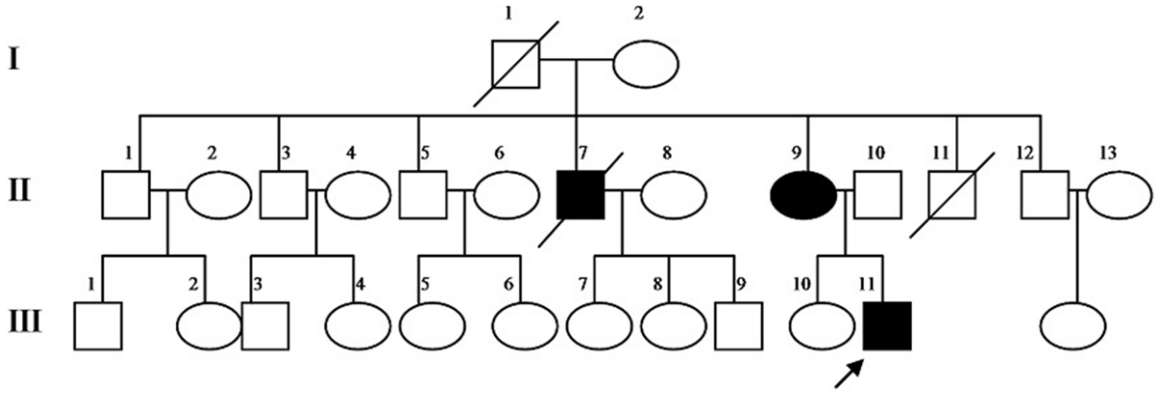

Figure 2. Pedigree of the family. □ male, ○ female, ■ male patient, ● female patient, ↗ proband.

1 U of Taq polymerase. Deionised water was added to the PCR reaction to a final volume of 50 μL. The mixture was placed in a T Professional standard gradient thermocycler (Biometra Co., Germany) and was subjected to the following amplification reaction program: 5 min of initial denaturation at 94°C, 30 cycles of 30 s at 94°C and 40 s at 72°C, followed by 10 min at 72°C. An aliquot of 5 μL of each PCR product was examined by 1% agarose gel electrophoresis and gel imaging acquisition analysis. The analyses were performed using 10 mL of DNA extract from Invitrogen Corporation. Then, gene sequencing was performed.

Results

Brain biopsies

The brain biopsy of the proband revealed several abnormalities, including extensive oedema, capillary hyperplasia, thickened capillary walls in a few arterioles, an increase in foam cells in the white matter and infiltration of small lymphocytes surrounding some small vessels. Electron microscopy displayed mild pyknosis of the neurons and glia and new endothelial cells with loose organelles (**Figure 3**).

Sural nerve biopsies

Biopsies of the sural nerves of the proband and his mother revealed moderate decreases in the density of the myelinated nerve fibres. Clusters of predominantly thin and small myelinated axons and "onion-bulb" formations were not detected. The capillary basement membrane was thickened. Electron microscopy displayed a thickened and multilayered appearance of the capillary basement membrane (**Figure 4**).

Muscle biopsies

A muscle biopsy revealed uniform size of the muscle fibres, scattered angular atrophic fibres, nuclear ingression in some cells and equally distributed fibre types. Muscle atrophy involved the type I and type II fibres. No fragmented red fibres were detected.

Genetic analysis

No mutations in the TREX1 gene were detected based on the genetic analysis of the proband, his father and his sister. Because his mother was died during the follow-up, unfortunately we did not do gene analysis for his mother.

Discussion

The proband initially presented with recurrent focal neurological deficits and visual loss with vascular attenuation based on fundoscopy, which indicated damage to the brain and the retina. Similar clinical characteristics were found in the proband's mother and uncle. A pathological examination of the brain revealed diffuse capillary hyperplasia in the cortex and white matter and thickening of the capillary basement membrane in the peripheral nerve, suggesting the mechanism of microvessel injury. The clinical and pathological findings were consistent with a diagnosis of hereditary endotheliopathy [1, 8, 9].

The onset of the disease in this pedigree has typically begun during young to middle age, although there have been reports of disease onset at approximately 40 years of age [8, 10]. Patients suffering from hereditary endotheliopathy present with a range of symptoms related

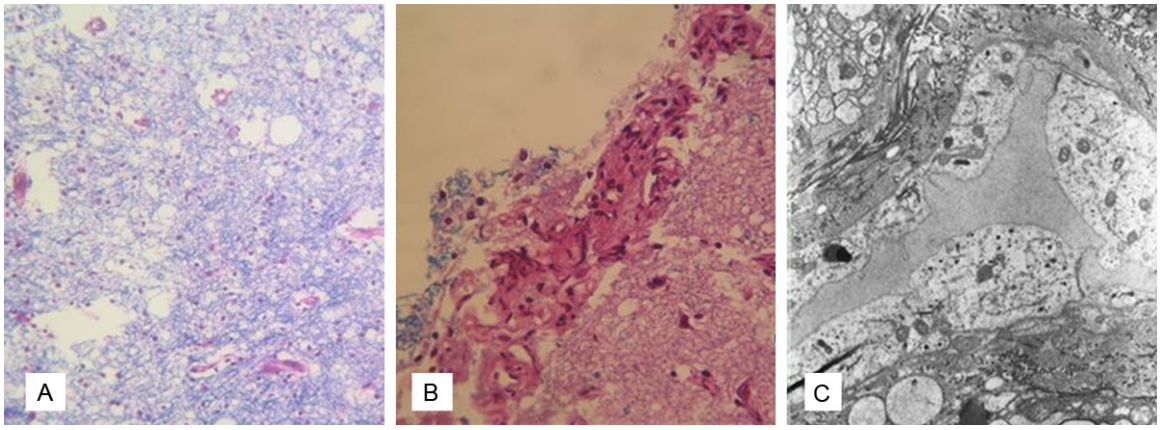

Figure 3. A. Spongy change and capillary hyperplasia in brain (LFB staining × 400); B. Increase of vessels on the brain surface of Subarachnoid space (LFB staining × 1000); C. Swelling of capillary endothelial cells (electron microscopy × 1000).

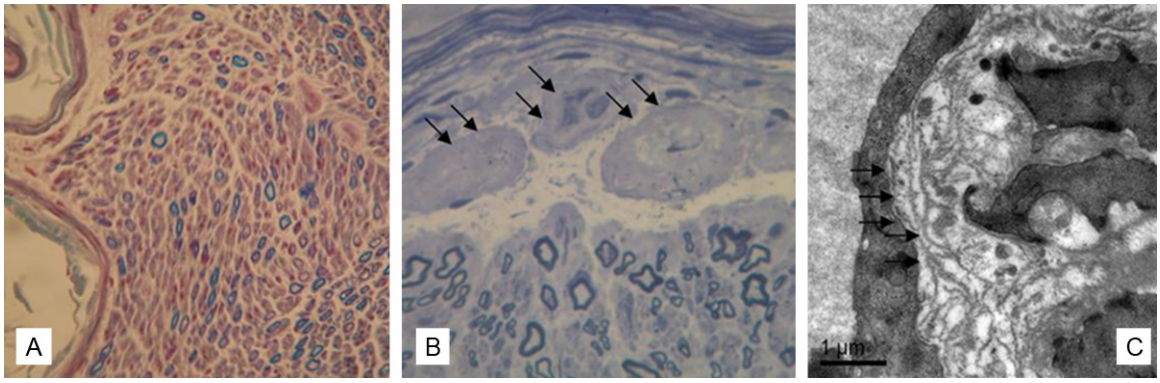

Figure 4. A. Moderately decreased density of myelinated nerve fiber (LFB staining × 400); B. Thickening of capillary basement membrane (arrow, Semi-thin section × 1000); C. Delamination of the capillary basement membrane (arrow, electron microscopy × 25000).

to their systemic involvement. Similar to stroke, the disease is characterised by recurrent migraines and haemiplegia. Fever is common and occurs with almost every onset. Although most of the clinical symptoms gradually disappear after disease onset, the intelligence decline is progressive. Persistent vision decline results from retinal damage and is detected as vascular attenuation via fundoscopy, along with tortuosity, exudation and microaneurysms in previous cases [8, 11]. We demonstrated a novel clinical finding of peripheral nerve injury associated with hereditary endotheliopathy. Although the clinical features generally resulted from the manifestations of brain lesions, peripheral nerve damage was detected based on an electrophysiological assessment. Although peripheral retinal involvement is a feature of hereditary endotheliopathy [2, 8], it was not detected in this case and should be monitored during follow-up.

The brain MRIs in this pedigree were characterised by diffuse oedema and contrast enhancement, consistent with the results found in previous cases. Midline shifts were detected, similar to the changes induced by brain tumours [11, 12]. Although extensive bilateral hemisphere lesions detected via MRI raised the possibility of viral encephalitis, the fluctuations in focus were not consistent with the course of viral encephalitis. This family displayed imaging alterations distinct from mitochondrial encephalomyopathy, lactic acidosis, stroke-like episodes [13] or Susac's syndrome [14]. That the distribution of lesions was not consistent with the law of vascular distribution distinguished these cases from vasculitis or vascular malformation.

The pathological examination revealed diffuse oedema and capillary hyperplasia in the brain, which are previously described characteristics

of hereditary endotheliopathy [12]. Focal necrosis and foam cells were found in some regions, indicating the mechanism of ischaemia. No pathological changes were detected in the muscles. Electron microscopic examination of the sural nerve demonstrated decreased density of the myelinated nerve fibres and thickening of the basement membrane. Capillary damage in the peripheral nerve fascicles indicates that pathological changes also occurred in the extracerebral tissue, ultimately leading to peripheral nerve injury.

Genetic analysis revealed no mutations in the TREX1 gene in the index case, his sister or his father. New genes must be identified.

There is no effective treatment available for patients suffering from hereditary endotheliopathy. Corticosteroids and mannitol, due to their dehydrating effects, provided some benefit for the clinical symptoms but did not alter the clinical course or prevent the progression of the brain lesions. The disease relapses as the number and scope of the lesions increase. Thus far, no drugs have proved effective [7]. Animal models could provide important insights into this disease and could be utilised to study the pathogenesis and treatment of these disorders [15].

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Disclosure of conflict of interest

None.

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